The Proceedings of XXV World's Poultry Congress 2016
—— Invited Lecture Papers

WPC2016

THE XXV WORLD'S POULTRY CONGRESS

September 5–9, 2016
Beijing, China

The Proceedings of XXV World's Poultry Congress 2016 —— Invited Lecture Papers

Editors: Ning Yang, Ling Lian, Jiangxia Zheng,
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Hosted by the World's Poultry Science Association,
and organized under the auspices of the World's Poultry Science Association
and the Chinese Association of Animal Science and Veterinary Medicine.

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L1 Global poultry production: current state and future outlook and challenges

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Abstract

The paper presents the current situation of the global poultry sector and the future trends, and discusses the challenges the sector is facing, with particular emphasis on food security, poverty alleviation and equity, animal and human health, and natural resources and climate change.

Poultry is the fastest growing agricultural sub-sectors, especially in developing countries. Poultry makes a substantial contribution to food security and nutrition, providing energy, protein, and essential micro-nutrients to humans, with short production cycles and the ability to convert a wide range of agri-food by-products and wastes into meat and eggs edible by humans. Poultry also contribute to poverty alleviation. Poultry birds are a major asset-representing capital and in many cases, a source of income. They can be sold in times of crisis and act as household insurance.

The global poultry sector is expected to continue to grow, as demand for poultry meat and eggs is driven by growing population, rising incomes and urbanization. In this context, the sector is facing unprecedented challenges.

Particularly for small holders and poor people, both in rural and urban areas, poultry is key to poverty alleviation, providing income and market participation. While its role in nutrition is recognized, it also represents a threat to human health, especially as a vector of infectious diseases and because of its role in antimicrobial resistance.

Poultry has a significant impact on the environment and uses large amounts of natural resources. While the sector is usually seen as quite efficient in converting natural resources into edible products, it uses large amounts of land, water and nutrients for the production of feed materials. It also contributes to climate change, mainly through feed production.

Keywords: poultry; production systems; trends; projections; natural resource use; efficiency

Introduction

The world has over 23 billion poultry birds-about three per person on the planet (FAOSTAT, 2016), and about 5 times more than 50 years ago. They are kept and raised in a wide range of production systems, and provide mainly meat, eggs and manure for crop fertilization.

Poultry meat and eggs are among the most common animal source food consumed at global level, through a wide diversity of cultures, traditions and religions, making them key to food security and nutrition. Within the livestock sector, poultry emerges as the most efficient sub-sector in its use of natural resources and in providing protein to supply a global growing demand.

Poultry is at the same time particularly important for small holders and poor rural and urban population and a commodity produced in large scale and intensive operations, making it one of the fastest growing agricultural sub-sectors. While most of the sector's growth has been driven by private investments, public concerns about the sector's impact on the environment and human health, its contribution to climate change and local and global economy is triggering governments' response and the development of public policies.

Current state of global poultry production and production systems

Poultry production is very diverse. Factors to consider to characterize this diversity include the feed base, breeds, the orientation and the type of housing. At global level, 3 main types of poultry production systems can be considered: broilers, layers and backyard (Table 1).

Table 1. Poultry production systems (Gerber et al., 2013)

System	Housing	Characteristics
Broilers	Broilers assumed to be primarily loosely housed on litter, with automatic feed and water provision	Fully market-oriented; high capital input requirements (including Infrastructure, buildings, equipment); high level of overall flock productivity; purchased non-local feed or on farm intensively produced feed
Layers	Layers housed in a variety of cage, barn and free range systems, with automatic feed and water provision	Fully market-oriented; high capital input requirements (including infrastructure, buildings and equipment); high level of overall flock productivity; purchased non-local feed or on farm intensively produced feed
Back - yard	Simple housing using local wood, bamboo, clay, leaf material and handmade construction resources for supports (columns, rafters, roof frame) plus scrap wire netting walls and scrap iron for roof. When cages are used, these are made of local material or scrap wire	Animals producing meat and eggs for the owner and local market, living freely. Diet consists of swill and scavenging (20 to 40 percent) and locally-produced feeds (60 to 80 percent)

Global production of eggs reaches 73 million tons and global production of poultry meat is close to 100 million tons (GLEAM 2, 2016). Backyard systems contribute to 8% of global eggs production and 2% of global poultry meat production. The majority (92%) of poultry meat production comes from specialized broiler systems and layers only contribute to 6% of the total. But these global figures hide significant regional differences. Backyard systems make significant contribution to eggs and poultry meat production in Eastern Europe, South Asia, Sub-Saharan Africa and to a lesser extent in East Asia and Latin America and the Caribbean (Figure 1).

Poultry is one of the fastest growing agricultural sub-sector. Demand for animal source food is increasing because of population growth, rising income and urbanization, and poultry meat has shown the fastest trend in the last decades. The average annual growth rate over the last 5 decades was 5% while it was only 1.5% for beef, 3.1% for pork and 1.7% for small ruminants' meat (Alexandratos & Bruisma, 2012). Global per capita consumption of eggs increased from 4.55 kg to 8.92 kg between 1961 and 2010, while global per capita consumption of poultry meat increased from 2.88 kg to 14.13 kg (FAOSTAT, 2016). Production has been particularly dynamic in developing countries, especially in East and South East Asia (Figure 2), with an annual growth rate in poultry meat production of 7.4%. The biggest poultry meat producers are the United States, with almost 20 million tons a year, followed by China, with 18 million tons, the EU and Brazil with about 13 million tons

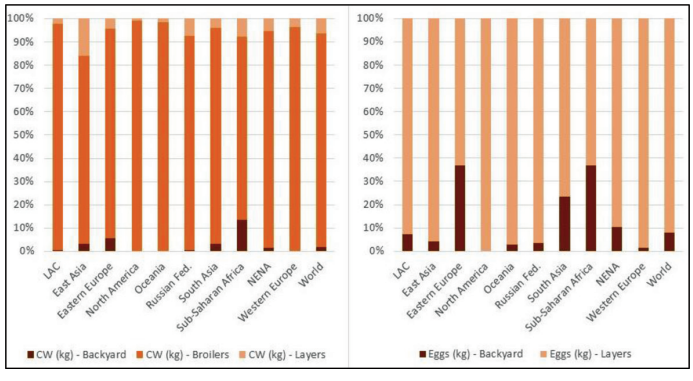

Figure 1. Eggs and poultry meat production by production systems and regions (GLEAM 2, 2016)

Technological changes in production practices were one of the main drivers of the sector's growth. The move from free-ranging to confined poultry operations dramatically increased the number of birds per farmer, facilitated the substitution of capital for labor, and led to a significant increase in labor pro-

ductivity (Narrod et al., 2012). For example, the same authors have shown that between 1985 and 1996, the share of poultry farms with more than 10,000 heads grew from 42% to 78% in the region Center West of Brazil. Advances in breeding to improve animal size, fecundity, growth rate and uniformity, have also contributed to increase outputs.

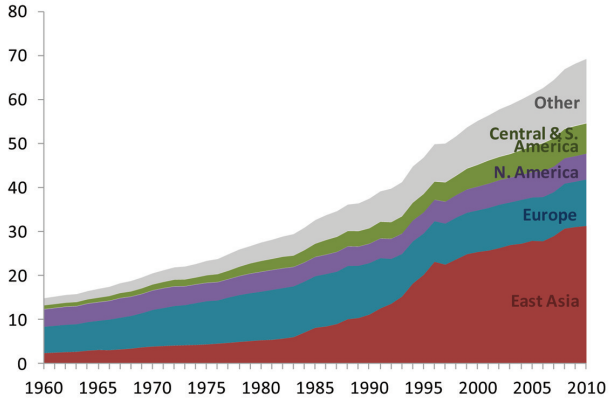

Figure 2. Global eggs production by region

Future trends

The growth of the global livestock sector is expected to continue. Global human population is estimated to reach 9.6 billion in 2050, with about 70% living in urban areas, while incomes could increase by 2% a year. In this context, Alexandratos and Bruisma (2012) projected that the demand for animal source food could grow by 70% between 2005 and 2050. While beef and pork demand could increase by 66% and 43% respectively, poultry meat is expected to have the highest growth, with 121%. Demand for eggs will increase by 65%. Global demand for eggs is also expected to keep on growing.

Still the fastest growing subsector, poultry meat production would however increase at a slower rate than in the past decades. By 2050, its annual growth rate is estimated to reach 1.8% at global level, and 2.4% in developing countries. This growth will be the result of strong regional differences.

Most of the growth will be driven by Asia. Average per capita consumption of poultry meat is still relatively low in Asia, with less than 10 kg per year, twice as less as in Western Europe and 5 times less than in Northern America, for example. OECD/FAO (2016) estimate that, in East and South Asia, meat production will expand by 1.8 Mt annually by 2025, with pork and poultry accounting for the bulk of this expansion.

In Sub-Saharan Africa, poultry consumption has expanded faster than any other meat and with domestic supply unable to match demand, almost 40% of the additional consumption was imported. For the period 2015-2025, OECD/FAO (2016) estimate that imports will supply 66% of the growth in poultry meat demand in the region, while this share will only be 16% for beef and veal, 2% for sheep and 45% for pork.

The same authors forecast a decrease in global meat prices in real terms due to a decrease in feed prices. Due to its short production cycles and high feed use efficiency, poultry is expected to experience an annual decrease in prices of nearly 1% between 2016 and 2025.

Globally 10% of meat output will be traded in 2025, up from 9% in 2015, with most of the increase coming from poultry meat.

Challenges

Food security

Using the same approach as Gerber et al. (2015), we can consider five main drivers of poultry's contribution to food security.

First, the feed ration of the animals, and whether the materials included in the ration are used or pro-

duced in concurrence with human edible food. This is for example the case of grains and fodder crop cultivated on arable land. On the contrary, crop residues, food by-products and swill produced on non-arable lands are not directly comestible by humans, although they could contribute to food production through fertilization and energy production. Figure 3 shows the estimated composition of the global feed ration of poultry. 58% of the global dry matter (DM) intake is from cereal grains, which represent a total of 348 million tones, or 14% of global cereal production. When adding cassava, soybeans and, pulses, rapeseed and soy oil, human edible feed material represent 64% of total feed intake.

Second, the efficiency with which the animal convert feed into edible products (kg of feed per kg of meat or eggs). This efficiency is driven by (i) the quality of the feed, (ii) the animal performances (e.g. growth rates, influenced by genetics and health conditions). Though more significant for ruminants, two more factors influence feed use efficiency: (iii) the proportion of meat supply from layers, since maintenance energy is diluted over the two products meat and eggs and (iv) the proportion of breeding stock in the herd (these animals need to be fed but do not contribute directly to the edible product output). Efficiency in poultry production systems is generally higher than in ruminant production. Layers and broilers require between 18.5 and 28.0 kg of DM feed to produce 1kg of protein, while backyard systems, which are less productive and use lower quality feed, require 81.4 kg in non OECD countries and 64.2 in OECD countries (Table 2). At global level, ruminants need an average of 134.8 kg per kg of protein. However, when only edible material are considered, layers and broilers systems appear less efficient than backyard systems or ruminants.

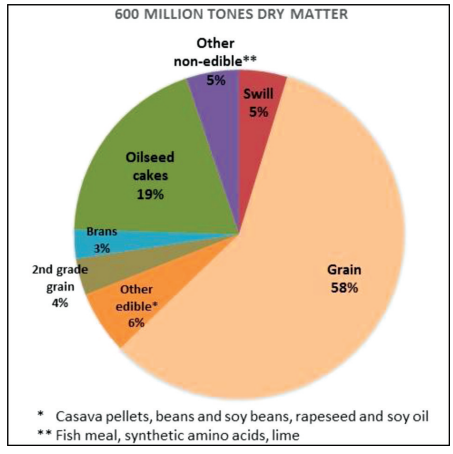

Figure 3. Global poultry feed ration (source: GLEAM 2, 2016)

Table 2. Feed conversion ratios by regions and production system (Mottet et al., 2016)

		Kg DM feed / kg protein product	Kg DM human-edible* feed / kg protein product
Non OECD	Backyard	81.4	2.6
	Layers	21.1	17.3
	Broilers	27.3	26.2
OECD	Backyard	64.2	0.1
	Layers	18.5	16.4
	Broilers	28.0	25.5
Ruminants		134.8	2,9

DM = dry matter
* Only from grains, pulses, roots & tuber

The global poultry sector has made significant gains in productivity. The average carcass weight increased by 30%, from 1.3 kg in 1961 to 1.66 kg in 2013 (FAOSTAT, 2016). The highest gains were made in South America (75%), Northern America (57%), Western Europe (33%) and Eastern Asia (32%). The highest gains in eggs production were made in East Asia (108%), Africa (75%), and Western Europe (59%).

Third, the contribution poultry makes to agricultural productivity through manure used in crop production. For example, in Europe, the share of animal manure input in total Nitrogen inputs was estimated at 38% and reaches 61% in the Netherlands (European Commission, 2012).

Fourth, the availability and affordability of other sources of foods and in particular protein and micro nutrients, and thus the exclusive or optional nature of eggs and poultry meat contribution to nutrition.

And fifth, the income generated by poultry production at household and national level. Today, an estimated 12% of total poultry meat and 4% of total eggs production are exported, with a few countries only (US, Brazil, EU and Thailand) generating substantial revenues (more than 85% of global exports). These share are still relatively low compared to other animal source food, such as beef (17%) or milk powder (over 50%). In parallel, least developed countries find themselves increasingly dependent on imports of poultry products: the share of imports in consumption increased from 2% in 1961 to over 28% in 2013. In 2013, least developed countries were net importers of nearly 1 million tons of fresh poultry meat (FAO, 2015). While poultry is key to poverty alleviation for small holders, least developed countries are becoming every year more dependent on imports to supply their increasing demand in poultry products.

Poverty alleviation and equity

There are 900 million poor people worldwide, living on less than US$1.9/day (World Bank, 2015). About half of them depend directly on livestock for their livelihoods. To poor people, farm animals are a major asset-representing both capital and, in many cases, a source of income. Livestock, which can be sold in times of crisis, act as household insurance. On the farm, poultry provide fertilization in addition to meat and eggs. Because of their short production cycle and their ability to convert swill and households wastes into edible products, poultry animals have a particularly important role to play for small holders (FAO-AGAL, 2016). They can contribute to three major pathways out of poverty by: (1) increasing resilience (2) improving smallholder productivity and (3) increasing market participation (ILRI, 2008).

Some two thirds of poor livestock keepers-290 million-are estimated to be women. They are largely involved in caring for small ruminants, poultry and dairy cows. But labor statistics may underestimate their role. That is because women are less likely than men to define their activities as work and less likely to report themselves as engaged in livestock management-while working, on average, longer hours than men. Despite women's major role in animal production and marketing, especially with poultry, however, they have less access to resources, land, and capital in particular. In order to help achieve gender equality in agricultural populations, priority should be given to improving the conditions of women working in the livestock sector (FAO-AGAL, 2016).

Animal and human health

Animal-source foods are important to nutrition and health, especially for children and pregnant women and for the elderly. They can help reduce mortality among children and the newborn. Animal source foods provide a wide range of micronutrients-such as vitamin A, vitamin B-12, riboflavin, calcium, iron and zinc-which are difficult to obtain in adequate quantities from plants source alone (Murphy & Allen, 2003). But livestock can also represent a threat to human health, and poultry has a significant role in this threat, given the intensity and frequency of contacts between humans and birds. A majority of recent pandemics such as H5N1, or "avian influenza", since 1997, are of animal origin. Of the known animal diseases, 61% are zoonotic, meaning that they can also infect humans (IFAH, 2012). Disease transmission between animals and humans occurs daily around the globe, both through agricultural practices and everyday activities. Again, as the main consumer of antibiotics (mostly used to speed growth), the livestock sector is a major contributor to global Antimicrobial Resistance (AMR)-a rapidly emerging threat to human health. Nonetheless anti-microbial consumption is expected to rise by almost 70% by 2030. Farm animals are also among the sources of some of the most severe but neglected tropical diseases

while, in economic terms, livestock diseases cause huge losses every year.

Animal welfare is also a significant challenge for the global poultry sector. For example, it is estimated that 61% of egg production come from industrial systems (Steinfeld et al., 2006) and around 60% of hens are kept in cages in the EU, with high frequency of beak trimmings.

Increasing international trade results in growing concerns about food safety. They in turn can translate into requirements to comply with standards and regulations (Narrod et al., 2012), which can represent a challenge for small producers and slow down market integration.

In order to increase livestock's positive contribution to human health, and reduce their negative impact, animal health should be made a priority in public policies, and a One Health approach, recognizing that the health of humans is connected to the health of animals and the environment, should be adopted (FAO-AGAL, 2016).

Natural resources and climate change

Feed production is the main activity through which poultry use land and water resources (Steinfeld et al., 2006). Land requirements per unit of edible product varies significantly between regions and production systems. Poultry production is the sub-sector that requires the most land for cereal production with an estimated 93 million ha in 2010 (Mottet et al., 2016), including 74 million in non-OECD countries and 19 million ha in OECD countries. This represent 44% of the total cereal area required by the global livestock sector. The area necessary to produce oil seed cakes fed to poultry represent 16 million ha, when land is allocated to the different co-products of the crops (oil and cakes).

Mekonnen & Hoekstra (2012) estimated that poultry needed an average of 4325 m3 of water per ton of meat and 3265 m3 per ton of eggs, which account for 11% and 7% of the total water footprint of animal production. The water footprint of broilers and layers are the lowest average among animal products. These estimations of the water footprint include different categories of water-blue water (diverted from surface and groundwater), green water (rainwater evaporated from soil and plants) and grey water (needed to assimilate the load of pollutants).

Nitrogen use efficiency, i.e. the percentage of nitrogen input retained in products, is relatively higher in poultry production that in ruminants. It is however very different between production systems and regions, and show some differences even within systems and regions. It ranges from 5% in backyard production in some countries in East Asia and Sub-Saharan Africa to 50% in certain broiler systems in Western Europe. None retained nitrogen is excreted as urine or faeces. Part of this manure recycled as it fertilizes pastures and crops but a large share is lost to the environment, as gaseous emissions and leaching, and contributes to air and water pollution and climate change.

Poultry contributes to climate change by emitting greenhouse gases (GHG) either directly (e.g. from manure management) or indirectly (e.g. from feed-production activities, conversion of forest into croplands). Based on a Life-Cycle Assessment approach (GLEAM 2.0), it is estimated that poultry supply chains emits about 836 million tons of CO_2 equivalent, about 11% of the total GHG emissions from livestock supply chains. Poultry is the smallest contributor to the global livestock sector's emissions.

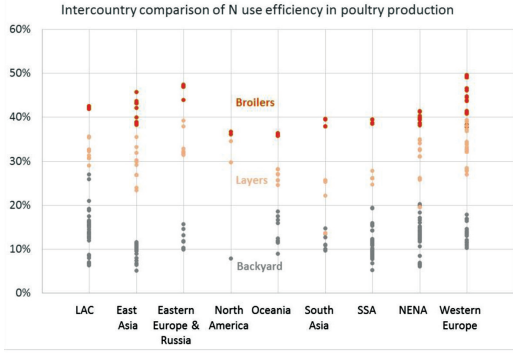

Figure 4. Intercountry comparison of Nitrogen use efficiency in poultry production (GLEAM 2.0)

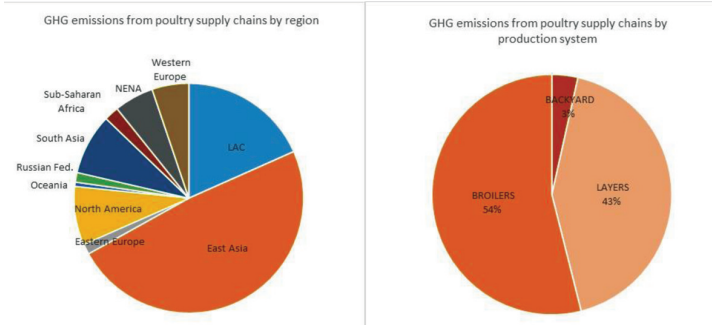

Figure 5. Greenhouse gas emissions from poultry supply chains (source: GLEAM 2.0)

When emissions are expressed on a per protein basis, poultry is the commodity with the lowest emission intensity (amount of GHGs emitted per unit of output produced), with an average of 40 kg CO_2-eq per kg of protein. Different agro-ecological conditions, farming practices and supply chain management explain the heterogeneity observed both within and across production systems, though the range of emission intensity is also smaller than for other species.

Manure management, energy used on farm and emissions from post farm processing and transport account for 38% of total poultry emissions. The rest of emissions result from feed production, including field work, fertilizer and manure application and land-use change for soybean and palm oil production, which account for 17% of total poultry emissions.

Poultry production uses an important area of land for the production of feed. This modifies many habitats. For example, the destruction of undisturbed habitats, as the conversion of the Amazonian rainforest to feed crops (soybean in particular) leads to important biodiversity losses. Increasing productivity and feed use efficiency in poultry production can contribute to reduce its negative impact on biodiversity by sparing land. Climate change, nutrient and pesticides pollution, to which poultry production contributes, are also important drivers of biodiversity losses.

Discussion and conclusion

The poultry sector needs to respond to the growing demand for meat and eggs and enhance its contribution to food security and nutrition. However, to be sustainable, it needs to consider its roles beyond just providing food. It needs to produce more with less, while benefiting all. It has a key role in providing secure livelihoods and economic opportunities for hundreds of millions of smallholder farmers. Enhancing this role requires a specific attention to market access. It needs to use natural resources efficiently, mitigate and adapt to climate change and reduce other environmental impacts. Finally it is necessary that the sector enhances human, animal, and environmental health and welfare.

Consequently, policies need to be designed in a holistic manner to best reconcile the various demands concerning productivity, sustainability and societal values. They should be tailored to regional/national specificities. They should not only consider the goods and services provided by the different production systems, their contribution to the economy and their environmental impacts but also producers' capacity to react and invest, the cost associated with reaching out to producers and monitoring change and the different entry points for public policies in the different production systems. Given the complexity of the challenges and the diversity of actors involved, multi-stakeholder initiatives have a key role to play in this process.

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L2 Future challenges and the need for poultry science research: a global perspective

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Keywords: poultry production, food security, animal well-being

Summary

Research over the past six decades resulted in dramatic increases in feed efficiency, product yield, and animal health in poultry. However, many challenges face global production in the coming decades. Estimates predict that global food production must double by 2050. Simultaneously, global warming will likely affect food production in many regions. Demand for antibiotic-free poultry products and concerns about poultry welfare are increasing. Approaches to address these concerns have implications on food safety. While poultry production is becoming more globalized, consumer preferences vary regionally. These issues present significant challenges that warrant equally significant investment in poultry science research worldwide.

Main text

Poultry products are the largest source of animal protein in the human diet worldwide. Consumption of poultry meat and eggs continues to increase globally, as the world population grows and the standard of living continues to increase in some regions of the world. Globally, per capita consumption of poultry meat is estimated to have increased from 2.7 kg/person in 1960 to 13.6 kg/person today. While consumption of chicken eggs has decreased in some Western countries, egg consumption has increased globally, particularly in developing countries. As the world population continues to grow and the standard of living increases in some parts of the world, particularly in Asia, increased demand for poultry products is likely to continue for the foreseeable future.

This increase in consumption of poultry products has resulted, in part, from reduced costs of poultry products due to increased production efficiency and increased consumer demand for lean meat. Feed efficiency of broiler chickens has increased substantially over the past 50 years (U.S. Broiler performance). In 1960, commercial broiler lines required 2.5 kg of feed for each kg of body weight gain. Today, that number is 1.6 kg of feed per kg of body weight gain. During this same period of time, body weight has increased from 1.5 kg to 2.9 kg, and the number of days required to reach market weight has decreased from 63 to 42 days of age (U.S. Broiler performance). Similar improvements in laying hen performance have also occurred (Pelletier, et al., 2014). In 1960, commercial laying hens required 3.4 kg of feed to produce 1 kg of eggs. Today, commercial laying hens require 1.9 kg of feed to produce 1 kg of eggs. Continued improvements in poultry efficiency are necessary to meet expected increases in consumer demand for poultry products in the coming decades and in the face of limited resources such as useable land available for crop production for animal feed.

Research over the past six decades resulted in dramatic increases in feed efficiency and product yield in poultry. These improvements have resulted primarily from research in the disciplines of poultry genetics and poultry nutrition. In the classic comparison of the 1957 broiler with the 2001 broiler raised on diets typical for the same years, it was estimated that 85-90% of the increase in body weight was due to genetic selection, while 10-15% was due to improvements in diet formulation (Havenstein, et al., 2003). Much of the effort in genetic selection of broiler chickens in the past has focused on growth and feed efficiency. However, genetics could be applied in the future to address well-being issues such as leg problems and animal health (Gonzalez-Ceron, et al., 2015; Swaggerty, et al., 2015). Research in poultry processing and products has decreased waste and increased stability of poultry meat. Research in the areas of poultry management and physiology have increased performance and reduced mortality. The use of vaccines and antibiotics have decreased mortality and improved performance substantially. In short, research in many disciplines over the past 50 years has resulted in dramatic increases in poultry efficiency and yield that have improved performance and reduced costs of poultry production. However, the need for poultry research is not finished.

Many challenges face poultry production globally in the coming decades. Estimates predict that global food

production must double by 2050 (Alexandratos and Bruinsma, 2012; Alexandratos, et al., 2006). This increase in food production cannot be restricted to countries that currently produce an abundant food supply. For practical, social, and political reasons, food production must increase worldwide, especially in regions with the greatest anticipated increases in population and standard of living. While it would be easily conceivable to double food production in some regions of the world, other regions are already failing to produce sufficient food for their current populations. Increasing food production in general and poultry production in particular in these regions will undoubtedly require future research on best management practices, best poultry breeds, best diets, etc., that are optimal for poultry production in these regions that are already under-producing food. Best management practices and environmental controls used in much of North America and Europe are not practical in all regions of the world where increases in the human population are predicted. Applied research aimed at maximizing poultry production within practical limitations in these regions is needed, as is increased investment in cooperative research projects between developed and under-developed nations. These research efforts should take into consideration the practical, social, and political limitations specific to each country in which increased poultry production is predicted to be most needed in the future. Success most likely will be achieved by teams of scholars working in multiple disciplines, including management, genetics, nutrition, physiology, animal health, economics, and sociology.

Concurrent with increased demand for poultry products, global warming will likely affect food production in many regions. Predicted effects of climate change include increased peak and average annual temperatures. The Intergovernmental Panel on Climate Change predicts an average temperature rise of 1.5 to 6°C over the next 100 years (Intergovernmental-Panel-on-Climate-Change, 2007). In addition to hotter average temperatures, increased frequencies of spikes in temperature (heat waves) are also predicted. These predicted changes in environmental temperatures will have ramifications on animal health, production and welfare. Heat stress in chickens can occur when temperatures reach more than 35° C. Notable effects of heat stress include increased mortality rate and decreased egg production, feed intake, and growth by the birds that survive (Mignon-Grasteau, et al., 2015). Increased ambient temperatures can severely affect poultry production, because high temperatures decrease feed consumption, which in turn decreases animal growth and egg production. The most common management approach to avoid mortality from heat stress is to increase ventilation and utilize misters or evaporative cooling pads in production houses. However, this is not always effective, especially during sudden and severe heat waves. In 2006, a heat wave in California resulted in the death of approximately 700,000 chickens and 160,000 turkeys (Severe california heatwave kills people, livestock and crops., 2006). In North Carolina in 2011, about 50,000 chickens died on a single farm after the power went off for less than one hour, stopping ventilation through the broiler house (Heat wave kills thousands of poultry, 2011). That same summer, more than 250,000 chickens died from heat stress during a heat wave in Arkansas (Chicken growers struggle to fight summer heat, 2011). These incidences of high mortality due to heat waves occurred in modern, tunnel-ventilated houses. The problem is not restricted to the United States. In 2003, more than 1 million chickens died during a heat wave in France (Heatwave kills 1 million french chickens, 2003). In 2012, more than 780,000 chickens died in South Korea due to heat stress caused by a heat wave (Record heat wave kills over 830,000 farm animals, 2012). In 2015, a heat wave killed more than 17 million birds in India (Indian chicken prices surge to record as heat wave kills millions of birds, 2015). In addition to the financial costs associated with loss due to morbidity and mortality during heat waves, heat stress in commercial poultry operations represents a serious issue of animal well-being. Further research is needed on approaches to mitigate the effects of heat stress, such as early life thermal conditioning (Loyau, et al., 2015).

Demand for antibiotic-free poultry products and concerns about poultry welfare are increasing. Public concerns about the welfare of laying hens has led to regulations governing housing of hens, and recent research has focused on effects of using conventional cages, enriched colony cages, and cage- free aviary housing (Heerkens, et al., 2015; Stadig, et al., 2016; van Asselt, et al., 2015; Zhao, et al., 2015b). Increased movement of birds in enriched caging and aviary systems can lead to improved bone strength and reduced lameness (Heerkens, et al., 2016; Regmi, et al., 2016; Yilmaz Dikmen, et al., 2016). However, enriched colony caging and cage- free aviary housing systems have been estimated to increase costs for egg production by 13% and 25%, respectively, over conventional caging (Matthews and Sumner, 2015), and air quality in aviary systems has been called into question (Shepherd, et al., 2015; Zhao, et al., 2015a; Zhao, et al., 2016). Furthermore, enriched colony caging and cage-free aviary housing systems can affect internal colonization of hens with pathogens and produce eggs with higher levels of coliform, *Salmonella* and *Campylobacter* contamination (Gast, et al., 2016; Jones, et al., 2015; Jones, et al., 2016). Therefore, approaches to address ethical concerns about how animals are raised for food have implications on food safety. However, the persistence of differences between conventional and enriched caging systems has been called into question (Gast, et al., 2015).

Public concerns about the use of antibiotics in poultry production have resulted in much investigation into the use of alternatives to antibiotics for improving animal health and performance, including vaccinations, probiotics and natural feed additives (Cengiz, et al., 2015; Diarra and Malouin, 2014; Diaz-Sanchez, et al., 2015; Gaucher, et al., 2015; Huff, et al., 2015; Park, et al., 2016; Saint-Cyr, et al., 2016). Results have been mixed. Probiotics and natural feed additives can potentially provide effective alternatives to antibiotics for increasing performance and improving animal health (Abdelrahman, et al., 2014; Cengiz, Koksal, Tatli, Sevim, Ahsan, Uner, Ulutas, Beyaz, Buyukyoruk, Yakan and Onol, 2015; Gracia, et al., 2016; Guyard-Nicodeme, et al., 2016; Huff, Huff, Rath, El-Gohary, Zhou and Shini, 2015; Park, Jeong, Lee and Kim, 2016; Upadhaya, et al., 2016; Wideman, et al., 2015). However, while performance and animal health in systems using alternative approaches is superior to the use of no treatment, drug-free programs do not consistently perform as well as programs using antibiotics (Gaucher, Quessy, Letellier, Arsenault and Boulianne, 2015). Therefore, research must continue on alternative approaches to improving performance and animal health in the absence of antibiotics.

The issues discussed above represent substantial challenges to poultry production that warrant equally substantial investment in poultry science research worldwide. Regional differences in climate, available feedstuffs, public concerns, and consumer preferences should be considered. A model that works well for poultry production in one country will likely not be the best model for all countries. Regional differences in availability and quality of feed ingredients will require research on diet formulations for feedstuffs available in each region. Regional differences in management practices and availability of affordable poultry housing will likely persist, and these differences will require research on best management practices for each type of region. Poultry breeds selected for best performance on one type of diet under one set of conditions will likely not perform best on other diets or under other conditions. Research will be required on genetic selection to maximize performance on different diets and under different conditions, if we are going to increase poultry production worldwide, including in underdeveloped countries. Environmental conditions differ around the world, and climate changes are predicted in the coming decades. Research will be required on the responses of different poultry species and different genetic lines to these existing and anticipated differences in environmental conditions that will undoubtedly affect poultry production. Local consumer preferences will affect market demand for poultry products, which might include local breeds of poultry. Research aimed at optimizing production to meet these demands will be required. Removal of antibiotics from feed and raising poultry under conditions that are ethically acceptable to the public in different countries will require research aimed at optimizing production under these conditions while maintaining the health of the birds and the safety of the resulting food products. Alternatives to antibiotics and alternative housing will likely have to be identified, if we are going to be able to increase poultry production worldwide. It has been predicted that we will have to double food production by the year 2050, and increased production of poultry will almost certainly have to occur to meet this demand. We should not assume that one model of production will work everywhere or that the same poultry breeds will perform optimally in all environments or meet the consumer expectations of all nations. Poultry science research over the past 50 years has resulted in amazing improvements in production efficiency, product yield and quality, and animal health. Equivalent investment in poultry research during the coming decades will be required in order to meet consumer demand and supply our growing population with high quality animal protein for human consumption.

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L3 The current situations and control strategies of avian influenza,Newcastle disease and infectious bronchitis in poultry in China

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The rapid development and growth in poultry industry in China in the past two decades have brought about several big challenges, one of which is the poultry disease problem. The inherited structural features of the poultry industry greatly affect the disease management. In this review we will discuss three most important diseases for poultry industry in China: the Asian H5N1 highly pathogenic avian influenza (HPAI), Newcastle disease (ND) and infectious bronchitis (IB). The epidemiology features and the pathogen evolution, the control strategies and vaccine development for each of these diseases will be addressed.

H5N1 HPAI

Although a number of subtypes of avian influenza viruses (AIVs) including H1, H3, H4, H5, H6, H7, H9, H10, and H11 have been isolated from poultry flocks in China in the past two decades, the H5N1 highly pathogenic avian influenza (HPAI) has the most important economic impact to poultry industry and imposes great threat to public health [3, 6, 9]. This Asian lineage H5N1 HPAI virus has spread to more than 63 countries in Asia, Europe and Africa since its first emergence in 1996 in Guangdong province, China [1, 3, 22, 24, 31] while its most recent descendants of H5N8 viruses have transmitted to more than 10 countries in Asia, Europe and North America since 2014 and generated novel reassortant H5N2 viruses in North America [3, 13, 21]. The Asian lineage H5 HPAI has become enzootic and persists more than ten years in poultry flocks in several countries including China [9, 23, 24].

Epidemiology features and virus evolution

The Asian lineage H5N1 HPAI virus was first detected from diseased geese in Guangdong Province in 1996 [31], and has been repeatedly detected in poultry in Southern China since then[3]. Up to Jan. 2016, nearly 200 outbreaks have been reported in more than 20 provinces covering most major poultry raising areas of the country and the H5N1 virus has become enzootic in poultry and wild birds since 2004 (http://www.oie.int/animal-health-in-the-world/update-on-avian-influenza/2016/). Vaccination has been an important component in the control strategy against H5N1 HPAI in poultry in China since 2004 [3, 9, 23, 24]. The H5N1 HPAI in China shares the following epidemiology features associated with the inherited structural and trading characteristics of the poultry industry. Firstly, outbreaks mainly occur in small-scale farms and backyard level raisings which are very popular and in huge number, because their vaccination coverage and biosecurity measures are relatively poor when compared with the medium to large scale operations [3]. Secondly, the domestic waterfowl which are in huge quantity (3.8 to 4.0 billion) and largely in free range, play a very important role in the transmission and maintenance of H5N1 HPAI in poultry since both ducks and geese usually demonstrate much lower immune response to vaccination and are less protected when compared with chickens [3]. In addition, almost all strains of Asian lineage H5N1 HPAI virus are highly pathogenic to chickens, while they vary greatly in virulence to ducks and geese, from avirulent to very virulent [3, 5, 8, 33]. So, domestic waterfowl can be asymptomatic carriers for some H5N1 viruses which are nonpathogenic for waterfowl but highly pathogenic for chickens. They can also be a good reservoir for AIVs to generate novel viruses by reassortment [5, 33]. Thirdly, mass trading in live poultry markets (LPMs), either wholesales or retails, which are very popular and scattered all over the major production areas contribute greatly to the persistence of H5 HPAI in poultry as evidenced by the fact that the virus detection rate in LPMs is much higher than on farms[3, 33]. Fourthly, outbreaks in vaccinated poultry flocks are mostly associated with the antigenic drift of the circulating field viruses from vaccine strains which become less protective as the former are constantly evolving [3, 5, 9, 23, 26, 29].

The HA gene of Asian lineage H5N1 virus changes much more rapidly in endemic area with selective pressures than that of any other subtypes of AIV in poultry [3, 24]. This genetic change is correlated with an-

tigenic drift [23]. To address all the genetic changes of the H5 gene, a revised nomenclature system, the clade system was proposed [26-29]. Each clade or subclade is at least 2% different in nucleotide sequence. The H5 virus has evolved into 10 clades (0-9) in China since 1996. As the time passed, some of the clades wan out while others persist and continue to evolve into subclades of different order [26-29]. The prevalent clades or subclades are dynamic scenarios that vary from year to year and from area to area. In the past five years, H5 viruses of fourth order subclades 2.3.2.1 and 2.3.4.4 and second order subclade 7.2 circulated concomitantly in poultry flocks in China while the prevailing one varied with time [29]. On the other hand, the H5N1 virus has reassorted with other subtypes of AIV to have generated a number of novel AIVs which bear the Asian lineage H5 HA gene but have different NA subtypes and internal gene constellation since 2008 [5, 9, 33]. Therefore, H5N2, H5N5, H5N6 and H5N8 subtypes HPAIVs bearing different subclades of Asian lineage H5 gene in addition to original H5N1 virus were frequently isolated from poultry in China in the past five years (10). Thus, the H5 HPAI outbreaks in China are very complicated: not only induced by H5N1 viruses of different subclades but also induced by H5 viruses bearing different subtypes of NA gene.

The control strategies and vaccine development

Because of the inherited structure features of the poultry production and the trading chain together with the insufficient veterinary service resources in China, the initial efforts targeted for the immediate eradication of H5N1 HPAI by culling infected and contact birds and an emergency vaccination program in buffer zones of the outbreak areas in 2004 proved to be unsuccessful [3, 23, 24]. Soon after, the control strategy was replaced by a compulsory routine (mass) vaccination program for all poultry in the country combined with biosecurity, surveillance and culling of infected birds and movement restriction, which has been lasted more than ten years ever since (http://www.oie.int/animal-health-in-the-world/update-on-avian-influenza/2004/).

A number of vaccines against H5N1 HPAI, either inactivated or live vectored, have been developed and used in China since 2004 [3, 23, 24]. For the inactivated vaccines, we have A/turkey/England/N-28/73 based H5N2 vaccine and recombinant Asian H5N1 vaccine of clade 0 (Re-1) and its updated versions for matching the prevailing clades with time from clade 7 (Re-4), clade 2.3.4 (Re-5), clade 2.3.2.1 (Re-6), clade 7.2 (Re-7) to clade 2.3.4.4 (Re-8) (www.ivdc.org.cn). For live virus vector vaccines, fowlpox virus vectored and NDV vectored vaccines have been in use since 2005 and 2006 respectively and the DEV (duck enteritis virus) vectored vaccine is in the process of licensing. A DNA vaccine (pCAGGopti-HA) was also licensed. These vaccines played a role in reducing the morbidity and mortality of the clinical disease and virus shedding of the infected animals but do not eliminate the virus while vaccination failure occurs frequently in different conditions. According to Swayne [23, 24], China accounted for 90.9% of the total vaccine used in the world for HPAI, in hundreds billions doses during 2002 to 2010. Experience in China emphasizes that vaccination alone is not enough to control the H5N1 HPAI, neither to eliminate the virus nor to reduce its evolutionary rate.

Perspectives

The final goal of the H5 HPAI control program in China is the eradication of the disease nationwide. The vaccination of poultry in the present control strategy against the disease is a double-bladed sword because of its negative aspects such as the potential for silent infections and subclinical shedding of the virus, difficulty in identifying infections in a vaccinated population, the high cost of vaccines and their labor-intensive administration, the delay in protection for at least seven to 14 days after administration and trade restrictions imposed by importing countries [23]. It is time for China to develop practicable vaccine exit strategies based on risk analysis of the present production system in poultry after more than ten years implementation of the compulsory vaccination program. We have a long way to go to reach the final goal of H5 HPAI eradication in China.

ND

ND is a devastating disease in poultry caused by the virulent Newcastle disease virus (NDV) as defined by OIE (http://www.oie.int/en/international-standard-setting/terrestrial-code/). The first report suspected of ND outbreak in China can be traced to late 1920s while the first ND cases in village chickens con-

firmed by virus isolation and identification occurred in 1946 [15]. ND has become one of the most important poultry diseases since the development and commercialization of poultry industry in 1970s. Intensive vaccination programs have been implemented to all commercial poultry sector since 1980 [17, 18].

Epidemiology features and virus evolution

ND is enzootic in most major poultry raising areas of the country. ND in China has the following epidemiology features. 1) Most outbreaks in vaccinated flocks are so-called atypical form of ND with low mortality and milder symptoms such as egg-drop, dilute droppings, and some respiratory signs while outbreaks in non-vaccinated backyard and village flocks are classic form with high mortality and morbidity [17, 18]. 2) Along with the emergence of genotype VII virus, ND has expanded its host range, not only pathogenic to chickens but also to domestic waterfowl, especially to geese since late 1990s [17, 30]. Just as for avian influenza viruses, domestic waterfowl are also a good reservoir for avian paramyxoviruses including NDV and play important role in their evolution. NDV, especially genotype VII can be transmitted mutually between chicken flocks and waterfowl flocks which are mostly in free range, so that the ND epidemiology in China is very complicated [17, 18, 25, 30]. 3) The phylogenetic and antigenic differences between vaccine strains and epidemic field viruses contribute greatly to the persistence of the field virus in intensively immunized poultry flocks as evidenced by the vaccination-challenge test [10].

The genotype VII NDV which induces more severe lesions in lymphoid organs than early virulent viruses has become dominant in poultry in China since late 1990s [12, 17, 18, 28]. According to the surveillance data in recent years in China, over 95% of the virulent NDVs isolated from both chickens and domestic waterfowl are genotype VIId while most strains isolated from pigeons and doves are genotype VIb [17, 18, 28]. Genotype VIId NDVs both from geese or chickens sharing high sequence homology, characteristic amino acid substitutions and very similar pathobiological features can infect both species and cause disease equally and no host-associated genetic phenotypic characteristics are found between them [25]. On the other hand, the most extensively used vaccine strain for both inactivated and live ND vaccines is La Sota which belongs to genotype II virus isolated in the US in the mid 1940s. The differences in sequence identity of F and HN genes between genotype VIId and La Sota are 84.1%～84.4% and 84.1%～84.4% respectively (10). The antigenic differences in F and HN epitopes are correlated to the corresponding genetic differences in sequence identity (10). Laboratory researchers and field practitioners argue that the La Sota vaccine does not provide full protection against the challenge of epidemic field strains, especially in terms of reducing virus shedding although NDV has only single serotype [10, 18, 19, 20].

The control strategies and vaccine development

Vaccination is an important component for the control of ND in China for both small scale production units with poor biosecurity and medium- to large- scale operations with high level of biosecurity [17]. There are indications that ND outbreaks still occur despite the fact that most of the commercial farms in poultry industry in China implement an intensive vaccination program with high frequency and high antibody titers against ND [17, 18, 30]. These situations suggest that it is the antigenic variation between vaccine strain and prevalent strain rather than poor flock immunity due to inadequate vaccination practices that may be responsible for outbreaks and spreading of virulent NDV field strains. Indeed, it has been shown that the extent of homology between vaccine and challenge strains is a critical factor in reducing the shedding of virulent virus [10, 18, 19, 20].

To improve the antigenic match between vaccine and currently circulating virulent strains in China, a genotype VII-matched vaccine (A-VII strain) based on reverse genetics has been developed and licensed in use since 2014 [10, 11]. Laboratory studies and field trials showed that this vaccine not only induces significantly higher (2-3 $\log_2$ HI titer) and quicker (one week) immune response but also significantly reduces the virus shedding (by 10-100 folds) after circulating genotype VIId virus challenge when compared with the mismatched conventional La Sota vaccine [10, 11]. The application of this novel matched vaccine proved to be useful in optimizing the ND vaccination schedule and reducing the vaccination frequency in commercial poultry flocks in China.

Perspectives

As for the H5 HPAI control, the final goal for ND control in China is also its eradication nationwide.

The present strategy for ND control in China mainly relies on the vaccination program which only reduces the clinical disease but by no means eliminates the virus. ND and H5 HPAI are the two most important poultry diseases which share similar epidemiology features and control strategies in China. It is time for China to develop a combined eradication program of both H5 HPAI and ND and work out the practicable strategies step by step from disease free with vaccination to disease free without vaccination.

IB

The first outbreaks of IB in poultry in China confirmed by virus isolation and identification were in late 1970s and early 1980s although suspected diagnostic reports were much earlier [32]. As in most other countries, IB is also one of the major diseases causing heavy economic losses in either vaccinated or unvaccinated poultry flocks in China [4, 7].

Epidemiology features and virus evolution

In China, IB always induced heavy losses when it accompanies with secondary bacterial infection or co-infection with other viral pathogens, especially H9N2 low pathogenicity avian influenza virus and some immunosuppressive viruses [7, 16]. IB itself manifests diversified clinicopathological patterns involving the respiratory system (inflammation of the trachea, bronchi, lungs and air sacs), renal systems (kidney damage) and/or the female reproductive tract (egg production drops and false layers) [4]. On the other hand, the IB virus (IBV) evolves continuously to generate new variants by random mutation and genetic recombination. Therefore, IB frequently occurs in some intensively vaccinated flocks. More than 15 genotypes/serotypes of IBVs isolated in China have been identified in the last two decades while the variant QX-like (LX4-type) maintains the dominant state [7]. According to Han et al. [7] and Zhang et al. (Zhang et al, personal communication), 54.1% (119/220) of the isolates during 1995-2009 and 74.5% (105/141) of the isolates during 2010-2015 are genotype/serotype QX-like whereas the average nucleotide and amino acid sequence identities of S1 between QX-like strains and the most extensively used Mass-type vaccine strains are less than 77% and 78% respectively. The minor genotypes/serotypes circulating in recent years include LSC/99I, LDT3/03, TW-1, TW-2, HN-08, Mass and 4/91 each of which accounts for only a small percentage less than 10. It is worth mentioning that China had never 4/91 type IBV strains before the unauthorized introductions of the 4/91 vaccine which contributed to the emergence of this type variant in the field [7].

The control strategies and vaccine development

Effective control of IB is not possible without vaccination although improved biosecurity and high standard management are essential. Currently we have three types of IB vaccines in use: licensed Mass type (H120, H52, Ma5, 28/86 and W93) and LDT3/03 type (strain LDT3-A) and unauthorized 4/91 type. Single Mass type or 4/91 type vaccine is less protective to the dominant QX-like field virus since the S1 gene sequence similarity between them is less than 78%. LDT3/03 type vaccine which was licensed recently is better than the Mass or 4/91 type vaccine while there is still room for improvement since the S1 gene sequence similarity between LDT3-A and QX-like is only 86%. More recently, a QX type vaccine with promising features in safety and efficacy is in the process of licensing (34). Laboratory experiments and field trials showed that this vaccine is very efficacious against the challenge of QX-like field viruses.

Perspectives

Licensing new vaccines matching serotypically and genotypically with the dominant variant types in addition to enhancing biosecurity and reducing co-infection and secondary infection will be conducive to the control of IB in China. The use of unauthorized vaccines should be banned to reduce the risks of generating new variants by recombination from vaccine strain and field viruses.

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L4 Update of non−antibiotic era in EU, new model of poultry production

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Summary

The overuse of antibiotics has created resistant strains of deadly bacteria (Nosocomial problems). The World Health Organization (WHO) is concerned with Antibiotic Resistance Genes (ARG). The main goal of WHO is to reduce the consumption of antibiotics without therapeutic prescription and also to improve the monitoring of antibiotic resistance.

The European Union has banned the use of antibiotic growth promoters (AGP) in 2006. Avilamycin, Avoparcin, Bacitracin, Flavomycin, Spiramycin, Tylosin and Virginiamycin are no longer in use for animal feeding in Europe as feed additives.

The ban of AGPs led to a reduction in antibiotic consumption. However, it is also known that the consumption of medicated feed, especially in piglets, is important. An important task has begun in order to find new substances or combinations of them with functionality as alternatives to AGP in practice, even before the ban of AGP. However, according to several revisions, none of the no-antibiotic AGP alternatives has shown to be a fully satisfactory replacer as AGPs.

Although the research should focus in discovering alternatives to growth promoters, it must be considered today that "All farms animals will experience some level of stress during lives". This means that animals will be under stressors which may influence the animal welfare conditions. Therefore new aspects or innovation dealing with the potential benefits provided by new substances or agents in animal feeding must be considered in order to promote a new model of poultry production based on good performance and better animal welfare conditions.

Main text

The overuse of antibiotics has created resistant strains of deadly bacteria (Nosocomial problems). The World Health Organization (WHO. 2014) is concerned with Antibiotic Resistance Genes (ARG). The main goal of WHO is to reduce the consumption of antibiotics without therapeutic prescription and also to improve the monitoring of antibiotic resistance.

The European Union territory banned the use of antibiotic growth promoters (AGP) in 2006. Avilamycin, Avoparcin, Bacitracin, Flavomycin, Spiramycin, Tylosin and Virginiamycin are no longer used in animal feeding in Europe as feed additive (EC 1831/2003).

Ban of AGPs led to a reduction in antibiotic consumption. No important consequences on the therapeutic use of antibiotics per animal have been detected in northern European countries except for piglets (DANMAP 2014). In other areas of Europe no reports have been produced. However, it is also known that the consumption of medicated feed especially in piglets is important. For instance, in southern European countries like Spain, Italy and France the volume of medicated feed reaches a percentage over the total production of feed higher than 4% (FCEC 2010). In contrast, poultry production has moved in the right direction.

Today one of the main challenges of animal production is to reduce the use of antibiotics in medicated feed. However, there are many difficulties to assess the problem because not all European countries have the same procedures. Therapeutic antibiotics through oral administration can be applied as medicated feed or through water or by top dressing/ mixing at the farm. UK, France, Italy, Poland, Portugal and Spain use medicated feed produced in the feed manufacturing plant. In other countries like Germany, Belgium or Denmark the most common route is top dressing/incorporation of ready-to-use products in the feed and mixing into water. In conclusion several routes of application are used today in the European Union. The replacement of antibiotics as medicated feed is not the only route of administration to consider in a monitoring process.

An important task has been initiated in order to find new substances or combinations with functionality as alternatives to AGP in practice, even before the ban of AGP. However, according to the several revisions avail-

able none of the no-antibiotic AGP alternatives has shown comparable effects as AGP (Huyghebaert, G. *et al.* 2011). The benefits of AGP according to recent research (Niewold, T. 2007) are based on the reduction of energy cost produced by the reduction of the immune reaction and consequently a non-antimicrobial concept is also accepted as new mode of action for AGP.

Still, the research should focus in discovering alternatives to growth promoters. Rostagno 2011 stated "All farms animals will experience some level of stress during lives". This means animals will be under stressors which may influence animal welfare conditions. Therefore new aspects or innovation involving the potential benefits provided by new substances such as enzymes, direct feed microbial (probiotics) and prebiotics in animal feeding must be important in order to improve the zootechnical performance as well the animal welfare conditions. In general one of the main objectives of the potential immunomodulatory additives should be the reduction of intestinal inflammation. An inflammatory response is typically accompanied by generation of free radicals, and the release of cytokines and nitric oxide. Inflammation is essential and is produced by macrophages, neutrophils and other cells responsible for innate immunity. However, systemic inflammation mediated by pro-inflammatory cytokines is responsible for decreased production performance in poultry and other animals. An inflammatory response decreases feed consumption and muscle protein accretion and mass while increasing metabolic rate, synthesis of acute phase proteins and liver mass (Klasing, 2007). Therefore, the elucidation of the immunological mechanisms which are relevant for the induction of tolerance and avoid gut inflammation is essential. All this background suggests that the study and control of immunological mechanisms involved with tolerance and homeostasis in chicken may be beneficial to fight against infections and stress to improve animal welfare. The approaches to drive the immune response towards anti-inflammatory and tolerance pathways should be accompanied by strategies to avoid pathogen persistence.

This presentation wants to update the process of AGP ban and how is enhancing the strategy for the reduction of antibiotics in poultry. The potential use of probiotics or prebiotics as alternatives to antibiotic application will be presented. This comparison is based on the concept of using pro- or prebiotics to modulate the gut microbiota and enhance animal health and growth. This balance should be measured considering the increased energy and nutrient cost to support the gut bacteria which could affect the needs for animals (de Lange et al. 2010). It should be noted that the probiotic effect is bacterial specific. According to Gong 2014, the selection of a novel probiotic is not only science, but also an art requiring a sophisticated design for selection and experience for handling. It will be also discussed why prevention using alternative products may be used under good conditions.

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L5 Nutrition for health of laying hens and egg quality

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Keywords: nutrition, laying hen, health, egg quality

Summary

The poultry egg industry is one of the fastest growing animal industries in the world and now facing new opportunities and challenges. Due to the demand for sustainable development and food safety, the egg industry has shifted towards improving healthy status of laying hens and egg quality. This paper reviews latest advances in poultry nutrition and relevant technologies in these areas.

Main text

Introduction

Poultry industry has made tremendous growth and brought a revolution in animal husbandry and food supply over the past decades. In 2013, the world egg production reached 68.26 MT, increased by 94.6% from 35.07 MT in 1990 (FAOSTAT, 2015). Traditional strategies in poultry nutrition have made vital contributions to the great growth in quantity. However, the productivity is not the only emphasis in the past. Now, the industry is facing new challenges, such as shortage supply of feed resources, environment pollution and other global issues. Societies and consumers are concerned about food safety and product quality. Although nutritional strategies alone could not address all these challenges, advances in poultry nutrition and development of modern biotechnology would provide some possible measures for developing more sustainable industry. Nutrition has been widely accepted as a strategy to influence health and diseases of laying hens. The maintenance of good health is an important prerequisite for improving productivity and egg quality. This paper reviews latest progresses in these areas, with a particular focus on dietary effects on healthy status of birds. In addition, the industry has a high expectation to produce egg products for meeting consumers' demands. Finally, diversified and special nutritional approaches that have been developed to minimize the incidence of egg defects and to improve nutritive values of eggs are also covered.

Nutrients and health of laying hens

Exploitation of nutrients with potentially health-enhancing benefits in laying hens

The impact of nutrition on health of animals can be reflected in immune stimulation, metabolic diseases, and illness arising from deficiencies of nutrients and harmful substances in diets. Recent focuses have been made on seeking some dietary components, which have potentially health-enhancing benefits. Some examples include vitamins, minerals or plant extracts could have protective effects against oxidative or heat stresses in laying hens. Much attention has been given to an exploitation of new bioactive substances, their efficiency and safety, and investigation of potentially physiological and biomedical mechanisms. Octacosanol [$HO\text{-}CH_2\text{-}(CH_2)_{26}CH_3$], for example, a long-chain aliphatic alcohol, is a main component of natural wax, and exists in wheat germ oil, rice bran oil, and fruits. Recent studies showed that dietary supplementation of octacosanol improved laying performance and egg quality during late stage of laying period. Its biological roles also include promoting the secretion of reproductive hormones and modulation of the development of reproductive organs in aged laying hens (Long et al., 2016). Another example is pyrroloquinoline quinone (PQQ), a newly discovered redox cofactor and a polyphenolic compound. PQQ disodium could be a potential antioxidant as vitamin E, and is effective against oxidized oil-related liver injury in laying hens. The protective effect of PQQ disodium on the liv-

er could be partially attributed to its ability of scavenging free radicals, inhibiting lipid peroxidation, and enhancing of antioxidant defense systems (Wang et al., 2016). These findings provide some examples for exploitation of novel feed additives potentially for layers.

Effects of non-traditional feed ingredients on healthy status of laying hens

The physiological responses of hens should be the primary concern in evaluation and use of non-traditional feed ingredients. The presence of anti-nutritional factors limits application of non-traditional feed resources to dietary formulation for laying hens. For example, free gossypol is a main anti-nutritional factor in cottonseed meal, and is toxic to the reproductive system, heart, and liver in monogastric animals (Nagalakshmi et al., 2007). However, when hens were fed diets containing low-gossypol cottonseed meal, the decreases in laying performance and egg quality were greater than birds fed diets containing an equivalent amount of low-gossypol (He et al., 2015), suggesting that there appears some other components that also contribute to adverse effects of cottonseed meal. Another examples is inappropriate replacement of soybean meal with rapeseed meal in diets for laying hens resulted in the negative effects on jejunum absorption and magnum secretory (Wang et al., 2015a). Thus, it is essential to know an optimally dietary inclusion level in the use of non-traditional feed ingredients. For *Moringa oleifera* leaf, dietary addition of 15% decreased egg production and induced histological lesions in the liver and kidneys (Lu et al., 2016), but dietary supplementation of less than 10% had no adverse effects on the health of the layers (Lu et al., 2016). These findings contribute to exploration of non-traditional ingredients as novel feeds for the poultry industry.

Nutrients and egg quality

Nutrients for eggshell quality and ultramicro structure

Egg quality is a big issue in the industry. Eggs with damaged shells account for 6-10% of all eggs produced worldwide, leading to a great economic loss (Roland, 1988). One major concern is a decrease of eggshell quality as the hen ages, and the incidence of cracked eggs can exceed 20% at the end of the laying period (Nys, 2001). Low efficiency of absorbing calcium in the gut and an unbanlanced increase of eggshell weight to egg weight in aged hens partly explain the deterioration in eggshell quality. The roles of calcium, phosphorus and vitamin D_3 in eggshell formation and function have been widely discussed. Trace elements, such as manganese, zinc and copper, could affect the mechanical properties of eggshells by influencing calcite crystal formation and modifying the crystallographic structure of eggshells (Mabe et al., 2003). A dietary manganese deficiency decreased eggshell thickness, leading to abnormal ultrastructure of the eggshell especially the morphology of mammillary knobs (Xiao et al., 2014). The deficiency could also inhibit the synthesis of glycosaminoglycan, the contents of which in eggshell membrane showed a strong correlation with breaking strength. In contrast, dietary supplementation of manganese improved eggshell quality, evidenced by the increments of breaking strength, thickness, and elastic modulus in response to dietary supplementation of 100 mg/kg of manganese (Xiao et al., 2014). The improvement in the mechanical properties of eggshell from manganese supplementation was not due to the increase in shell ratio, neither to a decrease in egg weight or production, rather it may be resulted from modification in shell ultrastructure (Xiao et al., 2014). Scanning electron photographs clearly showed that mammillary layer thickness and mammillary cone width decreased significantly by dietary supplementation of manganese (Xiao et al., 2014). A further study showed that dietary supplementation of either organic or inorganic manganese improved the eggshell mechanical properties and quality (Xiao et al., 2015). The bioefficacy of organic manganese is 357% (shell thickness), 406% (breaking strength), 458% (elastic modulus), and 470% (eggshell manganese), close to bioefficacy of inorganic manganese at equimolar levels (Xiao et al., 2015). Similarly, dietary supplementation of a combination of organic Mn, Zn, and Cu resulted in higher thickness of the palisade layer and lower mammillary density (Stefanello et al., 2013). Dietary supplementation of probiotics, organic acids and plant extracts also showed positive effects on eggshell quality, and increasing intestinal availability of Ca is one of mechanisms (Swiatkiewicz et al., 2012).

Of note, advances in analytical techniques, such as scanning and transmission electron microscopy and infrared spectroscopy, provide a possibility of deep understanding of the structure, morphology and

chemical compositions of the eggshell. The development of genomics, proteomics and metabolomics has also provide new approaches to identify key regulatory molecules in the process of eggshell formation. Further studies to understand the precise mechanisms of dietary actions on eggshell formation are needed.

Nutrients for egg albumen quality and proteomic profiles

Egg albumen (also known as egg white) accounts for about 58% of the entire egg mass, and egg albumen quality closely links to egg freshness. An observation of a watery, spread-out white usually indicates that the egg is stale when an egg broken onto a flat surface. Egg albumen quality is usually evaluated by the item of Haugh unit, which is based on the height of egg albumen.

Besides storage conditions and age of hens, egg albumen quality and proteomic profiles can be manipulated by dietary protein levels and sources. Reducing dietary protein level from 17.5% to 14.5% significantly decreased the albumen weight and height, and protein contents of egg albumen (He et al., 2016). Analyses of two-dimensional electrophoresis followed by matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometer on the egg albumen revealed that 22 egg albumen protein spots representing seven protein families were significantly changed in abundance and 16 proteins decreased, including ovalbumin, ovotransferrin, ovomucoid and ovoinhibitor; these proteins are involved in various biological functions that might relate to the changes in albumen height (He et al., 2016). Dietary compositions or feed ingredients, could be another major determinant on egg albumen quality. Cottonseed meal has been reported to have negative effects on performance and albumen quality. The Haugh unit and albumen height of fresh eggs were decreased when hens were fed a diet containing ⩾ 18.7% low-gossypol cottonseed meal as being compared with a soybean meal diet, even though the two diets were isocarloric and isonitrogenic and had similar amino acid profiles (He et al., 2015; Wang et al. 2015a). The cottonseed meal diets also increased the rate of development of watery whites when eggs were stored at refrigeration or room temperature (Wang et al. 2015b). The reduction of albumen quality by low-gossypol cottonseed meal was not only due to the presence of free gossypol in cottonseed protein (He et al., 2015), but also to possible deficiency of some amino acids (e.g., Phe, His, and Leu) or other undesirable compounds in cottonseed meal. The proteomic profiling showed that a total of 15 proteins that account for 75% of egg albumen proteins were reduced as the response of replacement of soybean meal with cottonseed meal in diets for laying hens, and these 15 proteins are identified to involve in antimicrobial activity, gelling characteristics, immune responses, inhibition of protein precipitation, protease inhibition, and metal ion binding or transportation (He et al., 2016). The decrease in egg albumen proteins may be partly explained by lower plasma progesterone and depressed growth of epithelial cells in oviduct magnum (He et al., 2016). These findings extend previous findings and further support roles of dietary protein levels and feed ingredients in egg albumen quality.

Nutritional modulation of functional ingredients of eggs

Eggs make vital contributions to covering the needs of protein for human. Egg protein supply per capita has increased since 1990 (FAOSTAT, 2015). Eggs also contain variety of bioactive compounds. For example, consuming one egg per day increased serum lutein and zeaxanthin concentrations in aged adults (Awilson et al., 2007), which reduced the risk of age-related macular degeneration. Eggs can also be enriched with some components for enhancing human's health. Nutritional strategies could be developed to produce eggs with multiple benefits, and to provide a choice of lifestyle to enhance health and to improve physiological well-being.

Great progress has been made in enriching eggs with n-3 polyunsaturated fatty acids (PUFA). Flaxseed, hempseed and marine products are commonly used feed sources for n-3 PUFA, which are rich either in α-linolenic (ALA, the parent n-3 PUFA) or long chain n-3 PUFA (mainly including eicosapentaenoic (EPA) and docosahexaenoic (DHA)). There is a limitation to production of n-3 PUFA-enriched eggs in consideration of transfer efficiency and cost control. Current research has been devoting to finding alternative sources (e.g. marine algae), rather than fish oils, for the production of DHA enriched eggs. Dietary supplementation of marine algae increased the level of total n-3 PUFA in a dose-dependent manner, and reduced the ratio of n-6 PUFA to n-3 PUFA correspondingly (Fredriksson et al., 2006). DHA is a main long-chain n-3 PUFA deposited in yolk lipids when laying hens were fed with diets con-

taining marine algae (*N. oculata*, rich in EPA but no DHA) and the maximum level of DHA in egg yolk reached at 21 mg/egg, but no substantial increase of EPA was observed in yolk phospholipids (Bruneel *et al*., 2013). A similar result was observed for docosapentaenoic acid (DPA) (Gładkowski et al., 2014). These results indicate that dietary EPA and DPA are largely converted to yolk DHA, and the conversion may be more efficient than ALA. In addition, enrichment of eggs with some vitamins (A, D, E, etc.) or minerals (I, Se, Zn, etc.) can be achieved by supplementing corresponding nutrients (Rooke et al., 2010; Schiavone et al., 2011), and concentrations of these vitamins and minerals in feeds are thus a mostly important factor that determines their corresponding contents in eggs. It has also been well demonstrated that some functional components in eggs, such as conjugated linoleic acids, xanthophylls, isoflavones, etc., could also be successfully manipulated by dietary means (Qi et al., 2011; Walker et al., 2012).

The consumer's desire for functional and value-added foods keeps increasing. In order to meet their demands, producers should review their approaches in producing egg products. Further studies are needed to explore factors that affect nutrient metabolism of laying hens and nutrient deposition in eggs. In addition, two things still need to be clarified: 1) fully understanding health benefits of the functional eggs, and 2) designing the functional eggs with clearly and specifically defined objectives.

Conclusion

Egg production and consumption have been globally increasing over the past twenty years. There will still be a growing trend in the egg industry in the near future. Much attention has been directed towards animal's health and product quality. Therefore, great efforts need to be on identifying precise roles of specific nutrients or dietary factors in metabolism of laying hens, providing new insights into the relationships between nutrition and animal's health and egg quality, and further developing innovative technologies and strategies to improve both poultry health and egg quality.

Acknowledgements

This study was financed in part by the China Agriculture Research System (CARS-41-K13), the China Agriculture Research System -Beijing Team for Poultry Industry, the National Natural Science Foundation of China (31172212，31572426) and the Agricultural Science and Technology Innovation Program (ASTIP).

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L6 Regulation of feed intake and body fat mass in chickens

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Keywords: appetite, adiposity, gut hormones, satiety

Summary

Broiler chickens eat more feed and grow faster than layer chickens. However, excessive accumulation of body fat has become a serious problem in the broiler industry. The mechanisms underlying the hyperphagia of broiler chickens is not fully understood, although the appetite regulatory system of chickens has been a focus of research in recent decades. Species specificity in the physiological role of peripheral hormones might make it difficult to understand the mechanisms underlying the central integration of peripheral adiposity and satiety signals. Here I provide an overview of recent findings in this field and propose promising strategies for reducing body fat mass in broiler chickens.

Main text

Broiler chickens, which are bred for rapid growth and high meat yield, do not adequately control their voluntary feed intake to meet their energy requirements. Consequently, their overconsumption of feed can lead to excessive accumulation of visceral fat, which is regarded as an animal by-product or as waste. In addition, excessive fat accumulation may lead to metabolic diseases, which are serious problems for the poultry industry (Julian 2005). Thus, the appetite regulatory system of chickens has been a focus of research in recent decades (Kuenzel 1994; Denbow 1994; Furuse 2002; Bungo et al. 2011). In mammals, appetite is regulated in response to the energy demands of the body. For example, adiposity signals, such as leptin and insulin, provide information about the body fat mass to the brain, and thereby suppress appetite (Schwartz et al. 2000). Satiety signals, such as cholecystokinin (CCK), peptide YY (PYY), and glucagon-like peptide-1 (GLP-1), provide information about meal intake to the brain, and thereby suppress appetite (Sam et al. 2012). However, lines of evidence suggest that the physiological roles of these signals are different between mammals and chickens. Here I hypothesize the role of adiposity signals, satiety signals, and other signals in chickens.

Adiposity signals

Body fat mass is controlled by appetite but appetite is controlled by body fat mass in mammals. For example, the adiposity signals leptin and insulin play a critical role informing the brain of changes in body fat mass (Schwartz et al. 2000). However, recent findings suggest that the physiological roles of leptin are different between mammals and birds: leptin is densely expressed in the brain but not in the adipose tissue in chickens (Seroussi et al. 2016) and zebra finches (Huang et al. 2014). Leptin receptor is densely expressed in the pituitary in chickens (Seroussi et al. 2016), rock doves (Friedman-Einat et al. 2014), zebra finches (Huang et al. 2014), and Japanese quails (Wang et al. 2016), suggesting that leptin might regulate the pituitary function in birds. In addition, although insulin shows appetite-suppressive effects in chicks (Honda et al. 2007), blood insulin levels were not correlated with either abdominal fat mass or the mRNA levels of appetite-regulating neuropeptides in the hypothalamus (Honda et al. 2015a). Birds need to fly. Therefore, birds may have developed so as not to increase their body fat mass for flying. The physiological roles of adiposity signals in the appetite regulatory system was lost in birds or developed subsequently in mammals.

Satiety signals

Satiety signals, such as CCK, PYY, and GLP-1, show appetite-suppressive effects, and their concentrations in the blood are elevated after meals (Sam et al. 2012). In contrast, ghrelin, a hunger signal,

shows an opposite effect: food intake is stimulated by ghrelin, and the plasma ghrelin level is decreased after meals (Sam et al. 2012). However, the role of gut hormones in appetite regulatory systems seems to be different between mammals and chickens. For example, ghrelin shows appetite-suppressive effects in chickens (Kaiya et al. 2013). We found that the gut hormone GLP-2 showed appetite-suppressive effects in chicks, suggesting that GLP-2 also functions as a satiety signal (Honda et al. 2015b, 2015c). Thus, the suppressive role of gut hormones on food intake in chickens might be physiologically more important than that in mammals. Birds need to fly. Therefore, birds may have developed not to increase intestinal content as much as possible. The supplementation of gut hormone secretagogues in feed, which adequately suppresses feed intake, may be effective for reducing body fat mass in broiler chickens.

Other signals

Birds need to have sufficient breast muscles for wing flapping. However, too much breast muscle increases the weight of body and therefore, might interfere with the ability to fly. Thus, birds may have developed to maintain an optimum weight of skeletal muscles for flying. Skeletal muscles produce and secrete myokines, which exert auto-, para- and/or endocrine effects (Schnyder and Handschin 2015). Insulin-like growth factor-1 and irisin, both of which are myokines, show appetite-suppressive effects in diabetic rodents (Lu et al. 2001; Duan et al. 2016). It is therefore possible that myokines provide information about changes in the skeletal muscle mass to the brain, and thereby suppress appetite. Skeletal muscle is also known to be a major site of energy expenditure. Taken together, increase of skeletal muscle mass can be a reasonable way to reduce body fat mass in broiler chickens.

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L7 Main factors affecting the nutritive value of poultry ingredients: cereals and soybean meal

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Keywords: amino acid digestibility, antintritional factors, energy evaluation, fat and fiber content, pelleting

Summary

Feeding represents 60 to 70% of the cost of production of eggs and poultry meat. Levels of apparent metabolizable energy (AMEn), standardized indispensable amino acids (Lys, Met+Cys, and Thr), and digestible P content of the diet are the main factors controlling the cost of poultry diets. In most countries, soybean meal, cereals and lipid sources are the main constituents of the diets, with increased interest for the use of crystalline amino acids and additives. Most feed companies formulate the diets based on the chemical composition and nutritive value provided by tables from different institutions throughout the world. In theory, values for key nutrients from all these tables should be similar and within a narrow range. However, this is not the case for many ingredients, including corn and soybean meal, the most traditional raw materials used in poultry diets. The reasons for the wide discrepancy observed among sources of information are not known but in most cases the differences reported, especially for AMEn content, are not justified.

Introduction

Poultry diets are based primarily in cereals and protein meals, with corn and soybean meal (SBM) as the main ingredients. Other raw materials used in practice are wheat, barley, sorghum and lipids as energy sources and sunflower meal, rapeseed meal, DDGS, and peas as protein sources. The world tends to globalization and consequently, the number of ingredients used in a given country and formal will increase.

Numerous factors affect the nutritive value of ingredients for poultry. Many of these factors are related to the animal itself with energy utilization that varied among birds (i.e., pullets vs. chicks vs. layers vs. turkeys) and health status. Also, the ingredient composition and the characteristics of the diets affect of percentage of the gross energy that birds can utilize. In this respect, particle size of the ingredient (fine vs. coarse grinding), feed form of the diet (mash vs. pellets), antinutritional factor (ANF) content, inclusion level in the diet, proportion of fat and fiber, and the use of enzymes and other additives, are of interest. For example, particle size might influence the utilization of the energy of the starch with more pronounced benefits with fine grinding in ingredients with highly protected starch, such as peas (Pisum sativum) than in ingredients with less protected starch such as rice (Parera et al., 2010). Also, pelleting might be of more benefit for these ingredients in which a high percentage of the lipid fraction is inside the espherosomes, such as corn and toasted soybean meal (Irandoust et al., 2012). The inclusion of moderate amount of insoluble fibers, such as oat hulls, might improve gizzard function and the utilization of nutrients and energy of the ingredients (Mateos et al., 2012; Jimenez-Moreno et al., 2016). Moreover, fat supplementation reduces rate of feed passage which in turn might improve nutrient and energy utilization of the diet (Mateos and Sell, 1980; Mandalawi et al., 2016). A key factor influencing the utilization of the nutrients and energy content of an ingredient is the content in ANF. In practice, trypsine inhibitor (TI) in soybean meal and peas, glucosinolate content in rapeseed meals, and non digested oligosaccharides in legumes and xylans and β-glucans in small grains are the main factors to control. In this respect, the use of adequate technologies (i.e., heat processing for TI and inclusion of enzymes for non starch polysaccharides in grains) might solve at a high extent the problems encountered. Finally, the improvement in energy content of the ingredients of the diet might improve because of the inclusion of adequate enzymes (or other convenient additives) has been well documented (Lazaro et al., 2003).

Energy and protein evaluation of ingredients

Different systems are available for evaluating the AMEn, and standardized ileal digestibility of the amino acids (AA). Moreover, numerous institutions (NRC, 1994, 2012; INRA, 2002; FEDNA, 2010; CVB, 2011; Rostagno et al., 2011; Premier Atlas, 2014; Russian Poultry Research Institute, 2014; Evonik, 2016) have edited comprehensive tables to evaluate the nutritive value of key ingredients in poultry diets.

In practice, the three main approaches used to evaluate the energy content of ingredients in poultry diets are based on values obtained from a) tables, b) predictive equations (in vitro studies, wet chemistry, or NIR technology), and c) in vivo experiments (research farms). Each of them has advantages and disadvantages and at present time, it is not easy to make a fair recommendation on which one is best (Mateos et al., 2015). Under practical conditions most feed companies will use table values often modified based on their practical experience or on analyses conducted in their labs by NIR technology. Consequently, this presentation will focus in this approach, using as examples determinations for SBM, cereals and grains in general.

Protein sources

SBM, rapeseed meal (RSM) and sunflower meal (SFM) are the main protein sources used worldwide. Usually, SBM will be the protein source of choice because of composition, protein quality and nutritive value (Van Eys, 2012).

The energy content of high protein SBM (47% CP), as recommended by different institutions, ranges from 2.21 Mcal/kg from CVB (2011) to 2.55 Mcal/kg for the RPRI (2014) (Table 1). The differences reported among Institutions are difficult to understand and indicate the need for a better approach in the presentation of energy values by the Institutions. As indicated by Frikha et al. (2012), Ravindran et al. (2014) and Garcia Rebollar et al. (2016), part of the differences might be due to differences in digestibility of the crude protein (CP) fraction as well as to the sucrose content among SBM samples.

Garcia Rebollar et al. (2016) in an extensive survey conducted during 8 consecutive years with SBM samples (n = 515) collected in Europe or in the country of origin of the beans, reported better uniformity and less fibre, more soluble sugars and higher indispensable AA content per unit of CP for the USA than BRA SBM, with SBM from ARG being intermediate. The USA SBM had higher TIA, KOH and PDI but lower HDI than the BRA SBM. In fact, SBM can be discriminated and classified by origin of the beans by NIR technology. These authors, reported different energy values for meals from the three major countries both in pigs, using the predictive equation developed by Noblet et al. (2003), and in poultry, using the predictive equation recommended by the WPSA (1989). In fact, the values reported using the WPSA equation were 2,621, 2,605 and 2,576 kcal/kg (on DM bases) for the USA, Brazil, and Argentina meals respectively. These differences, although important, do not explain the high range of values for SBM among sources exposed in Table 1.

Table 1. Energy content of soybean meal for poultry, Mcal/kg

Instit.	Country	Year	CP(%)	AMEn(Mcal/kg)
NRC	USA	1994	47.2	2.38
INRA	France	2002	47.2	2.34
NARO	Japan	2009	47.0	2.45
Fedna	Spain	2010	47.0	2.36
CVB	Netherlands	2011	47.1	2.21
Viçosa[1]	Brazil	2011	47.0	2.32
RPRI	Russia	2014	47.0	2.55
Evonik	Germany	2016	47.5	2.36

[1] Rostagno et al. (2011)

The variability in CP and AA content is also important among SBM samples with part of the differences depending on the processing conditions of the beans and the country of origin with lower CP content for Argentina meals than for USA or Brazilian meals. Also, CP content is reduced when higher amounts of hulls are added to the meal after processing. The AA profile of the SBM varied with the country of or-

igin and in general, the indispensable AA content was lower for the Brazilian meals than for the Argentinean and USA meals (Ravindran et al., 2014). In Table 2 we offered the Lys content (per kg of meal or per unit of CP) of high protein SBM according to different sources of information. Again, the variability reported is not easy to explain. When the profile of the protein fraction is studied, the values change, with less but still important differences among countries. In fact, Brazilian SBM showed a less convenient AA profile that the Argentina or USA meals.

Table 2. Lysine content of the soybean meal (% of the meal or %CP)

Institution	Country	Year	CP (%)	Lys (%)	CP (%)
INRA	France	2002	47.2	2.89	6.10
Fedna	Spain	2010	47.0	2.88	6.13
CVB	Netherlands	2011	46.4	2.88	6.20
Viçosa[1]	Brazil	2011	46.1	2.83	6.14
NRC	USA	1994	47.7	2.96	6.20
RPRI	Russia	2014	47.0	3.04	6.46
Evonik[2]	Germany	2016	47.5	2.87	6.04

[1] Rostagno et al. (2011) [2] Brazil

To notice, that similar variability or even greater in energy and CP content and AA availability has been reported for RSM (Table 3) and for SFM (Table 4). In the case of RSM most differences in protein quality are due to processing conditions and glucosinolate content. For SFM, the main reason for the difference in AMEn and CP content are the amount of hulls added to the meal.

Table 3.–Energy content of rapeseed meal for poultry (Mcal/kg)

Instit.	Country	Year	CP (%)	EE (%)	AMEn (Mcal/kg)
NRC	USA	1994	34.8[1]	3.8	2.00
INRA	France	2002	33.7	2.3	1.46
Premier	UK	2014	33.9	3.5	1.67
NARO	Japan	2009	37.3	2.9	1.74
Fedna	Spain	2010	33.8	2.2	1.70
CVB	Netherlands	2011	33.5	2.6	1.69
RPRI	Russia	2014	35.5	2.5	2.00
Evonik	Germany	2016	34.9	3.7	1.81

Table 4. Energy content of sunflower meal for poultry

Institution	Country	Year	CP (%)	AMEn (Mcal/kg)
NRC	USA	1994	32.0	1.54
INRA	France	2002	33.4	1.49
Premier	UK	2014	33.0	1.67
NARO	Japan	2009	32.0	1.59
Fedna	Spain	2010	32.1	1.53
CVB	Netherlands	2011	33.0	1.49
Viçosa	Brazil	2011	30.2	1.79
PS Inst.	Russia	2014	32.0	2.04

Cereals

When the AMEn of the cereals are compared, there is a lineal positive correlation between its non-starch polysaccharide content (NSP) and energy content. The highest AMEn among cereals corresponds to rice, followed by corn with the lowest value for oats (Table 5). Within a given cereal, the energy and digestible protein content will depend on the proportion of starch and fiber (closely and negatively related) as well as on the amount of viscous carbohydrates, mainly β-glucans and xylans. Consequently, one of the main reasons for the variability in nutrient content in samples from a given cereal, is its content in these complex oligosaccharides. Fortunately, the feed compound industry can utilize multiple enzymes complexes that permit to over-come this problem in all type of diets.

In table 6 we include data on the variability in AMEn of corn according to different Institutions, with values varying widely, from 3.20 Mcal/kg for INRA (2002) to 3.38 Mcal/kg for Rostagno et al. (2011). Again, these differences are difficult to understand and remained in many cases, even after removing the effects of moisture content. Similar type of problems are found for the energy evaluation of wheat (Table 7) and barley (Table 8). Rice is the cereal with the lowest ANF and the highest starch content. In addition, rice starch is easily digested because of the small size of the starch granule, simple protected matrix of the starch granules, and high relative content of amylopectin. As a consequence, rice is very easy to digest and contains more energy than any other cereal (Table 9).

Table 5. Relative energy values of main cereals in poultry

	Evonik (2016)		Fedna (2010)	
	AME (kcal/kg)	Relative value		
Corn	3,305	100	3,280	100
Rice	3,520	106	3,390	103
Barley	3,247	98	3,210	98
Wheat	3,084	93	3,150	96
Oats	2,576	78	2,730	83
Sorghum	2,701	82	2,800	85
Rye	2,612	79	2,500	76

Table 6. Variability in energy content of corn for poultry

Institut.	Country	Year	Moist. (%)	CP (%)	EE (%)	AMEn (Mcal/kg)
NRC	USA	1994	11,0	8.5	3.8	3.35
INRA	France	2002	13.6	8.1	3.7	3.20
NARO	Japan	2009	14.5	7,6	3.8	3.28
Fedna	Spain	2010	13.8	7.7	3.6	3.28
Viçosa[1]	Brazil	2011	12.5	7.9	3.7	3.38
CVB	Netherlands	2011	12.8	8.2	3.8	3.29
RPRI	Russia	2014	13.0	8.5	4.0	3.30
Premier	UK	2014	13.0	8.0	4.0	3.29

[1]Rostagno et al. (2011)

Table 7. Variability in energy content of wheat for poultry

Institut.	Country	Year	Moist. (%)	CP (%)	Starch[1] (%)	AMEn (Mcal/kg)
NRC	USA	1994	11.0	11.5	-	3.12
INRA	France	2002	13.2	10.5	60.5	2.98
Fedna	Spain	2010	11.4	11.2	59.0	3.15
Viçosa[2]	Brazil	2011	11.9	11.7	54.9	3.08
CVB	Netherlands	2011	13.2	11.1	55.7	3.09
Premier	UK	2014	13.0	10.7	60.0	3.11
RPRI	Russia	2014	12.0	11.5	54.9	2.95
Evonik	Germany	2016	12.0	11.7	60.2	3.08

[1] Starch analyses? [2] Rostagno et al. (2011)

Tabla 8. Variability in energy content of barley for poultry (Mcal/kg)

Institution	Country	Year	Moist. (%)	CP (%)	Starch (%)	AMEn (Mcal(kg)
NRC	USA	1994	11.0	11.0	-	2.64
INRA	France	2002	13.3	10.1	52.2	2.75
Fedna	Spain	2010	9.8	11.3	51.1	2.80
CVB	Netherlands	2011	13.1	10.4	50.9	2.79
Premier	UK	2014	13.0	11.0	51.5	2.82
PS Inst.	Russia	2014	13.0	11.0	49.9	2.67
Evonik	Germany	2016	12.0	11.2	49.7	2.70

Table 9. Comparative energy value of broken rice and corn (Mcal/kg)

Institution	Country	Year	Corn	Rice	%
INRA	France	2002	3.13	3.43	110
Premier	UK	2014	3.29	3.46	105
Fedna	Spain	2010	3.29	3.38	103
Rostagno	Brazil	2011	3.38	3.28	97
CVB	Netherlands	2011	3.29	3.48	106
Evonik	Germany	2016	3.31	3.52	106

The crude protein content of the main cereals used by the industry, according to the different institutions, is shown in Table 10. Again, to notice the high variability in CP among sources for all cereals, with a range from 7.5 to 8.5% for corn. Usually, the variability in CP content is lower for corn than for the small grains, specially in those countries, such as Spain, in which the small grain (wheat, barley, rye, triticale and oats) are produced in non-irrigated lands. As a consequence, the proportion of protein and starch (and fiber) of the seeds will depend on the raining conditions during the growing season.

Table 10. Crude protein content of major cereals

	INRA (2002)	Fedna (2010)	CVB (2011)	Vicosa (2011)	RPRI (2014)	Evonik (2016)
Corn	8.1	7.5	8.2	7.9	8.5	7.4
Rice	8.0	7.5	7.7	8.5	8.3	8.2
Barley	10.1	11.3	10.4	-	11.0	11.2
Wheat	10.5	11.2	11.1	11.7	11.5	11.7
Oats	9.8	8.7	10.4	-	10.5	10.5
Sorghum	9.4	8.9	9.4	9.0	9.5	9.2
Rye	9.0	8.7	9.8	-	8.2	8.6

Conclusions

In spite of the many years of research on the AMEn content and amino acid availability of the different ingredients used in poultry diets, still we do not have a simple method to evaluate with accuracy these values. Factors such as processing conditions, feed form and particle size, origin of the ingredient, diet characteristics, and use of additives will influence the energy and protein value of the ingredients and thus, tables values should be taken precaucioulsy. However, listed tables values, provided that the nutritionist applied "sound information to existing tables and the use of predictive equations" are useful tools to improve our knowledge on the nutrient content of ingredients.

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L8 Processing for profit and performance

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Keywords: processing, steam explosion, seed sorting, grinding, in-line NIRS

Summary

Processing of ingredients and feed contribute significantly to the cost of feed production but can also increase productivity and safety of the feed. Most feed ingredients are ground to reduce particle size thereby increasing surface area for digestion but this is a capital and energy intensive process. Recent studies have shown that poultry in particular may benefit from a course particle size as this promotes the development and function of the gizzard and increases exposure of the ingredients to pepsin and hydrochloric acid. Grinding, however, can also change the physical properties of the ingredient. Grinding of fibre reduces its ability to bind water while very fine grinding of cereals such as wheat can interrupt the crystalline structure of starch (starch damage) increasing water absorption likely resulting in more rapid digestion of a portion of the starch. On the whole however, it appears it is desirable to reduce the intensity of grinding to maximize performance and profitability. Quality control is important at the feed mill to ensure final diets are within specification but this usually only involves spot checks of samples upon receiving and shipping. In-line NIRS systems are now available that would allow the monitoring of incoming ingredients, processes and outgoing diets in real time. If properly utilized, these systems could ensure formulation is more accurate and safety margins minimized. Similarly, they could be used to optimize processing such as mixing, pelleting and drying to minimize cost, maximize throughput and ensure quality. Seed sorting technology is now available to remove contaminants in feed grains such as ergot and fusarium damaged kernels. This technology allows the industry to use a broader range of ingredients while minimizing the risks associated with using low quality feed ingredients. Steam treatments have been effectively used to reduce anti-nutritional factors in feed ingredients such as soybean for a very long time. However, more intense but short duration steam treatments such as steam explosion may be able to be used to increase the digestibility of high fibre by-products such as canola, cottonseed and linseed meals increasing productivity and profitability when using these ingredients.

Introduction

Approximately 70% of the cost of poultry production is associated with feed (ingredients, transportation and processing) and as a result the majority of research has focused on clearly defining the nutritional requirements of the bird at each stage of life and optimizing the use of available ingredients to minimize cost and maximize performance. The costs and impacts of feed processing have not received as much attention but recent research, especially in regards to particle size reduction is showing that processing can have a significant impact on the health and productivity of the bird. Particle size reduction also involves significant capital and operating expenses so maximizing performance while reducing costs associated with processing will increase overall profitability. There are some novel processing technologies that may be able to increase profitability of poultry production (in-line NIRS, seed sorting and steam explosion) as well. The objectives of this paper are to discuss current information on particle size and to discuss potential these novel processing technologies with a focus on profitability and performance.

Particle size reduction

Reduction of particle size through grinding has both a significant impact on performance and costs. The most commonly accepted dogma has been that it is desirable to reduce particle size as much as is practical and create as uniform particle size distribution as possible. The concept is that reducing the particle size increases surface area for digestions and therefore allows the animal to digest the feed more efficiently and quickly, therefore, resulting in increased feed conversion and growth rate. While this might be true in many species of livestock, poultry are unique in that they have a gizzard that allows them to grind the feed prior to entering the small intestine. One might argue that the role of the gizzard has been overlooked and traditionally the industry has relied more on mechanical processing to reduce particle size. Recently a review of the function of the giz-

zard and the impact of diet structure has shed some interesting light on this subject. Svihus (2011) convincingly argued that the gizzard is a valuable organ and a properly functioning gizzard is not only desirable but will result in higher profitability. He argues that by grinding too finely and minimizing the fiber content of the diet, the gizzard does not develop normally and therefore feed passes through the gizzard too quickly and may result in reduced exposure to pepsin and hydrochloric acid (which enters the gizzard from the proventriculus via reverse peristalsis) for sufficient time and possibly increasing passage rate resulting in reduced digestibility. In a recent study by Ruhnke *et al.* (2015), starch digestibility by laying hens was highest in course ground material supporting this hypothesis, however reductions in pH of the proventriculus were not observed in laying hens but this could be due to the high levels of limestone used in the laying diet. Naderinejad *et al.* (2016) examined the effect of particle size on digesta transit time, gizzard pH, apparent ileal digestibility of dry matter, nitrogen, starch and fat as well as AME in a mash diet of broiler diets, but they did not observe any differences due to particle size. However, in pelleted diets course grinding resulted in lower gizzard pH, and increased starch digestibility and AME. Given that the majority of broiler diets are pelleted, this would suggest that course grinding even when the diets are pelleting is likely beneficial. Lv *et al.* (2015) found that course grinding increased average daily gain and feed intake by broilers when fed a mash diet in the starter and grower phases but there was no effect of particle size when the diets were pelleted. Huang *et al.* (2006) showed that the death rate of Salmonella typhimurium was significantly higher in the gizzard *in vitro* when fed a course mash diet indicating the increase in gizzard function may have a positive impact on gut microflora.

Grinding also affects water absorption. Auffret *et al.* (1994) demonstrated that course fibre particles such as wheat bran are able to absorb and hold more water than finely ground material. They hypothesized that while intact fibre is able to absorb and bind water, fine grinding collapses the structure reducing its ability to bind water. Khanfas and Newkirk (unpublished data) showed that water binding capacity as measured by Solvent Retention Capacity (water) was significantly affected by screen size during hammer milling (Figure 1). The course ground wheat was able to absorb and bind more water than the finer ground materials likely due to the disruption of the fibrous matrix during grinding. However, very fine grinding increased water absorption likely due to starch damage. Physical damage of the crystalline structure of starch caused by intense grinding increases the ability of the starch granules to absorb water (Dexter *et al.* 1994). Fine grinding also increases the solubility of the fibre (Anguita *et al.* 2006). However, the overall effect of these changes in water binding capacity on poultry digestion and physiology is not clear. The increased starch damage and resulting rapid absorption of water will likely increase the rate of starch digestion for the damaged granules, while the impact of water absorption by fibre due to course grounding may increase bulk in the digestive track possibly reducing feed intake. However, overall it appears the beneficial effects on the gizzard and the lower processing costs associated with course particle size outweighs the potential benefits of fine grinding. Therefore, it might be logical to fine grind the ingredients high in starch to optimize digestion but course grind the more fibrous ingredients to stimulate gizzard development and function.

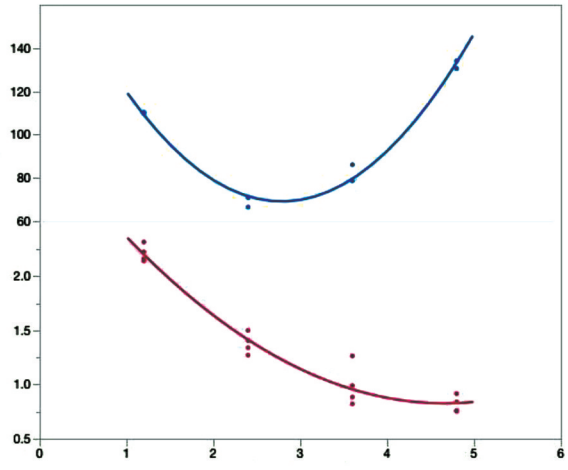

Figure 1. Impact of hammer mill screen size (mm) on the Solvent Retention Capacity (water) and Starch Damage in Canadian feed wheat (Khanfas and Newkirk, unpublished data).

In-line NIRS systems

Near Infrared Spectroscopy (NIRS) has been used for many years as a quality assurance tool in the feed industry. Typically, a bench top system is used and routine samples of ingredients and finished products will be collected and tested to ensure the products fall within a specified range of parameters. Overtime the average of the value from the received samples can be used to update the nutrient profiles of the ingredients used in the formulation package. The disadvantage of this system is it generally is used to catch mistakes in the process and diets are still formulated using semi-book values for nutrient content rather than individual ingredient nutrient specifications thereby requiring large safety margins in formulation. Other industries have adopted the use of In-line NIRS systems to gather real time information on their process and have automated it so changes to process and formulation occur automatically resulting in more consistent and safer products. For example, in pharmaceutical applications in- line NIRS sensors provide the manufactures with real time data allowing them to catch quality issues immediately. In the food industry, the fat or moisture content of products can be adjusted in real time. This technology has developed to the point that t is now readily available to the feed industry. Several companies offer inline systems that range from standard testing of moisture and protein content to some systems that provide very detailed information that can detect anomalies in the finished product and prevent the product from entering the market place.

Inline NIRS systems can range from a single detector on the receiving leg or finished product bin to systems with multiple locations in the plant. If the products are analyzed at shipping, the operator can monitor the consistency of the product, look for adulterants such as melamine or PCBs (de Jong *et al.* 2016), or update the formulation data base so diets can be formulated with actual nutrient composition rather than semi-book values, reducing required safety margins and therefore reducing the costs of production. In addition, the systems can be used to monitor quality through the process. For example, in-line NIRS should be able to examine the degree of homogeneity of the products in the mixer. This would allow for more efficient use of a mixer as well as preventing quality issues associated with insufficient mixing of diets. Similarly, the moisture content of the final product can be monitored to ensure the diets are meeting specific requirements and not either shipping too dry or too moist of product. In theory, one should be able to monitor the process to maximize the quality and efficiency of pelleting (Smillie unpublished data). Little to nothing has been published on the use of in-line NIRS applications in the feed industry for poultry feed, but based on the experiences of other industries, the potential to produce higher quality, more consistent, safer feed products using this technology seems realistic. The Canadian Feed Research Centre at the University of Saskatchewan has installed one system (Bruker) with 6 detectors in-line and are in the process of installing another system (Buhler) with 4 to determine how to most effectively use this technology in the feed industry and integrate it into a functioning feed mill and will be testing these systems extensively in the near future.

Seed sorting

Typically, the highest quality cereal ingredients produced are used for food applications and those products that do not meet the quality requirements of the food industry are used for the feed industry, but are sold at a discount. The causes of the downgrading can be a number of factors, many of which do not affect animal performance. For example, mildew, staining, broken seeds, sprouting can reduce the value of cereals for human food applications but have no effect on poultry performance. On the other hand, some degrading factors even if found at low levels can have a negative effect on animal performance, for example ergot or fusarium damage and the resulting toxins. The advantage of livestock production, is downgraded grains not suitable for human application due to aesthetics can effectively be turned into high quality protein and the feed industry utilizes as much of this product as possible. The challenge, however, is to produce a safe and consistent product with these low quality ingredients.

Seed sorting is not a new technology, farmers and the grain industry have been cleaning seeds by sieving, gravimetric segregation or aspiration for a long time. In the pulse industry, color sorters have been used for over 50 years to remove odd colored seeds. However, seed sorting technology has improved greatly in the last decade or so and now sorters that are able to sort not only based on color but size and shape (optical sorters), NIRS, and Near Infrared Transmittance (NIT, BoMill). Optical sorters have been used in Canada to effectively remove Ergot from cereals as well as remove obvious fusarium damaged kernels. The BoMill system is able to sort individual kernals of wheat, durum and barley for such things as protein and fusarium using NIT analysis of each individual seed. In a study at the University of Saskatchewan, Kautzman *et al.* (2012) was able to reduce the deoxynivalenol (DON) of durum samples from 8.4 ppm to 0.8 ppm by removing the fusarium damaged kernels (FDK) with a BoMill TriQ. In another more recent study by Newkirk et al (unpublished data) the fuarium damaged kernels and DON were reduced from 18.2 % and 20 ppm to 0.6 % when 40% of

the most infected grain was removed by sorting (Fig 2) using the same system. These type of technology will make it possible to use a wider range of ingredients at higher inclusion, lower cost and more safely than previously possible.

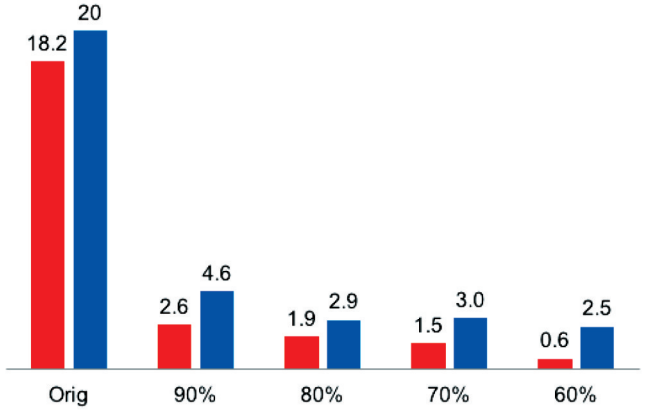

Figure 2. The Fusarium Damaged Kernel (%, red bars) and DON (PPM, blue bars) content of Canadian Western Amber Durum (CWAD) before and after sorting by NIT using a BoMill TriQ set to remove 10 to 40% of the grain.

Steam explosion

Steam treatments have been effectively used to enhance nutritional value of ingredients for over 100 years. Perhaps the best known of these treatments is the destruction of anti-nutritional factors (trypsin inhibitor and lectins) from soybean (Perez-Maldonado *et al.* 2003). However, more intensive steam treatments have been developed to change the chemical structure, particularly fibre and the associated lignin. However, the majority of the focus has been on treating highly lignified by products for cellulosic biofuel production (Horn *et al.* 20110 or increasing digestibility for ruminants (Viola *et al.* 2008). The process involves heating the product in a pressure chamber using high pressure steam and holding it at this pressure for a specified time to degrade the lignin and part of the hemi-cellulose, upon completion of the heating phase, the pressure is released suddenly resulting in violent rupturing of the cellulosic fiber. The process called steam explosion due to this violent depressurization, dramatically increases the fermentability of straws, stovers and wood products. However, due to the highly lignified nature, and high concentrations of cellulose, of the ingredients studied, the pressure and time can be excessive therefore making the process very costly. Many of the processes tend to be upward to 200° C and 2.5 minutes long in duration and therefore a batch system is employed. Continuous flow systems have been developed using a modified extruder but due to the time and pressure required the processes are relatively costly (Viola *et al.* 2008, Chen *et al.* 2014). Some of the ingredients (Canola meal, linseed meal, cotton seed meal etc.) fed to poultry have relatively high levels of lignin and cellulose and hemicellulose that if degraded would significantly increase the digestibility by poultry. However, the level of lignin and fibre and the complexity of this fibre is significantly less than the straw and wood products typically targeted by steam explosion. It is hypothesized that a modified steam explosion using a continuous flow system as described by Chen *et al.* (2014) but at much lower temperatures and processing times could increase digestibility, reduce anti-nutritional factors and therefore increase productivity and profitability of poultry production when fed these ingredients.

Conclusions

Ingredient and feed processing can be costly but can also significantly increase the digestibility of ingredients and affect the performance of the bird. Recent evidence suggests that ingredients should not be ground too finely prior to pelleting or fed as a mash in order to maximize the function of the gizzard and thereby improve the performance of the birds, while reducing processing costs. In-line NIRS systems, used in other processing industries, could be used in the feed industry to increase formulation accuracy, increasing processing efficiency and maximizing the quality of end products. Seed sorting technology can increase the safety of low quality feed grains such as wheat and barley by reducing infected kernals containing toxins. Steam explosion

technology may be able to increase the digestibility of high fiber/lignin ingredients such as canola, linseed and cottonseed meals thereby increasing productivity and profitability when using these ingredients.

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L9 Past, present and future of genomics in poultry breeding

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Keywords: genome, poultry, chicken, sequencing, gene

Summary

It's just over 10 years since the chicken genome was sequenced. This landmark event in poultry breeding formed the basis for the development of an array of tools for whole genome analysis of gene expression and genetic variation. Combined with recent developments in DNA sequencing there has been a surge of genomic applications in poultry breeding. In this paper the current status and future possibilities of poultry genomics will be discussed.

Introduction

Poultry is a billion dollar farming industry around the globe supplying a cheap source of animal protein for human consumption. According to the FAO 2011 statistics, each year 58 billion chickens are slaughtered for meat production (www.ciwf.org.uk/media/5235303) and over a trillion eggs laid by hens (www.ciwf.org.uk/media/5235021) are used for human consumption and production of hatchlings. A great deal of time, effort and funds by the poultry industry are being used to improve production, while maintaining sustainability and animal welfare. Genetic and genomic studies are playing a crucial role in achieving these goals by trying to unravel the genetic mechanisms underlying commercially important traits, so that this knowledge can then be applied in selective breeding programmes to enhance production, performance and animal health.

Chicken genome assembly

The chicken genome was originally sequenced by using the classic Sanger DNA sequencing technology, slow and expensive (International Chicken Genome Sequencing Consortium, 2004). But this genome assembly has moved from a draft to reference quality each year by taking advantage of new sequencing technologies and mapping resources (Burt and White, 2007). These sequencing methods included 454 and Illumina short reads, and the last assembly Gallus_Gallus-4.0 gave a genome assembly with a contig N50 of 280-kb and scaffold N50 of 12.9-Mb (*Table 1*). The latest genome assembly Gallus_Gallus-5.0, takes advantage of PacBio long reads of 8-kb or more, able to sequence through repeats and is less sensitive to GC-bias (Warren et al. *submitted*, 2016). This has produced an assembly with contig N50 of 2.9-Mb, ten-fold larger than the previous genome assembly (*Table 1*). With continued progress assemblies of contig N50 the length of whole chromosome arms are to be expected in the next few years. The chromosome level assembly and assignment to specific chromosomes has depended on physical mapping resources. The chicken has 38 pairs of chromosomes and the sex chromosomes, Z and W. The latter is small and repeat-rich and is sequenced poorly, and will only be completed one BAC clone at a time, as was the Z chromosome (Bellot *et al.,* 2010). The other autosomes can be divided into large, macro- (GGA1-5) and small, microchromosomes (GGA6-38), the latter are very GC-rich and compact. Chromosome 16, contains the MHC region, important in immune responses and disease resistance, but is also poorly assembled (10% of an expected 10-Mb chromosome), mostly due to the high repeat content and large numbers of gene families. The use of long reads and a physical map of BAC clones is likely to be the solution to this problem. Most of the tiny microchromosomes (GGA30-38) are not assembled and not identified. Further increase in long read coverage (50-fold or more) is likely to finish these and complete the chicken genome assembly. The chicken is very advanced and draft genomes are now available for the other poultry genomes, including turkey, quail, duck and many more avian genomes (Jarvis *et al.,* 2014). These can be improved using long reads and physical mapping tools like optical maps and long range mapping approaches (Burt *et al.,* 2015).

Table 1 Comparison of chicken genome assemblies Gallus_gallus−4.0 and −5.0

Global statistics	Gallus_gallus-4.0	Gallus_gallus-5.0
Total sequence length (bp)	1,046,932,099	1,230,258,557
Total assembly gap length (bp)	14,074,301	11,764,999
Gaps between scaffolds	915	397
Number of scaffolds	16,847	23,870
Scaffold N50 (bp)	12,877,381	6,379,610
Number of contigs	27,041	24,693
Contig N50 (b)	279,750	2,894,815
Total number of chromosomes	34	36

Gene annotation of the chicken genome

The next challenge is to be able to decode the instructions within these nucleotide sequences, both the regulatory and transcribed regions. Indeed, the key to realising the potential of the investment in the sequencing of poultry genomes lies in understanding the relationship between genotype ("from sequence") and phenotype ("to consequence"). Comparative genomics, including model organisms, with its underlying evolutionary framework facilitates the interpretation of data from one species on the basis of knowledge from related organisms. A high quality, richly annotated reference genome sequence for each genome is an essential framework around which to organise species-specific information. This annotation should include sequence variation plus details of coding and non-coding genes, and their regulatory sequences.

Until recently the annotation of genes and transcripts in the chicken genome was mostly through homology with other well annotated genomes, such as the human and mouse. This provided evidence on which to model most chicken protein coding genes but missed avian-specific proteins. Then further annotations used experimental data, such as RNA-seq to define genes and transcripts directly. The first time this was done was on Gallus-gallus-4.0 and predicted 17.1K genes (Ensembl v84, *Table 2*) and most (15.5K) were coding, a limitation of the prediction tools at the time. A recent annotation of the Gallus-gallus-5.0 assembly (NCBI v103, *Table 2*) used a larger dataset of RNA-seq short read data and predicted more genes, including 19.1K coding and many more non-coding RNA genes (6.6K), including long non-coding RNAs (lncRNAs).

Table 2 Comparison of chicken genome annotations for Gallus_gallus−4.0 and −5.0

Genome Feature	Gallus_gallus-4.0 (Ensembl v84)	Gallus_gallus-5.0 (NCBI 103)
Genes and pseudogenes	17,108	26,314
protein-coding	15,508	19,137
non-coding	1,558	6,550
pseudogenes	42	627
Gene transcripts	17,954	58,035
coding transcripts	16,396	46,409
misc_RNA, tRNA, rRNA and ncRNA of all classes	1,558	11,626

Comparison of the current number of annotated transcripts (e.g. Ensembl v84) in the human (~4 transcripts/gene) and mouse (~3 transcripts/gene) indicates that the complexity of the transcriptomes in the chicken (~1 transcript/gene), including transcript isoforms and alternative splicing, pseudogenes and lncRNAs is grossly under-estimated. The function of the latter is uncertain but lncRNAs are likely to be

important in the transcriptional and epigenetic control of gene expression. Using a phylogenomics approach Rands *et al.,* (2014) were able to show that evolutionary constrained sequences turnover at varying rates. In particular, constrained coding sequences are much more evolutionary stable than constrained non-coding sequences, and lncRNAs show the most rapid rate of turnover of all the types of non-coding elements. This and other studies empathise the need to define functional sequences in the target species, where for example only a fraction of the coding sequences from human can be found in chicken by comparative approaches alone, and very few regulatory sequences at all. Long read technologies are being used for the complete characterisation of the transcriptome of the chicken to overcome the limitations of short reads. The current knowledge of regulatory elements in poultry and other livestock genomes is almost non-existent. Currently lack of information of the location and sequence of regulatory sequences is a major obstacle in defining genetic variation that underpins phenotypic variation of complex traits, likely to be controlled mostly at the gene expression level. This is the aspiration and goal of the international "Functional Annotation of Animal Genomes" (FAANG) consortium (The FAANG Consortium, 2015).

These genome annotation projects are only able to "predict putative functional elements" in the genome, including coding and non-coding genes, and their regulatory sequences. Further work is required to show that these sequences play an important function at the cellular, tissue and whole animal level. Sequence conservation between multiple genomes can reveal if any of these putative functional elements show any evolutionary constraints. The recent assembly and comparison of 48 avian genomes (Jarvis *et al.,* 2014), including chicken (International Chicken Genome Sequencing Consortium*,* 2004), turkey (Turkey Genome Consortium, 2010) and duck (Huang et al., 2013), is a start and is able to define such elements to about 10 bp (Avian Genome Consortium, 2014). Further work to validate these functional elements needs to test the effect of genetic variants within these elements.

Genome variation within chicken populations

Annotation of a poultry genome is only the start, it is the genetic variation in the functional elements that are exploited in animal breeding programmes to alter specific phenotypes. Since the draft genome of the chicken genome resequencing projects have defined millions of genetic variants (Gheyas *et al.,* 2015). In the latest release of dbSNP (v147), the repository for genetic variants, 21 million SNPs were listed for chicken. Also, comprehensive catalogues for structural variants (CNV, ERVs, inversions, etc.) are very limited. This will change now whole genome sequencing can be done cheaply and at high coverage on single animals (e.g. using the Illumina X10 system). Most of these SNPs were characterised by Gheyas *et al.,* (2014) and indels by Boschiero *et al.,* (2014), with 15M and 883K, respectively.

Simple comparisons with the annotations of the reference chicken genome was able to classify these variants as coding or not. If coding, amino acid (non-synonymous) changes were predicted likely to alter protein function. Many stop or frameshift mutations were also predicted likely to be loss-of-function (LOF) mutations almost 50 per bird. The impact of non-synonymous codon changes was predicted using conservation of multiple sequence alignments of coding sequences from birds and other vertebrates. When used using either the software packages SIFT (Ng and Henikoff, 2003; Kumar *et al*., 2009) or PROVEAN (Choi *et al.,* 2012) there was a three-fold increase in the number of pathogenic predictions. By integrating selection signature analysis with known QTLs and functional annotation of variants, Gheyas *et al.,* (2015) identified potential candidate genes and causal variants for various traits found in broilers and layers. Many of these selective sweeps, however covered regions devoid of any genes suggesting an overlap with functional, non-coding elements with possible regulatory roles, yet to be defined. The ability to predict the effect of variants in non-coding regions, such as non-coding RNAs or putative promoter regions (or any other non-coding region) is almost impossible due to lack of annotations of regulatory regions.

Tools and resources for poultry genomics

International repositories, databases and genome browsers provide immediate access to a wealth of high quality genomic information on poultry genomes (Burt and White, 2007). These can be used to predict the impact of sequence variants in any genome resequencing or gene expression project. These resources have also been used to create rapid and accurate gene expression and genotyping assays

(Gheyas and Burt, 2013).

Gene expression tools have been important in a wide range of studies, such as developmental biology, animal physiology, host-pathogen interactions, etc. (Gheyas and Burt, 2013). The genetic control of gene expression is now an intense area of study linking putative functional elements in the genome such as coding and non-coding genes, with the regulatory sequences that control their expression.

Genotyping tools can be used at different densities depending on the application (Gheyas and Burt, 2013). Low density panels of perhaps 96-384 SNPs may be used for parentage or line identification. Higher densities of 60-600K SNPs may be used for GWAS or genome-wide-selection (GWS). A number of poultry companies (*Aviagen, Hy-line* and *Lohmann*) were active partners in a collaboration with the *Roslin Institute* to develop a chicken 600K high-density (HD) genotyping array (Kranis *et al.,* 2013) now available through the other partner *Affymetrix*. The HD array has found widespread usage as evidenced from the large number of citations (81 citations and 8754 accessions) since 2013. These genotyping panels can be combined through imputation methods so the genotypes of 10-100,000's of animals can be predicted genome wide using a mix of 384 (10,000's) and 600K (1000's) SNP panels. This can be taken further to impute the genome sequence of all by imputation from a smaller sample of 100's of whole genome sequences (WGS).

Applications and future prospects for poultry genomics

The main application of poultry genomics is in animal breeding, in particular to increase the accuracy, speed and diversity of traits subject to genetic selection. Traits are varied, a few are likely to be controlled by single genes (Mendelian traits, e.g. slow feathering) but most are complex, polygenic traits probably involving genetic variation in regulatory sequences controlling gene expression (Li *et al.,* 2015; 2016). Quantitative geneticists and animal breeders have been remarkably successful at developing statistical models that are effective predictors of an animal's future performance (Hill, 2014). The accuracy of these models has been increased by using HD SNP genotypes for the whole genomes in GWS (Meuwissen *et al.,* 2001). Further improvements can be achieved through the use of genome sequence data (Meuwissen *et al.,* 2010; Daetwyler *et al*., 2014) and by adding knowledge of the likely effects of the sequence variants whether coding or regulatory (Koufariotis *et al*., 2014). GWS will be of great benefit for expensive to measure traits, such as feed efficiency, egg production, disease resistance and welfare traits. Also sex limited traits, such as fertility and meat quality, and late in life traits such livability and egg production will also benefit from the application of GWS.

The future prospects for poultry genomics are good. The genome sequence of the chicken is likely to be completed (at least as far as chromosome arms) using long read technologies (Burt *et al.,* 2015). It is also likely whole genome assemblies of populations and individuals will be more common place. The imputation of genotypes and sequences is already in practice (Li *et al.,* 2009; Brøndum *et al*., 2014) but we will also see other types of imputations. For populations with whole genome sequences it will be possible to impute phenotypes for example from sub-populations (Dahl *et al.,* 2016). These phenotypes can be the traditional types such as performance, production, disease resistance, etc. or intermediate phenotypes of cells and tissues, based on costly assays, such as gene expression (Gamazon *et al*., 2015). These huge datasets open up a large number of possibilities for GWS, GWAS, understanding the mechanisms that control traits, etc. Finally, our original goal to predict the consequences (phenotype) of variation in genome sequence (genotype) is getting closer.

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L10 Genome−wide identification of regulatory elements in chickens

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Keywords: epigenetics, epigenomics, gene regulation, regulatory elements

Summary

The identification of regulatory elements is a key step in understanding how an organism's genome determines its phenotype. The technologies and assays developed in human and mouse ENCODE projects provide a solid foundation to functionally annotate chicken genome. We present the current progress in generating and analyzing data from this organism, including analysis of 16 RNA-seq libraries, 15 DNase-seq libraries, and 12 ChIP-seq libraries collected from two biological replicates (adult males) across eight tissues: adipose, cerebellum, cortex, hypothalamus, liver, lung, muscle and spleen. With a depth of over 200 million aligned RNA-seq reads per tissue, this study provides a robust gene expression profile that can be correlated with future regulatory regions identified from the other assays. The DNase-seq and ChIP-seq data for H3K4me3 histone modification identify active promoter regions of expressed genes, while H3K27me3 histone modification correlates with repressed gene expression. As more data are generated, an integrative analysis using machine learning methods can provide a comprehensive functional annotation for chicken at a tissue-specific level.

Introduction

Since the human genome sequence was published in 2000, DNA sequencing technology has become faster, cheaper and more powerful, which has allowed the generation of genome sequences for other species at an exponentially increasing rate. In the livestock industry, these genome assemblies have been invaluable in providing fundamental molecular and genetic knowledge for the enhancement of many agronomic traits including production efficiency, food safety, and animal welfare. However, the scientific focus on characterizing the genome has so far been on identifying genes or protein-coding portions of the genome, representing less than 2% of the sequence (Wallis *et al.* 2004; Elsik *et al.* 2009; Groenen *et al.* 2012), which are relatively easy to identify. As our understanding of biology grows, it is becoming increasingly evident that identifying genes and their products is insufficient to explain the genome-to-phenome relationship. Transcriptional regulation, which is a major mechanism behind complex traits, is driven not only by proteins in the coding regions but also regulated by other genomic regions such as promoters, enhancers, and silencers. The identification of these regions is critical to a full understanding of the regulation of gene expression, and therefore to understanding an organism's development and traits. Genome-wide association studies (GWAS) have found 88% of trait and disease-associated SNPs are in noncoding regions of the human genome (Hindorff *et al.* 2009; Andersson *et al.* 2014). The human and mouse ENCODE (Encyclopedia of DNA Elements) projects have produced annotations of functional elements for these genomes which have shown the importance of identifying these regulatory regions (Consortium *et al.* 2012; Mouse ENCODE Consortium *et al.* 2012).

As part of the FAANG (Functional Annotation of Animal Genomes) Consortium, we are working to produce annotations of functional elements for the chicken, cattle, and pig to further enhance our understanding of these highly relevant animal agricultural species (Figure 1). Because functional regulation is highly tissue-specific, we are targeting eight different tissues in order to provide a comprehensive annotation across the species, as well as identify regulatory elements specific to certain tissues which likely have important roles in the function of those tissues. Two male biological replicates were used from a cross of two highly inbred lines of chicken to ensure reproducibility of the data. A total of seven assays are planned for each tissue. RNA-seq will provide gene expression values which can be correlated with regulatory elements for potential functional roles, as well as to identify non-coding transcripts which may play a part in gene regulation. DNase-seq provides a picture of the open chromatin regions of the genome, which are highly correlated with gene expression and regulation. Finally, five ChIP-seq assays covering four histone modifications (H3K4me3, H3K27me3, H3K4me1, and H3K27ac) and the CTCF binding factor allow for the identification of promoter, silencer, and enhancer regulatory regions. The specific objective of this study was to identify regulatory elements in the chicken genome.

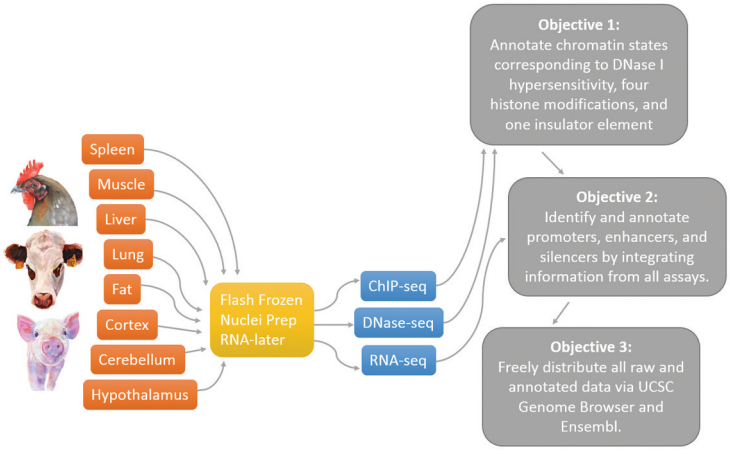

Figure 1 A flowchart showing the procedure for producing functional annotations for chicken, cattle, and pig

Materials and Methods

Lines 6_3 and 7_2, two highly inbred experimental White Leghorn lines at the USDA-ARS, Avian Disease and Oncology Laboratory (ADOL), East Lansing, Michigan that differ greatly in Marek's disease genetic resistance, was selected for this study and intermated to produce F_1 progeny. The use of the F1 progeny was advantageous for two reasons: minimized genetic variation between biological replicates; and allows for identification of allele-specific gene regulation. Eight tissues (adipose, cerebellum, cortex, hypothalamus, liver, lung, muscle, and spleen) were collected from each of two adult male chickens at 20 weeks of age (sexually mature). Depending on the assay to be performed, the tissue was snap frozen in liquid nitrogen, stored in RNA-Later, or process fresh to isolate nuclei then stored in liquid nitrogen.

Library Preparation

Total RNA (1 μg) isolated using Trizol method from tissues above was subjected to two rounds of hybridization to oligo (dT) beads (Invitrogen, Carlsbad, CA) to enrich for PolyA containing mRNA. Ribosomal RNA contamination was evaluated by RNA pico chip using a BioAnalyzer (Agilent, Santa Clara, CA). The resulting mRNA was then used to prepare cDNA libraries using a strand-specific RNA sequencing sample preparation kit (Illumina, San Diego, CA).

Nuclei were isolated from fresh tissues using the following protocol (http://www.mouseencode.org/media/protocols/data_assay/dnasei/01122011_nuclei_isolation_mouse_tissue_V2.pdf) and DNase-seq libraries were prepared and sequenced at High Throughput Genomics Unit at University of Washington (John et al. 2011; Thurman et al. 2012).

For ChIP-seq, chromatin cross-linking and immunoprecipitation were carried out using the Diagenode iDeal ChIP-Seq kit. Briefly, a 3 mm^3 section of liquid NO_2 frozen tissue was thoroughly homogenized in PBS and cross-linked using a 1% formaldehyde solution for 8-10 minutes. The sample was washed and then underwent cell lysis processing. A subset of the cell lysate was taken and stored for quality control purposes at -80 °C. The processed cell lysate then underwent chromatin shearing using the Covaris E220 system. Approximately 200 ul was placed into a Covaris shearing tube and exposed to 6-10 treatments of 60 second 200 cycle per bursts with a peak incident power of 140 W to obtain ~200-300 bp chromatin fragments. An aliquot of the sheared chromatin was treated to reverse the crosslinking followed by visualizzation using gel electrophoresis along with the pre-sheared chromatin to confirm product size. Samples were then frozen at -80 °C and stored until immunoprecipitation.

The sheared chromatin was then immunoprecipitated using protein A-coated magnetic beads and ChIP-seq confirmed antibodies obtained from Abcam. One ul (1%) of the sheared chromatin was set aside for use as an input sample. The chromatin was incubated with the magnetic beads and antibodies at 4°C overnight. Following incubation, the samples had the crosslinks reversed then the DNA purified using the IPure system included in the kit.

Purified immunoprecipitated (IP) DNA was then assessed for enrichment using quantitative PCR. Enrich-

ment was calculated using the equation: % recovery = $2(Ct_{input} - Ct_{sample})$. Efficient enrichment and immunoprecipitation was considered successful with a recovery value of > 5%. Furthermore, fragment distribution of IP samples was visualized and quantified using the Agilent High-Sensitivity system and Quant-IT dsDNA HS assay kit from Qubit, respectively. After successful quality assessment, the samples were prepared as Illumina sequencing libraries following the methods as described in of NEB's NEBNext ChIP-Seq Library Prep Reagent Set for Illumina.

Bioinformatics Analysis

Reads from all assays were first filtered to remove low quality reads, and low quality ends and adapter sequences using the trim-galore program with defaults parameters. For RNA-seq, filtered reads were aligned to the chicken (Galgal5) genome using Tophat. Alignments were filtered to remove those with an average alignment quality less than 15. This cutoff also acts to also remove multi-mapped reads, which are generally of low quality. Duplicate alignments were not removed.

For ChIP-seq and DNase-seq, filtered reads were aligned to the chicken genome (Galgal5) using the Burrows-Wheeler aligner (BWA) with the "-q 15" option to remove alignments with an average quality score less than 15, avoiding low quality and multi-mapped alignments. Duplicate alignments were removed using Picard Tools.

MACS2 was used to do peak calling for both ChIP-seq and DNase-seq. For H3K27me3 and DNase-seq, broad peak calling was performed with a q-value threshold of 0.05. For H3K4me3, a q-value of 0.01 threshold without the broad peak option. Peaks were combined between the two biological replicates for each tissue using the method described by Bardet *et al*. (2011). Briefly, peak calling was performed on one replicate, then each peak was validated in the other replicate by checking that there was statistically significant enrichment of the treatment over the input at the summit of the peak. This was repeated for the other replicate, then the two sets of validated peaks were combined, with overlapping peaks being merged into a single peak. Since DNase-seq does not have an accompanying input to act as control, the replicates were combined by doing peak calling on each replicate and keeping peaks that overlapped by at least 50% between the replicates.

Results and Discussion

Currently, we have completed the sequencing for all RNA-seq and DNase-seq assays, and have sequenced the H3K4me3 and H3K27me3 ChIP-seq libraries for six tissues. We have over 200 million reads per tissue for RNA-seq (Table 1), which is a recommended threshold for the identification of novel isoforms and transcripts with low expression, such as long non-coding RNA. Our DNase-seq and ChIP-seq data has allowed us to identify promoter regions correlated with expressed genes. Due to the difficulty of the DNase-seq assay and the small size of the hypothalamus in chickens, a high quality library for the hypothalamus tissue of bird A was unable to be generated before all material was used. As the remaining assays are generated, we will be able to combine the data using machine learning techniques to create a comprehensive functional annotation across the organism and at a per-tissue level.

Table 1 Aligned and filtered reads from completed assays

Tissue	RNA-seq	DNase-seq	H3K4me3	H3K27me3	ChIP Input
Adipose	198,929,564	76,848,739			
Cerebellum	242,807,223	131,632,284	41,023,866	45,384,874	42,692,478
Cortex	236,147,593	107,561,856	49,805,512	47,458,518	76,985,076
Hypothalamus	244,215,661	74,050,445	64,159,608	71,047,968	83,411,580
Liver	244,674,805	115,407,895	49,061,112	76,310,510	66,759,934
Lung	205,055,604	94,293,805	44,510,256	42,373,494	60,945,834
Muscle	238,435,618	89,981,350			
Spleen	201,084,991	355,071,073	36,701,468	49,291,330	73,482,690
Total	1,811,351,059	1,044,847,447	285,261,822	331,866,694	404,277,592

Table 2 shows the total peaks called per tissue using the methods described above. Note that the ChIP-seq assays for adipose and muscle are not yet completed, and an error in library preparation resulted in no peaks being called for spleen. As can be seen for an example gene in Figure 2, the DHS and H3K4me3 peaks are associated with expressed transcripts, while H3K27me3, which is a modification that silences transcription, is present at very low levels.

Table 2 Peaks per tissue for ChIP−seq and DNase−seq assays.

Tissue	H3K4me3	H3K27me3	DHS
Adipose			6,504
Cerebellum	13,222	21,838	23,037
Cortex	10,270	23,878	27,500
Hypothalamus	22,965	22,762	89,598
Liver	14,274	16,600	45,361
Lung	21,390	18,635	48,176
Muscle			21,861
Spleen	16,600	0	55,894
Total	82,121	103,713	317,931

Figure 2 Read depth of assays at the ADH5 gene. Note that the RNA−seq expression is supported by H3K4me3 and DNase−seq, which are associated with promoter regions, while the silencing H3K27me3 is present at very low read depth

Conclusions

A functional annotation for the chicken genome will lay a solid foundation in understanding genetic regulation of economically important traits. The knowledge gained can be used to improve production efficiency, disease resistance, food safety, and animal welfare of poultry. The RNA-seq, DNase-seq, and ChIP-seq data we have generated provides preliminary insights into the epigenetic landscape of this species, which tissue-specific enriched regulatory regions were associated with biological functions of specific tissues, and future data will expand this to allow the annotation of promoter, silencer, and enhancer regions. By targeting a wide range of tissues for this project, the resulting annotation will be more comprehensive as it will include regulatory regions that may only be present or active in certain tissues. Such an annotation will support even more advanc-

es in the understanding of complex traits and the genome-to-phenome relationship.

Acknowledgements

This project was supported by the USDA Agriculture and Food Research Initiative Competitive Grant no. 2015-67015-22940 from the USDA National Institute of Food and Agriculture and was partially supported by US. Poultry Genome Coordination Funds NRSP-8.

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L11 The current and future of epigenetics in poultry health

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Abstract

Epigenetics is one of important emerging fields in post-genomic era. These epigenetic regulatory mechanisms, including DNA methylation, histone modifications and noncoding RNA, are influenced by environmental factors. To facilitate epigenetics in poultry genetics and health, it is of interest to investigate how the epigenetics and genetics influence the development, health and production of poultry. With this aim, we simply review epigenetic progress and future research in the chicken.

Introduction

The term 'epigenetics' can be loosely defined as the study of changes in the phenotype of an individual caused by mechanisms other than underlying DNA sequence. One of the first indications that there was more to gene regulation than DNA sequence was the discovery of histone modifications and their possible effects on transcriptional regulation[1]. Epigenetics involving phenomena such as DNA methylation, chromatin modifications and non-coding RNAs constitutes a dynamic regulatory framework linking genotypes with environmental factors in differential disease responses among individuals having high genetic similarity[2]. The involvement of DNA methylation in various regulatory functions further confirmed the presence of significant epigenetic mechanisms in transcriptional control. Subsequent studies have shown that epigenetic mechanisms are associated with a multitude of critical biological processes. The advent of next-generation sequencing technology has revolutionized the field, making it possible to investigate histone modification profiles and DNA methylation in a genome-wide manner.

Epigenetics and poultry development

Chicken was one of the first few animal models on epigenetics, especially on DNA methylation[3-8]. In development, the expression of *ovalbumin* and *conalbumin* genes in chick oviduct, and digestions with *HhaI* differentiates between methylated and unmethylated *HhaI* restriction sites[3,9]. Then specific methylation sites of the chicken *alpha-globin* gene cluster were discovered in DNA from embryonic and adult erythroid cells as well as from brain and sperm cells[10-12]. It was reported that DNA methyltransferase activity is lower in meiotic cells containing undermethylated DNA than in immature testis, but enriched in spermatogonia with higher DNA methylation level[13]. Methylation of the upstream CpG island of the chicken *alpha-globin* gene domain may play an essential role in silencing the *alpha-globin* genes in non-erythroid cells[14]. The members of transforming growth factor-beta (TGF-beta) family and the fibroblast growth factor 2 (FGF2) have profound effects on skeletal myoblasts proliferation[15]. Differentially methylated regions (DMRs) may be associated with gene expression during early embryonic development and some epigenetic differences could be evolutionally conserved between mammals and birds[16,17]. Importantly, global DNA methylation levels decrease with the age of hens in the postembryonic stage[18]. In long-term effects, prenatal protein undernutrition by albumen removal leads to long-term alterations of the hepatic transcriptome in the chicken[19]. In addition, linking DNA methylation to the pathogenesis of fatty liver syndrome (FLS) was also specified in chickens[20,21]. *CEBPA* and *PPARgamma* are methylated in adipose tissue may regulate chicken early adipose development[22]. In management, feed condition and breed affect the methylation of *UCP3* in chicken breast muscle[23]. Interestingly, the inter-strain DNA methylation patterns were highly conserved in promoter region between the wild and domestic chicken breeds, indicating a global preservation of DNA methylation in either a genome-wide or locus-specific scale in chick muscle tissues[24].

As we know, Methylation of histones at specific residues plays an important role in gene regulation. Chromatin immunoprecipitation of H3K9Me2 on chicken beta-globin locus during erythropoiesis shows a complete correlation between regions of elevated lysine 9 methylation and acetylation. Lysine 9 is methylated most over constitutive condensed chromatin and to developmentally inactive globin genes, while lysine 4 methylation of histone H3 correlates with H3 acetylation. These results propose a hypothesis that by which the insulator in the beta-globin locus can protect the globin genes from being silenced by adjacent condensed chromatin[25]. Therefore, locus-wide chromatin remodeling and dynamic alterations of histone modifications are required for the

developmentally regulated activation of tissue-specific genes[26,27].

DNA methylation and poultry health

In poultry health, epigenetics in sarcoma-associated herpesvirus was initially studied, especially Marek's disease (MD)[6-8,28,29]. MD is a lymphoproliferative disease caused by Marek's disease virus (MDV) and characterized by T cell lymphoma. The MDV is a naturally oncogenic, highly contagious, and cell associated alpha-herpesvirus[30]. The disease is characterized by a mononuclear infiltration of the peripheral nerves, gonads, iris, various viscera, muscles, and the skin. For a long time, resistance to MD and disease risk have long been thought to be influenced both by genetic and environmental factors, the combination of which contributes to the observed outcome in an individual. To augment vaccination measures, host genetic resistant to MD becomes obviously more and more important. To efficiently control MD, we need to further understand the mechanisms of host-virus interactions. However, most of researches focused on the genetic differences between resistant and susceptible chickens to elucidate the mechanisms of MD resistance [31,32]. The more details of host-MDV interaction are not understood.

In our studies, with the aid of next generation sequencing, we took epigenomics approach in highly inbred lines to identify the mechanisms that contribute to the neoplastic diseases by utilizing the tractable and powerful combination of DNA methylation, histone methylation, noncoding RNAs (microRNAs and lincRNAs), statistical genomics and computational methods. The line 6_3 at the Avian Disease and Oncology Laboratory (ADOL) is relatively resistant to MD tumors but is susceptible to Marek's disease virus (MDV). However, another line 7_2 is susceptible to both MDV and MD tumors[33]. Therefore, these inbred lines with high degree of genetic similarity constitute unique models for epigenetic research because they make it possible to explore mechanisms of resistance and susceptibility to neoplastic diseases. In the three key methyltransferases *DNMT1*, *DNMT3a*, and *DNMT3b*[34], we first found two DNA mutations in *DNMT3b*[35] and a higher promoter methylation level of *ALVE* and *TVB* in the spleen of MD-susceptible chickens compared to that of MD-resistant chickens[36], and the methylation level in *CD4* promoter region was down regulated in the former but not in the later at 21 days post infection (dpi)[37]. To advance the understanding of functional patterns of DNA methylation in disease resistance or susceptibility, we extended the scope of examination to interested genes[38], which include genes related to immune that the expression levels of these genes are alterable upon MDV challenge[39,40]. We found DNA methylation heterogeneity between the MD-resistant and-susceptible chickens. Since MDV induces a dynamic expression change in DNMTs, differential methylation changes have been observed between resistant and susceptible chickens after MDV infection. To thoroughly ascertain the methylation variation induced by MDV infection in both chicken lines, we mapped the genome-wide DNA methylation profiles in each line. We first found that the methylation levels were reduced in chickens from the resistant line after MDV infection and 11,512 infected induced differential methylation regions (iDMRs) were identified. Importantly, we further demonstrated that *in vitro* methylation levels were associated with MDV replication, and found that MDV propagation in the infected cells was restricted by pharmacological inhibition of DNA methylation. The results suggest that DNA methylation changes in the host may be associated with disease resistance or susceptibility. The methylation variations induced by the viral infection may consequentially change the host transcriptome and result in diverse disease outcomes. All in all, the differential DNA methylation levels and its change induced by MDV challenge between the chicken lines suggested that DNA methylation may play a role in host resistance and/or susceptibility to MD[38,41]. In immune cells, the promoter region of *CD30* was hypomethylated and displayed a significantly higher expression in Marek's disease virus-infected tumor spleen tissues, suggesting that activation of *CD30* is possibly associated with the tumorigenesis of Marek's disease[42].

Histone modifications and poultry health

Although our study found that MD-resistant and susceptible birds with different DNA methylation levels on several candidate genes, indicating the potential functions of epigenetic factors in inducing different tumor incidence rates. However, little was known about the histone modification patterns in these two chicken lines before. Therefore, to gain more insights into the function of histone modifications in MD, we performed a histone landscape analysis using ChIP-Seq in the unique MD-resistant and -susceptible chicken lines both before and after MDV infection. Large number of line-specific H3K4me3 modifications and that their underlying genes in immune response and cell adhesion in $L6_3$ chicken were found. Interestingly, we also found that the virus-induced specific H3K27me3 patterns in chicken overlapped with some miRNAs whose target genes involved in novel pathways that may be related to MD-susceptibility [43]. Besides, WaveSeqR, a novel data-driven method of detecting regions of significant enrichment in ChIP-Seq data was developed. The distribution-free

method utilizes the wavelet transform, and is robust to diverse data characteristics such as low signal-to-noise ratios and broad enrichment patterns. The WaveSeqR can detect both narrow and broad peaks with a high degree of accuracy even in low signal-to-noise ratio data sets, and it is also suited for application in complex experimental scenarios, helping make biologically relevant functional discoveries [44]. From significant changed histone modifications, differential H3K27me3 and H3K4me3 marks were associated with immune-related pathways, such as MAP kinase signaling, focal adhesion and neuroactive ligand receptor interaction, and suggested varying degrees of silencing in response to infection[2,45].

Non−coding RNAs and poultry health

Besides static modifications, we also investigated temporal chromatin signatures induced by MDV at early cytolytic and latent phases of infection in the bursa of Fabricius. Immune-related microRNAs, e.g. *gga-miR-155* and *gga-miR-10b*, which suggested their contribution to MD-susceptibility. Finally, several members of the focal adhesion pathway, e.g. *THBS4* and *ITGA1*, showed marked concordance between gene expression and chromatin marks indicating putative epigenetic regulation in response to MDV infection. Our comprehensive analysis of chromatin signatures, therefore, revealed further clues about the epigenetic effects of MDV infection although further studies are necessary to elucidate the functional implications of the observed variations in histone modifications. The aberrant expression of *gga-miR-181a* and *MYBL1* in MD lymphoma might be involved in MD tumor transformation and played important roles [46]. We also found that *gga-miR-26a* inhibited MSB-1 cell proliferation. *Gga-miR-26a* and its direct target, *NEK6*, might play important roles in MDV infection[47]. We also discovered *gga-miR-199-3p* was down-regulated at 14 and 28 dpi, and the expression of *gga-miR-140-3p* was decreased at 14 dpi, which indicated that deregulation of these miRNAs appeared since early stage of MD tumor transformation[48].

Recently, long intergenic noncoding RNAs (lincRNAs) associated with a number of cancers and other diseases have been identified in mammals[49], but it is still formidable to be comprehensively identified and characterized. With this chicken model, we developed a precise pipeline for identifying lincRNAs and to determine the roles of lincRNAs in T cell tumourigenesis[50]. More than 1,000 lincRNA loci were identified in chicken bursa. Computational analyses demonstrated that lincRNAs are conserved among different species such as human, mouse and chicken. The putative lincRNAs were found to associate with a wide range of biological functions including immune responses. Interestingly, we observed distinct lincRNA expression signatures in bursa between MD resistant and susceptible lines. Thus, our results manifested that lincRNAs may exert considerable influence on MDV-induced T cell tumourigenesis and provide a rich resource for hypothesis-driven functional studies to reveal genetic mechanisms underlying susceptibility to tumourigenesis.

Future of epigenetics in poultry

Epigenetics is an active and exciting area of research. It is being driven by the massive amounts of new information being generated by next generation sequencing methods. Current epigenetics offers perhaps the greatest potential for the development, poultry health and welfare as well as production. Although we do not understand the mechanisms, we will identify unique epigenetic factors that could be potentially used as epigenetic biomarkers. The knowledge of host-pathogen interaction will provide a better understanding of epigenetic modifications at a 'systems level' and will serve as mechanistic studies aimed at defining epigenetic roles that underlie poultry health and production. Most importantly, we believe an improved strategy for epigenetically preventive measures against disease will subsequently pave the way for more focused and efficient application of marker-assisted selection (MAS) or genomic selection in poultry breeding program in the near future.

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L12 A review on changes observed in IBDV antigenicity

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Keywords: infectious bursal disease, IBDV, RNA genome, antigenicity, antigenic changes

Summary

Infectious bursal disease virus (IBDV) has a bi-segmented double-stranded RNA genome that allows for genomic reassortment, homologous recombination and point mutations. Not quite unexpected, significant changes in virulence and antigenicity have been observed since the first description of infectious bursal disease almost sixty years ago, causing severe problems in controlling this highly infectious agent by vaccination. Newly established molecular techniques and the elucidation of the structural basis of antigenicity have allowed get some insight into the nature of the most prominent antigenic changes. Here, attempts have been made for a brief review.

Main text

Introduction

Infectious bursal disease (IBD) or "Gumboro disease" is an acute and highly contagious disease in young chickens with great economic importance. The etiological agent is IBD virus (IBDV). Infections of susceptible young chickens with IBDV are characterized by lesions in the bursa of Fabricius, leading to immunosuppression or death. Direct economic losses are due to specific mortality, depending on the virulence of the infecting IBDV strain, the age and the breed of the animals, and the presence or absence of passive immunity; indirect economic losses are caused by secondary infections and failures in vaccination of chickens against other infectious agents due to severe and prolonged immunosuppression. Identification of a second IBDV serotype, appearance of antigenic variant strains and emergence of IBDV strains with an increase in virulence stimulated research on the structural basis of antigenicity. As the principle means of controlling the disease is by vaccination, the comparative antigenicity of field and vaccine viruses is an important issue (Snyder, 1990). Various methods and tools were developed which nowadays, together with the elucidation of structural details of the viral particles and a multitude of sequence data, may help to get some insight into the nature of antigenic changes and possible antigenic drift or even antigenic shift.

IBD emergence and prevalence

IBD emerged in 1957 in the intensively poultry producing Delmarva region in the USA. Between 1960 and 1964, the disease spread to most regions of the USA and reached Europe in the years 1962 to 1971. A high prevalence of IBDV-specific antibodies was observed in breeder flocks, acquired either following vaccination or sub-clinical infection. In the 1980s a second IBDV serotype was identified. Serotype 1 strains are pathogenic for chickens and are now subdivided (van den Berg et al., 2004) into four groups: classical virulent strains (cvIBDV), antigenic variant strains, cell-culture adapted attenuated strains, and very virulent strains (vvIBDV). Serotype 2 strains, isolated from turkeys, are apathogenic for chickens and are common in the field.

In the 1980s two major evolutionary events were observed. Before 1984, in most parts of the world IBD was essentially a subclinical disease, with high morbidity rates, but mortality rates less than 5% and satisfactory controlled by vaccination of breeders. In 1984, however, in the USA new "antigenic variant strains" emerged, causing only a slight increase in mortality, but rapid and severe bursa atrophy with lymphocyte depletion, and an increase in indirect losses due to immunosuppression. Standard serotype 1 IBDV vaccines did not provide good protection (at least 80%) in specific-pathogen-free broilers. Significant antigenic variation was also observed in Australia, where IBD first had been recognized in

1974. Prevailing strains were considered to be classical strains of low pathogenicity and disease was successfully controlled by vaccination with inactivated vaccines based on Australian classical strains. Some IBDV strains isolated during 1994/95, however, proved to be antigenic variants. In 1987/88, very virulent forms of IBD emerged in Europe, characterized by a high mortality of up to 80% in pullets and 25% in broilers. First described in Holland, Belgium and the UK, subsequently in Japan, China and South East Asia in the early 1990s, the disease has now spread all over the world with the exception of New Zealand. Remarkably, these strains, although having in general the same antigenic structure caused disease even in the presence of maternally derived antibodies against the classical vaccine strains, indicating an enhanced virulence. Molecular epidemiology indicates that all vvIBDV strains belong to the same genetic lineage. Nowadays, in the USA antigenic variant strains predominate, whereas in Europe, Africa, Asia and South America vvIBDV strains are predominant.

IBDV classification, morphology, genome organization and general functions of structural proteins

IBDV is a member of the *Birnaviridae* family and the type species in the genus *Avibirnavirus* (King et al., 2012). Virus particles are non-enveloped, icosahedral and single-shelled, with a diameter of 65 nm. The capsid has icosahedral symmetry and is formed by a single strand of the structural protein VP2, clustered in trimers arranged in T = 13 lattices and forming 260 projections of about four nm at the surface of the particle. The crystal structure revealed that VP2 is folded into three distinct domains, designated as base (B), shell (S), and projection (P). The domains B and S are formed by the N- and C-terminal stretches and well-conserved, whereas the domain P is highly variable (for a review, see Delmas, 2008).

The double-stranded RNA genome consists of the larger segment A and a smaller segment B. Segment B contains a single open reading frame (ORF) which encodes the viral RNA-dependent RNA polymerase and other enzyme activities required for transcription and replication of the viral RNA. Two ORF a present in segment A, a larger one encoding a 110 kDA polyprotein and smaller one, immediately preceding and partially overlapping the 110 kDa polyprotein gene. The polyprotein pVP2-VP4-VP3 is auto-catalytically and co-translationally cleaved into a precursor capsid protein pVP2, a viral protease VP4 and a "multitasking" (inner) capsid protein VP3. Processing of pVP2 generates the (outer) capsid protein VP2 plus 4 peptides associated with the virus particle. The smaller ORF encodes a non-structural protein VP5, the biological functions of which have not been elucidated convincingly until now.

Antibodies neutralizing IBDV are induced by a highly conformation-dependent antigenic domain, located on VP2 (Azad et al., 1987; Becht et al., 1988; Fahey et al., 1989). It is composed of at least three independent epitopes. VP3 carries non-overlapping epitopes inducing non-neutralizing antibodies; both serotypes have one of these epitopes in common, whereas the second epitope is distinct for serotype 1 and serotype 2.

Principles of IBDV genetic variation and evolution

The bi-segmented double-stranded RNA genome contributes to considerable genetic variation and to evolution. (i) Numerous *intraserotypic* reassortant IBDV strains have been generated *in vitro* by applying reverse genetics systems such as described by Mundt and Vakharia (1996), Qi et al. (2007) and Ben Abdeljelil et al. (2008), or were isolated from infected animals in the field (e.g., Le Nouen et al., 2006; Wei et al., 2006; Gao et al., 2007; Chen et al., 2012; Lu et al., 2015). Reassortant IBDV strains have been considered as effective vaccines (e.g., Mundt et al., 2003; Stoute et al., 2013; Qi et al., 2014). Reassortant i*nterserotypic* IBDV strains were generated *in vitro* and were investigated in susceptible chickens (e.g., Zierenberg et al., 2004), but were also isolated in the field (Jackwood et al., 2011). (ii) Homologuos recombination may naturally occur between different IBDV strains (Hon et al., 2008; He et al., 2009; Jackwood, 2012). It has to be kept in mind that for both, reassortment as well as recombination, the simultaneous infection of a cell with the parental viruses is a prerequisite. Unfortunately the isolation and unequivocal identification of reassortant IBDV following the experimental simultaneous infection of chickens with e.g., IBDV vaccine strains and vvIBDV have not been reported so far. (iii) Mutation rates of RNA viruses are in the range of 10^{-3} to 10^{-5} substitutions per nucleotide site due to the absence of efficient proofreading and post-replicative repair activities associated with RNA replicases (Domingo, 1997). Not all mutations introduced as the result of error-prone copying of viral templates is represented in progeny genomes. An important filter is provided by the acceptability of mutations with regard to ef-

fective genome replication and ability to complete an infective cycle (Domingo and Holland, 1994). Such mutant viruses have been designated as viral "quasispecies". On the other side, high mutation rates enable viruses to respond to selection pressure; e.g., specific mutations enable variant viruses to resist to neutralizing antibodies (Domingo and Holland, 1997). IBDV quasispecies have been identified in commercial vaccines and field isolates (Snyder at al., 1992; Jackwood and Sommer, 2002), or were generated experimentally as neutralization escape mutants (e.g., Öppling et al., 1991a).

The IBDV hypervariable region

A VP2 hypervariable region spanning from amino acid (aa) position 206 to 350 reflects the high mutation rates in the VP2 encoding region, with aa residues that determine tissue culture adaptation, virulence and antigenic properties. Aa positions 253 and 284 (numbering according to Bayliss et al., 1990) in the P_{DE} and P_{FG} loops of the P domain are involved in tissue culture adaptation, and aa residues at position 253, 279 and 284 are considered to be virulence determinants. Qi et al. (2013) implicated aa at position 249 and 256 as virulence markers. It has been demonstrated, however, that VP2 is not the sole determinant of virulence as segment B-encoded VP1 also determines virulence. The hypervariable region in the central domain of VP2 contains two major hydrophilic peaks designated A (aa 210 to 225) and B (aa 312 to 324). Two other smaller hydrophilic peaks named as 1 (aa 248 to 252) and 2 (aa 279 to 290) also have been identified (van den Berg et al., 1996). Using the algorithm of antigenic index (Jameson and Wolf, 1988), three minor antigenic sites had been identified in the hydrophobic matrix lying between the hydrophilic peaks A and B, representing "hot spots" for mutations in a number of strains, including escape mutants and variant strains (van den Berg et al., 1991; Schnitzler et al., 1993). The four most exposed loops of the P domain, P_{BC}, P_{DE}, P_{FG} and P_{HI}, are indeed located within the hydrophilic peaks. Using the web-based B-cell epitope prediction program ElliPro, Islam (2015) predicted four epitopes in the hypervariable region which were designated as antigenic site 1 (aa 211 to 225), antigenic site 2 (aa 245 to 256), antigenic site 3 (aa 277 to 289) and antigenic site 4 (aa 313 to 331). The predicted antigenic sites overlap with the hydrophilic peaks and each site includes one of the four VP2 projection loops. Antigenic sites 1 and 4, containing P_{BC} and P_{HI}, were predicted to form conformation dependent epitopes. Several amino acid substitutions in laboratory-generated neutralization escape mutants, antigenic variant strains and very virulent strains mapped in these antigenic sites (Vakharia et al., 1994; Eterradossi et al., 1998; Sapats and Ignjatovic, 2000; Islam et al., 2001b; Liu et al., 2002; Letzel et al., 2007).

Identification of a second IBDV serotype

Two distinct serotypes of IBDV are recognized in chicken and turkey flocks. A second IBDV serotype was identified (McNulty et al., 1979; McFerran et al., 1980; Jackwood et al., 1982) using neutralization and cross-protection tests. In one of these studies (McFerran et al., 1980), the isolates in serotype 2 consisted of an antigenically homogeneous group of viruses from turkey and fowl, while, within serotype 1, with isolates from fowl and ducks, some isolates showed only a 30% cross reaction with a vaccine strain. The serotype 1 isolates were from England, Northern Ireland and the USA. A common antigen shared by serotype 1 and serotype 2 viruses was detected using indirect immunofluorescence assay (Jackwood et al., 1982); later on epitopes common to both serotypes were confirmed using VP3-specific Mabs and Western blotting (Öppling et al., 199b). A sequence comparison of segment A ORF1 between the serotype 2 strain OH and classical virulent strains from Europe and Australia revealed that the sequence homology was 83% at the nt level and 89 to 90% at the aa level. In VP2, a larger hypervariable region was 151 to 152 aa long and a smaller had 37 aa residues (Kibenge et al., 1991). Another study (Vakharia et al., 1994) revealed that in serotype 2 strain OH is an insertion of an aa residue at position 249(Ser) and a deletion at position 680. OH lacks a serine rich heptapeptide SWSASGS located at amino acids 326 to 332 and initially considered to be conserved in all virulent strains (Heine et al., 1991). The second hydrophilic peak region was also missed, indicating a possible role of these residues in the serotype specificity or the pathogenicity of the virus. Schnitzler et al. (1993) considered a mismatch of four amino acids in this region responsible for an "antigenic drift" in IBDV. Antigenic relatedness among IBDV strains may be assayed in cross virus neutralization tests which correlate best with cross protection, but are laborious and expensive (OIE, 2008). Such tests have to be performed in embryonated SPF eggs when the viruses under investigation do not grow in chicken embryo fibroblast cell cultures

(e.g. vvIBDV).

Serotype 1− and serotype 2−specific monoclonal antibodies (Mabs)

Serotype 1- and serotype 2-specific monoclonal antibodies (Mabs) were produced (Becht et al., 1988) by injecting mice with cvIBDV strain Cu-1 (Nick et al., 1976) and strain 23/82 (Chettle et al., 1985), the latter isolated from turkeys and neutralized by antibodies directed against serotype 2 prototype strain TY89. Before, both viruses had been plaque-purified repeatedly and were highly purified by gradient centrifugation. Mabs were obtained that neutralized the homologous viruses with high specificity (e.g., 1/A6, 14/B5 and 41/A2 in the case of Cu-1, and 44/A1 of 23/82, respectively). Evidence was obtained that epitopes inducing neutralizing antibodies were located on VP2 and were highly conformation dependent. Epitopes inducing serotype-specific non-neutralizing antibodies were also identified on VP2. None of the antibodies which recognized VP3 had neutralizing activity. For both serotypes, two independent non-overlapping epitopes were demonstrated on VP3, one in common for both serotypes, and the other distinct for each serotype (Öppling et al., 1991b). By the generation of neutralization escape mutants (Öppling et al., 1991a) it was demonstrated that at least three overlapping epitopes are located on VP2. In one of these mutants, EM8, selected by Mab 1/A6, two aa were exchanged (Val313Glu, Gly322Arg); other mutants, e.g., EM300 and EM401, prepared in two selection steps employing Mabs 1/A6 and14/B5, the same aa exchange was observed, but others in addition (Gly233Asp and Ser251Arg, respectively). In a combined immunoprecipitation-immunoblotting experiment, neutralizing Mabs 1/A6, 14/B5 and 4/B4 were shown to bind to VP2 of vvIBDV isolated in 1988 in flocks with vaccination failure in Germany or in Nigeria (Öppling et al., 1991a). Mab 1/A6 conferred protective immunity to susceptible chickens, whereas VP3-specific antibodies did not have any protective effect.

Mabs suitable for the identification of vvIBDV

A panel of nine Mabs was prepared (Eterradossi et al., 1997a) using gradient-purified IBDV "intermediate" live vaccine strain Gumboral CT (Rhône Mérieux, Lyon, France). At least two distinct serotype 1-specific conformation-dependent overlapping neutralizing antigenic domains were shown to be present on IBDV-VP2, and were respectively probed by Mabs 3 and 4, and by Mabs 6 and 7. Whereas most mild or intermediate vaccine strains were efficiently neutralized by all Mabs, US variant A, European pathogenic strain F 52/50 and French vvIBDV 89 163 were not neutralized by Mabs 3 and 4. In an antigen-capture (AC)ELISA, these two Mabs were shown to bind to F 52/70, but not to the vvIBDV strain (Eterradossi et al., 1997b). Negative reactions with Mab 3 were associated with changes in the Proline-Glycine pair at aa positions 222-223 in the hydrophilic peak A (Eterradossi et al., 1998). In a large number of studies, these two Mabs proved useful for antigenic differentiation between European cvIBDV and vvIBDV isolates. Two other neutralizing Mabs, 6 and 8, were shown (Eterradossi et al., 1997b) to bind to both, cvIBDDV and most vvIBDV, but not to some atypical vvIBDV strains obtained from geographically and chronologically unrelated clinical cases due to aa changes at positions 318 to 324 (hydrophilic peak B). They exhibited different additional epitope modifications and were considered unlikely to represent successive steps in an antigenic drift.

Antigenic and genetic characterization of Polish IBDV isolates

The panel of Mabs established by Eterradossi et al. (1997a) was used for the antigenic characterization of IBDV strains isolated in the 1970/80s ("early IBDVs") or in the 1990s ("recent IBDVs") in AC-ELISA (Domanska et al., 2004). Recent polish isolates had the antigenic profile typical of vvIBDV, in accordance with results of aa analysis in VP2. However, two of the recent isolates proved to be similar to a French atypical IBDV strain as one epitope (Mab 8) had been lost; these isolates also share 99.8% identical nt sequences. It has been tempting to speculate, therefore, that the same IBDV strains were exchanged between the two countries. Unexpected results were also observed with early Polish viruses as their Mab reaction pattern indicated that they were different from both, the European reference strain F52/70 and the recent Polish vvIBDV isolates. This is in accordance with a report by Mato et al. (2001) showing that several early Hungarian IBDV isolates belong to a cluster different from the "classical" IBDVs.

Masbs prepared with vvIBDV isolates from Belgium

With gradient-purified viruses, vvIBDV strain 849VB and the vaccine strains PBG98 and D78 (van den Berg et al., 1996), Mabs were generated and four distinct epitopes were defined by neutralization and ELISA addition tests. Mab 7C9 was specific for mild vaccine strains, the relevant epitope being absent or modified on intermediate or pathogenic strains; for these strains, Mabs 1B2, 7C7 and 6F6 were specific and representative. These epitopes, however, were different from those recognized by Mabs 14/B5 and IV/B8 (Öppling et al., 1991a). No neutralizing Mab specific for vvIBDV strain 849VB was obtained, confirming that, antigenically, this strain still belongs to classical serotype 1. It became evident from this study that 849VB was not neutralized by Mab 14/B5, previously shown to neutralize another vvIBDV isolate (Öppling et al., 1991a), indicating that antigenic differences exist between vvIBDV isolates. It has been concluded, therefore, that typing of IBDV strains by Mabs for protection purposes could be questionable, as Mabs cannot differentiate between a minor (without implication in protection) and a major antigenic drift (van den Berg et al., 1996).

Mabs produced with virulent and attenuated Japanese IBDV isolates

Japanese IBDV isolate GBF-1 and the attenuated strain GBF-1E were used to produce Mabs that allowed the definition of at least three conformation-dependent serotype 1 specific virus neutralizing antigenic sites and a linear antigenic site located on VP2 and VP3, respectively (Yamaguchi et al., 1996). Mab recognition sites were mapped using recombinant *E. coli* clones which expressed N-terminal and (or) C-terminal truncated virus antigens, and competitive-binding assays. These showed that two of the virus neutralizing antigenic sites were localized in the central area of VP2 consisting of 156 aa residues. Another conformational virus neutralizing antigenic site recognized by a neutralizing Mab was not defined and it was supposed that the conformational antigenic site was composed of tertiary of quaternary protein structures which may not be constructed by recombinant *E. coli*.

Mabs used to identify linear epitopes

Mabs YNW17 and YNW29 (Yang et al., 2000) were used to identify IBDV neutralizing epitopes by phage-displayed heptapeptide library screening and synthetic peptide mapping (Wang et al., 2005). Both Mabs were able to neutralize vvIBDV strain H4 and the attenuated vaccine strain B87. Two linear epitopes were identified: D-X-P-R, located at aa position 201-204 recognized by Mab YNW17 is at the N terminal of the highly variable region of VP2, overlapping 4 or 6 aa with the N terminal of the conformational epitope; A-R-G, located at aa position 329-331 and identified by YNW29 is at the C terminal of the highly variable region and completely within the C terminal of the conformational epitope. Reactivity of overlapping peptides with the Mabs indicated linear epitopes at aa positions 179-209 and 329-337, respectively. These two linear epitopes were also recognized by chicken B cells as the corresponding peptides were reacted strongly with chicken anti-IBDV sera.

Identification of US IBDV variant strains

First US IBDV variant strains were isolated applying a Sentinel birds approach (Rosenberger et al., 1985) with chickens previously vaccinated with a mild, live IBDV vaccine. In contrast to cvIBDV, these isolates (designated as antigenic variants, Delaware A, D, G and E) produced a very rapid bursal atrophy associated with a minimal inflammatory response. In subsequent cross-challenge studies it became evident that commercially available killed vaccines derived from classic viruses did not afford as complete protection against these isolates as with challenge by the standard challenge virus (Snyder, 1990).

Mabs were produced by immunizing mice with one "or more" (several variant?) strains of IBDV (Snyder et al., 1988a). These antibodies are currently in use for analyses of IBDV field isolates by AC-ELISA. Mab B69 significantly neutralized only IBDV strain D78, whereas Mab R63 neutralized serotype 1 and serotype 2 strains. By results obtained with AC-ELISA, the observed antigenic change was due to an apparent deletion or alteration of the neutralization site defined by Mab B69. All cvIBDV field and vaccine strains isolated prior to 1985 had the B69 site while the variants isolated in 1985 did not (Snyder et al., 1988b). A second antigenic variant isolated shortly thereafter and designated GLS was characterized by the absence of both Mab-defined neutralizing sites, R63 and B69. Finally a panel of nine Mabs was prepared that included seven distinct neutralizing Mabs and two non-neutralizing Mabs.

It was concluded that at least five distinct, but closely linked neutralizing epitopes play a role in protection of chickens from challenge with cvIBDV (Snyder et al., 1992). A comparative study with reference (IM, STC, MD and Edgar strains) and live IBDV vaccine strains, employing these Mabs in AC-ELISA, revealed that nine vaccine and three reference strains had similar reaction patterns. All of those strains were isolated before 1985 and were designated as Classic viruses, although they varied in virulence and were neutralized differently. All of a total of 20 European isolates yielded reactivity patterns identical to US Classic viruses (van der Marel et al., 1990). The reference variant viruses provided three additional reactivity patterns denoted by the successive loss of Mab-defined neutralization sites (Snyder et al., 1992). In this study, a distinct geographic distribution pattern was observed, considered to reflect the antigenic evolution of these viruses. In isolated and less dense broiler growing areas, cvIBDV strains were predominant, whereas in the more densely broiler populated areas the isolation of cvIBDV was a more rare event. Areas with high broiler density yielded high numbers of variant IBDV isolates. Selective vaccination pressure by using cvIBDV strains in live vaccines may also play a role and, by the sequential loss of neutralization sites, the isolated variant strains were regarded as naturally occurring neutralizing Mab escape mutants (Snyder et al., 1992).

The molecular basis of antigenic variation of a number of isolates was determined. According to Vakharia et al. (1994), Gln at position 249 (Gln249) appeared to be critical in binding with Mab B69, and several other residues were identified to be involved in binding of individual neutralizing Mabs. Later on, however, the critical role of Gln249 was put in question (Letzel et al., 2007) and it was hypothesized that B69 could bind at a different region of VP2, away from loops P_{BC} and P_{HI}, probably to the S domain of VP2. Using some of the Mabs established by Snyder et al. (1992) and a reverse-genetics approach, these authors found that a few aa residues of VP2 contribute substantially to the antigenic properties of IBDV as only five residues located in two structural loops (P_{BC} and P_{HI}) at the tip of the VP2 P domain deeply modulate its immunoreactivity. It has been found that (i) the presence of a specific residue can be necessary for the recognition of an epitope, but others can contribute less critically to its reactivity, (ii) aa modulating binding of Mabs are adjacent in the 3-dimensional structure of VP2, although they are distant in sequence, and (iii) in some cases, the reactivity of an epitope excludes the binding of another antibody. Furthermore, Letztel et al. (2007) observed that replication of several IBDV recombinants was impaired relative to the recombinant D78 strain. Whereas some mutations, e.g., mutation of aa 222 (Pro to Ser) in loop P_{BC} showed no significant effect on replication in cell culture, others with aa substitutions in loop P_{HI} grew to significantly lower titers than the parental strain, possibly by a negative effect on binding to cellular receptors (reduced fitness for survival; Domingo & Holland, 11997)..

Two distinct genetic groups exist in Australia

IBDV strains endemic in Australia are characterized by an inability to cause marked signs or lesions, or death. Strain 002-73, isolated 1973 from a flock with mild disease is the typical representative of IBDV strains prevalent in Australia. In 1994/95, five out of six IBDV strains, isolated from broiler flocks with mild cases of IBD (Sapats and Ignjatovic, 2000) were antigenic variants, whereas one, isolated in a separate geographic location, was similar to classical-like vaccine strains (Ignjatovic and Sapats, 2002). Bursal homogenates were tested in a two stage antigen ELISA with a panel Mabs raised against strain 002-73 (Fahey et al., 1991). Three of these Mabs, 17-82, 39A and 9-6, are directed against viral neutralizing epitopes on VP2 and provide protection against virulent challenge; reactivity in an IBDV field strain indicates antigenic conservation and similarity with vaccine strains. Isolates identified as variants did not react with Mabs 39A and 44-18; others did not react with Mab 17-82, indicating a further antigenic change in the protective region of VP2. None of the 14 isolates under investigation shared sequence identity with any overseas IBDV strain. Phylogenetic analysis confirmed that Australian IBDV strains belong to two distinct groups, variant and classical strains.

A survey on antigenicity in recent Chinese IBDV isolates

In China, first cases of IBD were reported from Guangzhou Province in 1979. IBDV strain CJ801 was isolated in Beijing in 1981, and vvIBDV causing up to 100 % mortality in Harbin in 1991 (Zhang et al., 2001). Later on, however, atypical cases of IBD, characterized by transient depression, slight diarrhea and less or no mortality were also observed (Wang et al., 2001). As IBD had been well controlled before

by the use of conventional attenuated live and inactivated IBD vaccines, the new clinical forms of IBD indicated the emergence of highly pathogenic and/or antigenic variant IBDV strains. The analysis of strains isolated in the late 1980s and early 1990s at a molecular level showed that these were indeed vvIBDV and antigenic variant IBDV strains, closely related to vv European and variant American strains (Cao et al., 1998; Chen et al., 1998). Neutralization assays with 11 of such Chinese IBDV isolates (Liu et al., 2002) showed that 10 isolates belonged to the same subtype as attenuated strains while one (strain BX) belonged to a the subtype consisting of variant strain GZ902 isolated 1991 in Guangzhou. Remarkably, the early classical virulent strain CJ801 was assigned to a third subtype, different from those mentioned before. AC-ELISA using Mabs (Snyder et al. 1986; Tao et al. 1995) showed that nine out of 10 vvIBDV strains, isolated in a period from 1997 to 2000, lacked the neutralizing epitope B69 present in the classical vaccine strain D78. The variant strains BX and GZ902 were not recognized by Mabs 5B11, 2B8 and 2C4, reacting with an epitope present on VP2 of the classical strain Lukert. The results of a sequence comparison of the VP2 hypervariable region and a phylogenetic analysis suggested that the vv and variant strains isolated in China in the late 1990s share similar origins with the vv strains in Europe and Asia and the variant strains in the USA and might not have been derived from the early Chinese cv strain CJ801.

In a recent study (Li et al., 2015) one Chinese strain isolated in 1999 and three strains isolated between 2005 and 2011 were analyzed at the genetic, antigenic and pathogenic levels. Whereas early Chinese vvIBDV isolated in the 1990s exhibited intermediate pathogenicity, most probably due to the absence of a typical vvIBDV-like segment B, mortality caused by the recent vvIBDV strains was much higher, indicating a trend towards an increased pathogenicity of the Chinese vvIBDV in recent years, a feature that could be associated with a novel segment B, or on especially successful combination of their segments A and B. In AC-ELISA experiments using a panel of Mabs (Eterradossi et al. 1997), all Chinese vvIBDV exhibited antigenicity similar to the European typical vvIBDV strains. Based on the sequences of genome segments A and B, strain SH99, isolated in Shanghai in 1999, proved closely related to typical vvIBDV. However, the three more recent vvIBDV isolates showed several genetic changes in both segments and clustered in a lineage distinct from typical vvIBDV and the previously known Chinese vvIBDV. With regard to higher pathogenicity of the newer Chinese vvIBDVs, it was speculated that either by homologous recombination, or by reassortment, these strains might have acquired a "new" segment B from a reservoir unknown until now.

Concluding remarks

The characteristics of the IBDV bi-segmented double-stranded RNA genome allows for a number of genetic modifications: genomic reassortment, homologous recombination and point mutations, all of which might be of influence on IBDV virulence and antigenicity. Since IBDV had been isolated for the first time more than fifty years ago, a large number of IBDV variant strains have emerged which were of considerable impact to the poultry industries. Standard as well as newly established techniques have allowed the identification of changes in the clinical picture of IBD. Molecular techniques developed in the last decades allowed to analyze the genomes of the new variants and to elucidate the structural basis of antigenicity, both being of highest benefit for the production of diagnostic tools and effective vaccines.

As early as in 1993, Schnitzler et al. (1993) have proposed a general concept for antigenic changes in IBDV which is in accordance with the observations made by many others: "Exchange of one aa in the hydrophilic areas A and B is the motif for an antigenic drift; more exchanges in A and B may lead to the establishment of a serotype 1 variant strain, and replacement of four amino acids with a concomitant loss of hydrophilicity in A and particularly B can create a new serotype". With regard to point mutations, the accumulation of positive mutations may lead to a "gain of function", resulting e.g., in the establishment of escape mutants or, with regard to genome segment B, in an increase in polymerase and other enzyme activities of VP1. Meanwhile a multitude of sequence data on VP2 of a large variety of IBDV isolates has accumulated. As the structure of VP2 has been elucidated in many details, the localization of aa exchanges is now possible. With regard to significance, however, it has to be kept in mind that some epitopes are due to the tertiary (multimeric) structure of the capsomeres.

The Author regrets that due to space limitations only a reduced number of reports / publications could

be included in this brief review.

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L13 Classical and variant infectious bronchitis viruses: epidemiology, diagnosis and control strategies

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Keywords: infectious bronchitis virus, classical, variant, epidemiology, diagnosis and control strategies

Summary

Avian infectious bronchitis virus (IBV) causes respiratory, renal and reproductive diseases in chickens, leading to economic losses for producers and increased welfare concerns. With the use of traditional and molecular techniques, numerous variant strains have been identified globally, in addition to classical strains. In general, these strains can be divided into 3 main categories; i) classical strains, mainly the Massachusetts (Mass) and related, ii) variant strains of global importance, iii) variant strains of regional importance. Though the reason for the emergence of variant genotypes is unclear, it has been suggested that the RNA virus undergoes point mutation or recombination events which may allow emergence of new genotypes. Other than the Mass, currently 793B, QX and Q1 IBVs are considered to have significant global impact. Almost every country has their own population of IBVs, some examples being Arkansas, Delaware (GA 07, GA 08) and California in the USA. In addition, IS/885/00 and IS/1494/06 (variant 2) are commonly found in the Middle East, BR1 and BR2 in Brazil, It-02, D1466, B1648 in Europe, genotype I, II or III in Korea, and LDL, BJ, LHLJ and LDT3 in China. With regards to control of production losses due to IBV, live and inactivated vaccines are available. Though developments of new live vaccines are ideal for emerging variant IBVs, it is not possible to manufacture one for every variant IBV. Live vaccines of Mass, 793B, D274, QX, few regional vaccines, and inactivated vaccines of M41, D274, 793B and QX, are used worldwide. It has been reported that a combination of classical and variant live vaccines could increase and broaden the protection conferred against classical and variant IBVs. Whilst waiting for an innovative biotechnology-based vaccine, at present, it appears that strategic vaccinations using heterologous live classical and variant vaccines, alongside appropriate inactivated vaccines, is the best option available for many chicken producers.

Introduction

After avian influenza and Newcastle disease viruses, infectious bronchitis virus appears to be the most important cause of respiratory diseases in chickens. In addition, this virus also causes reproductive and renal infections and pathology. Co-infection with other pathogens tend to cause more severe disease outcomes. Damages to the respiratory, reproductive and renal systems lead to huge economic losses due to poor health, production and welfare concerns (Jackwood and de Wit, 2013, Ganapathy, 2009). The losses due to IBV are controlled with the use of live and inactivated vaccines. However, the virus continues to pose a great danger to the chicken industry worldwide, especially with the emergence of variant strains. This paper highlights some importance aspects of IBV epidemiology, variations in the disease caused by different IBVs, advances in the diagnosis, and current strategies in the control and prevention of IBV globally.

Global Epidemiology

In 1936, the causative agent of respiratory infections (Schalk and Hawn, 1931) affecting flocks of chicks was named infectious bronchitis virus (IBV), belonging to the Massachusetts (Mass) serotype. Thereafter a number of IBV strains divergent from the Mass were reported and classed as new serotypes (eg. Connecticut, Arkansas, D388) through serum neutralisation. By the 1990's, wider availability of molecular techniques advanced strain typing onto genotyping. Ease of use, speed, and high sensitivity and specificity resulted in IBV genotyping being widely used worldwide. IBV is a single stranded RNA enveloped virus with four structural proteins: the spike (S), membrane glycoproteins (M), a small mem-

brane envelope protein (E) and an internal nucleocapsid protein (N) (Lai and Cavanagh, 1997). The S protein consists of two subunits, S1 and S2. The S1 subunit plays an important role in inducing the release of neutralizing and haemagglutination inhibiting (HI) antibodies against IBV (Koch et al., 1990, Cavanagh et al., 1988). To date, rather than full S1 or whole genome sequencing, the majority of research laboratories only undertake amplification and sequencing of part-S1 gene for genotyping.

Though epidemiology has a wide application, this write up concentrates on the distribution of IBV strains globally. Based on serotyping and genotyping, the current population of IBVs can be grouped into three main segments; i) the classical strains, basically Massachusetts or related viruses that present worldwide, ii) the variant IBVs that are of global importance (eg. 793B, QX, Q1), and iii) regional variant IBVs that are of importance in a specific region or country (eg. Arkansas, Delaware (GA 07, GA 08) and California in the USA, IS/885/00 and IS/1494/06 (variant 2) in the Middle East, BR1 and BR2 in Brazil, It-02, D1466, B1648 in Europe, genotype I, II or III in Korea, LDL, BJ, LHLJ and LDT3 in China) (Jackwood, 2012). The main reason for the appearance of variant strains seem to be point mutations or recombination events when more than one genotype infects the same cell (Cavanagh et al., 1992, Lai and Cavanagh, 1997, Wang et al., 1994).

Changing disease manifestation (or severity)

The first description of the disease caused by IBV was reported by Schalk and Hawn in 1913, where primarily respiratory signs and pathology were described (Schalk and Hawn, 1931). In the 1960's, evidence of IBV causing renal disease emerged (Cumming, 1963, Winterfield and Hitchner, 1962). In laying birds, certain IBV strains can cause a drop in egg production and quality (Muneer et al., 1986, Cook and Huggins, 1986). Subsequently, a number of studies using different IBV isolates around the world demonstrated very similar pathogenesis. The only differences highlighted were the tropism of the new isolates, where some caused more severe respiratory, renal or reproductive diseases than others. There were few exceptions to this observation, wherein certain isolates tended to cause signs and/or pathology away from the traditionally affected tissues. For example, IBV 793B causes deep pectoral myopathy (Gough et al., 1992, Raj and Jones, 1996), QX is associated with proventriculitis (Wang et al., 1998, Ganapathy et al., 2012), and other reports have associated IBV with swollen head syndrome (Awad et al., 2016, Droual and Woolcock, 1994, Morley and Thomson, 1984) and enteritis (Villarreal et al., 2007). In general, the disease severity varies depending on the virulence and dosage of the strain, secondary agents, flock age, host immune status, management and environmental factors (Cavanagh and Naqi, 2003, Ganapathy, 2009).

Traditional to molecular diagnosis

Clinical diagnosis of IBV is difficult as the resulting signs and pathology are similar to those produced by many other diseases. History taking, clinical and pathological assessments must be followed with appropriate sample collection for demonstration of the antigen or antibodies against it (Ganapathy, 2009).

For antigen detection, an attempt is made for the virus to be traditionally grown in the SPF embryonated eggs and/or in tracheal organ cultures (Cook et al., 1976). In recent decades, the most frequently used tool for IBV detection is either the conventional or real-time reverse transcription polymerase chain reaction (RT-PCR). Subsequently, the PCR product is sequenced and analyzed to determine the genotype (Adzhar et al., 1996, Worthington et al., 2008, Awad et al., 2014) In most instances, this would allow genotype identification and to some extent, differentiation of the vaccine against virulent strains. Evidence of immune responses are mostly confined to detection of humoral antibodies by the enzyme linked immunosorbent assay (ELISA) and hemagglutination inhibition (HI) (Awad et al., 2014, Ganapathy, 2009, de Wit, 2000), and occasionally virus neutralization. In monitoring protection against virulent IBVs, it appears that IgA in tear and CD8+ populations in the trachea could be an important immune indicator (Chhabra et al., 2015). For an efficient diagnosis and surveillance of IBV in endemic regions/farms, it is best to use a combination of serology and virus detection by PCR, isolation and genotyping.

Evidence−based preventive measures

There are three main elements for control and prevention of losses caused by IBV. These are generally

management, biosecurity and vaccination, the first two forming the foundations for an environment that is minimal or free from virulent IBVs, and more importantly sustaining healthy and immunocompetent flocks. With these strongly established, an 'immunological shield' is formed in the flock(s) through the use of appropriate live and inactivated vaccines in the breeder, layer and broiler flocks. For a successful and effective IBV vaccination program, it should link the immunological shield in the breeders to the shield in the layer and broiler flocks. It is essential to remember that what provides the protection against losses caused by IBV is the immunological package developed in the flock(s). In inducing such immunological development, two important factors need to be considered, i) protection against which strain of IBVs, ii) what sort of live and inactivated vaccines could possibly induce a protective immunological shield.

In regards to evaluating vaccination against IBV, following vaccination and challenge, most studies concentrate on demonstrating freedom of disease (eg; clinical signs, macro and microscopic lesions), production losses and reduction in antigen concentration (de Wit and Cook, 2014). Studies to date have shown that strategic heterologous vaccination leads to higher and broader protection against classical and variant viruses (Cook et al., 1999, Awad et al., 2015, Chhabra et al., 2015). Chhabra and others demonstrated that the increased protection provided was mainly associated with IgA in the tears and CD8+ cells in the tracheal sections (Chhabra et al., 2015).

Conclusions

Historically, the main body systems affected by classical and variant IBVs remain the same -respiratory, reproductive and urinary systems. However, the disease severity varies according to the genotype, with some having a more severe impact on certain systems. It appears that the emergence of variant IBVs are unavoidable, as IBV is a RNA virus which tends to go through point mutations and possible recombination events. This is encouraged by complex chicken production systems and often inappropriate use of vaccines and vaccination programs that leads to partial flock immunization. Overall improvement in the chicken management and biosecurity is essential, and this needs to be supplemented with an appropriate strategic vaccination. Inclusion of more than one genotype of live and/or inactivated antigens is often preferred. A vaccine strain(s) that is closely-related to the circulating IBV should be included in the vaccination program for optimal protection. Control of IBV by vaccination needs to be coordinated, as programs in the breeders will most likely have some influence on the choice of vaccine and vaccination strategies in the layer and broiler flocks. To date, IBV vaccination is still relying on the use of conventional live and inactivated vaccines, and this would continue until an innovative biotechnologically based vaccine is found.

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L14 The impact to avian influenza epidemic by interventions to live poultry market

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In recently years, the general epidemic status of avian influenza was stable in China with the measures including compulsory immunization, surveillance + culling. However, because of the unstability of immune effect, viruses with different subtypes coexistence, the variation and recombination of the viruses, the prevention and control situation of AIV is still austere.

Live poultry markets were considered as the reservoir of avian influenza virus and the potential source of highly pathogenic avian influenza virus such as H5 and H7 subtype virus. Therefore, the prevention and control policy named "1110" has been implemented for all live poultry markets in Guangdong Province since March 2015, in an effort to prevent and control highly pathogenic avian influenza and reduce the risk of H5N1, H7N9 transmission to humans. "1110" policy measures include once-monthly rest-day, once-weekly thorough cleanup, cleaning and disinfection of each day and ban on overnight poultry storage of retail live poultry markets. In order to evaluate the effectiveness of the monthly rest-day measure, the environmental samples were collected and analyzed for the presence of virus before, during and after the rest-day (totally 11 rounds) in one wholesale market and two retail markets in Guangzhou, the capital of Guangdong. The study showed that although the positive rates of avian influenza virus declined dramatically immediately after rest-day, the rates increased significantly from the second day after the rest-day. This suggests that the measure is effective for reducing the virus, however, rest-day measure alone may not be sufficient in controlling and eradicating avian influenza virus in live poultry markets. In another study, for evaluating the effectiveness of ban on overnight poultry storage of retail live poultry markets for avian influenza control, two retail live poultry markets of Guangzhou were selected for the whole 4 weeks study. For the first and third weeks, non-ban on overnight poultry storage measure was taken; for the second and fourth weeks, ban on overnight poultry storage measure were taken. The environmental swabs and live poultry swabs were sampled 3 times every week and tested with real time RT-PCR for avian influenza virus. The study is the first systematic interventional epidemiological study for evaluate the effect of ban of poultry overnight storage in retail live poultry markets for avian influenza controlling. From our study, we suggest that the measure of ban of poultry storage in retail live poultry markets may be effective, however, the costs for measure implemented are relatively high and the measure implementation should be reconsidered. Therefore, the comprehensive measures including strengthen education for citizens, market managers and employees and other stakeholders, implementing the concentrated slaughter step by step, stop live poultry selling and slaughter in densely populated area should be taken for avian influenza prevention and control.

Acknowledgements

This work was supported by grants from the Provincial Natural Science Foundation of Guangdong Province(20140224), the National Natural Science Foundation of China (U1501212), the Earmarked Fund for Modern Agro-Industry Technology Research Systems (nycytx-42-G3-03).

L15 Impact of housing systems on welfare, health and behaviour of pullets and laying hens

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Key words: laying hen, pullet rearing, welfare, housing

Summary

Public concern for the welfare of laying hens is rapidly changing the ways that eggs are being produced and marketed around the world. Legislation and retailer requirements in many countries now require that laying hens are housed in enriched or cage free systems that provide more freedom of movement and opportunities to perform a greater range of natural behaviour. However, there can be welfare problems in alternative systems as well, including greater risks for injury and mortality. The objective of this paper is to present an overview of hen well-being in different housing systems and to introduce some new directions in research that are focused on optimizing design and management to improve hen welfare. One important area of research is focusing on rearing systems for pullets in order to improve our understanding of how early life experience affects behavioural and physical development of pullets and the life-long welfare of laying hens.

Introduction

Public concern for the welfare of laying hens is rapidly changing the ways that eggs are being produced and marketed around the world. Legislation and regulatory requirements for hen housing have been or are being adopted by many countries. The EU Council Directive 99/74 for laying hens prohibits the use of non-enriched cages systems since January of 2012. In the USA, several states have legislated bans on conventional cages that have already or soon will come into effect. In New Zealand, conventional cages are due to be phased out by 2022 under the Code of Welfare for Laying Hens, and recently Egg Farmers of Canada announced that no new conventional cage systems are to be installed after July 2016. Additionally, numerous food labeling and certification schemes have been developed for the purpose of marketing specialty "animal-welfare friendly" products to ethically conscious consumers. In North America, food processers, supermarket and restaurant chains are driving massive changes by requiring that egg suppliers adopt housing systems that consumers consider to be good for animal welfare. In 2015 and early 2016 an unprecedented number of restaurants, grocers and distributors made pledges to purchase eggs only from non-cage systems within the next 10 years, which will require new housing for over 150 million hens in the USA and Canada.

Although the broader community is placing more emphasis on freedom of movement and behavioural opportunities for laying hens, the scientific assessment of animal welfare involves multiple measures that capture different viewpoints on what constitutes a good quality of life for animals. These include measures of health and biological fitness (e.g. physical condition, mortality, production, indicators of stress), the emotional or subjective experiences of animals (e.g. conditions leading to pain, fear, discomfort or pleasure) as well as the ability to perform natural or species-typical behaviour patterns. It is often difficult to make broad comparisons of the welfare of animals in different types of housing systems since there is a wide range of alternative systems and the welfare of hens in those systems largely depends on details of system design and management. A number of literature reviews and scientific reports on the welfare of laying hens in different housing systems have been published (e.g. Lay et al 2011; Widowski et al 2013), and there are several recent reports comparing various welfare measures from data collected on large-scale commercial farms (Sherwin et al 2010; Petrik et al, 2015; Blatchford et al 2016; Weeks et al 2016). It is clear from these reports that there can be significant trade-offs in terms of different aspects of welfare for hens in different commercial-scale systems. Additionally, there can be trade-offs concerning other important factors affecting the sustainability of egg production such as environmental impacts, food affordability and worker safety (Swanson et al 2015; van Asseslt et al 2015). As new housing systems for laying hens are rapidly evolving, more research is aimed at identifying specific causes of welfare problems in these alternative systems and developing means to alleviate those problems. The objective of this paper is to present a brief overview of hen well-being in different housing systems and to introduce some new directions in research that are focused on optimizing design and management to improve hen welfare.

Welfare trade−offs in different housing systems

Conventional cages are criticized because the lack of space and barrenness of the environment in them impose a high degree of behavioural restriction for hens. It is well established that laying hens are highly motivated to gain access to a nest box to lay their eggs and to perform perching, foraging and dust bathing behaviour. Even basic activities such as locomotion, stretching and wing-flapping are significantly constrained in conventional cages, and the lack of load bearing exercise reduces bone strength (Widowski et al 2013).

Housing laying hens in cage-free systems with nests, perches and litter (or free range with access to the outdoors) offers substantial opportunities to perform a greater range of locomotion and engage in natural behaviours, but it can also significantly increase the risk of injury and poor health. Risks are highly dependent on the individual system or individual farm. Laying hens housed in non-cage systems such as single-tier floor systems and multi-tier aviaries have a greater risk of infectious diseases, broken bones, internal and external parasites and generally have higher rates of mortality than hens housed in cages (Lay et al 2011; Widowski et al 2013). A recent report based on data from over 3500 commercial flocks in the EU indicated that mortality rates were significantly higher and more variable in non-cage and especially in free-range systems compared to cage systems (Weeks et al 2016). The risks were considerably greater when hens were not beak trimmed, and were significantly associated with breed. Feather pecking and cannibalism are a major cause of poor welfare and higher mortality in non-cage systems and significant amount of research is on-going to identify underlying causes, management risks and means of prevention (Nicol et al 2013). Smothering, when birds mass together and pile on top of one another, is another cause of mortality in non-cage systems, which is only beginning to be investigated (Barrett et al 2014). Aspects of system design may be important as hens in group sizes of thousands can crowd together at different times of day to access different resources, for example to dust bathe on litter (Campbell et al 2016a).

Furnished and enriched colony cages outfitted with nest boxes, perches and foraging mats provide more space and greater opportunity to express motivated behaviour than conventional cages, and generally result in better health, hygiene and physical measures of welfare compared to non-cage systems (Sherwin et al 2010; Rodenburg et al 2012; Blatchford et al 2016). Nests are generally well-used by hens, although nest flooring, nest space and degree of enclosure can vary considerably across cage designs, and simple differences in design features have been shown to affect pre-laying behaviour (Hunniford et al 2014). Although turf nest floors seem to be most attractive to hens, they pose a hygiene concern for many producers, and optimal flooring that satisfies both the hen and the farmer still needs to be identified. Perches in furnished cages are also generally well used, depending on design and configuration in the cage; perching behaviour and/or increased space and locomotion results in greater bone strength in furnished cages (Regmi et al 2016; Casey-Trott, unpublished data). From a behavioural standpoint, provision of resources to support dust bathing and foraging pose the biggest challenge in furnished cages. Research on different sizes, surfaces and litter provision on scratch mats in furnished cages provide mixed results as to whether and how well they support these behaviours (Rodenburg et al 2012; Guinbretiere et al 2015).

One welfare problem in laying hens that is receiving considerable attention is the high prevalence of keel fractures and deformations (Harlander-Matauschek et al 2015). The keel bone extends from the sternum and is the major site of attachment for the pectoral muscles used in flight. Fractures of the keel have been shown to be painful for hens, may their limit their mobility in non-cage systems and are associated with alterations in behaviour in furnished cages (Casey-Trott and Widowski, 2016). The problem of keel damage is widespread and occurs in all types of housing systems including conventional cages (Petrik et al 2015), but prevalence increases in systems that offer more freedom of movement and particularly in complex aviary systems (Wilkins et al 2011). Reports of prevalence in non-cage systems are commonly over 80% of birds by end of lay (Wilkins et al 2011; Heerkins et al 2016). Although the etiology of keel damage is still not well understood, falls and collisions by birds attempting to navigate aviaries are commonly reported (Campbell et al 2016b). Perch placement (angles and distance between), perch material and aviary design can affect the ability of hens to fly or jump between different levels and contribute to the risk of keel damage (Wilkins, et al 2011; Heerkins et al 2016). The addition of ramps or ladders between levels has been shown to reduce falls and collisions and decrease the incidence of keel fractures (Strattmann et al 2015). Other aspects of system design and management such as flooring material and lighting need to be explored in order to design housing systems with reduced risk of injury to hens (Harlander-Matauschek et al 2015).

Rearing conditions affecting the welfare of laying hens

The majority of research on the welfare of laying hens has focused on housing and management of adult birds. However, the early experiences of chicks and pullets can have lifelong effects on the behaviour, health

and welfare of laying hens (Janczak and Riber 2015) and more research is needed to target these early life stages. This is particularly important when pullets are destined for alternative housing systems. The welfare of hens in non-cage systems requires that the hens housed in them are calm, that they adapt well to change and novelty and that they have few behavioural problems such as feather pecking or cannibalism. Fearfulness is particularly problematic in large groups since it is associated with feather pecking, and fearful responses to novel events can cause injury due to piling and smothering. Hens in multi-tier aviaries must also be able to negotiate complex environments where feed, water and nest boxes are located on different levels and do so without incurring injuries. Optimal rearing systems for pullets destined for furnished cages and enriched colonies also needs to be determined.

Practical experience indicates that it is critical that hens housed in complex aviaries also be reared in complex systems. Early experience with perches and more complex environments that include features such as ladders and platforms can affect both the cognitive and physical development of young birds. Early work indicated that there is a critical period of development during which birds must have access to perches in order for them to develop the spatial cognitive skills needed to be able navigate complex three-dimensional tasks (Gunnarsson et al 2000). More recent studies have shown that compared to pullets reared in conventional rearing cages, those reared in aviaries have better working memory, increased ability to perform spatial tasks, are less fearful and use three-dimensional space more fully (Brantsæter et al 2016). Rearing pullets with perches (Enneking et al 2012) or in aviaries (Casey-Trott, unpublished) also increases musculo-skeletal development and various measures of bone strength, and these effects persist through adulthood until end of lay.

The designs of rearing aviaries are evolving and currently there are a number of different variations that are commercially available. Some designs are similar to standard rearing cages outfitted with perches; chicks are started in the enclosed cage but as they grow the fronts of the cages are opened to allow access to the barn and litter floors. Other designs offer more vertical space to chicks within the starting cage and include different levels of perches and platforms during the first weeks of life; again, as the birds grow these cages are opened to allow them access to the barn and litter floors. Additionally, there are designs of pullet aviaries that provide significantly more vertical and horizontal space from 1 day of age (see Figure 1). As chicks grow, platforms within the system are raised and terraces are opened to provide access to the litter floor. These different designs of rearing systems facilitate different types of locomotion (e.g. running, perching, climbing, flying) at different ages. Currently little is known about the development of locomotory abilities in domestic chickens or how these different designs affect the learning process. New research is aimed at investigating how and when the different types of locomotion develop and at identifying strain differences in use of vertical space (Kozak et al 2016).

Figure 1. Rearing aviary for pullets (Farmer Automatic Pullet Portal, Clark AgSystems) that provides opportunities for locomotion, perching and flying from 1 day of age.
Chicks are started in the closed system (far left). As the pullets grow, platforms and perches are raised (center) and terraces are opened (far right) to give pullets access to a litter floor (not shown).

While rearing in enriched and more complex systems has been shown to have a number of beneficial effects on behaviour and physical condition of laying hens, there are questions concerning the long term effects of rearing system on hens destined for furnished cages, where space and freedom of movement are more constrained (Janczak and Riber 2015). Tahamtani et al (2014) observed that hens reared in aviaries exhibited more

alert and comfort behaviour when transferred to small furnished cages housing 7-9 birds, but had higher mortality compared to hens reared in standard cages. They concluded that hens reared in aviaries were better able to cope with environmental change but that long-term welfare might be compromised when moving from a large complex environment to a small-furnished cage. However, the effects of rearing may be different for hens destined for larger enriched colony systems.

My group recently completed a four-year longitudinal study on the effects of rearing pullets in a complex aviary system (as in Figure 1) versus conventional rearing cages using four rearing cohorts of birds. When subsequently housed in large (30 and 60 bird) enriched colony cages, hens reared in aviaries showed reduced fearfulness (e.g. lower latency to approach a novel object) than cage reared hens but there were no treatment differences in fecal corticosterone levels during the first few days or weeks following transition to cages (Prinold, unpublished data). Body weights of hens reared in aviaries were the same as those reared in cages at the beginning of lay but they were significantly heavier at 72 weeks. Over the entire laying period there were no treatment differences in hen-day egg production or mortality (Widowski, unpublished data). Quantitative computed tomography (QCT) analysis of bones excised from hens at end-of-lay indicated that hens reared in the aviary had significantly greater area and bone mineral content of structural bone in the radius, humerus and tibia compared to pullets reared in standard cages (Case-Trott, unpublished data). Behavioural studies indicated that rearing experience affected hens' perceptions of the furnishings in the cages but these effects did not appear to be adverse. For example, aviary-reared birds laid more eggs in the scratch area versus the nest, but their pre-laying behaviour was not much different than that of hens reared in cages (Hunniford and Widowski, 2016). Preference for dust bathing behaviour on different types of scratch mats was also affected, with aviary-reared hens preferring to dust bathe on turf mat and cage-reared hens performing more sham dust bathing on wire (Tayler et al 2014). Overall, these results suggest no detrimental effects and several beneficial effects of rearing in a complex environment on the well-being of hens in enriched colony cages.

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L16 Precision livestock farming: examples for poultry

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Introduction

The world population keeps growing and in several big countries the diets are changing since more people can afford animal products. The result is that the worldwide demand for meat and animal products could increase by 40% in the next 20 years. A question is how to achieve high-quality, sustainable and safe meat production that can meet this demand. At the same time, livestock production is currently facing serious problems such as animal health in relation to food safety and human health. Europe wants improved animal health and welfare and has made a significant investment in it. At the same time, the negative environmental impact of the livestock sector is far from being solved. Finally we must ask how the farmer, who is the central stakeholder in this process, will make a living from more sustainable livestock production.

One tool that might provide real opportunities for practical implementation is Precision Livestock Farming (PLF). PLF systems aim to offer to the farmer a real time monitoring and managing system based upon the continuous monitoring of the animals by using modern technology. This is fundamentally different from all approaches that aim to offer a monitoring tool without improving the life of the individual animal under consideration on that moment in the process. The idea of PLF is to provide a real-time warning when something goes wrong so that immediate action can be taken by the farmer. Continuous, fully automated monitoring and improvement of animal health, welfare, productivity and the environmental impact becomes a reality by applying PLF technology.

The objective of this paper is to show several examples of PLF systems for poultry that are operational today in commercial farms and products or services that soon will be available in commercial installations.

Moreover, the examples show how, like in the large European PLF project (EU-PLF) that we currently coordinate, that real time automated data analyses can generate added value for the farmer. PLF systems can replace the ears and the eyes of the farmer and work 24 h a day and 7 days a week to support him.

What is Precision Livestock Farming?

Precision Livestock Farming (PLF) is the use of modern ICT technology to improve livestock monitoring and efficiency of processes to grow meat and animal products like milk and eggs. PLF creates management systems based on continuous automated real-time monitoring and control of animal health and welfare, production/reproduction and environmental impact of livestock production.

Precision Livestock Farming is based upon the assumption that fully automated continuous direct monitoring of animals will enable farmers to detect and control in real time the health and welfare status of their animals. The farmer is already used to have modern technologies in order to measure a number of parameters on the farm. For example for climate control, financial programmes, equipment that automatically measure the feeder provided to the animals, programmes that quantify production outcome (e. g. milk production), etc. Most of these tools however do not focus on the central part of the production process: the animal.

Technological development and progress have advanced to such an extent that accurate, powerful and affordable tools are now possible. These include the intelligent use of cameras, microphones, sensors (such as 3D accelerometers, temperature sensors, skin conductivity sensors and glucose sensors), wireless communication tools, internet connections, cloud storage and many others. Modern technology makes it possible to use cameras, microphones and sensors sufficiently close to the animal so that they can replace the farmers' eyes and ears in monitoring individual animals and this during 7 days a week, 24 hours a day, 3600 seconds per hour.

The aim of PLF is to combine the appropriate hardware with intelligent software in order to extract information from a wide range of animal data and use this in real time in the management of the process. Precision Livestock Farming can indeed offer real time management tools that enable a farmer to monitor animals automatically and to create added value by helping to secure improved health, welfare, yields and environmental impact. This real time aspect and being part of the management system is quite different from other solutions like the use of so called Iceberg indicators (FAWC, 1979). Similar to an iceberg, in which the visible part is only a small part of what is hidden under the water, a bitten tail in the slaughterhouse is an indication of a bigger problem of tail biting during the fattening period. Another approach is the yearly visit by human experts to score the animal welfare in farms as proposed by the Welfare Quality approach (Welfare Quality). These are very fruitful concepts that help to create awareness of the problem of animal welfare. PLF however aims to help and adapt the process management on the spot in real time for the animal that is followed continuously during the production process and warn the farmer immediately.

Examples of PLF Technology: What is possible today for poultry?

Since 1991 we aim to develop real-time algorithms for monitoring and managing individual living organisms. We have started on insects, then mussels, fish, chicken, pigs, cows, horses and since 15 years humans. Meanwhile we have developed several techniques that can be used to collect data on poultry in a fully automated and continuous way and this in several stages of the development or the process. This is done by using sensors, camera and image analysis or by analysing the sound produced by the animals as shown in the examples described further.

Monitoring weight evolvement in the incubator.

Often in livestock production the biggest aim is to produce growth in a most economic way. The process in the incubator is one of the starting processes when producing broilers. To monitor the incubation process we have developed controllers to reduce the environmental differences between eggs in an incubator with 56.000 eggs. The control of micro-environment (temperature, humidity and gas concentration) is crucial for the embryo that is evolving from an endothermic to an exothermic living organism. To adapt the climate control continuously during the incubation period real-time monitoring tools have been developed for measuring the eggshell temperature and the weight loss of the eggs during the whole incubation period. This information is used as a basis to control the incubation process in a more efficient way. The aim is to improve hatching results and to reduce the hatch window (see Figures 1, 2 and 3).

Figure 1: The system for real−time monitoring of the egg shell temperature (OvoScanTM, Petersime)
during incubation (Berckmans et al., 2008, Romanini et al., 2013)

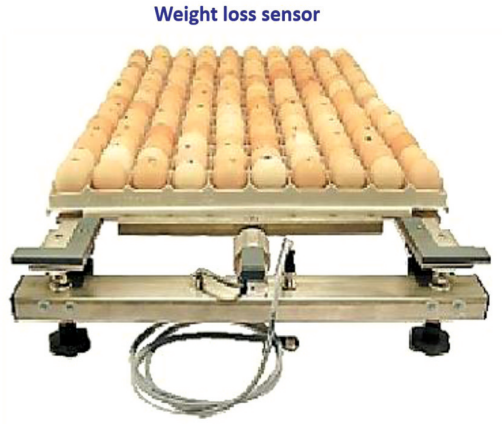

Figure 2: The system for real−time monitoring of weight loss during incubation. (Berckmans et al., 2008, Elibol et al., 2008)

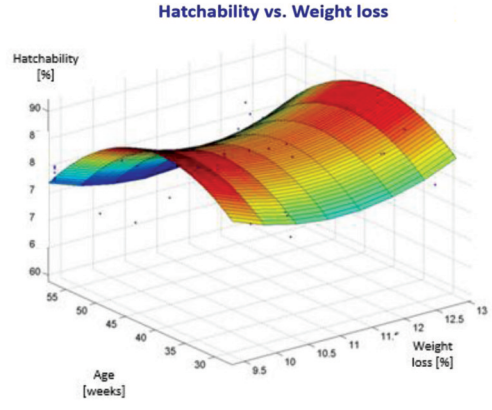

Figure 3: Hatchability versus weight loss as function of age (Elibol, 2008)

Such monitoring and control tool has the advantage for the farmer that the process is followed by quantitative data without opening the incubator since this last action is disturbing the whole process within the enclosed environment.

Continuous fully automated weighing system for broilers

In a broiler farm the growth rate and production of meat are of major importance. Since animal weight is the most important process output it is clear that one must try to monitor this in real time during the growth trajectory. Monitoring the most important process inputs (like feed) and process outputs (like weight) are fundamental principles in Precision Livestock Farming.

We developed a model based weighing system to improve the accuracy of weight measurements of broilers. Normally a fixed, population based, statistical relationship is used to connect the pressure or voltage as measured by the weighing scale to weight. In this approach that relationship is calculated on hourly basis for this group of birds (See Figure 4). Figure 5 shows that this allows to get rid of several wrong hits due to a bird jumping with only one leg, running over or pecking on the hanging weighing platform. Another problem is that at the end of the fattening period the heavy birds are jumping less on the moving scale. The software compensates for this.

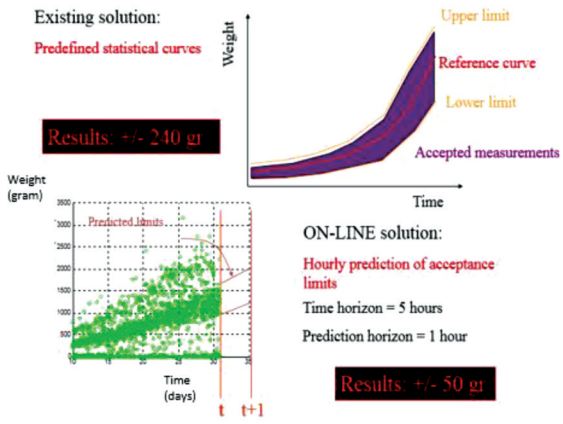

Figure 4. The limits of acceptable weights are calculated in real time.

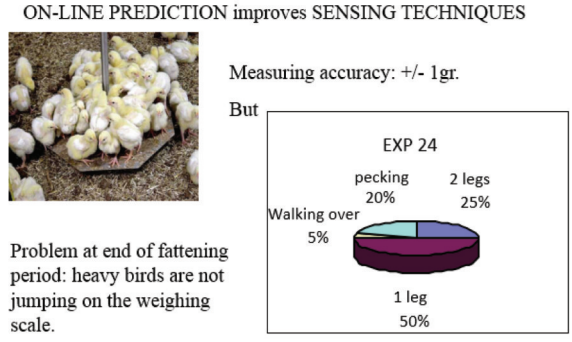

Figure 5. Only 25 % of all hits are useful.

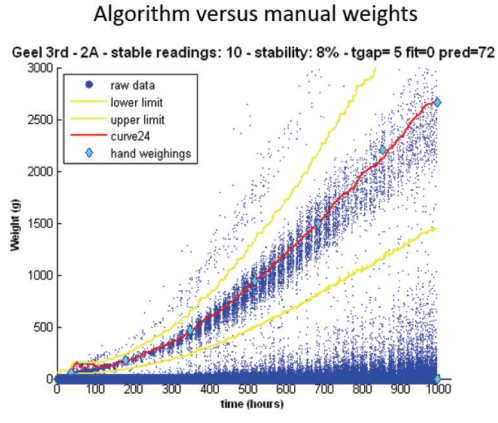

Figure 6. The algorithm detects the good hits.

Figure 6 shows that only 25 % of the hits are useful and the software does not accept other values. Most of the hits are not accepted as being useful and are rejected. The algorithm detects useful hits based upon the measurements of the days before.

Model-based growth trajectory control of broilers

Compared to ad lib feeding it might be interesting to actively control the growth trajectory (weight as function of time) of broilers in a more economical way to reduce mortality and leg problems.

When accurate data of the weight of broilers are available and one has a measure of the amount of feed given to the birds, then the dynamic relationship between the process input feed and the process output weight can be calculated every day. This means that we have a prediction every day on how the dynamic response of weight to feed supply will be. Based upon such prediction one can calculate how much feed is needed to get a certain weight response. Such a real-time model based controller shows to be able to realise a desired trajectory of growth in broilers (Figure 7).

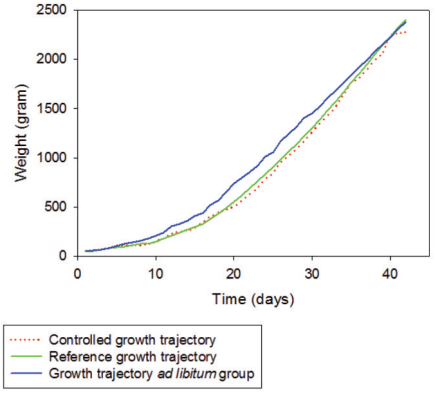

Figure 7: Resulting control of a model−based growth trajectory algorithm (Aerts et al. 2003)

Early warning system for Broiler Houses

Farmers with broiler houses are squeezed into a situation that they always need to grow more animals to make their living from this business. A broiler house with 30.000 animals in one house is not exceptional at all but it becomes very hard to observe such a high number of birds. Many problems can occur like animal disease, climate control problems, blocked feeder lines, electricity problems, dysfunctional drinking lines, failing lightning systems and others.

We have tested whether the PLF eYeNamic system (Fancom BV, the Netherlands) allows detecting most of the daily problems in broiler houses by just analysing the broilers' behaviour. The eYeNamic system consists of 3 or 4 cameras mounted at the ceiling that give pictures of the distribution of the birds (Figure 8 and 9).

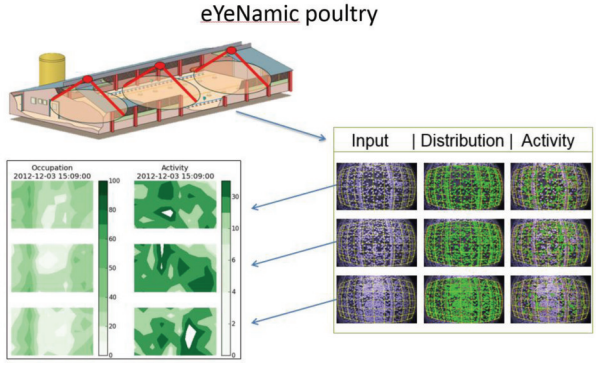

Figure 8. Three top view cameras and real time image analysis of broilers behaviour

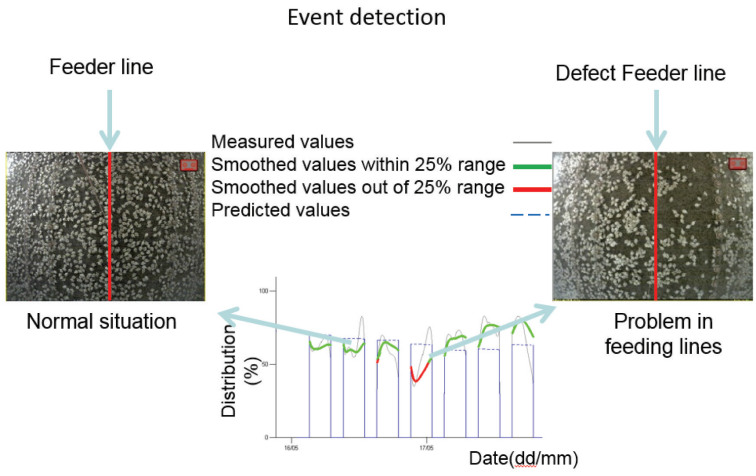

Figure 9. Image from the broilers as analysed in real time by the eYeNamic system

The setup of the experiment in a commercial farm was the output of the eYeNamic system that calculates in real time the activity number of the birds and the distribution of the birds: is the number of birds per m2 equally spread all over the ground surface. The farmer was asked to fill in a logbook where he noted down all problems that occurred during the whole fattening period. The PLF system used an algorithm that compared the actually measured distribution of animals with a predicted value at that time of the day. When the real measured value was more than 25 % different from the predicted value an alarm was given to the farmer (Figure 9). As can be seen in Figures 10 the behaviour of the broilers is quantified continuously and by measuring the distribution of the birds, indications of blocked feeder lines and other problems are given.

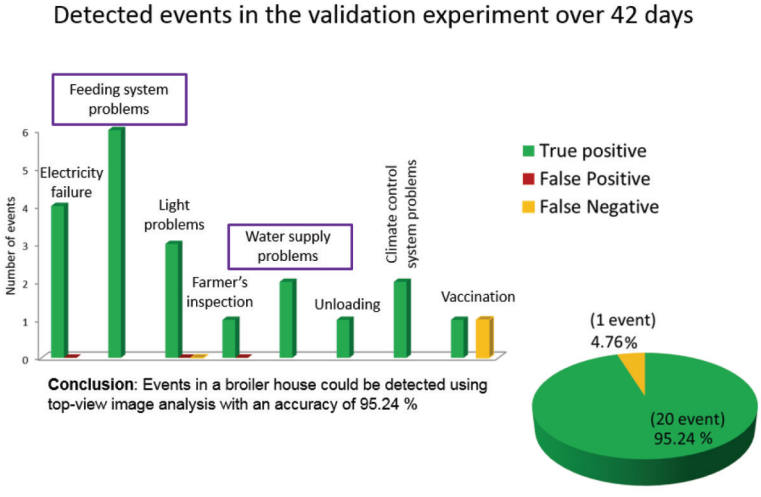

Figure 10. The eYeNamic system gives a warning for 95 % of the problems that have occurred

The PLF system shows that 95 % of all problems are detected from the behaviour of the birds (Kashi-

ha, 2013). It used a single parameter: the variation in time of the birds' distribution of the available space. This confirms again that the continuous measurement of animal responses is the way to go and there is no need to measure many variables to get systems that give added value. In this case the fact that most problems are detected means that the farmer can win working hours that he normally spends for controls. He can enter the building and disturb the birds to solve problems but there is no need to disturb them if no problems occur.

Continuous automated monitoring of feed intake of broilers by sound technology

The most expensive part of growing meat is the feed as the input variable in the process. It has been shown (see figures 11, 12 and 13) hat by using a microphone within the feeder pan of broilers, it is possible to select those pecking in which the birds indeed takes feeder to eat. By checking the amount of feeder that the bird is losing after taking it. The error of this measuring technology is very small, less than 1%. (Aydin et al. 2015).

- ROSS 308, male,28 days old
- Ad libitum feed, Constant lightning of 90 lux
- 15 minutes sound and video recording
- 15 frames (1024X768) per second

Data Set

Experiments

	Recording Times (min)	Number of Frames/Frequency
Sound	540	1428840
Video	540	486000
Weighing Scale	540	324000

Figure 11: Test to develop the feed intake sensor based on sound analysis

Data Set	Number of pecking (Algorithm)	Number of pecking (Video Labelling)	Accuracy of Algorithm (%)	True Positive	False Positive
1	113	105	93	105	8
2	99	95	96	95	4
3	109	106	98	106	3
34	107	101	94	101	6
35	98	91	93	91	7
36	95	88	92	88	7
Total-Average	3707	3447	93	3447	7

Figure 12: Results of the feed intake sensor

Chickens	Exp.	Minutes	Number of peckings per experiment	feed uptake per experiment (g)	Feed loss per experiment (g)	Feed intake per experiment (g)	Feed Intake Per Pecking (g)	Feed Intake Per Pecking (Mean±Std)	Feed loss per experiment (%)
	1	13	1193	28,63	0,325	28,31	0,024		1,14
1	2	12	759	18,98	0,198	18,78	0,025	0.025± 0.0015[a]	1,04
	3	10,3	895	24,17	0,222	23,94	0,027		0,92
	1	15	1250	32,50	0,236	32,26	0,026		0,73
2	2	13,5	1283	30,79	0,365	30,43	0,024	0.025 ± 0.0012[a]	1,19
	3	15	1460	35,04	0,348	34,69	0,024		0,99
	1	7,04	651	16,28	0,168	16,11	0,025		1,03
3	2	4,35	468	12,17	0,111	12,06	0,026	0.025 ± 0.0006[a]	0,91
	3	7,26	533	13,33	0,124	13,20	0,025		0,93
	1	6,54	583	13,99	0,145	13,85	0,024		1,04
12	2	7,43	654	16,35	0,165	16,19	0,025	0.025 ± 0.0015[a]	1,01
	3	6,65	573	15,47	0,155	15,32	0,027		1,00
Total-Average		300,10	25285	633,26	6,22	627,04	**0,025**	0.025 ± 0.0011[a]	**0,98**

Figure 13: The broiler is losing less than 1 % of the pecked feed

Conclusions

PLF systems are becoming available in products and are getting operational in commercial farms. From there we have to discover how they can create value for the animals and the farmers in the first place. So far, it looks that there are several ways that these system can create value. The fundamental advantage is that PLF systems are monitoring continuously and this can be 25 images per second. 20.000 sound samples per second for sound/vibration or 250 sensor samples per second for other variables and this 7 days a week and 24 hours a day. This is much more than what any farmer or human observer can do.

From examples today we see that value creation can be done in several ways: saving labour time, saving time in detecting problems, giving less stress to the farmer, solving problems on the spot immediately instead of later for other animals, giving social recognition to the farmer, giving quantitative numbers about what happens to the animals and others.

Collaboration between so called "animal people" (physiologists, veterinarians, ethologists, animal scientists, etc.) and "technical people" (bio engineers, software and hardware engineers, ICT people) is needed to make these systems to become successful support systems for farmers.

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BioBusiness Project, Training in Research, Product Development, Marketing and Sales in BioBusiness, Grant agreement no: 238296 (M3-BIORES, KU Leuven, Belgium; Stichting Dienst Landbouwkundig Onderzoek (DLO), Wageningen UR, Wageningen The Netherlands; The Agricultural Research Organisation of Israel-The Volcani Centre, Israel; DeLaval International AB, Sweden).
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FAWC: The Farm Animal Welfare Council, UK. Great Britain started first animal welfare council in the world in 1979. Website address: www.fawc.org.uk/freedoms.htm
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L17 Poultry genetic resources in China: conservation and utilization

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Keywords: genetic resources, poultry, China

Summary

Overview of Chinese poultry genetic resources was presented in which 201 poultry breeds from 15 species were included. Organizations and laws of conservation animal and poultry genetic resources given by the government were introduced. The names of special poultry breeds and gene pool for conservation were listed. The theory and technique for conservation animal and poultry genetic resources such as goal of conservation, generation interval, effective population size and sex ratio were presented in detail. The use of Chinese poultry breeds mainly was breeding new breeds and breeding commercial suite of lines for different needs of markets. Methods of selection and crossing were used in most of breeding program and got quite good results in egg and meat production.

Overview of Chinese poultry resources

Number of breeds

China is rich in poultry genetic resources. The numbers of poultry breeds in China are shown in table 1.

Table 1 number of poultry breeds in China*

	native	imported	improved	total
chicken	107	5	4	116
duck	31	1	0	33
goose	30	0	1	31
turkey	1	2	0	3
pigeon	2	1	0	3
quail	0	2	0	2
Muscovy duck	0	1	0	1

*Animal genetic resources in China: Poultry. Compiled by China National Commission of Animal Genetic Resources.

There are some rare poultry species such as ostrich (3), pheasant (3), emu (1), guineafowl (1), partridge (1), blue peacock (1), media duck (1), mallard (1). The number in the round brackets is the number of breeds.

Origin and domestication

Chicken (*Gallus gallus*) was original developed from the red jungle fowl. There are still two subspecies of red jungle fowl in China. One is DianNan subspecies (*G. gallus spadices*) in Yunnan province and the other is Hainan subspecies (*G. gallus jabouillei*) in Hainan Island which is now Hainan province. Chicken was domesticated in China about 7000 years ago at Neolithic period that was New Stone Age.

Duck (*Anas platyrhynchos domestica*) was original domesticated from "green head duck" (*Anas platyrhynchos*) and "speckle duckbill duck"(*Anas poecilorhyncha*) which domesticated in China about 3000 years ago at Western Zhou Dynasty. Among the world total, there is about 70% duck raising in China.

Gooes (*Anser domestica*) was domesticated from Hong Yan (*Anser cygnoides*) and Hui Yan (*Anser anser*) which domesticated in China about 3500 years ago at Shang Dynasty. Among the world total, there is about 90% goose raising in China.

Government organization and management

Government organization and laws

Government organizations have been set up for managing animal and poultry genetic resources. Such as:

China National Committee of Animal and Poultry Genetic Resources (1996), China National Experts Com-

mittee of Protecting Resources of Bio-species (2003).

Also China has laws and rules for conservation animal and poultry genetic resources. Such as:

Livestock Law of the People's Republic of China, Animal Epidemic Prevention Law of the People's Republic of China.

Statute and measure

In fact, China has quite a lot of rules for conservation genetic resources. Such as:

Management statute of conservation farms, protection areas and gene pool in animal and poultry genetic resources (2006), Statute of examining animal and poultry new breeds or a suite of lines, and of identifying animal and poultry genetic resources (2006).

Conservation breeds and gene pool

Names listed below are the national level poultry conservation breeds in China up to 2013 which including 11 chicken breeds, 5 duck breeds and 10 goose breeds.

Chicken: Beijing Oil, Dagu, Pudong, Langshan, Liyang, Xianju, Silkies, White-ears, Luxi Game, Qingyuanma, and Huiyang Beard.

Duck: Beijing duck, Youxian-ma, Gaoyou, Shaoxing, and Liancheng-bai.

Goose: Huoyan, Taihu, Wanxi-bai, Xingguo-hui, Lingxian-bai, Shi-tou, Wu-zong, Sichuan-bai, and Yili.

Gene pool, mainly keeps live birds of many breeds in certain numbers according to the conservation program. There are four poultry gene pools in China nowadays, two for Chinese native chickens in Jiangsu and Zhejiang provinces, and two for waterfowls in Jiangsu and Fujian provinces.

Conservation: Theory and Practice

First of all, we need to make a conservation program in which the goal of conservation of certain breed, type of conservation, suitable generation interval, effective population size and sex ratio should be considered.

Goal of conservation of certain breed

To take Beijing Oil chicken for an example, it is a famous quality meat type chicken in China and someone may call it Royal Yellow chicken. The goals of keeping Beijing Oil chicken are including body type and appearance, production performance, number of individuals keeping in the population, conservation period and utilization prospect.

Generation interval

It means the average period between upper and lower generations represented by the average age of the parents at birth of their offspring. That is $G_i = (t_m + t_f)/2$

G_i: generation interval, t_m: age of male when offspring were hatched, t_f: age of female when the offspring were hatched. Generation interval can be affected by mating age and mating interval of parents.

Generation interval of different species in poultry is suggested in table 2.

Table 2 Generation interval of poultry species

species	Breeding period(year)	Generation interval(year)
chicken	2-3	1.5
duck	3-4	2.5
goose	3-4	2.5
turkey	3	2
pigeon	5	3.0
quail	1.5	1.0
ostrich	20	10
emu	10	5
partridge	2	1
Guinea fowl	2-3	1.5
Muscovy duck	3-4	2.5
pheasant	2	1

Effective population size

If we assume that inbreeding coefficient of the conservation population should not exceed 0.1 within 100 years. Then the effective population size with different generation interval is shown in table 3.

Table 3 effective population size with different generation interval

Gene ration interval	Number of generations in 100 years	Effective population size
1.0	100	480
1.5	66	320
2.0	50	240
2.5	40	200
3.0	33	160
4.0	25	120
5.0	20	100
6.0	16	80
8.0	12	60
10.0	10	50

In order to calculate effective population size, we need to use other two formulae as follows:

$$\Delta F = 1 / 2N_e$$

ΔF: inbreeding increment

N_e: effective population size

and $F_t = 1-(1-\Delta F)^t$

F_t : inbreeding coefficient at t^{th} generation

t : the t^{th} generation

There $F_t = 0.1$ which assumed before. It is easy to calculate ΔF by logarithm, and then N_e.

Sex ratio in conserved population

In order to know the information of sex ratio in certain population that two formulae below are very common used in conservation of animal and poultry genetic resources:

$$N_e = 1/4N_m + 1/4N_f \quad (a)$$

and

$$N_e = 3/16N_m + 1/16N_f \quad (b)$$

Formula (a) is used in random mating conservation system while formula (b) is used in the system of remaining offspring equally in each family that means every male remaining a son and every female remaining a daughter in each family.

To take duck or goose for instance, we have known the generation interval is 2.5 years (see table 2.) and the effective population size is 200 heads (see table 3.). In random mating systems the number of males and females, the sex ratio are shown in table 4, while in the system of "equal remain" that related figures are shown in table 5.

If sex ratio, 1 male with 5 females, is suggested in a conservation population that need to keep 60 males and 300 females in random mating system and 40 males and 200 females in "equal remain" system.

Table 4 number of males, females and sex ratio in random mating system (Gi = 2.5, Ne = 200)

Number of males (N_m)	Number of males (N_f)	Total number(N_m+N_f)	Sex ratio(N_m:N_f)
100	100	200	1:1
80	134	214	1: 1.7
70	175	245	1: 2.5
60	300	360	1: 5
55	550	605	1: 10
52	1300	1352	1: 25
51	2550	2601	1: 50

Table 5 number of males, females and sex ratio in "equal remain" system (Gi = 2.5, Ne = 200)

Number of males (N_m)	Number of males (N_f)	Total number(N_m+N_f)	Sex ratio(N_m:N_f)
50	50	100	1:1
40	200	240	1:5
38	950	988	1: 25

Utilization

Breeding new breeds

Selection and cross breeding are very often used for improving performance of meat or eggs in native breeds. There are some examples showing below:

① New Langshan chicken was bred from original Langshan which was crossed with Australorp. After selection several generations of their offsprings, the growth rate and egg number were both improved.

② New Pudong chicken was bred crossing with White Rock and Red Cornish to original Pudong. The body size and growth rate were highly improved.

③ New Yangzhou chicken was bred from local chickens with clutter feather which crossed to New Hampshire. After selection, the egg numbers were increased and most of chickens were yellow feather.

④ Jinghai yellow chicken was selected from local chickens without crossing with foreign breeds. Family selection was used and got good results of growth rate and meat quality.

⑤Yangzhou goose was bred from three way crossing. Taihu, Sichuan-white and Wanxi-white goose breeds were crossed and reciprocal recurrent selection was used in the breeding procedure.

Breeding commercial suite of lines

From 2009-2014，there were 62 commercial poultry suite of lines passed examination by National Committee of Animal and Poultry Genetic Resources. Among them there were 54 chicken suites, 5 duck suites, 2 goose suites and 1 quail suite.

There are quite a lot of ways to breed a suite of lines for commercial use to meet different needs of market. To take mini-type layer "CAU No. 3" for an example, the suite combined with three lines: ① White Rock type chicken with early feathering and dwarf gene; ② Red Island type chicken with early feathering, cross ① and ② for producing male line A with both early feathering and dwarf gene regardless the colour of feather; ③ White leghorn with late feathering used as terminal female line B. Then to cross with A and B for producing commercial layer which is mini-type with dwarf gene less feed intake and can be auto-sexing at day old chicken.

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L18 Sustainable feed supply for worldwide poultry production

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Keywords: poultry industry challenges, poultry nutrition, sustainable nutrition, precision nutrition

Summary

The poultry industry is facing various challenges in its value chain. Production needs to be environmentally sound and social responsible with the prerequisite that it also has to be economically viable. Advances in poultry nutrition will contribute to meet these challenges. Targeted feed additive strategies can be applied to control microbial quality of feed and water and support gut health. It will contribute to establish a responsible, prudent use of antibiotics and will also fit in strategies to reduce the prevalence of food pathogens such as *Salmonella*. Recent advances in science also highlight the importance of early life nutrition for later life performance, health and product quality. The first days post-hatch is a period in which various epigenetic effects occur that may be modulated by nutritional interventions. Precision nutrition methods and tools, such as dynamic feed evaluation and animal models, can be implemented to economically optimize the feed program and reduce emissions into the environment. Sustainable feed supply meeting market demands is feasible and will require a multidisciplinary approach of all stakeholders in the value chain.

Introduction

Poultry derived food products, meat and eggs, are the most important animal protein sources globally and a significant increase is forecasted in global demand. The global production of egg mass and poultry meat in 2015 were estimated to be respectively 70 and 112 million tonnes (FAO 2003, 2015). The International Feed Industry Federation (IFIF) estimates that annual global feed production approaches 1 billion tonnes, of which 45% is for poultry. IFIF also cites FAO's outlook for poultry protein production to be over 200 MMT by 2050, greater than a 100% increase from 2010. The overall increase (2010-50) in animal and aqua protein production expectation is 60% ranging from 38% for pork to 104% for poultry (aqua by 90%).

But there is also a significant number of challenges facing the poultry and allied industries with respect to sustainable global production of poultry meat and eggs where market demands and consumer needs will put more constraints on our production systems and methods (Alders, 2016). These challenges are dynamic and diverse and solutions and opportunities will require development of appropriate technology and using and advancing our knowledge base. Sustainable livestock farming is based on three pillars: Environmentally sound, social responsible and economically viable. For all these pillars, innovation will be key and hence, advances in animal nutrition will play an important role, where we

have concrete challenges in economical optimization of the value chain and meeting product quality demands, whilst safeguarding animal wellbeing and human health.

Safeguarding animal wellbeing and human health

Responsible and prudent use of antibiotics to combat antimicrobial resistance is feasible

To supply safe and nutritious food is our licence to produce. Various quality and safety demands are applicable here, with absence of foodborne pathogens and multidrug resistant bacteria as top priority. The rapid development of antimicrobial resistance (AMR) urges the need for effective strategies to reduce antibiotic use in animal production. The World Health Organization (WHO, 2014) predicts that if

no additional measures are taken, the annual death toll attributable to AMR may rise to 10 million and exceed other causes such as cancer in 2050. Multidrug resistant Salmonella and E.coli are regarded by the WHO as priority pathogens to combat.

Antibiotics are applied in animal production as antimicrobial growth promoters (AGP) to improve efficiency of production and as prophylactic and therapeutic medical treatments for animal health. Van Boeckel et al. (2015) recently estimated that the total antibiotic usage in animal production of 228 countries in scope was 63,151 tons in 2010. Antibiotic use is expected to rise by 67% by 2030, and to nearly double in Brazil, Russia, India, China and South Africa if no additional restrictions on their use are taken. Especially the prophylactic use of antibiotics and their application as a growth-promotor are currently under pressure in many countries including the USA and China. These concerns overrule the contribution that AGP may have economically.

Stricter biosecurity programs, a more targeted administration of antibiotics to the animals via drinking water or individual treatment, and well-designed vaccination strategies are examples of best practices implemented by farmers to reduce antibiotic usage. Besides these measures, various strategies are followed to support animal health via drinking water and/or via the feed (Den Hartog et al., 2015). The application of acidifiers via the drinking water is an example of a commonly applied measure in antibiotic reduction programs in Europe. Besides its contribution to control of the microbiological quality of drinking water, the ingested organic acids also have a prolonged activity in the gut, which will assist the animals in reducing pathogen loads in the proximal intestinal tract. The use of water-acidifiers can be further supported and enforced by applying feed additives which have been developed for stabilization of the gastrointestinal microbiota and promoting immune competence. A large scale field study in Germany was recently finalized where we tested the use of water-acidifiers in a longitudinal study at 11 farms during a period of 5 years, with more than 35 million broilers being monitored in performance and health. The results of the first 2 cycles were taken as control or baseline level. In the year thereafter, various best practices were adopted in farm management and nutrition, followed by a three year period in which a feed additive strategy was applied including administration of water acidifiers. Antibiotic usage (mg active agent/kg broiler) was reduced by 60%, mortality decreased from 3.5 to 2.6% and feed conversion ratio decreased from 1.69 to 1.60 (unpublished results, Trouw Nutrition 2016) during the 3 year intervention period of applying best practices in management, nutrition and acidification of drinking water.

The package of measures of very strict policies for application of antibiotics, adopting best practices in biosecurity and farm management and the use of smart feed additive programs has led after the ban on AGP in 2006 to a further significant reduction in antibiotic usage of 58% in broiler production in the Netherlands from 2009 to 2014 (MARAN, 2015). Dutch broiler farmers apply in a similar way water acidifiers on a routine basis as has been described before in the German study. A very positive observation in relation to AMR is that decreased use in antibiotics indeed also reduced the prevalence of some AMR bacteria, including multidrug resistant *E.coli* and *Campylobacter.*

Salmonella can be beaten, Campylobacter is a challenge

Improvements in management and nutrition for reducing antibiotics, will in general also contribute to Salmonella control, which is becoming even more important on the political agenda because of emerging multidrug resistant *Salmonella*. To control *Salmonella*, more specific control measures may have be taken on top of best practices for antibiotic reduction such as decontamination of raw materials and feed to mitigate risks of *Salmonella* entry into the farm via the feed (Berge and Wierup, 2012). The use of formaldehyde is highly effective, but the application in the factory needs to be strictly controlled in order to minimize the risk of inhalation by operators. Alternatives may be found in the application of organic acids and thermal treatments. Further measures to reduce prevalence of *Salmonella* can be taken to prevent colonization and transmission with specific feed additive combinations that inhibit growth, block attachment of *Salmonella* to the mucosa and reduce the expression of specific virulence genes (Van Immerseel et al., 2005; Corujo et al., 2014, 2016). Moreover the host defense system can be promoted with immunemodulatory concepts. Strategies to reduce prevalence of *Salmonella* have been very successful, and various integrators operate nowadays at a prevalence level of less than 1% contaminated flocks.

The control of *Campylobacter* is more complex. From a human health perspective, *Campylobacter* is

more relevant than Salmonella. The European Food Safety Authority reported 267.000 confirmed cases of *Campylobacter* infections in humans and 89.000 Salmonella cases in 2014 (EFSA, 2015). Various measures and strategies have been tested to control *Campylobacter*, but most without success (Meunier et al., 2015). From a biosecurity point of view it may be key to control the horizontal transmission via insects. From a nutrition or feed additive point of view, it does not seem to be feasible to fully prevent or eliminate *Campylobacter*, but it may be possible to reduce the caecal *Campylobacter* numbers of the birds at slaughter, which is expected to be correlated with the *Campylobacter* levels at broiler carcasses. Carcass treatment with antimicrobials is not allowed to date in the EU, but is an effectively applied measure to lower *Campylobacter* levels in various countries outside EU.

Mycotoxin risks can be monitored and controlled by rapid diagnostics and mycotoxin solutions

In relation to feed safety, mycotoxins are probably one of the most important risk factors that need to be controlled. Rather effective strategies have already been developed to reduce the risk and impact that for example aflatoxin may have on birds health with the use of mycotoxin solutions (Murugesan et al., 2015). It is also encouraging to note, that rapid diagnostics are now more widespread globally adopted for quality control to take appropriate measures once mycotoxin contamination in raw materials is detected. It is an essential part of feed quality assurance and with the right measures the risks can be mitigated, which will prevent unexpected performance losses and health problems.

Wellbeing of birds from start to end

Birds are confronted with various stressful events during their life, especially in critical transition periods such as hatch and transport. An example here is the welfare concerns of early hatched chicks not having access to feed and water for up to 2 days. This has a negative impact not only on body weight loss, but also on important early life developments. Various important conditions for life performance are already being determined during the embryonic development and in the very first days and weeks of life post-hatch, partly mediated via epigenetic effects (Uni et al., 2012; Berghof et al., 2013). Nutrition and the host-microbiota interactions in early life seem to play a significant role in development of the gut, immune competence and muscle and skeletal cell development (Muir et al., 2015). Recent information for example suggests that newly hatched layer chickens that have been deprived of food had a distinct development of innate and adaptive immunity and responded differently to a non-infectious lung challenge (Simon et al., 2015). Similar to food deprivation, antibiotic treatment of day-old chicks may have significant impact on early-life microbiota which is not beneficial for the birds in relation to develop appropriate immune-competence (Simon et al., 2016). Evidence is accumulating that newly hatched chicks having delayed access to food and prophylactic antibiotic treatments are undesirable challenges and interventions in early life in our production systems.

Provision of nutrition and water during the immediate post-hatch period (Careghi et al., 2005; Willemsen et al., 2010) and during transit from hatchery to farm has shown promising effects on broiler performance and health in the first days and weeks of life (Bergoug et al., 2013; unpublished results, Trouw Nutrition 2016). Early life interventions do not per se result in higher market weights or improved feed efficiency in each flock, but it will contribute to more stable and consistent performance and a reduced risk of birds developing health problems.

Economical optimization of the feed program

Precision nutrition as approach to optimize feed economics

From an economical point of view we need in general to meet nutrient requirements of the birds in the most efficient and economical way and assure that animals are in good health to exploit their potential. Precision nutrition and modelling are here promising fields of research where recent advances have shown promising effect (Pesti and Miller, 1997; de los Mozos et al., 2015b). Precision nutrition requires accurate and detailed insight in the nutritional value of the various feed ingredients and matches nutrient supply as closely as possible with nutrient requirements of animals of different ages and production stages. The progress in growth potential, feed efficiency and breast meat yield has changed dramatically nutrient requirements of broiler chickens in the last decades. Tools such as growth models are nowadays ap-

plied to assess the dynamic relation between genetic potential, nutrient supply and growth with accurate predictions of nutrient requirements (de los Mozos, 2016). More feed phases have been introduced in broiler nutrition to meet requirements more accurately and more economically. The dietary amino acid and energy supply is optimized and safety margins in feed formulation can be reduced leading to cost-savings and reduced N-excretion. The same counts for phosphorus, where more dynamic approaches have been introduced taking into account more accurate estimations of phosphorus digestibility in broilers, calcium availability and the non-linear efficacy of phytase (Angel et al., 2015; Navarro-Villa et al., 2015 and 2016). Application of this knowledge can lead to significant reduced P-output into the environment.

A very nice example of precision nutrition in layers or breeders is the split or oviposition feeding program (de los Mozos 2012a,b; 2015a). As the name implies two diets differing in nutrient (energy, amino acids and/or minerals) levels are offered the bird using a single feed line. The morning (07:30-14:30h) and afternoon (14:30 to 07:30) diets are formulated to be better suited to hourly nutrient demands of egg production (i.e., matching nutrient required for yolk that is relatively constant; and albumen and shell that are more variable). Unlike energy, animals have limited capacity to maintain pools of amino acids and calcium in reserve and optimizing their availability through the diet at the right moment is crucial. Split feeding has been shown to significantly improve feed utilization, health as well as production of eggs with sound egg shells. Based on Life Cycle Analysis there were significant improvements in sustainability with split feeding as compared to conventional feeding practices. Split feeding program is more economical, N- and P-emission can be reduced (by resp. 10.0 and 4.1%) and egg shell quality is improved.

NIR to facilitate flexible and adequate use of ingredients

Efficient use of resources e.g. feed ingredients will benefit environmentally sound production. In this respect, use and conversion of co-products from the food and biofuel industry to highly nutritious animal products is contributing to sustainable production as well. De Vries (2015) reviewed the area of increasing use of fibre in poultry diets and addressed the question of whether fibre was a bonus or a burden. The impact of altering feed structure by using different sources of fibre as well as modifying feed particle size has been widely studied by many authors targeting the development of the proventriculus and gizzard and thereby improving gut health, reducing litter moisture associated problems, and increasing nutrient utilization (Hetland and Svihus, 2001; Gonzalez-Alvarado et al., 2007; Biggs and Parson, 2009; Svihus, 2011, Navarro-Villa et al., 2015). One of the challenges in our industry is to be flexible with our raw material usage in order to manage higher use of low quality ingredients and anticipate on fluctuations in raw material prices, whilst at the same time we need to have grip on variation in raw material quality and assure that the feed delivers the same high performance. Here, NIR can be a useful tool for rapid and accurate estimation of the nutritional value of feed ingredients. Besides rapid estimation of the gross chemical composition of ingredients and recalculation methods to adjust the nutritional value, direct NIR based estimations of metabolizable energy content may be feasible for specific raw materials (Valdes and Leeson, 1992; Garnsworthy et al., 2000; Owens et al., 2009; Losada et al., 2009 and 2010). Recent advances in our research program also indicate that it is feasible to estimate the amount of reactive lysine in specific feedstuffs by NIR. Reactive lysine is the proportion of lysine that can be utilized by the animal for protein deposition (Moughan and Rutherfurd, 1996; Rutherfurd, 2010; Kim et al., 2012). Non-reactive lysine may be formed by Maillard-reactions during heat treatment of protein sources and cannot be utilized by birds. It is digestible, but has no biological value. Such rapid methods can be applied to discriminate protein sources that may have been under- or over-processed, for example soybean meal, rape seed meal and meat and bone meal.

Product quality demands

The genetic progress is the main success factor for the improvements we have seen the last decades in productivity in broilers and layers. However, the enormous increase in productivity may also have negative side effects. For example, there are increasing concerns about muscle myopathies in broiler chicken, in particular what is termed ‘White stripping’ and ‘Wooden breast’ (Petracci, 2015). The deviating visual appearance and impaired storage and cooking quality may result in downgrading and condemna-

tion. The two myopathies are both connected with rapidly growing birds and are more common when larger body weights are required for further processing. Incidence is higher as expected in the faster growing male and the high breast yield genotypes. The solution will require an integrated approach in breeding, nutrition and management.

Sustainable feed supply for poultry production

The importance of using a holistic approach to enable successful conversion of feed into high quality poultry protein in a sustainable way is evident. These high producing animals have to be able to consume, digest, absorb and convert sufficient nutrients to meet their genetic potential, and do this consistently from flock to flock regardless of season. In order to do this successfully and achieve high consistent production with acceptable risk will require increased use of existing technology, developing new technology and expanding our knowledge and information network.

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L19 Current and future challenges of the poultry industry

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Keywords: consumer expectations, animal welfare, food borne diseases, prevention, legislations

Summary

Several factors and challenges are currently as well as in future will influencing the poultry health and production. Beside the loss of consumer confidence and trust in the quality and safety of poultry meat and poultry products the emergence and re- emergency diseases will remain a continuous major challenge. Several human foodborne infections are linked to poultry. Control and/or elimination of these food borne pathogens present a great challenge for poultry industry. In addition, the increase of antibiotic resistant bacteria will also be a continuous public health hazard.

The future concept of animal health will cover not only the absence of disease in birds, but also the relationship between the health of animals and their welfare. It will also take into account social, economic and ethical considerations, as well as support the achievement of a high level of environmental protection. This means that farmers, veterinarians, stockholders and all other partners involved in the production chain need to share more responsibilities. The present paper explores these points.

Introduction

The modern poultry industry aims at high production and better quality at a low cost. This, in addition to an increase in the demand for poultry meat and products, necessitates constant, efficient and goal-oriented healthcare to prevent the development of diseases and to reduce the use of antibiotics.

These will include: established of programmes to control of emergence and re-emergence infectious diseases, to face the continuous changes in social, political and consumer perceptions with regard to food safety, animal welfare and environmental protection issues as well as the steady increase of the food and feed costs. In addition, the emergence of new and unforeseen diseases, and new legislations in several countries will remains an important issue. The present paper describes the main challenges face the poultry production.

Changes in social, political, and consumer perceptions

Food safety

The loss of the consumers trusts and confidence in the quality and safety of poultry meat is a further challenge. Poultry meat can harbour different food borne pathogens. Many reports from recent years have shown that different *Salmonella* serovars and *Campylobacter spp.* are the most common causes of human food borne bacterial diseases linked to poultry. In addition, the development of antibiotic resistance in bacteria, which is common in both animals and humans, is also an emerging public health hazard. Controlling this food borne organisms requires a broader understanding of how microbial pathogens enter and move through the food chain, as well as the conditions that promote or inhibit growth for each type of organism (Hafez, 1999, 2005).

In November 2003**,** the European Parliament Council Regulation 2160/2003/EC (EC, 2003) on the control of salmonella and other specified food- borne zoonotic agents was passed. This regulation and further several regulations covers the adoption of targets for the reduction of the prevalence of specified zoonoses in poultry populations at the level of primary production, in layers, broilers and turkeys. After the relevant control programme was approved, food business operators must have samples taken and analysed for zoonoses and zoonotic agents. In addition, the flocks should be sampled also by the competent authority. Currently, in the EU strong reduction of specific Salmonella serovars such as *S*. Enteritidis and *S*. Typhimurium were achieved (EFSA-2015)

A further problem related to food safety is *Campylobacter*, which is the leading cause of zoonotic enteric infections worldwide. Campylobacter infections in humans are mainly transmitted by contaminated food. No evidence has been found either for vertical transmission or for horizontal transmission from one flock to the next via persistent house-contamination. However, since the organism has been detected in the intestines of most slaughtered poultry, the major route for campylobacter contamination of poultry appears to be the horizontal transmission from the environment. Investigations indicated that the external campylobacter load per chicken is increasing during transport, de-feathering and evisceration (Hafez et al., 2001), and decreasing at the other processing steps studied, with an overall reduction of the mean load from production-to-consumption of about 4 to 5 logs. Good hygienic practice protocols should be prepared and strictly followed in all stages of production. Biosecurity should be improved throughout the production chain. Since campylobacter is found in the environment, hygienic barriers should be constructed to keep them outside the house (Anderson et al., 2003).

To protect consumers, the EU has adopted an integrated approach to food safety from the farm to the fork. The approach consists of both risk assessment and risk management measures involving all key actors; EU Member States, European Commission, European Parliament, European Food Safety Authority (EFSA), the European Centre for Disease Prevention and Control (ECDC) and economic operators. The approach is supported by timely and effective risk communication activities. These help European decision-makers in setting policies and making decisions to protect consumers in the European Union. The risk assessment as defined by WHO and the FAO means scientific evaluation of known or potential adverse health effects, which is an integral part of risk analysis, and which also includes risk management meaning evaluating, selecting and implementing different courses of action. These should by followed by risk communication, which meaning exchanging information among all interested parties. The four steps of risk assessment are a) hazard identification; b) exposure assessment; c) hazard characterisation and d) risk characterisation (Schlundt et al., 2004).

The "General Food Law" came into force on 21 February 2002 (Regulation EC/178/2002 and after a transition period the law is in force since 1st January 2005 (EC, 2002) to provide a framework covering animal feed, animal health and welfare, hygiene, contaminants and residues, novel food, additives, flavourings, packaging and irradiation. The food law aims at ensuring a high level of protection of human life and health taking into account the protection of animal health and welfare, plant health and the environment.

The aims of the EU several legislations toward food safety can be summarized according to Mulder (2011) as follows:

1-Safety (consumer health): by new methods to reduce the use of antibiotics /medicines; improve disease resistance; zoonosis control; traceability of animals and products

2-Safety (product safety): stimulate and control hygienic processing, traceability of products and materials intended to come into contact with food

3-Animal welfare: animals kept according to rules/systems

4-Product quality: improved quality and composition; quality and chain control systems; traceability of animals and products

5-Environment: reducing environmental contamination, Nitrogen and Phosphorous. There is a critical look at the use of by-products of human food production. The re-use of by-products for non-food applications (feathers) should be encouraged.

6-Rural impact, economic effects and bio-diversity

In addition, the failure of consumers to apply hygienically acceptable food handling and cooking practice, and the fact that the processing plants are not able to reduce the level of pathogenic bacteria in poultry products, mean that every effort must be made to reduce the contamination of the live birds before despatch to processing plants (1).

New approaches to the problem of contamination must be adopted and the discussion on the decontamination of the end product must be re-evaluated carefully and without emotion.

Antibiotic resistant and associated problems

The development of antibiotic resistance in bacteria, which is common in both, animals and humans, is and will also be a continuous public health hazard. It is generally known, that supplementation of poul-

try feed with antibiotic growth promoters (AGPs) improves performance of livestock. The effect of AGP on gut flora results in improvement of digestion, better absorption of nutrients, and a more stable balance in the microbial population. As consequence the prevalence and severity of intestinal disorders are reduced. However, AGPs also can increase the prevalence of drug-resistant bacteria. Based on „Precautionary Principle" and experiences made in some European countries, the EU completely banned the use growth-promoting antibiotics in feed of food producing animals by January 2006. Field observations in Europe showed that the poultry industry faced several problems after the ban of AGPs. The impact of the ban has been seen on the performances (body weight and feed conversion rate) as well as on the rearing husbandry (wet litter and ammonia level), animal welfare problem (foot pad dermatitis) and general health issues on the birds (enteric disorders due to dysbacteriosis and clostridial infections.

Multi-resistant bacteria are increasingly posing a hazard to human and animal health worldwide, impeding successful antibacterial treatment (Arias et al., 2010). In addition, the development of novel antibiotics does not keep step with the emergence of antimicrobial resistance in bacteria (García-Rey, 2010). Increasing application of antibiotics for the treatment of humans and animals and the use of the glycopeptide avoparcin in subtherapeutic levels as a growth promoter in the past have been generally held responsible for a progressive deterioration of the resistance situation in bacteria.

Among multi-resistant bacteria, vancomycin-resistant enterococci (VRE) have been estimated as one of the most common bacteria causing a rise in cases of nosocomial infections in humans in the last few years (Arias et al., 2010). The prevalence of vancomycin-resistant enterococci (VRE) in 20 turkey flocks reared in the southwest of Germany was recently investigated (Sting et al., 2013). The VRE could be isolated by means of a procedure combining bacterial cultivation in an enrichment broth and on a selective solid media. Enterococci were identified biochemically and subsequently tested on the presence of the vancomycin resistance genes vanA, vanB (B1/B2/B3), and vanC (C1/C2/C3) using real-time PCR assays. Vancomycin-resistant enterococci were detected in 15 (75%) of the 20 turkey flocks investigated. In 5 flocks, all animal samples and environmental dust samples taken were VRE-negative. In a total of cultivated 68 isolates from birds and dust samples, enterococci bearing *van*-genes were detected. Of these, 12 isolates carried the *vanA* gene (17.6%) and 56 isolates carried the *vanC1* gene (82.6%). Neither *vanB* (*B1*, *B2*, *B3*) genes nor the *vanC2* or *vanC3* genes could be detected.

In addition, Livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) have been isolated from a number of livestock species and persons involved in animal production. Infections with MRSA often prove difficult and expensive to treat. During the last few years LA-MRSA have been isolated from a number of farm animal species including pigs, veal calves, dairy cattle and broilers (de Neeling, et al. 2007; Hasman et al., 2010, Persoons et al., 2009). Also turkey meat was shown to be contaminated with MRSA (de Boer et al., 2009), however, only limited information is available concerning the situation in turkey flocks with respect to prevalence, site of colonization or infection and involved strains. Recently, Richter et al. (2012) investigated the prevalence of LA-MRSA in fattening turkeys and people living on farms that house fattening turkeys. Eighteen (90%) of 20 investigated flocks were positive for MRSA. All female flocks were positive, while 8 male flocks were positive. On 12 of the farms 22 (37.3%) of 59 persons sampled were positive for MRSA. None of them showed clinical symptoms indicative of an MRSA infection. People with frequent access to the stables were more likely to be positive for MRSA. In most flocks MRSA that could be assigned to clonal complex (CC) 398 were detected. In five flocks MRSA of spa-type t002 that is not related to CC398 were identified. Extended-spectrum beta-lactamase (ESBL) bacteria, are also found in poultry.

Animal welfare

Currently, there is great concern that serious animal welfare and health problems might have been caused already due to genetic selection practices within the poultry industry. Fact is that genetic selection practices within the poultry industry have achieved significant progress in terms of growth rate, better feed conversion, better meat yield and low production cost. All the time this was accompanied by continuous improvement in husbandry practices, nutrition, and disease control. The most outstanding and visible changes in modern poultry compared to their ancestors is the rapid growth and the higher percentage of breast muscle. As a consequence, it is important to understand the relationship between genetic selection pressures and other factors that may have a subsequent impact on the health conditions.

According to a published report on a new Animal Health Strategy for the European Union (2007-2013), the concept of animal health covers not only the absence of disease in animals, but also the relationship between the health of animals and their welfare. It will also take into account social, economic and ethical considerations, as well as support the achievement of a high level of environmental protection. (EC, 2007).

Global movement of poultry and poultry products

Strong global competition and varying production costs in various regions will lead to an increase in the global movement of poultry and poultry products. This, however, increases the risk of introducing diseases to areas that are now considered to be free from such diseases. Poultry diseases will remain a major challenge to the industry. Once an outbreak of a given disease occurs, it can explode into an epidemic and may have a significant negative effect on trade in a specific country, a continent or even globally.

The steady increase in the cost of feed will accelerate the global trade. In addition, the increase of biofuel and biogas production will reduce the available land for food grains and feed production, leading to a considerable increase of feed costs for animal production. In the future, the feed industry, however, will also be forced to take more responsibility not only for the quality of the feed ingredients, but also to ensure that no poultry pathogens and unwanted contaminants and residues are present in the feed. In addition, Climatic changes and limited water resources also need to be seriously considered, as they will have an influence on the cost of production (Hafez, 2009).

Emergence and Re-emergence of poultry diseases

Several factors can precipitate and/or predispose to disease emergence. These include changes in the structure and development of the poultry industry, strong global competition and varying production costs in different continents and countries, leading to an increase of the global movement of poultry and poultry products. This could also increase the risk to introduce infections to areas which were previously considered to be free from such diseases (Hafez, 2009). Re-emerging and resurging infections are those that existed in the past but are now rapidly increasing either in incidence or in geographical or host range.

Health disorders and infectious diseases of poultry are mostly associated with severe economic losses. Several pathogens are incriminated as possible causes of many disease complexes of poultry poults either alone (mono-causal) or in synergy with different other micro-organisms (multi-causal) or accompanied by non-infectious factors. Non Infectious means all factors which influence the bird health and include house structure, climatic conditions (ventilation, temperature, and litter condition), stocking density, feed and water supply, hygienic condition as well as the knowledge and qualification of the stockman. These factors affect each other and can promote or inhibit the health condition of the flock. In aim to achieve desired performance results, managers of poultry flocks should integrate good environment, husbandry, nutrition and disease control programs. The rearing management must be directed to satisfy the bird's requirements, to promote the production and to prevent diseases condition. Any disturbance will cause stress, which will reduce the resistance of the birds, increase their susceptibility to infections and reduce their immune-response to vaccines. Infectious diseases caused by several infectious agents such as viruses, bacteria, fungus and parasites are involved in many disease conditions. These infectious agents can be introduced and spread in poultry farms by different routes. It occurs by vertical and/or horizontal route. At early days of age, the main disease problems are related to vertically transmitted infections and improper hatchery eggs sanitation (Yolk sac infection/ Omphalitis) with salmonella, E. coli, mycoplasma, aspergillus, staphylococci, streptococci, pseudomonas and avian encephalomyelitis, inclusion body hepatitis. Those and other infectious agents can also be transmitted horizontally (laterally) by direct contact between infected and non-infected birds. Currently, the most important problems of poultry are respiratory diseases, possibly caused by avian influenza, Newcastle disease, infectious bronchitis, avian metapneumovirus and /or *ornithobacterium rhinotracheale* and E. coli. Furthermore, enteric disorders caused by several viral agents such as coronavirus, astrovirus, and rotavirus or due to parasitic infestation such as coccidia are common problems.

The severity of clinical signs, duration of the disease and mortality are extremely variable and are in-

fluenced by kind, virulence and pathogenicity of the infectious agent as well as by many environmental factors such as poor management, inadequate ventilation, high stocking density, poor litter conditions, poor hygiene, high ammonia level, concurrent diseases and the type of secondary infection.

Future expectations Disease diagnosis, treatment and control

In future improvements in laboratory diagnosis, such as diagnostic micro array and other technologies, will allow faster, more sensitive and more accurate diagnosis of infectious diseases, and early interventions will become a reality.

However, only a few authorised pharmaceutical veterinary products will be available for the treatment of poultry as food producing animals. Future scientific findings on the pathogenic mechanisms of bacteria will help to improve the treatment of bacterial infections, and instead of non-specific antibiotic therapy, new drugs will be able to target the signalling mechanisms, which are able to disrupt the pathogenic effects of the pathogen bacteria. Vaccination is regarded as one of the most beneficial biopharmaceutical interventions due to its ability to induce protection against infectious diseases through targeted activation of the immune system. Many valuable new vaccine production technologies have been developed as a result of rapid progress in various areas. The use of future progressive vaccine production technologies, such as recombinant, subunit, reverse genetic and nucleic acid vaccines, can significantly reduce the cost of vaccines, ensure better efficacy, and allow easy and rapid intervention to face the steady mutation of the microorganisms. Furthermore, the development of efficient vaccines against bacterial infections will lead to a reduction of the use of antibiotics and subsequently of the development of resistant bacteria. Genetic resistance and selective breeding to improve production traits and health is a long-standing goal of the industry. The desire to enhance breeding strategies through the use of molecular techniques (genetic linkage maps) will lead to the characterisation of genome structure and genes that are associated with production traits and disease susceptibility and resistance. This will allow selecting bird lines that are genetically resistant to several pathogens. In addition, improvement of rearing technology, management and nutrition will help to maintain bird comfort.

Conclusions

In the future, the global cooperation and trade will force the governments to harmonize the existing different legislations related to trade, animal disease control, animal nutrition as well as the licensing of drugs and vaccines for veterinary use.

Last but not least the consumer expectations for high standards quality of poultry products will strongly influence the production methods. This means that farmers, veterinarians, stockholders and all other partners involved in the production chain will have to share more responsibilities and that cooperation will be intensified.

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L20 Impact of animal welfare on worldwide poultry production

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Keywords: poultry, welfare, production systems, competitiveness, production costs

Summary

Animal welfare organizations have developed contemporaneously with the industrialization and urbanization of the human society in the 19th century. In the 1960th the interest of welfare was focuses on farm animals kept under "industrial" conditions. Since laying hens experienced the most rapid transition from unlimited free range towards battery cages, they attracted most interest of welfare organizations, public media, politicians and scientists. Consequently the management conditions for laying hens in Europe were regulated in detail by national laws and EU-Directives, and these were used as templates for welfare regulations in broilers, turkeys and even other farm animal species. Conventional cages have been banned in the EU and discussion on welfare of caged hens is going on in the USA as well. Activities in Europe are now directed towards other issues, such as beak-trimming and killing day-old chicks of layer lines. All measures which are considered to improve the welfare of animals increase the cost of production. Hence, differences in national welfare regulations are expected to favor the production in countries with low welfare standards. There is a tendency that important retailers use welfare as a marketing argument and establish high price premium labels. Since standards which are established and controlled by stakeholders of the poultry market, they are independent of the welfare legislation of the countries of production. This will, to some extent, attenuate the differences in production costs and contribute to harmonization of welfare standards across countries.

Introduction

Welfare has become one of the most important issues in livestock farming in industrial countries. In ancient agricultural societies the treatment of animals was regulated by traditions. Although management conditions in most cases were not animal-friendly by the present welfare standards in Europe and other industrialized countries, there was consent within the societies on appropriate animal treatment. Industrialization and urbanization in Western countries lead to reduction of the rural population. In industrial countries the rural population represents only 3% of the total population, while 40 to 50% of the population in developing countries lives in rural areas (Ribbekk, 2005). Industrialization of farming systems and animal production was the consequence of the migration of manpower from rural to urban areas. This development was more dramatic in poultry than in any other livestock species. Within a time span of 20 years only there was a transition from this system to restricted free range to battery cages. The extreme changes provoked animal welfare organization to opposition. Arguments against the industrialization of farm animals have been published in Ruth Harrison`s book "Animal Machines" (Harrison, 1964). The outstanding role of freedom in the debate on animal welfare is reflected by the "Five Freedoms" (FACW, 1995), which are used to define adequate husbandry systems for farmed animals. "Five freedoms" not only comprise freedom to move, but also freedom from hunger, thirst, discomfort, pain, injuries and distress. This shows that the perception of animal welfare is closely related to the prevailing conditions of the human society. For several decades the discussion on poultry welfare was limited to European countries. Only a few years ago the conditions of laying hens and broilers have become subject of public discussion in the USA. As the market of poultry eggs and meat is highly internationalized poultry welfare is now global issue.

Definitions, criteria and assessment of animal welfare

Definitions

Theoretically, animal welfare is understood as subjective state of animals, ranging from extreme suf-

fering to total wellness. There exist numerous definitions of animal welfare (LayWel, 2006), which cover a wide range, from physical damages, diseases, physiological and psychological disturbances towards adverse experience and positive feelings. Since under practical conditions positive and negative events occur simultaneously the state of welfare can be considered as the balance of the positive and negative influences (Mench, 1998). Providing the inputs which are required to cover the biological needs, such as feed, water, shelter, protection against adverse climatic conditions, disease and predators does not necessarily produce positive feelings. The latter is expected from provision of environmental enrichment objects. Increased awareness of cognitive competences of animals (Duncan and Petherick, 1989; Meyer et al., 2010) will lead to a further extension of welfare definitions and requirements of adequate husbandry conditions.

Criteria of welfare

Regarding the variation of welfare definitions it is extremely difficult to assess welfare in poultry production practice. Physical damage, such as wounds produced by cannibalism, bone breakage, and disease can easily assessed by established scoring systems and veterinary diagnosis. It is generally accepted that these conditions impair the wellbeing of animals. Physiological and ethological criteria are more difficult to measure and their relationship with wellbeing is often not fully understood. Stress as physiological response to environmental stimuli is frequently mentioned in the context of welfare (Rushen, 1991). Release of corticosterone and epinephrine and the change in the Heterophile- Leucocyte ratio (H/L) (Maxwell, 1993) are usually measured as indicators of stress. The validity of these criteria as indicators of poor welfare has been demonstrated under extreme environmental conditions and under experimental situations. Their application under practical husbandry conditions, however, is difficult and cannot be clearly related to the state of wellbeing. Behavioural criteria are used to assess the psychic conditions of animals. Indicators of psychic disturbances are changes in the normal behavior, the occurrence of displacement activities (e.g. displacement preening), damaging behaviors (e.g. feather pecking and cannibalism, aggression, fear, and stereotyped behaviors). Positive psychic conditions are assumed to be expressed through comfort behaviors, such as dustbathing, stretching and play behavior. There exists, however, a great variability of behaviors and it is difficult to distinguish between normal and abnormal or disturbed behavior. Only damaging behaviors, such as feather pecking and cannibalism are unanimously accepted as indicators of poor welfare. The assessment of a positive emotional state of animals is even more difficult. Preening, dustbathing, playing and positive social contacts are performed when the animals are not threatened by adverse stimuli. However, a high uncertainty remains when we try to interpret these behaviors with regard to the animal`s positive feelings. Functional Magnetic Resonance Imaging (fMRI) provides more reliable information on positive and negative emotions (Montaigue and Berns, 2002). The application of this technique under practical conditions is not possible at present.

Welfare assessment

In spite of the above mentioned problems of welfare definition various assessment schemes have been developed (Sundrum, 1997). The most elaborated and comprehensive scheme for on farm welfare assessment of broilers and turkeys has been elaborated by the Welfare Quality project (EFSA, 2012). This scheme is based on 4 principles, good feeding, good housing, good health and appropriate behavior. There exists a voluminous assessment protocol which comprises environmental and animal based criteria. All criteria are aggregated in a welfare score, which can be used to appraise among farms and production systems. There is an increasing number of studies where the scheme has been applied (Keeling, 2009). Its applicability however needs further development (de Jong et al., 2016).

Comparison of welfare regulations

Comparison of welfare regulations in different countries have been reported in recent studies (van Horne and Achterbosch, 2008; Bracke, 2009; ECON; 2010; Lichter and Kleibrink, 2016). These studies show that welfare regulations for farm animals and particularly for poultry are more elaborated in EU countries and in Switzerland than in other countries. EU (1999) regulates in detail stocking density, lightning, climate, noxious gases, feed and feeding systems, perches, nests, health care, inspection, hygiene measures, and mutilations (e.g. beak trimming). Welfare of broilers is regulated in similar detail (EU,

2007). Since the EU tolerates deviation from the Directives, provided minimum standards are followed. Some countries apply stricter rules. Legislation related to poultry welfare in countries outside the EU has been reported from Switzerland, Argentina, Australia and Brazil. Mandatory Codes of Recommendation exist in New Zealand, voluntary Codes of Practice in Canada, China and USA. Van Horne and Achterbosch (2008) analysed a large number of countries for their level of welfare legislation, using scores from 1 (low standard) and 5 (high standard). Switzerland and Northern European countries reached highest scores, followed by other Western European countries, South European countries, Australia, Japan, Middle East, South America, East Europe, and Asian countries. The authors pointed out, that their scoring system was based on "selected standards of welfare, which are not, on their own, good indicators". In a study on legislation of broiler and turkey welfare in different countries, Lichter and Kleibrink (2016) identified four clusters with Germany, Austria and Sweden exceeding the minimum requirements of the EU regulation (EU, 2007), followed by Brazil, USA and Russia. USA and Brasil are in a special situation. Though there is no legal basis for welfare in broiler production, the recommendations of the National Chicken Council (NCC) are implemented under the control of the large scale broiler integrations (Robins and Phillips , 2011), and large broiler production enterprises produce for the EU market and comply with EU regulations. Relevant welfare standards for broilers are laid down in a specific law in Russia. In most other countries there are no official standards for broilers.

Welfare standards

Broilers and turkeys

Under the influence of different welfare regulations prevailing management conditions diverge considerably among countries. Most regulations provide a basic stocking density which can be exceeded when special good management practices are ensured. The basic stocking density for EU countries is 33 kg/m². Under improved management conditions up to 42 kg/m² can be allowed. Nevertheless in some European countries the allowed maximum stocking densities is lower than 42 kg/m². In Sweden for instance, basic stocking density is 20 kg/m², but up to 36 kg/m² can be allowed when the farmers take part in a control scheme where housing and animals are regularly inspected. Maximum stocking density in Germany is 39 kg/m². There is no regulation for stocking density for broilers in Brazil, but due to high ambient temperature only 10 to 12 broilers (approximately 35 kg/m²) are kept per m². There is also no legal limit of stocking density in the USA. Recommendations of several producer organizations vary from 32 to 42 kg/m². In organic production the maximum stocking density is generally lower than in conventional production: 21 kg/m² in the EU, Canada and New Zealand and 10 kg/m² in Australia. The EU Directive (EU, 2007) regulates further details of broiler management such as light intensity (20 lux), duration of the darkness (8 hours), and level of ammonia (20 ppm). During recent years numerous welfare agreements with higher welfare standards have been established among welfare-oriented NGOs, retailers and farmers. In most cases stocking density is reduced and other criteria, which are not regulated by laws, are included. In the "Tierwohl Initative" (animal wellbeing initative) in Germany broilers have to be provided with enrichment materials, such as pecking blocks or hay baskets. According to some schemes for organic broiler production maximum growth rate is restricted and perches have to be provided. There exists no EU regulation for turkey production. The only country where turkey production is legally regulated is The Netherlands (NL, 2006). Recommendations for turkey production are considered in the European Convention for the Protection of animals kept for farming purposes (EU, 2001). This document contains a comprehensive list of welfare criteria, including behavior, integument, performance, health and mortality. Other recommendations are provided by the Farm Animal Welfare Council in the UK (FACW, 1995), by the WelfareQuality Consortium (EU, 2012) and by some other NGOs. Turkey production in Germany is regulated through a Voluntary Agreement, where producer associations, welfare-oriented NGOs and the ministry concerned officials signed an agreement on maximum stocking density and other details of good management practice and animal care.

Laying hens

Even grater abundance of welfare standards exist for laying hens with the most detailed laws and regulations in Switzerland and the EU. As with broilers stocking density plays a central role. Maximum

stocking density in furnished cages in EU countries is 750 cm²/bird (EU, 1999). There are, however, EU countries with stricter regulations. In Germany maximum stocking density is 890 cm²/bird. There is a strong resistance of welfare organization to furnished cages in Germany and retailers have decided not to sell eggs from any type of cages. Therefore most egg producers replaced conventional cages through barn, aviary or free range systems. After conventional cages were banned in the EU, the debate started in the US where most layers are still kept in this system. The United Egg Producers (UEP) recommend space allowance of 430 cm²/bird. Higher density (350 to 400 cm²/bird) is practiced in most cases. In California conventional cages have been banned in 2015, and important fast food chains and retailers recently announced that they would stop using or selling cage eggs (Windhorst, 2008). The USA follows obviously the trend in European countries in providing laying hens more freedom to move. Special welfare labels for conventional and organic production exist in many countries. ECON (2010) listed voluntary welfare schemes of 10 European countries. There exist, however, many others welfare labels of regional interest which have not been considered in this review.

Beak trimming, forced molt and killing day-old male chicks of layer lines are intensively discussed. Beak trimming as a means to reduce damage through feather pecking and cannibalism is allowed by EU regulation in chicks up to 10 days of age. In Switzerland, Sweden, Norway and Finland beak trimming is prohibited. In the UK beak trimming is allowed only using infra-red treatment. Several European countries announced a ban of beak trimming recently. Egg producers in Germany anticipated a ban of beak trimming through a voluntary renunciation as of 2017 and launched various research and extension projects which should assist egg producers to manage intact-beak birds.

The problem of force molt or induced molt is being discussed as welfare issue mainly in the USA. Forced molt is not prohibited, but a fast food chain has stopped using eggs from molted flocks. Killing of day-old chicks of layer lines has become a major welfare issue in Germany. So far this procedure was not prohibited under the condition that it is carried out using adequate methods so that the chicks would not suffer. There exists, however a general paragraph in the German law of animal protection that no animal should be killed without sound reasons. The fact that male chicks of layer lines cannot be economically raised for meat production is not generally accepted as "sound reason" and attempts are underway to formulate a law to ban killing of day-old chicks generally.

Economic aspects of welfare

Improving welfare in layers and broilers is related to increased space, provision of enrichment devices and intensified control. Transition from cages to aviaries and barns bears increases the risk for feather pecking and cannibalism (Sherwin et al., 2010). This effect will be more pronounce when birds are not beak trimmed (Hartcher et al., 2015). Free range production increase energy requirement for exercise and compensation for low ambient temperature under moderate and cold climatic conditions. In broilers there is a linear increase of production cost with decreasing stocking density (Shanawany, 1988) and the profit per m² surface decreases correspondingly. The main effect of stocking density on the economic result is based on the decreased cost for housing, heating and labor. Costs further increase when material for enrichment is provided. According to own unpublished calculations costs increased by 0.03 € per kg live weight when stocking density was decreased from 39 to 35 kg/m² in broilers. Installation of commercial pecking blocks lead to further increase of costs of 0.003 €/kg live weight. In caged laying hens high stocking density of 350 to 400 cm² has proved to produce optimum economic results (Bell, 2000). There is a linear increase in production cost when space per bird increase from this level to greater space allowance in conventional cages, furnished cages under EU standard (750 cm²/bird) and barn systems (1111 cm²/bird) under EU standards (van Horne and Achterbosch, 2008). According to Matthews and Sumner (2015) cost per egg increase by 13% in furnished cages and 36% in aviaries as compared to conventional cages. Using intact-beak birds as compared to beak trimmed birds results in increased costs of 0.22 to 0.49 € per hen and year (Damme et al., 2013). The scenario of a ban of day-old layer chicks and the use of dual purpose breeds instead of conventional layer and broiler lines has been reported by Damme et al. (2015). Change of the production system would lead to extra cost per egg of 2.6 to 3.9 cts.

The relative increase in production cost seems to be small and consumers in Europe are supposed to be willing to pay for higher welfare standards. Retailers, however, respond to extremely low price differences and extra costs for welfare are not accepted if there is no special label which ensures higher con-

sumer prices. In the EU the housing system (cage, barn/aviary, free range and organic), is stamped on the eggs as a numbered code. Consumers are generally informed about the code and pay higher priced for eggs free range and organic production. Additional welfare measures, like enrichment materials and renouncing of beak-trimming, force molt and killing day-old male chicks need still to be communicated to the consumers.

Driving factors in animal welfare development

Requests for improvement of welfare standards are traditionally brought to the attention of legislative authority which incorporates them into laws or directives. Since legislation is usually a compromise of interests between welfare organizations, farmers, retailers and consumers, the resulting legal acts does in most cases not fully satisfy the particular interests of all stakeholders involved. Consequently different coalitions of shareholders developed their own welfare standards. In Germany there existed "Voluntary Agreements" between poultry farmer organizations, welfare organizations and government authorities which regulate minimum requirements in turkey and broiler production on the national level. The Voluntary Agreement for broilers has been replaced by a legal regulation. For turkeys an updated version (Voluntary Agreement, 2013) and is still used. Retailers are the most important driving forces in many non-government welfare regulations, and welfare organizations are involved in the development of the standards. This protects the retailers from adverse campaigns of welfarists in public media or in shopping centers. Farmers, however, who were not involved in the discussion, had to bear the higher costs of welfare-friendly production. Recently poultry farmers organizations in Germany decided to initiate own welfare labels in direct cooperation with retailer chains (www. Initiative Tierwohl.de). The retailers pay 0.04 Eurocent per kg of poultry meat into a central welfare fond. Participating certified farms are paid through the fond a gratification for the higher costs of production. Taking a leading role in setting welfare standards not only grant regulations which are scientifically sound and economically viable, but also improve the image of poultry farmers in the public. In addition it generates awareness of retailers and consumers on the costs of welfare.

Conclusions

Perception of welfare differs largely among countries and continents. Definitions of welfare not only depend on the cultural background but also on the living standard of the human population. This is reflected in different levels of welfare legislation and welfare standards among countries. The main welfare issues in egg production are cage rearing, stocking density, beak trimming, force molt, and killing of day- old chicks of layer lines. In broilers stocking density, light programs, litter conditions and leg problems are the main points of welfare concern. Higher levels of welfare lead to considerable increase of productions costs. Under competitive international market conditions the different legal welfare standards will lead to a shift of production towards countries with lower levels of welfare. At present retailers of poultry products establish in cooperation with welfare-oriented NGOs and/or poultry producer organizations special welfare standards which are independent of the legal situation of the country of production. These standards will not only attenuate the differences in welfare-born production costs but also lead to harmonization of welfare standards among countries.

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L21 Feeding broilers of the future

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Keywords: precision nutrition, AGP free diet, ingredient quality, phase and sex feeding, particle size and pellet, enzymes

Summary

The future of broiler feeding will mean a paradigm shift for nutritionists. While they are continually challenged to come up with low-cost feed formulations,. that provide best-performance results, society is demanding more transparency on how production animals are being fed and treated. The trend toward environmental accountability and sustainability innovation will continue to be strong market drivers. In the future, nutritionists will need to participate in sustainability initiatives; give more attention to ingredient quality; work on phase and sex feeding differentiation; concentrate on particle size and pellet quality, and use additives more efficiently, paying special attention to enzymes. In addition, more AGP- free broilers will be required by consumers. So, new methods of feeding will need to be developed to meet demand.

Introduction

In the last 50 years, there has been fantastic development in the poultry industry all over the world, and the progress made has been significant. The most important scientific advances are focused on poultry production knowledge; on quick adaptation of backyard production to industrialized business; on low cost of broiler meat and hens' eggs and on non-cultural and religious restrictions of chicken products. Data from the Food and Agriculture Organization of the United Nations (FAO) (2015) confirms broiler consumption is growing relative to other proteins. However, the future will come with new growth challenges, with cost of production remaining a central issue. Producers must be profitable enough to continue working with full efficiency, while remaining competitive and delivering affordable products.

There are other aspects on broiler production that nutritionists will need to address, and biosecurity is one of the top concerns. With the onset of avian influenza, there is increased attention on broiler losses and farm biosecurity. In addition, many consumers are confused about how avian pathology (*Salmonella, Campylobacter, Newcastle disease*) can affect human health. To avoid compromising farm biosecurity, governments, industry professionals and producers must work together to establish and to follow best practices, such as controlling the presence of backyard/wild chickens living close to production farms. In addition, farms must be as clean as possible, and employees must implement sanitation programs, established according to veterinary global recommendations.

In the last 20 years, consumers have been more active in expressing concerns related to what production animals eat, why they grow faster than in the past and how the industry treats them. Society is demanding more information on food safety and animal welfare, and there is an emphasis on sustainable production.

So, how can broiler nutrition and feeding innovate and advance while respecting society concerns and improving technical procedures for sustainability? First, an improvement in sustainability must be defined. At its base, producers must improve efficiency on broiler feed conversion (use less feed), and reduce excreta (pollutant) production. Additionally, producers must reduce water use, an input that will become increasingly scarce, restricting production in many regions of the world. In these areas, where animals will compete with humans for resources, there will be no room to feed broilers with a large nutrient specification. The only way to maintain production is to improve the understanding of precision nutrition so that producers can feed animals with low or no margin for error. This article will offer some suggestion for nutritionists who need to revisit some concepts and innovate for the future of poultry production.

Feeding broilers of the future sustainable

Broiler production free of antibiotic growth promoters (AGP)

Producing broilers free of antibiotic growth promoters (AGPs) has been considered for many years. The first proposal on producing broilers AGP free came from Sweden in 1986 (Cogliani et al., 2011). Initial reactions to the proposal focused on a loss of broiler production efficiency, wherein the cost of production would increase. After many years of research, however, these assumptions are no longer accepted in the industry. Societal concern in Europe promoted the development of research on non-antibiotic additives (probiotics, prebiotics, essential oils, organic acids, antioxidants, etc.) and a more efficient use of enzymes, which preserves gut health, with minimal or no reduction on broiler performance. Simultaneously, technical broiler advisers reinforced the implementation of management best practices, feeding and biosecurity care, all improving infection prevention and minimizing production inefficiency.

While the production of AGP-free animals is now an option in many countries, the speed of implementation varies. In 2015, the U.S. finally joined the European movement. The move to AGP-free started in 2010, when the Food and Drug Administration (FDA) called for a strategy to phase out production use of medically important antimicrobial products and to bring the remaining therapeutic uses under the oversight of a veterinarian. This had an important consequence. Food chain and supermarket enterprises accepted the challenge and began publicizing that the ingredients used in their products were AGP-free. Consumers picked up on the news, and the AGP-free movement kept growing. Today, reducing antibiotics in production remains an important innovation priority for nutritionists.

Ingredient analysis

Knowing ingredient composition, with the help of qualified laboratory support, is mandatory if the nutritionists want to formulate feed with a lean safety margin. Historical data shows that many nutritionists underestimate ingredient nutrient values, which does not guarantee better broiler performance. Instead, it means an incremental nutrient loss and a possible increase on pollution (nitrogen and phosphorus). With the progress of NIR technology, mills have no excuse for not having constant nutrient evaluation on their ingredients (Black et al., 2014). Here are examples of proper nutrient end energy valuation and its benefits:

- Pirgozliev et al. (2009) identified the importance of looking at the amylose:amylopectin ratio of different cereals (corn, wheat and rice) and how they can affect broiler performance.
- Zhou et al. (2010), working with ducks, similarly observed that amylose:amylopectin ratio is one of the main factors that determine true metabolizable energy in corn, and can be used to predict the available energy.

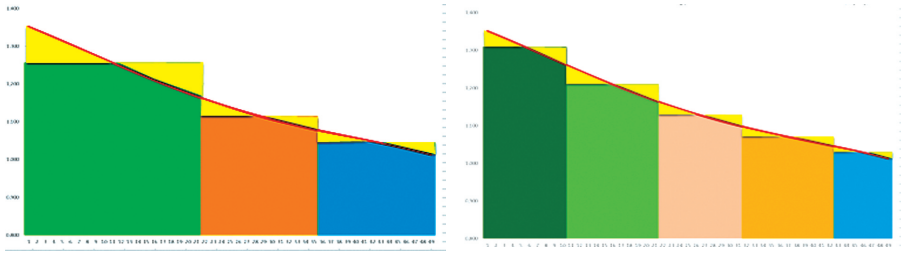
These examples show that ingredients used in the diets are not just "commodities" when used in feed formulation, but important nutrient drivers of production. New technologies like "in-line feed formulation" are pushing the bounds of precision nutrition. The technology allows a NIR to be installed in the ingredients transportation line, before the mixer, which allows an immediate reading and consequent formulation and dosing, according to the nutrient composition of the ingredients that were read. This and other advancements are helping feed mills to deliver feed with exacting specification to produce better poultry, more sustainably.

Digestibility and phase feeding

The digestibility of the nutrients and energy vary in different production phases. For example, younger chicks are less efficient than older chicks (Noy and Sklan, 1995; Batal and Parsons, 2002 a,b; Thomas et al., 2008). In addition, composition and digestibility of ingredients can differ in various years and global regions. Knowing these digestibility differences and correctly calculating the required ingredient composition for the various phases will be an important skill for nutritionists in years to come. Getting digestibility calculations, before feed formulation, means a lessened diet cost, better host digestive tract health and increased environment sustainability.

In addition to improving digestibility calculations, increasing the number of phases during production ensures less nutrient waste. For example, using five feeding phases, compared to three phases in broiler feeding, increases lysine delivery precision (Graph 1). Brewer et al. (2012b) worked with three different

genetic strains and fed broilers with three different diets (0-18, 19-32 and 33-40 days of age) or, after 19 days of age, phase-fed the birds, changing the diets every two days. Phase feeding, during the overall period (19 to 40 days of age) did not affect weight gain but improved feed efficiency on two of three strains and did not compromise the fillet weight in all strains. Phase feeding significantly reduced protein and amino acid consumption, making the cost of feeding cheaper than the conventional system. Similar results were seen when working with four different strains, phase feeding one group every two days, from 17 to 58 days of age (Brewer et al., 2012a). The main benefit of phase feeding was the reduction of production cost due to lower nutrient and energy consumption. These findings confirmed what was observed by Angel et al. (2006). Moving from four to six broiler phases (0 to 42 days of age) and supplementing the six diets with amino acids (lysine, methionine, threonine, isoleucine, valine, arginine and tryptophan), the nitrogen excretion was reduced by 40 percent when compared to the control proposal (4 phases).



3 phases 5 phases

Graph 1. The effect of different phase feeding (3 and 5) on digestible lysine supply (yellow space represents the amount of digestible lysine that is over or under offered to broilers, per phase).

The scientists are concern with the best use of nutrients and energy but, in the future, more research will be done using ingredient net energy instead of conventional metabolized energy, another method that will favor environmental sustainability.

Sometimes phase feeding is limited because of the feed mill structure. This new understanding of phase importance must be considered when a new feed mill project is developed and logistics are defined, so that the mill does not become the bottle neck of production.

Sex feeding

Generally, sex feeding is a controversial subject among nutritionists, hatcheries, feed mills, broiler production and slaughter plant managers. But, in the future, an increase focus on sustainability will require that all areas of production contribute logistically to improvement. The differences between male and female growth speed, body composition, nutrition requirement and behavior are sufficient to justify separate sex production. This strategy promotes reduction of feed cost and, most importantly, reduction of slaughter weight variability. In mixed-sex production, males will need to stay in the barns longer to push the weight up to a specific average body weight that cannot be achieved by females. That procedure can double body weight variability, making male feed efficiency worse and increasing mortality, because of the extra days in the barn. The Aviagen manual for broiler 308 (2014) and the Cobb manual for broiler 500 (2015) do not offer information on sex nutrient requirement differences. However, Aviagen (2014) suggests that in the case of using separate sex feeding, modifications of nutrients and energy requirements should be considered. Both manuals show differences in the speed of weight gain and feed efficiency of different sexes and they differ on nutrients and energy recommendations. Therefore, broilers of each of these strains require different feed and nutrients. These considerations were confirmed by Faridy et al. (2015) when they evaluated data research from Cobb and Ross strains. Using meta-analysis, they concluded that males require more lysine than females and there is a difference of lysine requirement according to the strain. Also, lysine requirement increases with the increase of crude protein in the diet.

Particle size and pelleting

The nutritionists and feed mill managers must look more to particle size and feed pelleting. Feed with

larger particle size promotes better gizzard development, gastric motility and gastro duodenal reflux in poultry. It improves digestion and reduces the entrance of pathogens in the intestine (Amerah et al., 2008; Gabriel et al., 2008, Svihus, 2011). Large particles require less energy during feed apprehension, as birds require fewer pecks to ingest the same amount of feed (Amerah et al., 2007) and feed mills save energy from reduced grinding (Reece et al., 1986).

Broilers fed pelleted diets showed better performance than those fed mash diets, with improvement directly related to pellet quality (McKinney and Teeter, 2004). Poultry fed pellets have higher dietary density; higher feed intake; reduction of energy for consumption; better starch and protein digestibility and reduction of feed waste (Amerah et al., 2007; Dozier et al., 2010). Zang et al. (2009) added that pellet improves intestinal function, as shown by increase in villi height and in villi height to crypt depth ratio. Most of these earlier findings were confirmed lately by Naderinejad et al. (2016), who reinforced that coarse corn particle size and pellet diets improve gizzard development and function and improve the use of nutrients and energy that promote better broiler performance.

Additives -enzymes

Sometimes enzymes are wrongly called additives. They are proteins. They can improve digestion of nutrients and energy but also offer nutrients and energy to the host. New enzyme technology has been growing very quickly in the last 15-20 years and it will continue to do so in the future. In 1996, Cowan et al. said that this technology improves ingredient digestibility and nutrient absorption. Also, Penz Jr. and Bruno (2010) reinforced that enzyme technology reduces pollutant excretion in animal waste. There are many enzymes available to help the digestion of phosphorus, calcium, carbohydrates, proteins and lipids of diets offered to the animals, especially poultry and swine. However, enzymes will not always work with 100 percent efficiency because they are exogenous sources. Their efficient use will depend on correct technical decisions, correct mixing and substrate availability.

There is a vast wealth of information available describing different modes of action and different enzyme products and inclusions, and nutritionists should look at enzyme use as another way to make poultry production more environmentally sustainable. Even 10 percent improvement on enzyme-promoted nutrients and energy digestibility might reduce waste disposal and nitrogen and phosphorus pollution. In addition, with the appeal of producing AGP-free broilers increasing, gut health is more important than ever. It is well known that around 70 percent of poultry immune response is provided by digestive tract cell stimulation, and well-used enzymes have a unique, positive effect on broiler immune response from the gut.

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L22 Natural antioxidants and stresses in poultry production: from vitamins to vitagenes

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Keywords: antioxidants, poultry, stress, vitagenes

Summary

Commercial poultry production is associated with various stresses leading to decrease of productive and reproductive performance of growing chickens, parent birds as well as commercial layers. In general, there are four major types of stress in poultry industry: technological, environmental, nutritional and internal stresses. Growing body of evidence indicates that most of stresses in poultry production at the cellular level are associated with oxidative stress due to excess of free radical production or inadequate antioxidant protection. Therefore, dietary antioxidants are considered to be the main protective means to deal with various stresses in poultry production. Indeed, the development of the effective antioxidant solutions to decrease negative consequences of commercially- relevant stresses is an important task for poultry scientists. One of such approaches is based on possibilities of modulation of vitagenes, a family of genes responsible for animal/poultry adaptation to stress. The new concept of fighting stresses is based on an idea that supplying birds with various antioxidants via the drinking water could help them to effectively deal with stress conditions. In fact, it was proven that inclusion of vitagene-regulating compounds (carnitine, betaine, vitamin E, etc.) in water, as well as some minerals, vitamins, electrolytes and organic acids could be effective in fighting various stresses.

Stresses in poultry production

Commercial poultry production is associated with various stresses leading to decrease of productive and reproductive performance of growing chickens, parent birds as well as commercial layers. In general, there are four major types of stress in poultry industry: technological, environmental, nutritional and internal stresses (Surai and Fisinin, 2016a; 2016b; Table 1).In fact, a list of commercially-relevant stresses in poultry production could be quite long, but the main point is the most of the stresses suppress reproductive performance of parent birds including reduced fertility and hatchability. Furthermore, stresses are associated with impaired feed conversion, reduced average daily weight gain, immunosuppression and increased mortality in growing birds. Growing body of evidence indicates that most of stresses in poultry production at the cellular level are associated with oxidative stress due to excess of free radical production or inadequate antioxidant protection (Surai, 2002; 2006; Surai and Fisinin, 2016a; 2016b). According to the recent literature review, heat and diet are among main means causing oxidative stress in domestic birds that may lead to biological damage, serious health disorders, lower growth rates, and, hence, economic losses (Estevez, 2015). Therefore, dietary antioxidants are considered to be the main protective means to deal with various stresses in poultry production (Surai, 2002; 2006)

Antioxidant systems of the body

During evolution, living organisms have developed specific antioxidant protective mechanisms to deal with ROS and RNS (Surai, 2002). Therefore it is only the presence of natural antioxidants in living organisms which enable them to survive in an oxygen-rich environment. The general term "antioxidant systems" describes these mechanisms, which are diverse and responsible for the protection of cells from the actions of free radicals. These systems include: Natural fat-soluble antioxidants (vitamins A, E, carot-

enoids, ubiquinones, etc.); water-soluble antioxidants (ascorbic acid, uric acid, carnitine, betaine, taurine, etc.); antioxidant enzymes: glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD); thiol redox system consisting of the glutathione system (glutathione/glutathione reductase/glutaredoxin/glutathione peroxidase and a thioredoxin system(thioredoxin/thioredoxin peroxidase/thioredoxin reductase). The protective antioxidant compounds are located in organelles, subcellular compartments or the extracellular space enabling maximum cellular protection to occur. The antioxidant system of the body is responsible for prevention of damaging effects of free radicals in stress conditions. Therefore, dietary supplementation of antioxidant compounds is a way to improve efficiency of poultry production in commercial conditions associated with various stresses. Dietary-derived antioxidants related to poultry nutrition can be divided into several groups:

• Vitamin E -main chain-breaking antioxidant in the cell, located in biological membranes and proven to be effective in antioxidant protection. The main reason for vitamin E dietary supplementation for poultry and farm animals is to maintain their optimal health and high productive and reproductive performances. It includes positive effects of vitamin E on male and female reproduction, immunocompetence, effective growth and development, high quality of eggs and meat, decreased negative consequences of various stresses (Surai, 2002; 2006, 2014). Extensive research and wide commercial application for a number of years clearly shows essentiality of vitamin E in animal/poultry nutrition. Recently it has been shown that vitamin E recycling in the cell is key for its antioxidant activity. Ascorbic acid, selenium, vitamins B1 and B2 are important elements of vitamin E recycling. Therefore, if recycling is effective, even a low vitamin E concentration, for example in the embryonic brain, can prevent lipid peroxidation *in vivo*. Dietary vitamin E supplementation is an important part of poultry nutrition (Surai, 2002). The main source of vitamin E for poultry is a premix providing the vitamin usually in excess of dietary requirement. There is a range of anti-stress premixes with an increased vitamin E content. After 90 years of extensive research in the field of vitamin E we greatly appreciate its unique role in biological systems, in maintaining growth, development and general health of humans and animals.

• Selenium -an integral part of 25 selenoproteins participating in various antioxidant reactions in the body. It is supplied with the diet in various forms and optimal Se status is a key for effective antioxidant protection (Surai, 2006). It has been shown that organic Se in the form of Selenomethionine (SeMet) is the Se form of choice for poultry breeders (Surai and Fisinin, 2014). Generally speaking, there are two major Se sources for poultry, namely inorganic selenium (mainly selenite or selenate) and organic selenium in the form of selenomethionine (SeMet; mainly as Se-Yeast or SeMet preparations). Advantages of organic Se in poultry nutrition have been shown (Surai, 2006; Surai and Fisinin, 2014) and Se-Yeast receive a lot of attention as an effective source of organic selenium in poultry/farm animal nutrition. However, it seems likely that the level of SeMet in the yeast is the major determinant of its value as a Se source. However, that value is still variable (usually in a range 50%~70%) and it is a difficult task to stabilise and guarantee it at the level of production of Se-Yeast. Pure SeMet has some problems related to its stability and therefore an introduction of the new stable supplemental form of Se (Seleno-hydroxymethionine) could be considered as a next step in improving Se nutrition of poultry.

• Carotenoids -important elements of the antioxidant system, possessing antioxidant activities and participating directly or indirectly (for example, by recycling vitamin E or regulating expression of various genes) in antioxidant defences. There are more than 750 carotenoids in nature and their efficiency varies considerably. Recently, an important role of canthaxanthin in breeder nutrition has been described (Surai, 2012a; 2012b). Indeed, there is a growing evidence indicating that canthaxanthin has a special role in antioxidant defences of the developing embryo and its dietary supplementation of the breeder's diet is a way of improving hatchability.

• Vitamin C (ascorbic acid) - an important antioxidant synthesized in chickens. Its dietary supplementation is shown to be effective only in stress conditions, when its requirement substantially increases. The role of vitamin C in vitamin E recycling is a topic of great interest.

• Polyphenolic compounds -a group of various plant-derived compounds comprising more than 8,000 various compounds possessing antioxidant and pro- oxidant properties in various conditions (Surai, 2014). The main problem with polyphenols, including flavonoids, is their low bio-availability. Their concentration in the diet could be very high, but their levels in blood is low and their concentration in target tissues (liver, muscles, egg yolk) usually is negligible. Therefore, the main site of flavonoid action is the gut where they can have health-promoting properties by participating in maintaining antioxidant-prooxi-

dant balance (Surai, 2006). Emerging findings suggest a large number of potential mechanisms of action of polyphenols in preventing disease, which may be beyond their conventional antioxidant activities. Therefore, it seems likely that antioxidant activity is not a major mechanism for possible beneficial effects of flavonoids in poultry nutrition. Indeed there is a shift in polyphenol-related research from testing their antioxidant activities in vitro to deeper understanding their molecular mechanisms of action including cell-signalling and gene expression. In particular, silymarin (SM), an extract from the *Silybum marianum* (milk thistle) plant containing various flavonolignans (with silybin being the major one), has received a tremendous amount of attention over the last decade as a herbal remedy for liver treatment. In fact, SM has been the gold standard drug to treat liver disorders of different aetiologies and milk thistle extracts have been used as traditional herbal remedies ("liver tonics") for almost 2000 years. Indeed, protective roles of silymarin in poultry production deserves more attention (Surai, 2015d)

• Carnitine is an important element of the antioxidant system of the body (Surai, 2015b) and it has positive effects on chickens when added to the feed or supplemented via water. In fact, carnitine could be considered a new type of antioxidant, regulating the mitochondria, a major site of free radical production. Effects of carnitine on vita-gene expression deserves more attention. Carnitine also has immunomodulating properties in chickens and needs further investigation. Indeed, carnitine is synthesized in animals, including chickens, however, in stress conditions its synthesis is most likely not sufficient and there is a need for its supplementation. In general, carnitine supplementation via drinking water is considered an effective means of improving carnitine status of the birds and their performance.

• Betaine is another new entrant into the antioxidant family participating in osmotic balance regulation. It seems likely that as a source of methyl groups in the body betaine plays important roles in many physiological processes related to stress biology.

Vitagene concept development

Since at the molecular level most stresses are associated with overproduction of free radicals and oxidative stress, the development of the effective antioxidant solutions to decrease negative consequences of commercially-relevant stresses is an important task for poultry scientists. One of such approaches is based on possibilities of modulation of vitagenes, a family of genes responsible for animal/poultry adaptation to stress. The term "vitagene" was introduced in 1998 by Rattan who wrote "Our survival and the physical quality of life depends upon an efficient functioning of various maintenance and repair processes. This complex network of the so-called longevity assurance processes is composed of several genes, which may be called *vitagenes"*. Later vitagene concept has been further developed in medical sciences by professor Calabrese and colleagues in 2004-2016. In accordance with Calabrese et al. (2007; 2009; 2014) the term vitagenes refers to a group of genes that are strictly involved in preserving cellular homeostasis during stress conditions and the vitagene family includes heat shock proteins (HSPs), including heme oxygenase-1 (HSP32, HO-1) and HSP70, the thioredoxin system and sirtuins. The list of potential candidates to vitagene family was extended. In particular, SOD, a major inducible enzyme of the first level of antioxidant defence, has been included into the vitagene family (Surai, 2015a; Surai and Fisinin, 2015b). The products of the mentioned genes actively operate in detecting and controlling diverse forms of stress and cell injuries. The cooperative mechanisms of the vitagene network are reviewed in recently published in the aforementioned comprehensive reviews with a major conclusion indicating an essential regulatory role of the vitagene network in cell and whole organism adaptation to various stresses. Indeed, cellular stress response is mediated via the regulation of pro-survival pathways and vitagene activation with the following synthesis of a range of protective antioxidant molecules is the central event in such an adaptation. The vitagene concept helped in developing effective strategies to fight oxidative stress in various human diseases, including neurodegenerative disorders, neuroprotection, aging and longevity, dermatology, osteoporosis and Alzheimer pathology, and other free radical-related diseases (for review and references there see Surai and Fisinin, 2016b). Indeed, HSPs, including heme oxygenase-1 (HO-1) and HSP70, are responsible for protein homeostasis in stress conditions of poultry production (Surai, 2015c), while the thioredoxin system is the major player in maintaining redox status of the cell involved in protein and DNA synthesis and repair as well as in regulation of expression of many important genes (Surai and Fisinin, 2016b). Furthermore, sirtuins regulate the biological functions of various molecules post-translationally by removing acetyl groups from protein substrates ranging from histones to transcription factors and orchestrate cellular stress response by maintenance of genome integrity and

protein stability. Finally, SODs belong to the first level of antioxidant defence preventing lipid and protein oxidation at the very early stages (Surai, 2015a). All the aforementioned vitagenes operate in concert building a reliable system of stress detection and adequate response and are considered to be key elements in stress adaptation.

Vitagene–based concept of fighting stresses in poultry production

Recently, he vitagene concept has been successfully transferred from medical sciences to poultry science (Fisinin and Surai, 2011; Surai and Fisinin, 2012a; 2012b; Surai, 2015a; 2015b; 2015c; 2015d; Surai and Fisinin, 2015b). The new concept of fighting stresses is based on an idea that supplying birds with various antioxidants via the drinking water could help them to effectively deal with stress conditions. Indeed, a decreased feed consumption at time of stress is observed and existing feeding systems do not allow to include anything into the feed loaded into the feed storage bins. Therefore, water-soluble additive supplementation via drinking system is shown to be a valuable option. In fact, modern commercial poultry houses are equipped with water medication systems, which can be effectively used for the aforementioned supplementations. It was proven that inclusion of vitagene-regulating compounds (carnitine, betaine, vitamin E, etc.) as well as some minerals, vitamins, electrolytes and organic acids in water, could be effective in fighting various stresses (Fisinin and Surai, 2011; Surai and Fisinin, 2012a; 2012b). This helps at chick placement, when the antioxidant system is crucial for the digestive and immune system development (Fisinin and Surai, 2012a; Surai and Fisinin, 2015a). In particular it was proven that inclusion of an anti- stress composition (PerforMax) into the drinking water at the University trial improved chicken growth and feed conversion ratio (FCR; Fotina et al., 2011; Fotina et al., 2014). Using the same anti- stress composition in commercial conditions improved FCR during a 39 day broiler growth trial. At the end of the trial, the improvement in FCR due to the anti-stress composition during the first three days post-hatch as well as before and after vaccination was highly significant (Velichko et al., 2013; Velichko and Surai, 2014). The importance and efficacy of the anti-stress composition for rearing birds and adult egg type parent stock (Hy-Line) at one of the biggest egg producing farms in Russia (Borovskaya poultry farm, Tumen region) have been recently reviewed (Shatskich et al., 2015).In particular it was shown that usage of the anti-stress composition with drinking water at specific periods of the increased stress can improve breeder's performance. In particular there was an increase by 2% of the egg peak production and peak plateau lasted about 50 days longer than that in the control birds. It is interesting to note that hen housed egg production in the control group (260.8 eggs) was higher than the target for the line (253.4 eggs) and in the experimental group it was increased by 6 eggs. Furthermore, improved egg production was associated with increased weight of the oviduct in the experimental layers. It is also important to mention that FCR (feed per 10 eggs) was also improved by usage of the anti-stress composition and was better than the target for the line. Notably, shell strength at age 26, 36 and 56 weeks was improved in the experimental group by 2.8, 5.6 and 5.6%, respectively. The most interesting finding was related to a significant increase of the carotenoid level in the egg yolk of experimental birds. Since carotenoids were not supplied with the anti-stress composition, this increase could be due to improved absorption of nutrients resulting from anti-stress composition usage. This can also explain improved FCR in the experimental birds. Vitamin A level in the egg yolk from the experimental layers was also increased probably reflecting its transfer from the anti-stress composition. In particular, anti-stress composition usage was associated with improved fertility at 16, 40, 48 and 56 weeks by 2.5; 2.7; 2.8 and 3.7%, respectively. In the same experimental group the hatch of condition chicks improved at 26, 32, 40, 48 and 56 weeks by 3.6; 2.1; 3.4; 4.9 and 4.3%, respectively (Shatskih et al., 2015). In addition, it was shown that the anti-stress composition had an immune-modulating effect in broilers (Fotina et al., 2011) and growing ducklings (Surai et al., 2012). Improvement of the antioxidant system via supplying an antioxidant composition via the drinking water could also help dealing with various mycotoxins in feed, including DON (Fisinin and Surai, 2012b; 2012c), ochratoxin (Fisinin and Surai, 2012d; 2012e) and T-2 toxin (Fisinin and Surai, 2021f; 2012g). Furthermore, such a technology could help fight heat stress (Surai and Fotina, 2013) and immunosuppression (Fisinin and Surai, 2013a; 2013b). However, further work is required to understand molecular mechanisms of the interactions of vitagenes with various signaling systems and transcription factors in the cell to build an adequate adaptive response to minimize detrimental consequences of commercially-relevant stresses in poultry production.

Table 1. Stresses in poultry production (Adapted from Surai and Fisinin, 2016a)

Technological stresses
Chick placement
Increased stocking density
Weighing, grading, group formation, catching, transferring to breeder houses
Prolonged egg storage, egg transportation, inadequate egg storage conditions, incorrect incubation regimes
Environmental stresses
Inadequate temperature
Inadequate ventilation and increased dust
Inadequate lightning
Nutritional stresses
Mycotoxins
Oxidised fat
Toxic metals (lead, cadmium, mercury, etc.)
Imbalance of minerals (Se, Zn, Cu, etc.) and other nutrients
Low water quality
Usage of coccidiostats and other drugs via feed or water
Internal stresses
Vaccinations
Microbial or virus challenges
Gut dis-bacteriosis
Pipping and hatching

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L23 Feed resources: limitation, exploitation and utilization

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Keywords: novel feed sources, insect protein, algae as feed, feed production

Summary

Meat and eggs are essential staples for humans. As population grows, the need for food production, and hence for meat and eggs, increases. Despite the tremendous advances in animal production and feed technology that have occurred to date, the capacity of the world to keep producing feed by using the traditional feed ingredients in a traditional manner has serious limitations. Thus, application of emerging technologies and exploitation of novel feed resources will be required to address future limits in the production of food of animal origin.

Limitation to feed supply

Introduction

Feed production is driven by meat consumption, which, in turn, is driven by population increase, urbanisation and GDP growth. The world population is expected to reach 8 billion by 2024 and the average GDP by around 30% over the period between 2014 to 2024 (World Bank, 2015). According to the OECD-FAO Agricultural Outlook (FAO, 2016), meat production increased by 20% between 2004 and 2014 and is expected to increase by 17% by 2024. Poultry meat is the single most important driver for this increase. The demand for eggs is also large. In 2000, the total amount of eggs (in shell) produced in the world was just over 51 million tonnes (Mt) but in 2010 it was over 64 Mt, or a 25.5% increase over ten years (FAO, 2016). If the same trend continues, then by 2020 the total egg production will reach 80.3 Mt.

A decreasing availability of arable land combined with an increasing environmental constraint for livestock production means that much of the increase in meat consumption will have to come from intensive industries. According to the Global Feed Tonnage Survey 2015, the global production of feed in 2014 reached 980 Mt, with pig and poultry feed accounting for more than 2/3 of the total. The Survey showed a 2% increase on the previous year. If this trend continues, then the total global feed production should reach approximately 1.2 billion tonnes in 2024. It is obvious that feed grain production alone will not match this demand.

Cereal grains

According to the FAO, the total coarse grain production is projected to reach 1,313 million tonnes in 2016, of which 750 million tonnes are destined for feed use. Corn accounts for 77.2% of the coarse grains, i.e., reaching a total production volume of 1,014 million tonnes in 2016. However, an important complicating factor for the availability of feed grains in the future is the biofuel mandate of various jurisdictions around the world. For instance, ethanol and biodiesel volumes are predicted to reach 134.5 and 39 billion litres, respectively, by 2024. Currently, less than 4% coming from feedstock other than food crops (EU Biofuels Technology Platform, 2016). Due to lack of investment in R&D, and hence any significant breakthroughs in the use of non-grain feedstock, a large proportion of coarse grains will be used in biofuel generation. The production of 134.5 billion litres of ethanol is equivalent to a net use of 361.8 Mt of corn (1 L of ethanol production requires approximately 2.69kg corn, Pimentel, 2001). Thus, it is reasonable to speculate that biofuel production will continue to complete directly with feed use of coarse grains, forcing the feed industry to exploit other means to meet its growing demand.

Oilseed meals and cakes

The main oilseed crops include soybean, rapeseed/canola, sunflower, palm kernel, copra, linseed pea-

nut and sesame seed. After the oil is extracted, the remaining residue is used as a feed ingredient. The world oilseed meal/cake production was 263 Mt in 2013 and is projected to reach 297 Mt in 2020 (FAO, 2016). Oilseed meals make up 20-25% of a poultry diet. Inclusion levels vary among formulations for different species and for the same species in different regions.

Summary

Feed grain supply faces a number of challenges. Chief among these is the increased production of biofuel from grain feedstock as many countries mandate biofuel blending in transport fuels. What will make up the gap in feed grain supply? Three strategies come to mind. Firstly, a move to increase feed grain production. Despite a capacity to increase production in some parts of the world, this strategy is completely unpredictable due to geopolitics, availability of arable land and climate variability. This strategy will not be discussed. Secondly, a strategy to boost productivity increase through the use of biotechnology, applied to both feed and animals. This strategy will involve the use of feed additives to reduce the cost of production and application of genetic selection to enhance feed efficiency. In this paper, only the feed additives part will be covered. Thirdly, a strategy to explore novel sources of feed. This strategy will examine the use of ingredients that are not traditionally used in animal feed. For example, worms, insects, fly larvae, aquatic plants (algae, seaweeds and duckweed) and microbial proteins.

Exploration of novel feed resources

Introduction

Research has been very active in exploring novel feed and food resources to achieve a sustainable balance between food security and environmental protection. Opportunities and threats of novel sources of feed ingredients such aquatic plants (algae, seaweeds, duckweed), insect meals and microbial proteins were analysed by van der Poel et al. (2013). The potential for some of these ingredients, for example, algae, is massive.

Algae

Algae refer to a very diverse species of organisms ranging from single cell forms, such as Chlorella, to massive multicellular forms like the giant kelp, which is a large brown alga that grows up to 50 meters in length. According to Guiry (2012), estimates for the number of algae vary from 30,000 to 1,000, 000 species. Of great interest to academia and industry is the potential of using microalgae as anything from a source of wonder foods to feedstock for biofuels; from medicine to animal feed. Indeed, more than 15,000 novel compounds originating from algal biomass have been chemically determined (Cardozo et al., 2007). The compounds include not just macro nutrients such as carbohydrates, proteins and lipids, but also carotenoids, antioxidants, enzymes, peptides, toxins and sterols. In recent years, a great deal of research has occurred in the field of biofuel production. It is also an active area for the animal nutrition industry where microalgae have been trialled for animal feed (Yaakob et al., 2014). Further exploration of the use of algae as poultry feed remains an important area of research and application in the future as they represent a resource currently untapped.

Insect protein

Humans have consumed insects since pre-history (Bodenheimer, 1951). In fact, there is a recent report by the United Nations entitled "Edible insects- future prospects for food and feed security" (van Huis et al., 2013). It presents evidence that insects form part of the traditional diets of at least two billion people with more than 1,900 species of insects used as food. Józefiak and Engberg (2015) showed the nutritional composition of selected insects at different stages of growth. They are all rich in protein and lipids, and some insects contain antimicrobial peptides. For instance, the nymph meal of Turkestan cockroach (*Blatta lateralis*) contains 53.4-73.4% protein and 17.6-26.1% fat. Likewise, the black soldier fly larvae contain 41.1-45.0% protein and 15.0-35.0% fat. Since insects take up very little space, they offer a huge advantage over other intensive systems in amassing large amounts of protein in a very small area of land. Józefiak and Engberg (2015) highlighted that it is possible to produce 180kg black soldier larvae in one square meter area within 42 days. Despite all these advantages, massive regulatory barriers

exist in developed countries for the use of insect protein in food or feed.

Cassava

Cassava is not necessarily a novel feed ingredient in some countries and regions, but it is novel to use it as feed in most countries. (Morgan and Choct, 2016). The global production of cassava reached 256 Mt in 2012 (FAO, 2016) with Nigeria being the leading producer. Whilst much of the African production (45% of total) goes to food, the Asian production (23% of total) is used primarily for feed use. The remainder is produced in Latin America and the Caribbean. The fluctuation in cassava production has been large over the past ten years, but the average increase in cassava production between 2001 and 2011 was 4% per annum. This means that by 2020, world cassava production could reach 350 Mt with 80.5 Mt going to feed use. Assuming Asia's share of cassava production remains the same in 2020, and all its production goes to feed, 80.5 Mt (350 Mt x 23%) will be available for the feed industry. However, on a dry matter basis, it is equivalent to 32.2 Mt (the average dry matter content of cassava is 40%). This is still a sizeable contribution to the feed ingredient pool and understanding the nutritional attributes of cassava as a feed ingredient is therefore important.

Indeed, a great deal attention has been paid to the nutritive value of cassava for poultry in recent years. Table 1 shows the nutrient profile of three types of cassava products used in the feed industry in Asia.

In general, in broiler chickens, the apparent digestibility of cassava starch was high (97.6% to 98.6%) for all three products (Tang et al., 2012). The apparent digestibility of NSPs was around 20%, according to the same study.

Table 1 The carbohydrate contents and starch profile of cassava products

Parameters	Chips	Pellets	Pulp
Total starch(%)	75.14	67.8	37.4
Amylose(%)	17.4	18.0	11.3
Amylopectin(%)	57.8	49.8	26.1
Amylose / Amylopectin	0.29	0.36	0.43
Free sugars (up to 10 monosaccharides) (%)	1.9	2.6	1.3
Soluble NSPs (%)	8.3	8.3	2.8
Insoluble NSPs (%)	4.2	5.3	9.7

Tang et al., 2012 unpublished data

Summary

Algae and insects are two emerging sources of novel feed that are attracting a great deal of attention throughout the world. Numerous hurdles remain for both sources to be used widely as feed ingredients. For algae, good production processes exist today thanks to the interest in using them as feedstock for biofuel production, but the logistics of massive production need to be fine-tuned further so that algae can be cheap enough to be used in the livestock industries. For insect protein, on the other hand, processes for intensive production will require more investigation. In addition, despite there is a lot of interest in the use of insect protein for feed and food, there are regulatory obstacles to overcome and public acceptance to gain in developed countries before serious momentum for research and intensive production of insect meals can occur.

Utilisation

Introduction

Approximately 15-25% of feed is not digested depending on the ingredients used, the processing of the feed and the age of the animal. The undigested components consist of starch, protein, fibre (mainly non-starch polysaccharides, NSPs), lipids and minerals. Most of this undigested part of feed is fibre, which his poorly utilised in poultry. However, there are technologies such as enzymes can be deployed to increase the utilisation of these by-products.

Milling by-products of wheat and corn

There are numerous types of by-products available at large quantities. Although by-products of plant

and animal origins make up the bulk, the amount of by-products originating from the food industry by-products can also be considerable. This paper will cover by-products of cereal grain and the biofuel industry. Other important by-products, such as meat and bone meal, will not be discussed.

The food use of corn and wheat is estimated to reach 996 Mt by 2020 (FAO, 2016). The milling process for wheat and corn produces between 25 to 30% by-products. Therefore, 249-299 Mt of by-products would be available for feed use in 2020.

It is important to stress that not all cereal by-products will go to the feed industry. A significant proportion of brans and pollards is used in high fibre breakfast cereals or is fed directly to animals such as horses.

Biofuel by-products

As shown in Section 1.2, bioethanol production will reach 134.5 billion litres by 2024, requiring approximately 361.8 Mt of coarse grains. Bioethanol production yields ethanol, CO_2 and DDGS in approximately equal proportions (1/3 each). Therefore, the production of 134.5 billion litres of ethanol will generate 120.6 Mt of DDGS. DDGS is high in NSPs but is now widely used in feed formulation as it has become a reasonably consistent ingredient in terms of chemical composition. Choct and Petersen (2009) analysed 6 batches of DDGS from different ethanol plants in the US. They reported that the average nutrient values were: 29.4% crude protein, 18.2% starch, 17.7% NSPs, 4.4% free sugars and 10.4% fat.

Likewise, the global production of biodiesel is projected to reach 39 billion litres by 2024 (FAO, 2016). This will consume 13% of all vegetable oils produced in the world by 2024. The by-product from the process is glycerol. Biodiesel:glycerol is approximately 10:1, thus 39 billion litres of biodiesel will yield 3.9 billion litres of glycerol. Glycerol, which is often referred to as glycerin in the feed industry, is highly digestible in chickens with an ME value of 18 MJ/kg (4300kcal/kg) (Kerr et al., 2009).

The use of fibre as an energy source

Cereal grains and vegetable protein sources contain between 10-75% of NSPs. NSPs in cereals form part of the cell wall structure and in vegetable proteins, such as legumes, may also play a role as an energy storage material. As far as monogastric animal nutrition is concerned, NSPs are either poorly digested or anti-nutritive. This is because (a) the soluble proportion of NSPs elicits anti-nutritive activities, and (b) the utilisation of insoluble NSPs by non-ruminant animals is very poor to zero. Most by-products and alternative ingredients are very high in NSPs. However, as largely characterised chemical entities, NSPs present exciting opportunities. It may be possible in the future to degrade at least some of these polysaccharides into their constituent sugars, which can be utilised either as a source of dietary energy or feedstock for biofuels. Table 2 shows the NSP contents of selected ingredients.

Table 2 The levels of NSPs present in cereals and cereal by−products (% dry matter)

	Wheat	Barley	Rye	Oats	Triticale	Sorghum	Corn	Rice*
Soluble	2.4	4.5	4.6	3.8	1.7	0.2	0.1	0.3
Insoluble	9.0	12.2	8.6	24.5	14.6	4.6	8.0	0.5
		Wheat pollard		Wheat bran			Rice bran	
Soluble		1.7		3.2			0.5	
Insoluble		33.6		38.4			21.3	

Choct, 2010.

The two important vegetable protein sources, soybean meal and canola meal, contain 21.7% (Bach Knudsen, 1997) and 19.8% (Kocher et al. 2000) total NSPs, respectively. Other by-products such as copra meal (53% NSPs), palm kernel cake (75% NSPs; Düsterhöft et al., 1991), and sunflower meal (55% NSPs; Düsterhöft et al., 1991) are very high in NSPs. The digestibility of NSPs varies enormously depending on the animal species. Bach Knudsen and Hansen (1991) reported that the digestibility of pea NSPs was 28% in chickens and 84% in pigs. The chemical structure of NSPs plays a major role in determining their digestibility. For example, in pigs ß-glucans were 64-75% digested in the ileum whereas cellulose, arabinoxylans and uronic acids were totally undigested, illustrating the importance of solubility and structure in determining NSP digestibility. Furthermore, the digestibility of NSPs is affected by

age of the animal, in general, with increasing digestibility as the animal gets older.

Since apparent digestibility measures the disappearance, rather than utilisation, of a nutrient from a section of the gut or the entire GI tract, it is difficult to know the respective proportions of NSPs that are degraded to monomers and reduced to low-molecular weight carbohydrates. But it is well known that carbohydrases degrade NSPs to yield some monomers as well as low-molecular weight carbohydrates. Indeed, the enzyme technology, for instance, has been applied in practice for more than two decades with outstanding results. However, the potential for the technology is far from exhausted. In fact, even enzymes, such as carbohydrases, which have been studied extensively, have a long way to go when it comes to increasing the utilization of insoluble NSPs as energy sources (Bedford and Partridge, 2010).

Currently, most NSP-degrading enzymes are used to deal with the anti-nutritive effects of soluble NSPs, rather than depolymersing insoluble NSPs, such as cellulose and mannans, to produce significant amounts of low-molecular weight carbohydrates for utilisation as energy or as a source of prebiotics. Furthermore, when considering the use of the end products of enzyme degradation, two key technical aspects need to be borne in mind: (a) the utilisation of low-molecular weight carbohydrates as energy can only occur through fermentation and the capacity of poultry to attain dietary energy via fermentation is very limited (Svihus et al., 2013), and (b) two of the most abundant sugars present in feed NSPs, arabinose and xylose, are poorly utilised in poultry and pigs with as low as a 5% inclusion of these sugars seeming to have adverse outcomes (Schutte, 1991). Thus, a total depolymerisation of arabinoxylans is perhaps not desirable for poultry.

All in all, it is reasonable to assume that a productive use of 20% of feed NSPs could occur by 2020. This means, more cereal grains will be spared, adding to the pool of available ingredients.

Summary

Approximately 300 Mt of milling by-products of wheat and corn may potentially be available although a large proportion of wheat by-products are used by the food industry as high fibre breakfast cereals. On the other hand, 120.6 Mt of DDGS and 3.9 billion litres of glycerin will be available to use in the feed industry.

Although cereal by-products and DDGS contain a high level of NSPs, the potential for efficiency gain through the use of additives and processing technologies is appreciate in the future as the enzyme technology develops further.

Conclusions

There is no doubt that the gap between demand and availability of traditional feed resources will widen. This is due primarily to population growth and climate change. Competition for food, fuel and feed will also make traditional raw materials more expensive. However, there are near ready solutions that will fill the gap. On one hand, many by-products are currently under-utilisation in terms of nutrient digestibility, which will change as more advanced technologies emerge. On the other hand, through global effort in frontier research, industrial development and good policy settings, the production and utilisation of novel ingredients will become reality.

Acknowledgments

Part of this paper was presented at the "Poultry Beyond 2020" conference held in Queenstown, New Zealand in 2014.

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L24 Challenges to starch digestion in poultry

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Keywords: amylase, broiler, enzymes

Summary

Starch is quantitatively the most important nutrient in poultry diets, and will to a large extent be present as intact starch granules due to very limited extent of gelatinization during pelleting. Despite the fact that native starch is difficult to digest due to a semi-crystalline structure, even fast-growing broiler chickens appears to be able to digest this starch more or less completely during passage through the jejunum. However, a reduced starch digestibility has been observed, particularly in pelleted diets containing large quantities of wheat. Although properties of the starch granule such as size and components on the granule surface may affect digestibility, the entrapment of starch granules in cell walls and a protein matrix may be even more important factors impeding starch digestion. In that case, this and the fact that amylase secretion is normally very high in poultry may explain the lack of convincing effects of exogenous α-amylase added to the diet. However, few well-designed experiments assessing mechanisms of starch digestion and the effect of α-amylase supplementation have been carried out, and thus more research is needed in this important area.

Introduction

This paper is based on a recent review (Svihus, 2014). Full paper and a reference list can be found there.

Among the nutrients in poultry diets, starch is quantitatively the most important. Diets may contain up to 50 % starch on a dry matter basis, and starch is the most important source of energy. It is also well known that the capacity for digesting fat is limited, at least in young birds (Wiseman *et al.*, 1998), and thus starch must be the main energy source in the diets. The main source of starch is seeds from plants in the grass family, where starch is stored and used as an energy source by the offspring during germination. Although this source in the form of maize, wheat and other cereals are dominating, starch from tubers such as cassava may also be used in poultry diets.

Digestibility of starch

Since starch gelatinization is limited in the pelleting process, starch in poultry diets is to a large extent present in the form of native starch granules, and keeping in mind the complex digestion process as will be discussed, a low starch digestibility, at least for fast-growing broiler chickens which consumes large quantities of starch, would be expected. However, a very high starch digestibility is commonly observed even in young broiler chickens. A number of studies have shown that chicks are rapidly adapting to starch digestion when fed at hatch, as indicated by high activity levels of disaccharidase (Mahagna and Nir, 1996) and α-amylase (Sklan and Noy, 2000) two days after hatch. Accordingly, Zelenka and Ceresnakova (2005) found that the total tract starch digestibility coefficient already exceeded 0.96 at three days of age for broiler chickens. The same authors also found that starch digestibility decreased ($P < 0.01$) linearly with increasing age in fast-growing broiler chickens but not in slow-growing layer chickens. Thomas *et al.* (2008) observed that starch digestibility in broiler chickens dropped from five to seven days of age, but was restored to a normal high level at 14 days of age. Even when measured on material collected from the ileum of broiler chickens, starch digestibility has often been observed to be above 0.95 even for pelleted diets (Svihus, 2001; Hetland *et al.*, 2002; 2003; Svihus *et al.*, 2004; Hetland *et al.*, 2007). This high capacity of poultry for digesting starch is truly impressive, not the least in broiler chickens, where pelleted diets and a high appetite results in material passing through the digestive tract in less than 5 hours (Svihus *et al.*, 2010; Svihus *et al*., 2002). This means that in less than five

hours, the starch granules must be released from the surrounding protein and cell walls, become completely moistened followed by complete degradation by a cascade of amylases, and finally the resulting glucose must be absorbed. This is particularly impressive since *in vitro* studies have shown that intact normal starch granules after a pretreatment imitating the pre-intestinal human digestion process are incompletely digested even after four hours under conditions resembling the small intestine (Shrestha *et al.*, 2012). The capacity of broiler chickens to digest even unprocessed starchy ingredients is further illustrated by the fact that even when whole untreated cereals have been used in large quantities, starch digestibility has been observed to be very high. Svihus *et al.* (1997) found ileal starch digestibility to be 0.98 in a mash diet containing 70 % untreated whole barley as the only cereal source, and Svihus and Hetland (2001) found that 50 % of the birds exhibited an ileal starch digestibility above 0.94, even when given a pelleted diet with 38.5 % whole wheat. Even more astonishing were the results by Hetland *et al.* (2002), who observed an ileal starch digestibility of 0.98 in diets with 44 % whole wheat mixed with pelleted other ingredients. The starch digestion process in such cases cannot commence before grinding in the gizzard, and since digestibility was based on analyses of contents collected from the ileal segment between Meckel's diverticulum to the ileo-cecal junction, this means that the whole starch digestion and glucose absorption process has taken place during the short retention time in the duodenum and jejunum, estimated by Rougiere and Carre (2010) to be around one hour.

Despite the above indications of high starch digestion capacity, starch digestibility values below 0.9 (measured either on ileal and/or total tract level) have also been seen in a large number of experiments (Wiseman et al., 2000; Maisonnier *et al*., 2001; Marron *et al.*, 2001; Svihus, 2001; Svihus and Hetland, 2001; Weurding *et al.*, 2001; Carré *et al*., 2002; Hetland *et al.*, 2002; Carré *et al*., 2005; Zimonja and Svihus, 2009). In several of the reports, a considerable variation among cereal species, among varieties within species and between individual birds, has been observed. This indicates that factors intrinsic to cereals and birds alike are affecting starch digestibility.

Factors Impeding Starch Digestibility

It is clear that properties of the starch ingested will affect digestibility, because numerous experiments have reported that glucose response and digestibility of starch vary with starch source (Svihus *et al.*, 2005). However, there is still a lack of knowledge on the exact causes for these variations in starch digestibility. Factors such as the ratio between amylose and amylopectin, granule size and content and properties of proteins, lipids and phosphates on the surface of starch granules were identified as potential causes for low starch digestibility (Svihus *et al.,* 2005; Singh *et al.*, 2010; Tester *et al.*, 2006). Although the complexity of this issue and the lack of experimental data preclude firm conclusions, these reviews indicate that a small granule size may explain the high digestibility of starch from oats and rice, and that a large gluten matrix may contribute to low digestibility of starch from wheat.

As pointed out before (Svihus, 2011), wheat appears to be predominant in papers reporting low starch digestibility. Also, wheat has been shown to result in consistently lower starch digestibility when used at a high inclusion rate and when being compared with other cereals such as barley and oats (Svihus 2001; Zimonja and Svihus 2009). Addition of xylanase has sometimes been shown to improve starch digestibility of wheat diets (See Svihus (2011) for an overview of literature), indicating that fibers may affect starch digestibility. Although this is correlated with reduced viscosity (Murphy et al. 2009), the rather moderate correlation coefficient indicates that other effects of enzyme addition are also contributing. It is possible that accessibility of the starch in the wheat endosperm is an issue, and that one potential beneficial effect of enzyme addition is that enzymes degrade cell walls and thus increase access to starch and other nutrients in the endosperm cells, as discussed by Murphy *et al.* (2009). This hypothesis is supported by results by Amerah *et al.* (2009), where xylanase addition improved metabolizable energy content in hard wheat, but not in soft wheat. Carré *et al.* (2005) found that low starch digestibility was associated with hardness of wheat, and investigated this further to elucidate causes for low starch digestibility in hard wheat varieties. On the basis of particle size analysis and microscopy of ileal contents, they found that a large part of the undigested starch was entrapped in cell wall material, particularly from areas of the endosperm close to the aleurone layer (Péron *et al.* 2007). The same authors did not find a large number of particles in the size class of starch granules in the ileum, which indicated that the low digestibility observed with hard wheat was not caused by structural arrangements of the starch granules. In another

experiment, it was shown that very fine grinding corrected the very low starch digestibility observed with a normal particle size distribution (Péron *et al.* 2005). As stated by Carré *et al.* (2007), this supports a conclusion that the cause for a low digestibility of starch in wheat diets is partly that starch granules are entrapped in cell walls and/or protein matrix.

It is possible that for broilers, the short time available for digestion may be one of the causes for impaired starch digestion under some circumstances. Results have shown that digestibility of a diet with a low digestibility increases when feed intake is reduced by changing diet form from pellets to mash (Svihus and Hetland 2001), and this indicates that feed intake may be inversely correlated with starch digestibility. Several studies have shown a significant negative correlation between feed intake for individual birds on identical diets and starch digestibility or apparent metabolizable energy (AME) value (Svihus, 2006; Svihus, 2011). In data from one of these experiments, AME and total tract starch digestibility for individual birds were very strongly correlated (r = 0.984), and starch digestibility was inversely related to feed intake (Svihus, 2011). In these data, 4 out of 10 *ad libitum*-fed birds on a finely ground pelleted wheat diet showed signs of being feed over-consumers, characterized by a normal weight gain, a higher than average feed intake and an AME value < 2462 kcal/kg (Svihus et al. 2010). The hypothesis that feed over-consumption leads to an overly fast feed passage that results in poor starch digestibility, is consistent with observations by Hughes (2008), who showed that AME of broiler chickens increased with increasing transit time.

Effect of Supplemental α−Amylase

Published work assessing the extent to which exogenous α-amylase may improve starch digestibility is scarce. Mahagna *et al*. (1995), Shapiro and Nir (1995) and Ritz *et al.* (1995) did not observe any improvement in starch digestibility when α-amylase was supplemented to broiler chickens during the first 14 days of age, and Moran (1982) stated that "unlike most mammals, the ability of fowl to release sufficient amylase is never a problem". This corresponds with observations by Svihus and Hetland (2001), where digestibility was not improved by adding pancreatin to the water of broiler chickens exhibiting low starch digestibility of a wheat diet. Conversely, Gracia *et al.* (2003) observed a significant increase in starch digestibility when α-amylase was added to a maize-based diet, thus indicating that α-amylase secretion may be a limiting factor. Jiang *et al.* (2008) added increasing levels of α-amylase to broiler chicken diets and observed an increase in weight gain at the highest supplementation level. Interestingly, endogenous α-amylase production seemed to be reduced at this high level. Corroborating this, other experiments where amylase has been included in the enzyme cocktail have shown improved nutrient utilization (Cowieson and Ravindran, 2008; Cowieson *et al.,* 2006; Olukosi *et al.*, 2008), although the fact that other enzymes were used together with amylase results in that these latter reports are of limited value in this context. Interestingly, Hughes *et al.* (1994) separated chicks from two breeds based on genetic variants of the pancreatic α-amylase followed by a growth trial, and concluded that one of the α-amylase genotypes from one of the breeds resulted in higher feed/gain, thus indicating that birds of certain α-amylase genotypes may cause suboptimal starch digestibility. If an impaired starch digestibility is dependent both on specific properties of the diet such as cereal type and inclusion level, and on bird-related factors such as appetite and digestive tract development, this may explain the conflicting and inconclusive results. However, the very high starch digestibility observed even under the most demanding conditions indicate that broiler chickens have the capacity to digest starch completely, and thus that Moran's (1982) statement that the bird releases sufficient amylase in most cases may still be true.

The very few reports published on addition of α-amylase to poultry diets warrants further research into this very important area of poultry nutrition. Experiments should be carried out with pure α-amylases tested with pelleted diets based on both wheat and maize, and in different bird phases as we have seen that feed intake can play an important role. Detailed assessment of starch digestibility throughout the small intestine should be also carried out. Furthermore, experiments are warranted which addresses the causes of the effective starch digestion in broiler chickens, including a comparative effectiveness of intestinal chyme from broiler chickens compared to other animal species in releasing glucose from starch.

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L25 The effect of monochromatic photostimulation on growth and reproduction of broiler birds

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Abstract

Source, spectra, intensity and regimen of light supplementation became major factors in modern poultry management, enabling us today to use targeted illumination in order to increase productivity. Broilers in-ovo or post hatch photostimulated with green light (GL) were heavier from dark incubated birds. Furthermore, we defined the cellular and molecular events associated with the effect of in ovo and post hatch GL photostimulation on muscle growth. We found that GL photostimulation have a stimulatory effect on proliferation and differentiation of satellite cells and a promoting effect on the uniformity of the muscle fibers in the early post-hatch period. We gathered some evidence to support these findings; in ovo GL photostimulation increased plasma growth hormone (GH) and prolactin (PRL) levels, as well as hypothalamic growth hormone releasing hormone (GHRH), and both the liver growth hormone receptors (GHR) and insulin-like growth factor-1 (IGF-1) gene expression. Both retinal and extra-retinal photoreceptors are active during embryogenesis and can be first detected at E14. We found that In-ovo green light photostimulated chicks have significantly higher retinal and hypothalamic green opsin mRNA gene expression levels compare to control. In contrast in ovo blue light BL photostimulated chicks have significantly lower retinal and hypothalamic green opsin mRNA gene expression levels compare to dark control group. Many avian species are photoperiodic and respond to long photoperiods with an activation of reproductive axis. Photoreceptors were suggested to be involved in the detection of daily or seasonal changes in photoperiod. Photoreceptors locates in the eyes (retinal photoreceptors), pineal gland and hypothalamus (extraretinal photoreceptors). In birds subjected to a gonad stimulating photoperiod, long wave radiation (630-780 nm) penetrates the tissue and directly acts on hypothalamic extraretinal photoreceptors to stimulate reproductive function. In contrast to the stimulatory effect of long wave radiation on reproductive activity, activation of retinal photoreceptors by visible radiation by the green-yellow bands of the light spectrum (545-575 nm), appears to be inhibitory to reproduction. In a study conducted on female turkey and broiler breeders we found that extraretinal photostimulation combined with nonphotostimulatory conditions to the retina caused a significant elevation in reproductive activity manifested by increase mRNA expression of hypothalamic GnRH-I pituitary LH and FSH, plasma LH and gonadal steroids, and increase in egg production.

The role of monochromatic light on growth of meat type birds

The effect of post hatch monochromatic photostimulation on growth and development of broilers

Broilers raised under blue or green fluorescent lamps gained significantly more weight than birds reared under red or white light, whereas feed conversion and mortality were not affected[1]. There are, however, conflicting reports regarding the effect of monochromatic light on birds' growth. Whereas green light (GL) was found to stimulate growth[1-4], mature female Japanese quail had lower body weight (BW) when reared under GL and blue light (BL) as compared to those reared under red (RL) or white light (WL)[5]. Turkeys grew better under BL until 16 wk of age but by 24 wk those reared under RL and WL were heavier[6]. Pink light depressed chicken hatching weights with reversal by WL at 4 wk of age[7]. The possible causes of contradictory findings were probably due to variability in light sources[8], methods of measuring light doses[9], species, breed, gender and age of experimental birds[3-5].

In order to eliminate some of these controversies, a light emitting diode (LED) was used for broiler photostimulation. LED is a pure monochromatic light source. Early studies conducted in our laboratory[10].

on broiler post hatch chicks have shown acceleration in growth rate in birds photostimulated by green and blue light during incubation, this acceleration in growth rate was also associated with increase in breast muscle weight. Green light enhanced growth rate significantly more than white and red light three days prior to hatch. Growth was also enhanced by blue light, but the onset of this effect was at an older age. These results were in agreement with Wabeck and Skoglund (1974), who demonstrated it in broilers, and Phogat et al., (1985) who found it in quails[11].

Chicks reared under green and blue light, by five days of age, had 2-2.4 times as much breast muscle satellite cells compared to white and red photostimulated chicks. Since satellite cells are members of myoblast family, any rise in their level may indicate stimulation of muscle growth, and a possible mechanism explaining the elevation in BW associated with green and blue monochromatic photostimulation [12].

The effect of in ovo monochromatic photostimulation on growth and development of broilers

In commercial hatcheries, broiler eggs are set in the dark; however, several studies have shown that photostimulation accelerates embryonic development. In first study we investigated the effect of monochromatic green light on egg core temperatures. Photostimulation of broiler eggs with monochromatic green light caused a significant, time-dependent elevation in yolk temperature. Setting the eggs under an intermittent light regimen (15 min on, 15 min off) eliminated the rise in egg yolk temperature, and as a result, prevented early hatch associated with in ovo photostimulation [13].

Green light photostimulation enhance embryonic BW, calculated as percentage of egg weight, and % pectoralis muscle weight. The positive effect of light stimulation on muscle growth preceded its effect on BW in embryos and was evident on almost all days during incubation, suggesting a specific effect of green light on muscle growth [14].

At hatch (d 0), BW of the chicks did not significantly differ between the 2 in ovo treated groups. However, during the first week post-hatch, BW of chicks that were in ovo photostimulated with green light was significantly higher than that of those hatched in the dark. The pectoralis muscle weight as percentage of BW of the chicks that hatched under green light was significantly higher than that of the chicks that hatched in the dark, both on hatching day and at 6 d of age.

What are the cellular and molecular basis underlying skeletal muscle developments in embryos and post hatch chicks in response to in ovo green-light illumination?

We found evidence for the mechanism behind the elevation in body weight and muscle growth associated to in ovo green light photostimulation[13].

1.The number of satellite cells per gram of muscle was higher in the green group compare to dark group [14].

2.A stimulatory effect of in ovo green light illumination on muscle cell proliferation was further observed by immunohistochemical staining for PCNA in muscle sections [14].

3.Expression Levels of Pax7 and myogenin at early post hatch period are elevated by in ovo green lighting. Our earlier findings that the percentage of pectoralis muscle of total body weight in in ovo illuminated chicks is significantly higher than that in chicks incubated in the dark at all post hatch days analyzed (through *day 42*; 32), together with higher Pax7 levels (i.e., higher reservoir of quiescent/proliferative satellite cells), imply an enhanced hypertrophy potential of muscle because of in ovo green-light illumination.

4.We have shown a higher expression of growth hormone (GH) receptor mRNA in satellite cells derived from green light illuminated chicks[12].

Photoreception and stimulation of muscle growth in broilers

It is possible that the monochromatic green light penetrates the eggshell and has a direct effect on muscle in the embryo. Although short wavelengths are more likely to be effective at the dermal level, blue light has been found to penetrate the abdominal wall of rats to a depth 2 mm[15]. However, we were unable to detect any proliferative effect of monochromatic green light on cultured myoblasts derived from standard (no illuminated) *E17* embryos and 3-day-old chicks (Halevy O, Piestun Y, and Rozenboim I, unpublished data). A more likely explanation is that green light indirectly affects myoblast proliferation via the endocrine system; the latter receives photic cues from the retinal or extra retinal photoreceptors.

Recently we studied the expression of retinal and hypothalamic green opsin in related to in ovo photostimulation with green, red and blue lights (Rozenboim unpublished data). Findings yielded from this study, shows that *in ovo* green light photostimulated chicks have significantly higher retinal and hypothalamic green opsin mRNA gene expression levels compare to control. In ovo blue light photostimulated chicks have significantly lower retinal and hypothalamic green opsin mRNA gene expression levels compare to control group. Post hatch green and blue light photostimulated elevated retinal and hypothalamic green opsin mRNA gene expression levels compare to control group.

Brain photoreceptors were not detected during late embryogenesis, suggesting that the enhancement of growth and muscle development in meat type birds might be governed by retinal photostimulation. Thorough and extended research is required in order to reveal the molecular mechanism by which the light affects the chicken's genome, and the indirect way it influences its reproductive performances.

The role of monochromatic photostimulation of reproduction

Many avian species are photoperiodic and respond to long photoperiods by activating the reproductive axis. The neuroendocrine response to photostimulation is reflected by a significant release of GnRH-I [16.] followed by pituitary secretion of gonadotropins [17-19], resulting in gonad recrudescence [20].

Photoreceptors have been suggested to be involved in the detection of daily or seasonal changes of photoperiod [21]. It has been demonstrated that domestic fowl subjected to a gonad-stimulating photoperiod responded to the longer wavelengths of the spectrum [22]. The sensitivity of the bird to long-wave radiation (630-780 nm) is a result of deep tissue penetration (hypothalamic extraretinal photoreceptors) stimulating the reproductive axis [23,24]. In contrast, activation of retinal photoreceptors appears to be inhibitory to reproduction [25-27]. The response to visible radiation is probably mediated by the green-yellow bands of the light spectrum (545-575 nm), where the avian retina is in relative peak sensitivity [18,28,29]. We study the effect of retinal photostimulation by green light (14 h) while maintaining the extra-retinal photoreceptors in a nonphotostimulatory condition by red light (6 h) and the effect of extra-retinal photostimulation by red light (14 h) while maintaining the retinal photoreceptors in a nonphotostimulatory condition by green light (6 h) on reproduction of female turkeys and broiler breeders. Our results indicate that extra-retinal photostimulation combined with nonphotostimulatory conditions to the retina caused a significant elevation in reproductive activity of turkey and broiler breeder hens. This elevation was manifested by elevation in mRNA gene expression of hypothalamic GnRH-I, pituitary LH and FSH, reduction in hypothalamic VIP and pituitary prolactin mRNA gene expression, elevation in plasma LH and gonadal steroids, and significantly increase in egg production.

We investigated the mechanism behind the debilitating effect of retinal photostimulation with green illumination, by studying the involvement of serotonin and vasoactive intestinal peptide. We found that blocking the serotonergic axis by parachlorophenylalanine (PCPA) improved reproductive activities to the control illuminated group, however, active immunization of VIP did not improve reproduction. Collectively, the results suggest that retinal photostimulation inhibits the reproductive axis through serotonin and not through vasoactive intestinal peptide[30].

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L26 Improving geese out−of−season breeding performances by integration of environment control zootechnics

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Keywords: goose, out-of-season breeding, photo-program, environment control

Summary

In order to increase the economic efficiencies of goose production, and to reduce seasonal fluctuations so to support the sustainable development of the industry, out-of-season breeding techniques that fundamentally relied on alternating geese breeding season by using artificial photoperiod have been developed and extended in China. For both short and long breeding geese, specific photo-programs have been used to induce sound breeding activities during the summer non-breeding season. For geese to fully manifest production performances, control of stocking density, environment and biological control measures must also be adopted in order to eliminate stress factors, and to create a high standard bio-security low in bacteria and endotoxin loads in geese living environment. Integrations of proper design and ventilation of the geese house, separation of geese from contamination from feces by use of slated floor, use of probiotic microbes to reduce Gram-negative bacteria excretion and growth, have enhanced geese health status and production performances to new levels.

Introduction

China boasts the world's largest goose industry with an annual output of greater than over 600 million geese that accounts for greater than 90% of the world's total production (Shen et al., 2011). In recent years, caused by the high economic returns brought about by the out-of-season geese production (Sun et al., 2007), and also by weakened chicken and duck consumption markets due to the economy down turn, poultry farmers have increasingly invested into goose production. These have lead to further expansion of goose industry production as seen by increased stocking levels of both breeding geese and growing flocks (Sun et al., 2007, Shen et al., 2011), and also by establishment of start-up goose farms. Accompanied with such intensification process of goose industry saw the frequent outbreaks of diseases, such as *avian influenza, paramyxovirus* disease, Goose *parvovirus infections, riemerella anatipestifer infections,* and most common the *colibacillosis* (Liu et al., 2011; Zhang, 2012). Many of the diseases derive from poor farm environment, and it is also common that mixed infections of more than one type of pathogens the diseases were caused by (Liu et al., 2011). Not only the diseases of pathogens affect or endanger goose health and cause considerable mortality, but also severely reduce geese production performances, both the reproductive performances in the breeders, and the growth performances in the goslings (Shi et al., 2011). For the healthy development of the Chinese goose industry and to improve economic efficiency of goose farming, a series of studies have been conducted in the past several years in order to identify mechanisms that environment quality affect goose health and production efficiency, and also tried to integrate environment management techniques so to provide geese with clean and comfortable living environment.

Photo−programs for inducing geese out−of−season breeding

Goose production in China is based on predominantly the native geese breeds, with the a few breeds imported from Eurpoean countries taking a small portion. There are approximately 30 indigenous breeds nationwide, which can be classified into short and long day breeding types (Shi et al., 2008). The short day breeding geese are represented by the Magang goose in southern province of Guangdong, while the

long day breeding type represented by Yangzhou geese in Jiangsu Province. Though these breeds differ in the photoperiodic and endocrine mechanisms entraining their reproductive seasonality, their non-breeding season basically occurs in the summer months. Therefore, to achieve out-of-season breeding in short day breeding geese, a "long and short" photoperiod program is implemented, which consists of a period of 75 days of long photoperiod (18 h) starting from winter times (December to January) to stop egg laying, and the subsequent period of 11 h short photoperiod to induce out-of-season breeding. Under such photo-program, good laying performances and egg fertility can be achieved during the traditional summer non-breeding season (Sun et al., 2007; Huang et al., 2008).

For the long day breeding Yangzhou geese and the exotic Hungarian Hortobagy breed, a specific photo-program is also used that inhibits reproductive activities during spring time, but stimulates egg laying in summer months. It is a three-phase, consisting of an initial 3 to 4-week first phase of long photoperiod (18 h) starting in winter (early January), an 8-week second phase of short photoperiod (8 h), and the third phase of 10 to 11 h daily photoperiod (Shi et al., 2015). The first 2 phases function to inhibit reproductive activities of the geese, and to set them sensitive to long or increases in daily photoperiod, so to initiate out-of-season breeding during late spring and summer months. If photo-intensity is also controlled not to exceed 100 Lux in the third phase, which can only be achieved with using artificial lighting and by confining geese entirely in the house, excellent reproductive performance of high laying rate greater than 45%, a long peak production lasting for as long as 4 months, and a total number of over 80 eggs laid in one season can be achieved (Shi ZD, unpublished data).

Improving water bio−safety in "goose−fish" integrative system

Potential bio-risks in "goose-fish" integrative system

The great majority of goose production in south of Yangtze river in China is in the form of integrated "goose-fish" production. This system has long been established as an ecological and economical animal production model, that waterfowl waste can be fully utilized by fish to produce valuable high quality protein for human consumption (Chen 1992). In this integrated system, fish ponds also serve as the important site for geese activities, such as mating and preening, besides providing drinking water for the geese. Since the advent and extension of out-of season laying technique, geese breeding has increased significantly the economic earnings, which helped the farmers to further expand flock sizes (Sun et al. 2007). This in most cases lead to increases in stocking rates as the farm or fish pond sizes are not easily expanded for small holder goose farms. Reductions in egg fertility, and especially egg hatchability from the norm of greater than 80% to 60% and even lower soon followed in, together with reductions of growth speed and elevated mortalities of the newly hatched goslings (Jiang et al. 2009). In severe cases, *E. coli*. and *Salmonella* infections in geese reproductive tract often caused atresia of ovarian follicles leading to vitelline peritonitis and death of geese (Wen 2004). It was found that as stocking density increased, so did excretion of goose feces into the water, as well as the Gram-negative enterobacteria such as *E.coli, Salmonella* and *Shigella*, et al. These bacteria thrived in pond water on rich supply of nitrogen and phosphorus nutrients of fecal origin, especially during the summer month when water temperature was high enough to support active bacteria proliferation. LPS released after death of these Gram negative bacteria is absorbed through goose gut and enters into blood circulation, severely impairs wide ranges of physiological activities, damages reproductive organ tissue integrities (Subedi et al., 2007; Ozoe et al., 2009) that leads to reduced reproductive performances (Jiang et al., 2011). Furthermore, as a lipid soluble substance, LPS is also readily deposited into the egg yolk and albumen during ovarian follicle development and egg formation. During incubation, especially at the later stage of embryo development when the yolk starts to be utilized, LPS is mobilized which may kill goose embryo leading to reduced hatchability. The yolk LPS, after goslings are hatched, also impairs gosling early growth and health that leads to increased mortalilty or higher susceptibility to pathogen infections resulting in diseases.

Reduction of bacteria and LPS pollutions to improve breeding performance

Based on the above findings, the primary task of improving goose health and production performance is to maintain hygiene, or to suppress accumulation of Gram-negative enterobacteria and LPS in their living environment. Apart from maintaining proper flock size or stocking density, and using fresh water

sources whenever possible, probiotic microbes are used that can competitively reduce Gram-negative bacteria growth and LPS pollution in the environment (Jiang et al., 2011). Probiotic microbes such as *B. subtilis, Lactobacillus, Bifidobacterium, Saccharomycetes,* and *enterococcus faecalis*, et al., individually or synergistically function to inhibit growth of Gram-negative bacteria growth by one or more of four actions, namely, the creation of a restrictive physiological environment, the competition for bacterial receptor sites, the secretion of antimicrobial compounds, and/or the competition for essential nutrients (La Ragione et al., 2001). Therefore, in research and practical production, inclusion of *B. subtilis* spore products into goose feed can reduce gut growth and fecal excretion Gram-negative bacteria such as *E.coli, Salmonella* and *Shigella*. Further treatment of pond water with photosynthetic bacteria (also a mixture of several probiotic bacteria), which absorbs nitrogen and phosphorus nutrients for growth, functioned to deprive Gram-negative bacteria of essential nutrients for growth. The combined uses of B. subtilis spore products and photosynthetic bacteria maximally reduced Gram-negative bacteria proliferation in water, reduced LPS concentrations in water, geese blood circulation, and improved gosling growth rate, and also geese breeding performances by increasing egg fertility and hatchability (Yang et al., 2012).

The principle of controlling environmental bacteria and LPS pollutions had been extended to develop out-of-season breeding in the largest goose in China, the Shitou goose of Guangdong Province. Because of its large body size, Shitou goose had high level feed intake and also fecal excretions of environment exacerbating agents such as enterobacteria and their required nutrients nitrogen and phosphorus. These made Shitou goose out-of-season breeding impossible for it caused considerable environmental problems that severely reduced breeding performances, and inflicted disease outbreaks. By integration of stocking density management, i.e. maintaining 1 bird per square meter of water surface area, feed inclusion of *B. subtilis* spores and photosynthetic bacteria treatment of water, out-of-season breeding in summer months was successfully realized in Shitou geese (Yang et al., 2015). It was also clear that when bacteria and LPS pollution were well controlled, summer out-of-season breeding out-performed natural season production that lacked proper environment control measures, and also produced goslings that grow better (Yang et al., 2015). The importance of providing clean environment is critical to maintain high level production in both breeding and the subsequent progeny geese.

Improving goose production performances in confined housing system

Risks when confining geese flocks in house

In central and northern China, water sources are limited, and intensive goose production takes place in confined houses. In some operations, 24 h mechanical ventilation is required to control the in house environment. Geese performance and health can be affected by in house air quality, which in turn, is determined by stocking density, ventilation rate, humidity of the air, types of floor bedding, types of drinker and waste water drainage. Poor design and construction of the goose house, and poor ventilation, together with high stocking density, would lead to high concentrations of moisture, ammonia, fecal and airborne bacteria and virus, increase incidences of *airsacculitis*, *pneumonia* and *septicemia* caused by *E. coli*. infections, and also incidences of avian influenza and the recently emerged new disease, the Tambusu virus or flavivirus disease (Huang et al., 2011). In addition, during the raining seasons in June and November, high level moistures stimulates growth of bacteria such as *E.coli., Riemerella anatipestifer* and *Salmonella* increases incidences of geese paralyses, poor egg fertility and severe egg drop, and in severe cases vitelline peritonitis.

While in the hot summer month, the high ambient temperature causes heat stress to the geese, and inhibits appetite, which also decreases egg laying rate, egg fertility and hatchability.

Environment control measures for improving breeding performances

The fundamental prophylactic measures to safeguard breeding geese health and reproductive performances in via creating a clean and comfortable environment that reduces pathogen and toxin load, ammonia and humidity concentrations, and also provides a temperature suitable for geese physiological activities. To meet these demands, goose house must possess properties of providing clean non-toxic atmosphere, as well as maintaining comfortable thermal status. Mechanical ventilation with cooling functions keeps in house concentrations of pathogens, LPS, ammonia and humidity low, as well as alleviates heat

stress during the summer time out-of-season production. For reduction of airborne pathogen concentrations or disease risks, tunnel ventilation house should be limited not to exceed 60 meters in length, so to avoid air quality deterioration in the areas near the exhaust fans (Dai et al., 2015). Other measures to reduce pathogen concentrations and disease risks including limiting goose stocking density in the house, and taking preventive measures to reduce pathogen growth and accumulation. In house stocking density should be limited to 2 birds/m^2 for semi-open houses, and 1 birds/m^2 for completely confined houses. Besides, nipple drinkers must be fitted in a partially isolated drinking bay in house, which can drain the spilled water to outside. This reduces atmospheric moisture in the goose house, and reduces pathogen proliferation and disease risks. Installing high rise slatted floor functions to prevent geese contamination of feces, enterobacteria and viruses, which also improve in house hygienic standards.

Apart from the above stocking density and physical environment control measures, biological control techniques using probiotic microbes are other very important measures used to reduce pathogenic Gram-negative bacteria and LPS loads in the geese house. Apart from direct supplementation into the complete feed of *B. subtilis*, *lactobacillus* and *Clostridium butyricum* types probiotic microbes, pre-fermentation of feed with these probiotic microbes increased feed nutrition value and numbers of active microbes. Feeding such fermented feed to geese substantially improved gut health, reduced fecal pathogenic bacteria discharge, suppressed incidences of *E.coli* and fowl cholera, and improved consistency of egg laying, and egg fertility.

Integration of the above physical and biological control techniques will no doubt to create healthy environment that functions to improve geese welfare, health and performances.

Acknowledgements

This work was supported by the National Science Foundation of China Grant (31372314), the Jiangsu Agriculture Science and Technology Innovation Fund (CX(15)1008) , and the China Agricultural Research System Grant (CARS-46-13).

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L27 Regulation of the sexual phenotype in chickens

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In addition to the obvious differences in reproductive systems, male and female vertebrates differ in behaviour, physiology and in disease susceptibility. Male and female chickens display a number of obvious sexually dimorphic features such as comb, wattle and spurs, but they also differ in size, with broiler males larger than females at the same age and for the same nutrient intake, reflecting a sex difference in muscle mass. In chickens the number of myofibers in muscle is fixed by hatch, and so it seems that this sexual dimorphism in muscle mass is established during embryonic development.

It is known that hormones play a major role in establishing and maintaining sexually dimorphic characteristics and, until relatively recently, all extra-gonadal differences between male and female vertebrates were thought to be due to the action of gonadal hormones. However, we have shown that, in birds, the sex-specific development of many tissues is partly due to inherent cellular factors, and analysis of mixed-sex chimeric (gynandromorph) birds indicated that somatic cells have an inherent sex identity. In a series of experiments where undifferentiated cells were transplanted between early chick embryos of different sexes, and the resulting chimeric embryos allowed to develop to an advanced stage, the transplanted donor cells retained their donor sex identity. It is now widely accepted that, in vertebrates, sexually dimorphic features and behaviour are due to a combination of gonadal hormones and the direct effects of genes encoded on the sex chromosomes. We have now identified a number of markers that are ubiquitously expressed in a sexually dimorphic fashion, and have identified a likely mechanism for the increased muscle mass seen in male birds. The novel sex differences that we have identified were insensitive to sex reversal, demonstrating that these particular sexual dimorphisms are independent of the organizational and activational influences of gonadal hormones.

L28 A comprehensive introduction about small-scale poultry production systems and the current developing status from a world-wide vision

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Abstract

Poultry are domesticated avian species that are raised for eggs, meat and feathers. Poultry can include a wide range of birds from indigenous and commercial breeds of chickens to muscovy ducks, mallard ducks, pigeons, guinea fowl, geese, quail and turkeys. Chickens constitute about 90.55% of the poultry population and are, by far, the most important poultry species in all parts of the world. The term "poultry" is therefore often used synonymously for chickens. Chickens are found everywhere around the world; every culture knows them and how to husband them. Over the last decade, poultry population has grown spectacularly throughout the world: 23% in developed and 76% in developing countries, respectively. The term "Family poultry" used to describe the full variety of small-scale poultry production systems that are found in rural, urban and peri-urban areas of developing countries. The term is used to describe poultry production that is practised by individual families as a means of obtaining food security, income and gainful employment. Being called 'Family poultry', 'Small-scale poultry', 'Small-holder poultry', 'Scavenging poultry', or "Village poultry" the different systems of poultry rearing with various levels of intensification are now adopted by poor, marginal as well as richer members of the society with intensification according to their economical status and requirements. Small-scale poultry production can also be considered as less intensive or 'alternative system'. There are four broad well recognized small-scale poultry production systems. They are (1) Free-range extensive system, (2) Backyard extensive system, (3) Semi-scavenging system, and (4) Small-scale intensive system. Characteristics of these systems met 14 criteria. Small-scale poultry production is everywhere in developing countries and has economic and social significance in the rural areas. Hence, it can be used as a tool in the eradication of poverty, as a means of economic empowerment, as a way of ensuring food security for rural families, as a vehicle for demonstrating the appropriate application of science and technology to solving problems, and as a unique opportunity for technical cooperation among developing countries. Therefore, increased production of poultry, both commercial and small-scale production, is a vital contribution to food security at both the household and community levels in many countries. Small-scale poultry is thus a valuable asset to the local human population in many countries located in the tropical and sub-tropical environments.

Keywords: a compressive introduction, small-scale family poultry production systems, current developing status

Introduction

It is well-known that poultry are domesticated avian species that are raised for eggs, meat and feathers. Poultry can include a wide range of birds from indigenous and commercial breeds of chickens to muscovy ducks, mallard ducks, pigeons, guinea fowl, geese, quail and turkeys. Chickens constitute about 90.55% of the poultry population and are, by far, the most important poultry species in all parts of the world (Table 1). The term "poultry" is therefore often used synonymously for chickens (FAO, 2014). Chickens are found everywhere around the world; every culture knows them and how to husband them. Over the last decade, poultry population has grown spectacularly throughout the world: 23% in developed and 76% in developing countries, respectively (Dadheech and Vyas, 2014). They are the world's major source of eggs and are a meat source that supports a food industry in virtually every country. They are extremely useful on a worldwide basis because they offer great potential for improving the nutritional levels of all the world's peoples. They have been utilized for so many centuries that in most so-

cieties their use is ingrained (Henuk, 2015). Chickens are most widely known for their use as food (meat and egg), commercial products (feather products, vaccines etc.) and experimental animals in developed countries. However, chickens in developing countries have more diverse uses and benefits to households. The use of indigenous chickens in the majority of developing countries in the tropics varies from region to region and from community to community within a region (Dessie *et al*., 2011). Improved small-scale poultry production has been demonstrated to increase the number and percentage of school age children attending school in participating households. Poultry are also involved in human recreational activities in many parts of the world, from pigeon racing to cock fighting (Alders, 2012). A comprehensive introduction about small-scale poultry production systems and the current developing status from a world-wide vision are described in the following sections.

Table1. Distribution of poultry species by region (%)

Region	Chickens	Ducts	Geese and guinea fowl	Turkeys	Other poultry
Africa	96.03	1.10	0.85	1.21	0.81
Americas	93.95	0.45	0.01	5.58	0.00
Asia	88.07	8.99	2.70	0.10	0.14
Europe	91.30	2.65	0.89	5.03	0.13
Oceanea	96.45	1.60	0.07	1.88	0.00
World	90.55	5.53	1.67	2.09	0.15

(Source: FAO, 2014: 3).

A comprehensive introduction about small-scale poultry production systems and the current developing status from a world-wide vision

In general, for a husbandry system to be considered as less intensive or ‘alternative system’, it should be: (1) less confining-birds kept in cages should have more room to get up and lie down fully; (2) less crowded-birds in pens should be kept in smaller groups and with more floor area per bird; and (3) better able to meet the bird’s food and perching requirements. In other words, small-scale poultry production can also be considered as less intensive or ‘alternative system’ (Figure 1; Henuk, 2015). The term “Family poultry” used to describe the full variety of small-scale poultry production systems that are found in rural, urban and peri-urban areas of developing countries. The term is used to describe poultry production that is practised by individual families as a means of obtaining food security, income and gainful employment. Being called ‘Family poultry’, ‘Small-scale poultry’, ‘Small-holder poultry’, ‘Scavenging poultry’, or “Village poultry” the different systems of poultry rearing with various levels of intensification are now adopted by poor, marginal as well as richer members of the society with intensification according to their economical status and requirements. It is also well-known that the term “Poultry farming” refers to the raising of domesticated birds such as chickens, turkeys, ducts, and geese for the purpose of farming meat or eggs for food. Small-scale poultry production is everywhere in developing countries and has economic and social significance in the rural areas. Hence, it can be used as a tool in the eradication of poverty, as a means of economic empowerment, as a way of ensuring food security for rural families, as a vehicle for demonstrating the appropriate application of science and technology to solving problems, and as a unique opportunity for technical cooperation among developing countries. Therefore, increased production of poultry, both commercial and small-scale production, is a vital contribution to food security at both the household and community levels in many countries (Alders, 2012; FAO, 2014; Henuk, 2015; 2016).

In some tropical countries there are no identified ecotypes and therefore, chicken populations are simply called indigenous, local, native etc. For example, in most Arabian countries native chickens are called ‘Balady’ which means native whereas native chickens in Malaysia and Indonesia are called ‘Kampung’, which means ‘countryside’ or ‘village’ (Dessie *et al*., 2011). Indonesia with its 34 species of native breeds, for example, native chickens are often called “non-breed chickens”-“or “ayam kampung” or “ayam buras” to differenciate local chickens from commercialized chickens breeds such as widely known strains of Cobb, Hubbard, Hybro, Isa, Hyline and Hisex (Henuk, 2015; 2016). Native

chickens are historically the result of years of domestication of four wild chicken species: red wild (*Gallus gallus*); Indian grey wild (*Gallus soneratti*); green wild (*Gallus varius*); red wild chicken (*Gallus gallus*); Indian grey wild (*Gallus soneratti*); and Ceylon orange wild (*Gallus lavayetti*). The red wild, which is believed to be the progenitor of the domesticated chicken, has its widest distribution in east Asia, from Pakistan through China, Eastern India, Burma, most of Indo-China, and on the islands of Sumatra, Java and Bali. Existing poultry varieties comprise of a wide range of breeds and strains that have evolved in the process of domestication and breeding. Breeding of poultry for commercial purposes using highly efficient selection programmes has resulted in a few highly specialized lines dominating today's world market (Henuk, 2015; 2016; Henuk *et al*., 2016).

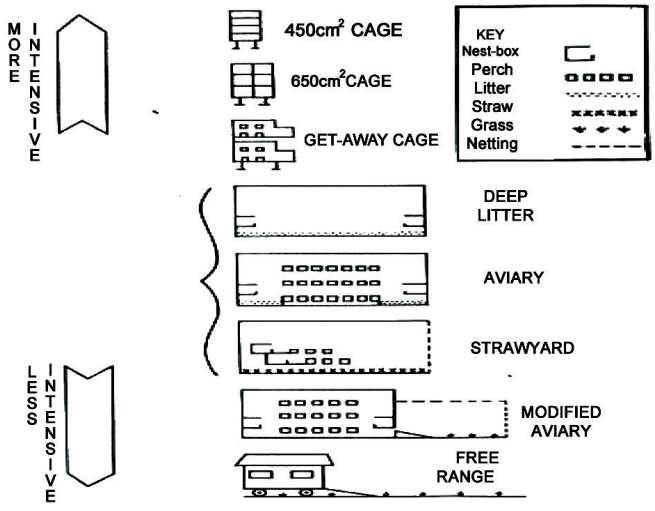

Figure 1. Housing systems for ´alternative systems´ of poultry husbandry (from Henuk, 2015: 251).

Small-scale poultry production is ideally suited to rural areas where the conditions for a successful commercial poultry sector are rarely met. Indigenous poultry breeds are excellent scavengers, transforming feed resources considered unsuitable for human consumption into high quality products uch as poultry meat and eggs. The ability of indigenous breeds to scavenge, to flee predators, to lay and hatch their own eggs and to contribute to pest control results in a production system that complements other farm activities without directly competing with humans for cereal crops (Alders, 2012). From an economic point of view, prices of local chickens are often higher than those of commercial broilers, creating a great incentive for small-scale poultry producers and reflecting consumer preferences for local chickens raised on the free range. While imports and commercial poultry products sometimes saturate the urban markets with devastating effects on prices for domestically produced commercial broiler and eggs; the prices for small-scale poultry are often relatively stable throughout the year although prices may peak during festive seasons (FAO, 2014; Henuk, 2015; 2016). Small-scale poultry production makes a substantial contribution to food security and poverty alleviation in many countries around the world and thus represents a major contribution towards achieving Millennium Development Goal (MGG) (halve the number of poor people in the world by 2015). It also contributes to achieving the MDGs with respect to gender equity and women's empowerment and promoting the well-being of rural populations (Henuk, 2015). The distance of the small-scale producer from market affects the availability of inputs and services for production and the opportunities and ways of selling products. This is expressed in the relative importance accorded to poultry production for either food security or income generation. Table 3 provides a schematic description of this relationship (FAO, 2014). There are four broad well-recognized small-scale poultry production systems. They are (1) Free-range extensive system, (2) Backyard extensive system, (3) Semi-scavenging system, and (4) Small-scale intensive system (Table 3). Characteristics

of these systems met the following 14 criteria listed in Table 4.

Table 2. Influence of site effects on small−scale poultry production (FAO, 2014: 6)

Location	Main purpose	Poultry production system
Remote village	↓ Food security ↑ Income generation	↑ Small extensive scavenging
Village with access to rural markets	Food security = Income generation	↑ Extensive scavenging ↓ Semi-intensive
Peri-urban village with access to urban markets	↓ Food security ↑ Income generation	↑ Semi-intensive ↓ Small-scale intensive

Note: ↑ : higher importance; ↓ : lower importance; = : equal importance.

Table 3. The four broad well recognized small−scale poultry production systems (Henuk, 2015: 250; Henuk et al., 2016).

Production systems	General description
(1)Free-range extensive system	The birds are not confined and can move over a wide area for scavenging. Shelters may or may not be used. The birds usually roost in trees and nest in the bush. It is nowadays getting less common.
(2)Backyard extensive system	Poultry are housed at night and are allowed to scavenge during the day. Farmers usually provide grains, grain by-products and kitchen waste etc. in the morning and/or evening to supplement scavening. This is the most widely followed by farmers of Asia, Africa and Latin America.
(3)Semi-scaveng-ing System	Birds are confined to a certain area with access to shelter. They are allowed a part of the day, for instance, 6-8 hours for scavening. Supplementary feeding is a must which is usually carried out with homegrown grains, grains by-products, kitchen waste etc. It has become an issue for debate since achieving biosecurity of the birds reared under the system is difficult and they may contribute to the spread of diseases like Avian In-
(4)Small-scale intensive system	Birds are totally kept confined under this system. Home- made feeds or commercial feeds are supplied in the poultry house. Small scale commercial layers and broilers are produced within this system. In some countries, productive native breeds or cross-breeds are reared. This system is important for self-employment, maintenance of livelihood and to ensure food and nutrition security. The number of birds to be raised (flock size) in this system varies depending on perception and priorities, financial capacity and facilities of the poultry producers.

Small-scale poultry is thus a valuable asset to the local human population in many countries despite its relatively low productive performance of 40 to 60 eggs per year and 1.5 to 1.7 kg body weight at maturity (Akinola and Essien, 2011). Although local chicks are slow growers and poor layers of small sized eggs, they are, however, ideal mothers and good sitters, excellent foragers and hardy and posses natural immunity against common diseases. The small body size of indigenous chickens is a desirable characteristic in many countries located in the tropical and sub-tropical environments. One of the most important positive characters of native chicken is their hardiness, which is the ability to tolerate the harsh environmental conditions and poor husbandry practices (climate, handling, watering, and feeding) without much loss in production (Dessie *et al*., 2011). In Asia, for example, village chickens provide manure and feed for fish when raised on top of ponds as part of an integrated system. They also have an important cultural value and are used in many festivals and ceremonies. Birds can also be kept as pets, controlled by different members of the family, or as fighting cocks, where they are under the control of a male family member. They can also play an important role in providing households with people living with HIV/AIDS with additional resources There is evidence that investments in small-scale poultry farm-

ing generate handsome returns and contribute to poverty reduction and increased food security in regions where a large share of the population keeps some poultry birds. This is the case for South Asia. Governments in South Asia recognize that increasing the productivity of small-scale poultry farms can contribute to alleviating poverty and reducing malnutrition on a broad scale ((Dadheech and Vyas, 2014).

Table 4. Charateristics of the four small−scale poultry production systems (FAO, 2014: 5; Henuk, 2015: 252; Henuk et al., 2016)

Criteria	Small intensive scavenging	Extensive scavenging	Semi-intensive	Small-scale intensive
(1) Production/ farming system	Mixed, poultry and crops, often landless	Mixed livestock and crops	Usually poultry only	Poultry only
(2) Other livestock raised	Rarely	Usually	Sometimes	No
(3) Flock size (adult birds)	1-5	5-50	50-200	>200 broilers >100 layers
(4) Poultry breeds	Local	Local or crossbreed	Commercial/ crossbreed/local	Commercial
(5) Source of new Chicks	Natural incubation	Natural incubation	Commercial DOC or natural incubation	Commercial DOC or pullets
(6) Feed source	Scavenging; almost no supplementation	Scavenging; occasionally supplementation	Limited scaveng-ing; regular supplementation	Commercial balanced ration
(7) Poultry housing	Seldom; usually made from local materials or kept in the house	Sometimes; usually made from local materials	Yes; conventional materials; houses of variable quality	Yes; con-ventional materials; good quality houses
(8) Access to veterinary services and vete- rinary pharmaticals	Rarely	Sometimes	Yes	Yes
(9) Mortality	Very high >70%	Very high >70%	Medium to high 20% to >50%	Low to medium < 20%
(10) Access to reliable electricity supply	No	No	Yes	Yes
(11) Existence of covnentional cold chain	No	Rarely	Yes	Yes
(12) Access to uban markets	Rarely	Rarely or indirect	Yes	Yes
(13) Products	Live birds, meat	Live birds, meat, eggs	Live birds, meat, eggs	Live birds, meat, eggs
(14) Time devoted each day to poultry management	< 30 minutes	< 1 hour	>1 hour	>1 hour

Conclusions

The term "Family poultry" used to describe the full variety of small-scale poultry production systems that are found in rural, urban and peri-urban areas of developing countries. There are four broad well recognized small-scale poultry production systems. Being called 'Family poultry', 'Small-scale poultry', 'Small-holder poultry', 'Scavenging poultry', or "Village poultry". They are (1) Free-range extensive system, (2) Backyard extensive system, (3) Semi-scavenging system, and (4) Small-scale intensive system. Characteristics of these systems met 14 criteria. Small-scale poultry is thus a valuable asset to the

local human population in many countries despite its relatively low productive performance of 40 to 60 eggs per year and 1.5 to 1.7 kg body weight at maturity. Although local chicks are slow growers and poor layers of small sized eggs, they are, however, ideal mothers and good sitters, excellent foragers and hardy and posses natural immunity against common diseases. The small body size of indigenous chickens is a desirable characteristic in many countries located in the tropical and sub-tropical environments.

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L29 USAID Feed the future Innovation Lab for Genomics to Improve Poultry: increasing food security in Africa by enhancing resistance to Newcastle Disease and heat stress in chickens

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Keywords: chicken, Newcastle disease, genomics, Africa, smallholder farmer

Smallholder poultry production has great potential for combatting food insecurity and poverty in rural Africa. The production of chicken meat and eggs provides important nutrients, crucial for alleviating malnutrition. Chickens also provide important economic opportunities to households with few sources of income. Newcastle disease (ND) is the most significant hurdle for raising poultry in rural Africa, causing widespread mortality in affected flocks. The USAID Innovation Lab for Genomics to Improve Poultry program uses advanced genetics and genomics to sustainably improve innate resistance to ND and heat stress in indigenous African chickens. Relatively ND-resistant and susceptible highly inbred chicken lines in the US were challenged with a high titered lentogenic ND virus strain, in the presence or absence of heat stress. Six African chicken ecotypes and one commercial layer widely used in Africa were also challenged with the lentogenic ND virus strain. Genes and signal pathways associated with genetic resistance to ND infection, evaluated through viral load and antibody level, and heat stress were identified by RNA sequencing. The chicken 600K SNP chip was used to identify genomic regions associated with resistance to ND and heat stress. An economical low-density SNP panel will be developed to select birds that are more resistant to ND, which will be considered for breeding and distribution to smallholder farmers. The expected results will contribute to the USAID Feed the Future Program goals to reduce poverty and strengthen food security in Africa.

L30 Overview of commercial production poultry manure management options

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Keywords: poultry, broiler, layer, manure, nutrient management, land application

Summary

This paper provides an overview of the predominate housing and manure management systems currently used in commercial meat and egg poultry production systems. Poultry manure is managed as a solid in both the meat poultry (chicken and turkey) and layer (shell egg and breaker) industries. Litter-based housing systems dominate the poultry broiler and turkey industry. Housing systems that utilize under-cage belts for manure removal are the current systems of choice within the layer industry. Manure belt system are used in conjunction with a variety of layer housing systems including conventional cage, aviary house and enriched colony systems. Over the past decade social pressure has resulted in a move from conventional caged systems to aviary house and enriched colony systems within the egg industry. In both meat and egg poultry production systems the management of in-house atmospheric ammonia levels is extremely important and is linked directly to manure management and building ventilation practices. Dry broiler poultry litter and layer manure are typically stored under roof until land applied as a fertilizer to either forage or row crop-production fields. Because poultry litter and manure typically provides far more phosphorus than crops require when applied on a nitrogen basis, poultry nutrient management plans must adequately address phosphorus management. While the majority of poultry manure is surface broadcast, systems that allow low moisture content poultry litter and manure to be applied sub-surface have been recently developed and commercial proto-types are currently being developed for the retail market.

Introduction

Different housing and manure management systems have developed for meat and egg poultry production systems. The primary functions of a poultry manure management system are collection, handling, storage and the land application of the manure nutrients generated by the poultry production system. Because all of the poultry production systems reviewed in this paper collect manure and store it for some period of time in the animal housing area, the housing system and manure management system operate as an integrated system. As such, the housing system selected dictates how poultry manure will be handled and collected within the system. The management of manure stored within the poultry housing system is directly linked to the overall system management and the well-being and productivity of the birds in both meat and egg production systems.

Meat poultry broiler and turkey systems producing birds for harvest typically use litter-based housing systems where poultry manure is mixed with bedding and stored within the poultry house for one or more flock production cycles. Most egg production systems collect manure on belt systems which are used to transport it to a separate manure storage facility on a daily to weekly basis. Poultry manure is managed as a solid manure in the majority of commercial poultry meat and egg production systems. Managing poultry manure as a dry solid has several advantages over liquid-based manure management systems. Advantages of solid manure management systems include reduced odor, reduced nitrogen loss in stored manure and lower risk of manure spills. The greatest disadvantage of solid manure management systems is the inability to transport manure hydraulically via a pump and pipe system to nearby fields and land apply it via sub-surface injection. If manure nutrients need to be transported long distances to application fields however, handling manure as a low moisture solid is advantageous since the transport of excess moisture is minimized.

Poultry and turkey manure is excreted with a moisture content of approximately 74% to 75% moisture on a wet basis. This is considerably drier than cattle and swine manure which is typically excreted in the range of 88% to 90% moisture content. Because of the lower as excreted moisture content of poul-

try manure it is feasible to handle it as a solid with a limited amount of additional moisture removal accomplished through either in-house ventilation management or with purpose designed air drying systems. Because of the wide variation in ventilation management, in-house drying schemes and manure storage conditions it is difficult to provide a characteristic moisture content for poultry manure. However poultry manure needs to be dried to a minimum of 70% moisture content in order to be managed as a solid during the handling process. While poultry manure at 70% moisture will behave more as a solid than a liquid, it is still difficult to handle as a solid at 70% moisture and drying to a lower moisture content is recommended prior to land application. Broiler and turkey litter is far drier than as excreted poultry manure due to the absorbent qualities of the bedding. Broiler poultry litter typically ranges from 25% - 35% moisture content by the end of a flock cycle. Litter that is allowed to dry over time in a roofed storage facility can reach moisture levels below 20% prior to land application. Layer manure must also be dried to lower moisture levels to allow land application through solid manure application systems. The drying of layer manure is accomplished through the use of air blown across the manure during collection, or through the use of air exchange through the manure storage facility. The final moisture content of layer manure can vary from 20% - 70% depending if a drying system is used, the type of drying system, and or the amount of air exchange provided during storage.

Poultry production manure management systems

Litter Based Systems

Litter based poultry production systems house birds in an enclosed production house with some type of absorbent bedding material on the floor. After bedding is mixed with poultry feces, feathers, spilt water and feed it is called litter. Litter based-systems are predominately used with meat broiler and turkey production systems. They are also used in broiler-breeder egg production systems. Many different types of materials are used as poultry bedding. Bedding serves the purpose of cushioning and insulating birds from the house floor and absorbing manure and excess moisture. The moisture management within a poultry house and hence the management of litter moisture is critical within litter based poultry production systems. Commonly used bedding materials include soft-wood and hard-wood sawdust, rice hulls, soft-wood and hard-wood chips, paper and sand.

Litter management varies between integrator and location. Due to the cost of litter materials it is typical to use litter for multiple flocks. Litter will typically be used for one or more consecutive flock production cycles or “grow outs” in the United States. In contrast, some broiler producers in European Union counties will replace litter with each flock of broilers however. The practice of producing multiple flocks of birds on the same litter is commonly referred to as using “built-up” litter. Between flocks, producers will remove clumps of litter that have formed in areas of high moisture in a process called “de-caking”. A de-caking machine is typically used to remove these clumps from the litter by skimming them off of the surface of the built-up litter. Figure 1 shows the placement of chick on built-up litter.

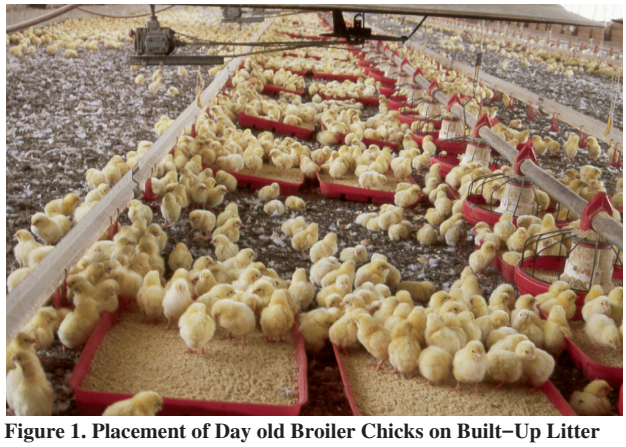

Figure 1. Placement of Day old Broiler Chicks on Built−Up Litter

Some producers also add a thin layer of new litter over the built-up litter before placing new birds. Ammonia generation from poultry litter is one of the primary litter management concerns. Built up litter typically generates more ammonia during the first week of the flock production cycle when compared to a flock grown on new litter. As the use of built-up litter has increased in the United States, so has the use of ammonia control amendments. Various compounds such as aluminum sulfate, ferric sulfate and sodium bisulfate have been successfully used as litter amendments. These amendments work by decreasing litter pH and therefore reducing ammonia emissions. They are typically effective for two to three weeks following application. Between flocks and prior to the addition of amendments it is a common practice to increase the broiler house temperature to drive off ammonia. Maintaining poultry litter at or below 25% moisture content is a good management practice to help reduce in-house ammonia levels. Figure 2 shows low moisture content broiler litter stored and ready for land application.

Figure 2. Stored Broiler Litter

High Rise House

High rise housing systems are used to house layer chickens for shell (table) egg and breaker (fluid) egg production systems. These systems are called high-rise houses because they are built two levels high. Layers are housed in conventional cage systems in the top level of the house. Manure is deposited by gravity and stored in the lower level of the high-rise system. Note that other poultry broiler and layer housing systems are sometimes built in multiple levels to reduce overall construction costs but should not be confused with high-rise layer housing systems where the birds and manure are stored on two different housing levels. High-rise systems typically use a conventional A-Frame cage system. These cages allow for multiple layers of birds to be housed in the cages and the manure from each level to fall to storage in the lower level without contacting birds housed below them in the cage system. Some conventional cage systems use backing boards to direct manure away from birds in the lower levels and some do not. Poultry manure is stored in the lower level of the high-rise house for up to a year. In addition to ventilation fans in the bird housing level, banks of fans are used in the lower level of the house to exhaust ammonia generated by the stored manure. In addition to reducing in-house ammonia levels this air exchange also serves to dry the stored manure prior to land application. The amount of air exchange provided in the lower level of high-rise house and the storage time will determine the final manure moisture content when it is removed for land application. Depending on these variables high-rise poultry manure can typically range from 70% - 30% moisture on a wet basis when removed from the house. High-rise housing systems were the dominate layer production system in the United States for many years. Over the past 10 years the layer industry has moved away from high-rise houses in favor of belt battery housing systems due to the improved air quality associated with belt manure management systems.

Belt Battery House

Belt housing systems utilize belts placed underneath bird cages to remove manure from the poultry house. These systems use conveyor belt technology originally developed for use in the rock and gravel industry to collect and transfer poultry manure to storage. The belts are placed below the poultry cages

and manure is deposited directly on a cross conveyor belt directly under the cage bottom. Birds are housed in multi-tier cage systems to make the most efficient use if in-house space and every tier of birds will have a cross-conveyor belt below the cage bottom to collect manure. Cross-conveyor belts are typically made from 1 to 1.5 mm polypropylene and are used transfer the collected manure to the end of the cage tier. At the end of the cage tier the cross-conveyor belt passes under a scraper that forces the manure to separate from the cross-conveyor belt. The separated manure then falls onto a belt that collects the manure from each tier of cages. Figure 3 shows the cross-conveyor belts as the manure is scraped off at the ends of the tiers. These belts are typically constructed of multi-ply abrasion resistant rubber or PVC that is used to transfer the manure out of the poultry house and into a roofed storage facility.

Belt manure removal systems are used with different types of layer housing configurations. Belts are used to remove manure from underneath battery cage systems, enriched colony layer cages and under aviary house layer cages. While the bird stocking densities vary in each of these three housing systems, each of these systems utilize multiple, vertical tiers of cages and a similar system of cross-conveyor belts is used below each tier of cages to collect manure. The aviary house system differs from battery and enriched colony cages in that the birds are allowed to access a bedded floor area outside of their cages during certain times of the day. Typically 90% of the layer manure will be deposited while the birds are in the cage system and collected on the manure removal belt. The remaining 10% of the manure is deposited on the bedding on the floor system and must be manually collected. Belt manure removal systems utilize various degrees of manure drying prior to transfer to manure storage. Belt removal systems that do not provide additional forced air drying transfer manure in its as-excreted state to storage or to further treatment or processing. Many belt manure removal systems utilize air blown down the cross conveyor belts to dry manure to around 35% moisture before transfer to manure storage facilities. Figure 4 shows a perforated cross-conveyor belt with air drying of poultry manure while on the belt.

Figure 3. Cross−conveyor Belts at Cage Tiers Ends.

Figure 4. Perforated Cross−conveyor Belt

Additional drying systems can be added to belt manure removal systems that use warm air exchange to dry manure to around 20% moisture prior to transfer to storage. The final moisture content of the poultry manure removed for land application depends on if air drying is utilized on the cross-conveyor belts, if an additional drying system is used and how much air exchange is provided in the storage facility as well as the length of storage. Because of these many variables belt-house poultry manure moisture levels can range from 70% to 20% moisture on a wet basis.

Land Application

Poultry layer manure and broiler and turkey litter is usually surface broadcast using tractor towed manure spreaders or spreader trucks. Handling poultry manure as a low moisture solid typically increases the distance that manure and litter nutrients can be economically transferred to land application fields when compared to liquid manure slurries with high moisture content. Traditionally the primary disadvan-

tage to land applying poultry nutrients as a dry solid is that they were required to be surface broadcast. Following the surface broadcast of poultry manure and litter it will continue to lose nitrogen through atmospheric volatilization and generate odor unless it is incorporated below the soil's surface. Unincorporated surface applied manure nutrients are vulnerable to being transferred to nearby surface waters during rainfall events that generate run-off. When poultry manure is applied to row-crop systems, incorporation into the soil via disking or tilling is viable. For poultry manure and litter applied to forage pasture or hay fields (which is the case with the majority of broiler poultry litter produced in the United States) mechanical incorporation is not a viable option.

Over the past decade systems have been designed to apply poultry litter below the soil's surface. Different proto-type sub-surface litter application systems have been developed by teams within the United States and Canada. At this time some companies are developing commercial sub-surface proto-types systems for testing that is expected to lead to these systems becoming commercially available. These systems operate similar to no-till planters in that they utilize a coulters with double-disk openers followed by a closing wheel. A system of parallel augers is used to pulverize the litter and transfer it to be dropped into the soil. These systems typically require that litter or poultry manure be at a 25% moisture content or lower to be sub-surface applied.

Conclusion

While the majority of poultry broilers and turkeys are currently produced in litter based systems, there are examples of meat bird production in a wide variety of systems. Examples of broilers produced in a variety of cage systems, including belt houses, over slatted floors and in open range systems are readily found. Layers are also housed in a wide variety of systems for egg production that range from traditional floor systems to newly developed systems such as the Roundel system in the Netherlands. However, the majority of layer production systems are currently using a belt manure removal system under either conventional cages, enriched colony or aviary housing systems as described in this paper.

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L31 Long−term effects of poultry manure application on water quality under a corn−corn system

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Keywords: nitrate, poultry manure application, tile drainage, water quality

Summary

The Upper Midwestern United States is an agriculturally productive region of the United States but also contributes high loads of nitrogen and phosphorus to the Gulf of Mexico. A long-term study was conducted to assess the effects of poultry manure (PM) application to a continuous-corn cropping system on nitrogen losses from 2010-2014. Soil test samples at 0-15 cm and 15-30 cm depth were collected each spring, prior to fertilizer application including poultry manure at 112 kg · ha^{-1} (PM) and 224 kg · ha^{-1} (PM2), and urea ammonia nitrogen at 224 kg · ha^{-1} (UAN). Tile drainage water samples were collected weekly and following precipitation and flow was recorded. PM2 application rates resulted in similar NO_3-N load (43.4 kg · ha^{-1}) and concentration (38.7 mg · L^{-1}) to tile waters in comparison with UAN (51.9 kg · ha^{-1}, 43.5 mg · L^{-1}). The PM application resulted in lower NO_3-N load (13.8 kg · ha^{-1}) and concentration (13.5 mg · L^{-1}). PM was found to be the best treatment for both acceptable crop yields and the lowest nutrient losses with the NO_3-N concentration closest to the 10 mg· L^{-1} NO_3-N Maximum Contaminant Level (MCL) for drinking water set by the U.S. Environmental Protection Agency (EPA).

Introduction

Iowa, U.S. leads national agricultural production of commodities including corn, soybeans, and eggs (USDA-NASS 2015). Approximately 1.2 billion pounds of poultry manure were generated every year from 2010 to 2014, with an average 2.5% annual increase. Due to the increase in poultry production in recent years, great opportunities as well as big challenges accompany the fast expansion of the industry. Land application of manure is one of the solutions to process the manures generated each year (Moore et al. 1995). Poultry manure is valuable and economical as an organic fertilizer from the poultry industry in Iowa and other regions in the U. S.

Poultry manure application may also contribute to non-point source (NPS) pollution. Risks of poultry manure entering water bodies and causing water quality impairments do exist, especially when over-application of fertilizer occurs or manure is applied prior to precipitation. Nitrogen and phosphorus are important for plant growth but are also recognized as important NPS pollutants. In the Upper Midwestern U.S., nitrate primarily enters waters through subsurface leaching while the majority of phosphorus export is thought to be associated with surface runoff and sediment; although recent studies have also documented subsurface transport of phosphorus to surface waters. Bundy and Andraski (2005) found that about half of the nitrogen applied to the soil is subject to leaching into groundwater. Studies have been conducted to determine the environmental mechanism affecting the N leaching, such as cropping systems, hydrology, or fertilization rates (Hansen and Djurhuus 1996; Morecroft et al. 2000; Bakhsh et al. 2002). For Iowa, a state where subsurface drainage systems are widely implemented, the risk of nitrate leaching to tile drainage systems is high. Studies have revealed nutrient losses from agricultural land through the drainage systems, where the nitrate concentrations measured are reported in the range of 10 mg· L^{-1} to 70 mg· L^{-1} (Hansen and Djurhuus 1996; De Vos et al. 2000; Kladivko et al. 2004). In the U.S., the MCL for nitrate nitrogen in drinking water is set at 10 mg· L^{-1}.

Current efforts are being implemented to reduce nutrient fate and transport, including Federal Mississippi River/ Gulf of Mexico Hypoxia Task Force and the Iowa Nutrient Reduction Strategy. The goal of this study is to define the effect of poultry manure application and hydrology on nitrate water quality under a corn-corn cropping system, specifically to determine 1) how different application rates of poultry manure affect tile drainage NO_3-N load in comparison to commercial fertilizer; and 2) how different application rates of poultry manure affect tile drainage NO_3-N concentration in comparison to commercial fertilizer. Results of this study are useful for understanding what factors affect nutrient losses through tile drainage.

Materials and Methods

A long-term study of the effects of poultry manure on water quality was initiated in 1998, with 11 chisel-plowed plots under a corn-soy rotation (Nguyen et al. 2013; Hoover et al. 2015). For 12 years, fertilizers were applied in the spring, only to the half of each plot planted in corn. The center tile drain was taken as a dividing line for planting, with corn planted in the north half part and soybeans in the south half part of each plot in even years, while the opposite planting of corn and soybean in odd years (Nguyen et al. 2013; Hoover et al. 2015). In 2010, all plots were converted to a corn-on-corn rotation.

Within the 8 field plots (plot 1, plot 2, plot 3, plot 4, plot 5, plot 7, plot 8, and plot 10 (Fig 1), area ranging from 0.19 ha to 0.40 ha) in the experiment, three treatments, including two applications of poultry manure (PM and PM2) and one application of urea ammonia nitrate (UAN), laid out in a split-plot design with target N-basis application rates for PM of 112 kg · ha^{-1}; PM2 of 224 kg · ha^{-1}; and UAN of 224 kg · ha^{-1}. The drainage system under plots 6 and 9 has not functioned since the beginning of this study period. The check plot was also not included in analysis because it lacks replicates. The application rates are based on N-basis poultry manure application rates assuming a 60% availability of N in poultry manure (Iowa State University Extension 2008).

Subsurface drainage water samples were collected approximately weekly and after major rainfall events. Samples were acidified with sulfuric acid and stored at 4 degrees Celsius. Unfiltered water samples were analyzed using a Seal Analytical AQ2 discrete auto-analyzer in the Water Quality Research Laboratory for the combine of nitrate-nitrite. Nitrate-nitrite was determined using the cadmium reduction method with method detection limit (MDL) 0.03 mg · L^{-1}. Weekly volume basis flow was calculated from weekly flow meter reading. Weekly drainage on a depth basis was calculated by dividing flow by plot area. Nitrate load was calculated by drainage volume multiplied by concentration. Missing concentration data were assumed to equal to the concentration value of the nearest sampling date.

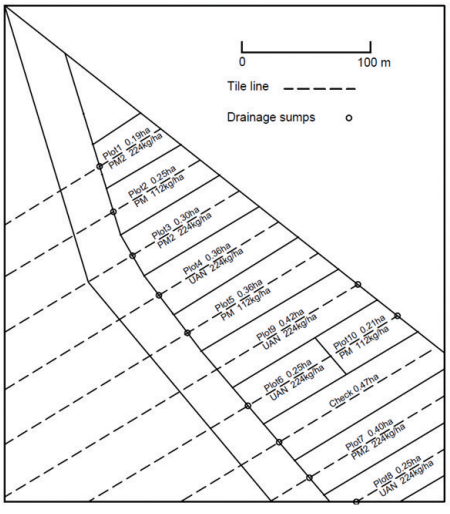

Fig 1. Field 5A research site at Iowa State University Agronomy Research Farm. Each plot is labeled with area and fertilizer or manure application rate. Plots 6, 9, and Check were excluded from data analysis.

Results and Discussion

The treatment mean flow weighted NO_3-N load (Table 1) during the research years (2010-2014) are 43.4 kg· ha^{-1} for PM2, 13.8 kg · ha^{-1} for PM, and 51.9 kg · ha^{-1} for UAN. For different treatments, flow weighted NO_3-N loads follow the order UAN>PM2>PM in 2010, 2012 and 2013. Though plots from both UAN and PM2 treatments are applied under similar N application rate (about 224 kg · ha^{-1}) in 2010, 2012 and 2013, mean yearly drainage is 164 mm for UAN, 1.47 times the drainage of PM2, which is 112 mm. The flow weighted NO_3-N load from UAN in 2010, 2012 and 2013 is 44.8 kg · ha^{-1}, 1.5 (similar to 1.47) times the flow weighted NO_3-N load from PM2, which is 29.8 kg · ha^{-1}.

Table 1. Yearly nitrogen load by treatment from 2010 to 2014

Year	PM (kg · ha^{-1})	PM2 (kg · ha^{-1})	UAN (kg · ha^{-1})	Mean (kg · ha^{-1})
2010	16.6(±8.4)[1]	32.8(±20.2)	51.3(±3.6)	33.6(±18.6)a
2012[2]	11.8(±13.3)	16.5(±8.2)	25.6(±2.7)	18(±10.2)a
2013	12.3(±4.4)	40.2(±11.6)	57.4(±3.2)	36.6(±20.5)a
2014	13.9(±3.2)	83.8(±18.1)	73.2(±26.3)	56.9(±37)b
Mean	13.6(±7.4)a[3]	43.4(±29.1)b	51.9(±20.9)b	

[1]the mean yearly nitrogen load is an average of all plots for each treatment with standard deviation in parentheses

[2]little drainage in 2011, no samples were collected in 2011

[3]mean NO_3-N loads by treatment for all years follow the order UAN>PM2>PM. The Tukey pairwise comparison test shows that the mean yearly N load of treatment PM is significantly lower than that of the other two treatments, and there is no significant difference between the mean N loads for PM2 and UAN. In the last row and the last column, values with the same letter are not significantly different at the p=0.05 level.

Differences were observed in 2014 when the flow weighted NO_3-N loads follow the order PM2> UAN > PM. This is because in 2014, UAN plots produced about 119 mm tile drainage, 0.82 times the amount of drainage of the PM2 plots (145 mm). This ratio is close to the ratio of mean flow weighted NO_3-N load for UAN (73.2 kg · ha^{-1}) over mean flow weighted NO_3-N load for PM2 (83.8 kg · ha^{-1}) in 2014, which is 0.87. Therefore, the variation of treatment mean flow weighted NO_3-N load can be best explained by the variation of yearly tile drainage.

Statistical differences between the mean NO_3-N loads in different treatments are found according to ANOVA (p=0.0226). The Tukey pairwise comparison test also shows that the mean NO_3-N load for the PM plots is significantly different from the other two treatments. There is no significant difference between the mean NO_3-N loads for treatment PM2 and UAN, although it is reported that the mean NO_3-N load for UAN is larger than that of PM2.

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L32 Broiler breeder protein turnover, de novo lipogenesis, heat production and body composition changes

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Abstract

A study was conducted to evaluate the effect of four different feeding regimens on breast muscle protein turnover in broiler breeder Cobb-500 parent stock (PS) pullets and breeder hens. The four feeding regimens based on BW curves utilized for the study were as follows: Everyday feeding(ED), skip-a-day feeding (SKIP) (Cobb Standard BW curve), lighter BW (LBW) (BW curve 20 % under) and heavier BW (HBW) (BW curve 20% over). Each pullet feeding regimen (Treatment) consisted of 150 d-old pullet chicks that were provided a different feeding regimens from 4 wk to 20 wk of age. Protein turnover was determined in PS pullets/breeders at 6, 10, 12, 16, 21, 25, 31, 37, 46, and 66 wk of age. A completely randomized design was used with a 4x10 factorial arrangement (four feeding regimens, 10 ages), each pullet represented a replicate. Five pullets/breeders at each age were given an intravenous flooding-dose of ^{15}N-Phe (150 mM, 40 APE (atom percent excess)) at a dose of 10 ml/kg for the determination of fractional synthesis rate (FSR). After 10 min, birds were euthanized and the breast muscle (*pectoralis major*) excised for protein turnover. Excreta was also collected from each pullet or breeder for 3-methylhistidine (3-MH) analysis. All birds were scanned with a dual energy x-ray absorptiometry (DEXA) The Fractional synthesis rate (FSR) in breast muscle of pullets significantly increased from 6 wk to 12 wk and then decreased significantly for 31 wk-old breeders. FSR in breeder breast muscle increased significantly from 31 wk to 66 wk. Breast muscle fractional degradation rate(FDR)significantly increased from 21 wk to 31 wk(peak egg production) (P-value < 0.001), then significantly decreased at 66 wk (P-value < 0.0001). There was a large increase in breast muscle FDR during the transition for the pullet to sexual maturity with continuing increases in breast muscle FDR through peak egg production

Changes in heat production and body composition in modern broiler breeders can provide means to understand nutrient utilization and an opportunity to improve feeding strategies. Twelve Cobb 500 fast feather breeders were evaluated every 3 weeks from 26 to 59 weeks of age. Heat production, and respiratory exchange ratio were determined by an indirect calorimetry and body lean and fat composition using a dual X-ray absorptiometry (DEXA). Feed allocation was 123 g/d (352 kcal) at 26.3 wk. and changed to 136 g/d (390 kcal) at 29.6 wk until the end of production. Light program was 16L: 8D from 29.6 wk. HP increased with age (d) in 0.28 kcal/d. During the light period, hens consumed more VO_2 (+17.5 L/d) (P<0.01) than in the dark. HP during the dark period was 83 kcal/kg$^{0.75}$ which could be considered resting metabolic rate and during the light period was 115 kcal/kg$^{0.70}$. RER decreased with age in -0.1x10^{-3} per day suggesting more fat being used later in production. Lean body mass changed from 642-783 g/kg reaching the lowest at 37 and 50 wk and the highest at the beginning 26-33 wk (P<0.001). Fat body mass changed from 168-261 g/kg with the lowest at the beginning of production 26-33 wk and the highest at 50 wk of age (P<0.001). Broiler breeders may be using body fat reserves for energy from 50 wk because lean mass increases when the egg production has reduced below 50%. Broiler breeders change nutrient fuel use along egg production.

The de novo lipogenesis (DNL) is important in fat deposition and efficiency of animal production. Two experiments were conducted using Cobb 500 hens for the purpose of gaining a better understanding of DNL with non-lipid precursors and to evaluate the differences between young and older breeder hens. Hens (25wk old) in experiment 1 were dosed (50mg/d) with U-^{13}C Glucose, L-^{13}C Alanine, or L-^{13}C Leucine for 14d. Hens (28 and 40wk of age) in experiment 2 were dosed with 40mg/d of U-^{13}C Glucose for 14d. Eggs and abdominal fat samples from the breeder hens were saved at different times. The enrichment and concentrations of labeled palmitic acid (LPA) were analyzed. In experiment 1, the enrichment of LPA in the yolk, derived from glucose and alanine significantly decreased (P<.0001) from d1 (0.578 vs. 0.381%) to d10 (0.032 and 0.047%), respectively, whereas the enrichment of LPA derived from leucine significantly increased (P<.0001) from d1 (0.112%) to d10 (0.355%). In experiment 2, young hens showed significantly lower concentration of LPA in the yolk on d1

(3.774 vs 2.706 mg/egg; P<.05), but significantly higher on d7 (0.042 vs. 0.178 mg/g; P<.05) as compared to older hens, respectively, while there was no difference on d14. LPA was only detected on d1 in abdominal fat of young hens in a noticeable amount (1.139 mg/g) and the concentration decreased to lower levels on d14 (0.188 mg/g). These results indicate that breeder hens use amino acids as precursors for DNL and the rate in young hens is higher than the older hens.

Keywords: fractional degradation rate, fractional synthesis rate, protein turnover, calorimetry, lean mass, fat mass, de novo lipogenesis

Introduction

Skeletal muscle protein turnover in broiler breeders has been studied by Ekmay et al. (2012, 2013). The researchers showed that the fractional degradation rate (FDR) of breast meat increased at sexual maturity and then the fractional synthesis rate (FSR) and FDR declined with additional egg production. The objectives of the present study were: 1) To determine the effect of four different feeding regimens: skip-a-day (SKIP), everyday (ED), heavier BW (HBW) and lighter BW (LBW) on breast muscle protein turnover in pullets and hens from broiler breeder parent stock during and after sexual maturity.

Meat-type hens or broiler breeders have been intensively selected for growth rate, feed efficiency, and breast meat yield traits, but not necessarily for reproductive traits; in fact, these hens have less eggs than table-egg producing hens (Robinson et al., 2003). Therefore, management and nutrition of the broiler breeder is the most complex piece of the poultry production (Kleyn, 2013). Body composition has changed over time resulting in leaner breeders being lean protein very important at the onset of sexual maturity (De Beer and Coon, 2007). Calorimetry can explain the nutrient oxidation, and DEXA body synthesis. T the objective of the present study is to follow the same breeder during production to evaluate calorimetry parameters: VO_2, VCO_2, RER, HP, along with body lean and fat mass.

The de novo lipogenesis (DNL) and the synthesis of triglycerides are important factors in fat deposition and efficiency of animal production. The precursors for DNL can be either glucose, acetyl-CoA, or amino acids which can come from both food and breakdown of muscle tissue by protein turnover (Murphy, 2006). Fractional protein degradation rate in breeder hens has been shown to increase significantly at sexual maturity, and gradually decrease after peak production (Vignale, 2014), suggesting that young breeder hens may use muscle protein for both yolk and albumen formation more than older hens. The objectives of the present study were to determine if non-glucose precursors can be used for fatty acid synthesis and to investigate difference in DNL between young and old breeder hens.

Materials and Methods

The effect of four different feeding regimens on breast muscle protein turnover in broiler breeder Cobb-500 parent stock (PS) pullets and breeder hens was evaluated. The four feeding regimens based on BW curves utilized for the study were as follows: Everyday feeding (ED), skip-a-day feeding (SKIP) (Cobb Standard BW curve), lighter BW (LBW) (BW curve 20 % under) and heavier BW (HBW) (BW curve 20% over). Each pullet feeding regimen (Treatment) consisted of 150 d-old pullet chicks that were provided a different feeding regimens from 4 wk to 20 wk of age. Protein turnover was determined in PS pullets/breeders at 6, 10, 12, 16, 21, 25, 31, 37, 46, and 66 wk of age. A completely randomized design was used with a 4x10 factorial arrangement (four feeding regimens, 10 ages), each pullet represented a replicate. Analysis of variance was performed using JMP pro 11 software (second edition, 2014). Five pullets/breeders at each age were given an intravenous flooding-dose of 15N-Phe (150 mM, 40 APE (atom percent excess)) at a dose of 10 ml/kg for the determination of fractional synthesis rate (FSR). After 10 min, birds were euthanized and the breast muscle (pectoralis major) excised and frozen in liquid nitrogen for protein turnover. Excreta was also collected from each pullet or breeder for 3-methylhistidine (3-MH) analysis. All birds were scanned with a dual energy x-ray absorptiometry (DEXA). Protein synthesis and degradation was determined via GCMS.

A mixed model was used to evaluate calorimetry parameters HP kcal/d, VO2, VCO2 L/d and RER by age (10 points of evaluation), time of day (2 levels: light and dark), and hen as random because of repeated measurements. Heat production was calculated after determining oxygen consumption and carbon dioxide production (HP kcal/d = 3.866 VO_2 L/d + 1.233 VCO_2 L/d) (Brouwer, 1965). A complete randomized design, CRD - one way ANOVA (age) with hen as random effect was used for body composition, lean and fat gain g/d. Means were separated by Tukey-HSD test. Egg production was recorded daily and averaged at every week of evaluation. Hens were scanned alive one day before calorimetry. Hens were scanned using a dual energy X-ray absorptiometry, DEXA scanner (GE, Madison, WI) with small animal body software module (Lunar Prodigy from GE encore version 12.2). No chemicals or anesthesia were used during the scan.

The first experiment, 18 Cobb 500 hens (25wk of age) were assorted into three groups of six hens each. Four hens in each group were dosed (50 mg/hen/d) with U-13C glucose, L-13C alanine, or L-13C leucine for 14d, and two hens were used as control. After 14d of dosing, enrichment in eggs collected on d1, d5 and d10 were analysed. All hens were euthanized on d10 and abdominal fat samples were taken. The second experiment compared the rate of DNL between young (28wk) and older (40wk) breeder hens. Fifteen hens from each age group were used; twelve hens were dosed with 40mg/hen/d of U-13C glucose for 14d, and the remainder of hens were used as control. After 14d of dosing, the enrichment in eggs saved on d1, d7, and d14 was determined. For each sampling time, three hens from each treatment and one hen from control group were euthanized and fat samples were taken. Fatty acid methyl esters were prepared and the percent enrichment and concentration (mg/egg) of labelled palmitic acid analysed using gas chromatography equipped with flame-ionization detector and mass spectrometer.

Results and Discussion

Protein turnover in broiler breeder parent stock

FSR in breast muscle of pullets significantly increased from 6 wk to 12 wk (3.62 %/d to10.93 %/d; P- value = 0.01) and then decreased significantly for 31 wk-old breeders (5.77%/d; P-value = 0.01). FSR in breeder breast muscle increased significantly from 31 wk to 66 wk (5.77 %/d to 11.76 %/d; P-value = 0.002). Breast muscle FDR) significantly increased from 21 wk (5.70%/d) to 25 wk (13.81 %/d, first egg) and 31 wk (22.46%/d, peak egg production) (P-value < 0.001), then FDR significantly decreased at 66 wk (6.53%/d; P-value < 0.0001) (Figure 1). The present results are in agreement with Ekmay et al. (2012, 2013). The researchers showed that the fractional degradation rate (FDR) of breast meat increased at sexual maturity. There was a large increase in breast muscle FDR during the transition for the pullet to sexual maturity with continuing increases in breast muscle FDR through peak egg production and a simultaneous loss in breeder lean mass during this same time period. Since protein turnover is energetically expensive, it is believed that broiler breeders rely on skeletal muscle tissue as a source of nutrients for egg production.

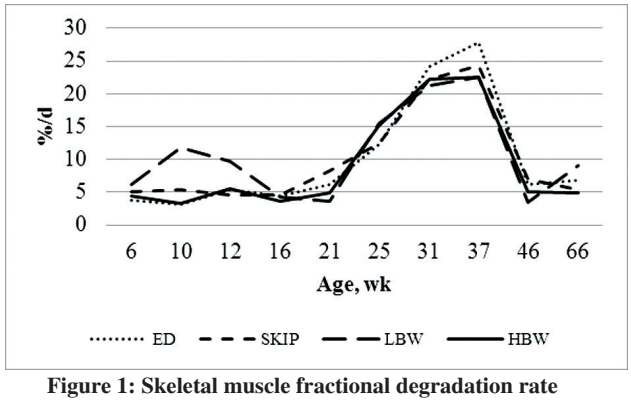

Figure 1: Skeletal muscle fractional degradation rate for broiler breeder pullets and hens by age and feeding regimen

Breeder heat production and body composition change during egg production

Heat production increased 0.28 L/d because the hen kept increasing body weight during production. Past research have reported 14.6 L/kg$^{0.75}$ for oxygen consumption (Waring and Brown, 1965) which is lower than the value reported in the present experiment, 20 L/kg$^{0.75}$. This may be due to modern breeders having more lean tissue per kg body weight than birds in 1965 (L/kg$^{0.75}$). This increase represents 37% more oxygen in 2015 compared to 1965. RER reached the lowest point at 40-43 wk of age which could mean fat oxidation is higher compared to beginning of production. Salas (2001) reported hens using glucose for egg production at the beginning of production and fat at the end of production. Lean tissue mass reached the lowest point at 37 and 50 wk (Figure 2) which is in full agreement with data found by Salas (2011). Protein degradation rate was found to be the highest at peak production (30-37 wk) Vignale (2014), suggesting the hen is using breast protein for egg synthesis. After 50 wk, egg production decreased and the hen started increasing lean tissue at the expense of fat oxidation.

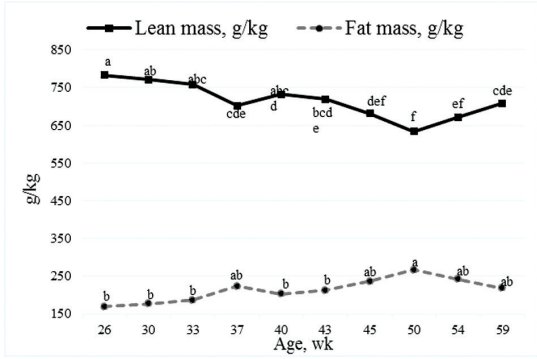

Figure 2. Body composition changes in breeders during production

De novo lipogenesis in broiler breeder hens

In experiment 1, the concentration of labelled palmitic acid (LPA) in the yolk, which was derived from U-13C glucose or L-13C alanine significantly decreased (P<0.0001) from d1 (5.578 and 4.041 mg/egg) to d5 (0.234 and 1.768 mg/egg) and were both almost negligible on d10 (0.338 and 0.085 mg/egg), respectively, whereas the concentration of labelled palmitic acid derived from L-13C leucine significantly increased (P< 0.0001) from d1 (0.713 mg/egg) to d10 (2.466 mg/egg) (Figure 3). These results show that young hens use amino acids not only for protein synthesis but also lipid synthesis. The conversion of glucogenic amino acids (alanine and glutamic acid) into lipids in fat and lean chickens was reported by Geraert et al. (1990). The different conversion patterns of leucine and alanine into LPA in present research could be because leucine is a ketogenic amino acid which can be converted into acetyl-CoA or acetoacetate which can either be used for energy purpose or fatty acid synthesis (Margaret and John, 2013). In experiment 2, on d1 the total amount of LPA per egg was significantly higher in 40wk hens compared to 28wk hens (3.774 vs 2.706 mg/egg; P<0.05). However, on d7, the concentrations of labelled palmitic acid in the yolk were significantly lower in 40wk old hens compared to 28wk hens (1.842 vs. 3.018 mg/egg; P<0.05), while there was no difference on d14. This data suggest that DNL for egg lipid in young hens is higher than in older hens, which is consistent with results from Salas (2011) who proposed that young breeder hens use DNL as a main source of egg yolk lipids while older breeder hens utilized more dietary and body fat to make the egg yolk. Unlike the yolk, abdominal fat of young hens contained 10 fold labelled palmitic acid (1.139 vs 0.138 mg/g on d1; P<0.01) compared to 40wk hens, which decreased continuously by d of sampling, and was almost negligible on d14 (0.188 vs 0.032 mg/g; P<0.001). The total amounts of LPA in the abdominal fat of the hens is shown in Figure 4 based on concentration per gram. These results were in agreement with Buyes et al. (2004) who proposed that the rate of glucose oxidation increased with age of broilers, indicating less DNL in older chicks. These results indicate that breeder hens use amino acids and glucose as precursors for DNL, and rate of DNL in young hens is higher than in the older hens.

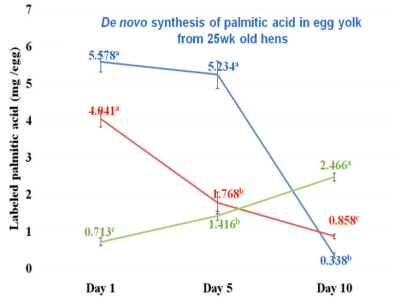

Figure 3. Concentration of palmitic acid derived from labelled glucose, alanine and leucine

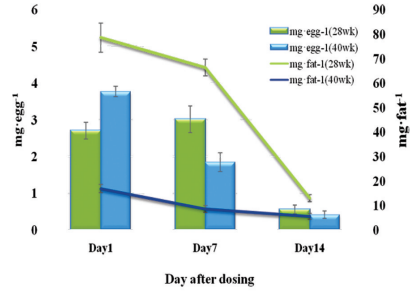

Figure 4. Concentration of labelled palmitic acid in total egg yolk and abdominal fat pad of young and old breeder hens

Acknowledgements

The authors thank Cobb-Vantress for their financial support for research, feed and breeders. The authors also want to thank the staff, graduate students and farm workers at the University of Arkansas for their help.

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L33 The potential of perinatal nutrition

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Keywords: perinatal nutrition, *in ovo* feeding, adaptive conditioning, gene expression,poultry meat

Summary

Based on Darwin's theory of adaptive evolution and Mendel's fundamental laws of heritability, breeding and genetic selection for increased growth rate and meat yield has dramatically advanced the production efficiency of poultry during the last 50 years, and this trend is expected to continue well into the future. Now, the period of embryonic and neonatal development is approaching 50% of the productive life of modern broilers, turkeys, and ducks. Although genetic selection does dictate how maternal and paternal genes that are inherited by their progeny, we are becoming more aware that nutrition and management may influence how those inherited genes are expressed. Epigenetics is the rising science of programming gene expression during critical developmental periods, which subsequently allow an animal to metabolically or physiologically adapt to specific dietary or environmental conditions. In poultry, epigenetic programming can occur during two critical periods: during the period of gametogenesis when breeding stock are adolescents, and during egg formation when egg nutrients are consumed by the embryo *via* amniotic fluid prior to hatch and yolk through to the first few days after hatch. Adaptive conditioning can be advanced further by nutritional or physiological imprinting during the first days after hatch. This paper discusses implications of nutritional and physiological stress of breeders on the epigenetic response of progeny, and how it can be modified by perinatal nutrition, including amniotic fluid supplementation by in ovo feeding and early feeding technologies. Epigenetic and adaptive conditioning of neonatal nutrition will also be discussed in the context of a "programmed nutrition" strategy to increase production efficiency and meat quality. As new molecular biology tools to measure gene expression become increasing affordable and robust, the study of epigenetic programming by perinatal nutrition will become an increasingly popular field of research. Moreover, emerging perinatal nutrition technologies for hatcheries will likely make programmed nutrition a commercial reality in the future as genetic selection for performance efficiency continues.

Introduction

Modern agriculture constantly strives to maximize biological performance of food production in an effort to optimize economic efficiency, profit potential, and sustainability. Commercial poultry production is among the most efficient and progressively successful of all food production sectors. What factors does it take for continued success in efficient poultry production? It takes the right genetics, combined with optimum health and management practices, and an optimized nutrition and feeding program. Efficiency and sustainability depends on the ability of a poultry production company to achieve competitive production indicators, including average daily gain, days to market weight, feed (caloric) conversion, livability, flock uniformity, and processing yields. However, profitability largely depends on how well a poultry production company meets consumer demand. Consumers want wholesome, safe, and affordable food. They want poultry products that look good, and are enjoyable to eat. Moreover, the most affluent consumers also want to buy their food from companies that excel in environmental stewardship and animal welfare. After proper management of commercial genetic stock, nutrition and feed is the most variable component of economic efficiency and profitability, as it represents 70 to 80% of live production costs.

Genetic selection is continually changing the "playing field" of production potential for the poultry industry; but it is the expression of this genetic potential that drives growth performance, health, and ultimately the profitability of poultry production. Growth performance and meat yield has improved linear-

ly by about 1% each year, and 85% of this improvement is attributed to genetic selection of broilers (Havenstein et al., 2003) and turkeys (Havenstein et al., 2007). One may argue that nutritional advancements have not kept pace with genetic selection as metabolic disorders and apparent nutritional deficiencies continue to arise, which mandate diet formulation constraints to be updated. However, the time has come to close this pace gap as we learn to harness the power of perinatal nutritional imprinting and adaptive conditioning to program the expression of genes associated with socioeconomically important traits.

The ancient Greek philosopher, Aristotle, theorized an individual's traits are acquired from their parents and contact with their environment. In simple terms, Aristotle's theory of developmental destiny means that all life on this planet is programmed to succeed in its given environment! Just a generation before Mendel's time, Jean Lamark was a proponent of the inheritance of acquired characteristics. The so-called Lamarckism theory emphasized the use and disuse of organs as the significant factor in determining the characteristics of an individual, and it postulates that any alterations in the individual could be transmitted to the offspring through the gametes. Despite many attempts, this inheritance of acquired characteristics has never been experimentally verified. Furthermore, many of Lamarck's examples, such as the long neck of the giraffe, can be more satisfactorily explained by means of natural selection.

The academic discipline of genetics has followed Mendel's basic concepts for over a century, and it was reinforced by the discovery and sequencing of DNA. Genetics describes the inheritance of information on the basis of DNA sequence. As the DNA sequence fragments were scientifically associated with certain biological traits, genomic scientists began to realize the importance of the gene expression. It was not until the recent introduction of molecular biological tools that the science of epigenetics and conditional imprinting has emerged. We can now study gene expression by mRNA up-regulation, proteomics and metabolonics. Now, many molecular geneticists agree that gene expression in response to environmental cues can be passed on to future generations. The old Greek philosophers and Jean Larmarck may have been right after all; they just did not have the scientific tools to prove it! There is now growing evidence that nutrition and environmental stimuli of parent stock and their progeny during the perinatal period may literally program how an animal's genes are expressed as an adaptive response to increase the chances of survival. This new science of "gene expression programming" is Epigenetics; it is the inheritance of information on the basis of gene expression, or inherited adaptation.

What is Epigenetic or Adaptive Conditioning?

I am the son of immigrants who came to Canada in 1956 to farm as my ancestors did before them. My parents left the Netherlands so their children would not experience what they had experienced when they were children during World War II. They occasionally spoke of how the Nazi soldiers took nearly all the food their family farm produced, leaving barely enough of the food they toiled to produce to eat for themselves. Towards the end of World War II there was a national famine that caused over 30,000 people to starve to death because of scarce food supplies from war-torn agricultural lands, and an unusually harsh winter. Detailed birth records collected during that "Dutch Winter Famine" provided scientists with useful data for analyzing the long-term health effects of prenatal exposure to famine. The children of this famine to 3 generations have unusually high incidence of developmental and adult disorders, including low birth weight, short body height, diabetes, obesity, coronary heart disease, and cancer, (Pray, 2004). In another study, Kaati et al. (2002) correlated grandparent's prepubertal access to food with diabetes and heart disease. Remarkably, a pregnant mother's diet can affect the expression of her genes in such a way that not only her children, but her grandchildren and possibly great-grandchildren inherit the same health problems. Using data from a small Swedish community, Pembrey et al. (2006) observed that epigenetic effects are sex-linked. Grandfathers who had access to surplus food during their slow growth phase (9 to 12 years of age) begot more diabetic grandsons than grandfathers who did not have as much food available to them before they reached puberty. In contrast, the biggest effect of food supply in grandmothers occurred when she was a fetus and infant, and it affected the mortality rate of their granddaughters. These responses suggest that information is being captured at key stages of egg and sperm formation, and is passed on to the offspring, possibly as modifications to the epigenome.

Epigenetics literally means "on genes", and refers to all modifications to genes other than changes in the DNA sequence itself. DNA within each cell is wrapped around proteins called histones. Both the DNA and histones are covered with chemical tags, to form what is called the epigenome. These chemical tags react to signals to the outside world, such as diet and stress. Some parts of the epigenome are

wrapped and unreadable, and other parts are relaxed and readable for expression. A good instructional video that describes the basic concepts of epigenetics can be viewed at http://learn.genetics.utah.edu/content/epigenetics/intro/.

Epigenetic imprinting of genes occurs most often by differential methylation of DNA at the promoter regions of specific genes that can permanently modulate an organism's adaptive response to adverse stimuli during critical periods of development. Particularly, early-life programming can turn on "Thrifty" genes that permanently reprogram normal physiological responses to survive environmental stressors, including moderate nutrient deficiency, and thus increase the chances of passing on their genes to the next generation. Evidence for epigenetic programming is demonstrated by swarming locusts: the swarming phenotype is environmentally influenced by drought conditions and the trait is passed onto the next generation until the population finds better conditions.

Transgenerational epigenetic or adaptive conditioning may explain some of the blessings and curses observed as a result of our system of commercial poultry production. Consider how we manage the weight of broiler or turkey breeders before and during egg production: this is during the critical epigenetic period of gametogenesis. Broiler breeder nutrition and feeding management likely has an important epigenetic effect on progeny. Consider how we manage and incubate commercial hatching eggs: this is during the critical epigenetic period of *de novo* methylation of somatic cells in the embryo. Environmental conditions (*i.e.* temperature and oxygen concentration) in the incubator may program epigenetic responses that affect subsequent metabolism. Consider how we manage chicks during the first few days after hatch. Feeding behavior, nutrition, and brooding conditions can affect metabolism and the development of breast muscle, the skeleton, and immune system.

In Ovo Feeding Jump−Starts Perinatal Development

Phenotypic characteristics that are programmed or imprinted to succeed in it's given environment and diet happen most effectively when the animal is young, and it is the first few meals that usually make the difference. For example, all honeybees are genetically similar, but what predestines a bee to become a worker or a queen is what the larvae are fed. Likewise, poultry may be programmed to succeed with the desired phenotypic traits by nutritional modification during the perinatal period: the 3 days before hatch and the 3 days after hatch. The chick's first meal occurs when it imbibes the amnion prior to hatch, and so this is the first opportunity for nutritional programming. By in ovo feeding (Uni and Ferket, 2003; US Patent No. 6,5692,878), nutrient balance and key metabolic co-factors of the amnion meal can be modified and influence subsequent phenotypic traits of economic importance for the poultry industry.

The benefits of in ovo feeding on early growth and development of broilers and turkeys have been demonstrated by several experiments in our laboratory (Uni and Ferket, 2004). In ovo feeding has increased hatchling weights by 3% to 7% ($P<.05$) over controls, and this advantage is often sustained at least until 14 days post-hatch. The degree of response to in ovo feeding may depend upon genetics, breeder hen age, egg size, and incubation conditions (i.e. the epigenotype). Above all, IOF solution formulation has the most profound effect on the neonate. Positive effects have been observed with IOF solutions containing NaCl, sucrose, maltose, and dextrin (Uni and Ferket, 2004; Uni et al., 2005), β-hydroxy-β-methyl butyrate, egg white protein, and carbohydrate (Foye et al., 2006ab), Arginine (Foye et al., 2007), zinc-methionine (Tako et al., 2005), butyric acid (Salmanzadeh et al, 2015), IGF-1 (Liu et al., 2012), and L-glutamine (Shafey et al., 2013). In addition to the increased body weights typically observed at hatch, the positive effects of in ovo feeding may include increased hatchability (Uni and Ferket, 2004; Uni et al., 2005); advanced morphometic development of the intestinal tract (Uni and Ferket, 2004; Tako et al., 2004) and mucin barrier (Smirnov et al., 2006); enhanced expression of genes for brush boarder enzymes (sucrase-isomaltase, leucine aminopeptidase) and their biological activities, along with enhanced expression of nutrient transporters, SGLT-1, PEPT-1, and NaK ATPase (Tako et al., 2005; Foye et al., 2007); increased liver glycogen status (Uni and Ferket, 2004; Uni et al., 2005; Tako et al., 2004; Foye et al., 2006a); enhanced feed intake initiation behavior (de Oliveira, 2007); increased breast muscle size at hatch (Uni et al., 2005; Foye et al., 2006a), breast muscle growth and meat yield (Kornasio et al., 2011), and improved skeletal development (Yair et al., 2015). In ovo feeding clearly advances the digestive capacity, energy status, and development of critical tissues of the neonate by about

2 days at the time of hatch. Using scanning electron microscopy, Bohórquez *et al*. (2008) observed that *in ovo* feeding significantly increased functional maturity and mucus secretion of goblet cells of villi of ileum and ceca of turkey poults. Associated with these goblet cells was the colonization of lactobacilli. Therefore, *in ovo* feeding may help improve the colonization resistance of enteric pathogens of neonatal chicks and poults. Based on the rapidly growing number of peer-reviewed publications from around the world, in ovo feeding consistently shows promising benefits, especially if applications can be done without compromising hatchability.

In ovo feeding offers promise of sustaining the progress in production efficiency and welfare of commercial poultry. Although selection for fast growth rate and meat yield may favor the modern broiler to become a more altricial, proper early nutrition and *in ovo* feeding may help these birds adapt to a carbohydrate-based diet and metabolism typical of a precocial bird at hatch. Our original research on *in ovo* feeding has established a new science of neonatal nutrition that many other scientists are now pursuing. As a result, we are all gaining greater understanding of the developmental transition from embryo to a juvenile bird. Now more work on in ovo feeding application technology and hatchery logistics must be done before *in ovo* feeding can be widely adopted for commercial practice.

Potential of Post−Hatch Nutrition on Nutritional Imprinting

The first few days post-hatch is the second part of the perinatal period that can imprint production traits by adaptive conditioning of gene expression. Chicks can be imprinted to enhance their tolerance to immunological, environmental, or oxidative stress. Nutritional programming during the perinatal period can also influence energy and mineral utilization or requirement, while other bioactive dietary components may "program" enteric microflora colonization that affect gut health and food safety. For example, Yan et al. (2005) reported that conditioning broilers fed a diet low in calcium and phosphorus for 90 hours post-hatch improves intestinal calcium and phosphorus absorption at 32 days of age, and increases the expression of the gene for the mineral transporter protein throughout the life of the bird. Angel and Ashwell (2008) demonstrated that broilers fed a moderately deficient conditioning diet for the first 90 hour post-hatch were more tolerant to a P-deficient grower and finisher diet, but they were also heavier, had better feed conversion, and they had higher tibia ash and P retention. The work of Angel and Ashwell demonstrate that epigenetic imprinting and nutritional adaptation to low dietary Ca and P is indeed possible and likely for other minerals as well.

Based on the concepts of epigenetics, imprinting, and adaptive conditioning presented above, several experiments has been done to test various nutritional programming strategies at the Alltech-University of Kentucky Nutrition Research Alliance Coldstream Farm and Alltech's Center for Animal Nutrigenomics and Applied Animal Nutrition. By evaluating the expression patterns of key functional gene groups, dietary amounts of nutrients that affect homeostatic balance were discovered to depend on the form of the nutrient, levels of and interactions among nutrients, and the timing of administration. Feeding chicks a specifically-formulated diet during the first 72 hours post-hatch has been developed to "condition" the gut for better nutrient utilization and program metabolism that ultimately affects production efficiency, carcass composition, and meat quality. Chicks that have been fed the appropriate conditioning diet, followed by a complementary growing and finishing diet, have improved growth performance and feed efficiency through to market age, and over 70% higher calcium and phosphorus digestion than controls. A programmed nutrition strategy can literally change the nutrient requirement and production efficiency, and may yield a response greater than any single feed additive on the market. Not only can programmed nutrition increase production efficiency that is so important to poultry producers, there is evidence that it improves the meat quality consumers demand, which yields greater potential profits from the poultry products produced. Broilers that have been raised on a programmed nutrition strategy have reduced carcass fat and produce breast meat that has more appealing color, less drip losses during storage, improved oxidative stability, and lower cooking losses.

Although feeding broilers a special nutritional conditioning diet for just 72 hours after hatch presents great opportunities, it is logistically difficult to accomplish in practice using current production systems. Moreover, variation in the time and stress exposure between hatch-pull and placement will affect the effectiveness of the 3-day nutritional conditioning period. However, recent hatch-brood technology (http://www.hatchbrood.nl/hatchbrood/product.php) offers a practical means to deliver specially formulated di-

ets during the first 2 or 3 days post-hatch in the controlled environment of a hatchery. The hatchery of the future will be a place that will do much more than simply hatch and vaccinate chicks: it will also be the place where the chicks will be conditioned better tolerate the challenges of life, and be programmed for optimum nutrient efficiency. Nutritional science is no longer a matter of supplying minimally required nutrients in the ideal balance to achieve desired production and welfare goals. We now know that nutrition is a process that can be programmed to succeed by strategic perinatal diet manipulation by in ovo and post-hatch feeding.

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L34 Broiler gut microbiota−the role in host nutrition, performance and energy metabolism

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Keywords: energy utilisation, feed utilisation, intestinal bacteria, poultry, production

Summary

An important industry measure of broiler performance is the capacity of the bird to convert feed into carcass with the greatest efficiency. Understanding the gut microbiota of poultry is important in maximising production, animal welfare and food safety outcomes, while at the same time minimising the environmental impact of livestock production. It is well accepted that nutrition, environment, host genetics, age and management all influence bird performance and health, but these factors also influence the host's gut microbiota. The gut microbiota is a complex ecosystem with vast metabolic activity. With the advent of genomics, transcriptomics, proteomics and metabolomics we have gained higher resolution tools to investigate the impact of diet and environment on the gut microbiota and associated microbial metabolic activities. Knowledge gained should aid in the developing of targeted feeding strategies for improved industry, animal welfare and consumer outcomes.

Main text

There is growing evidence that gut microbiota and energy metabolisms are linked in humans, animal models, and poultry and ruminant livestock (Turnbaugh et al. 2006, Torok et al. 2008, Turnbaugh et al. 2009, Hernandez-Sanabria et al. 2010, Torok et al. 2011). In chicken, the gut microbiota has been shown to have both a buffering (reducing energy loss when the host is in a fasted state) and counterproductive (reducing dietary energy utilisation) action on energy metabolism (Muramatsu et al. 1994). Although, chicks raised in a germ-free environment grow faster than their counterparts reared in conventional environments, germ-free chicks tend to exhibit physiological abnormalities, such as reduced intestinal motility, lower body temperature and poorly developed immune system (Coates et al. 1963, Niba et al. 2009). However, all these abnormalities can be improved following the addition of normal gut microbiota (Niba et al. 2009).

The gut microbiota aid in the digestion of feed and feed compounds which otherwise may remain unavailable to the host and provide essential amino acids and vitamins. Furthermore, by-products of bacterial metabolism (short chain fatty acids) fuel epithelial cells and suppress expression of pathogen virulence factors (Rehman et al. 2007). In turn, the gut microbiota are affected by the flow of nutrients from the diet, host derived substrates, immunological responses of the host and the gut anatomy (Rehman et al. 2007). Any disturbance to the gut microbial balance brought about by changes in diet composition, host immunity or gut physiology can lead to dysbiosis and/or enteritis. Dysbiosis may impact host metabolism by either altering the ratio of beneficial and detrimental bacterial species, hence affecting the host's ability to harvest energy and respond to energy intake, or by increasing the bacterial load and bacterial derived products, resulting in an innate immune response and low grade inflammation (Kogut 2013).

Therefore, a favourable gut microbiota is important for the optimal growth and performance of chickens, while an unfavourable microbiota may promote enteric infections, leading to decreased growth rates and increased mortality. However, gut bacteria need not be pathogenic to impact negatively on bird performance and production. Hydrolysis of conjugated bile acids by some members of the genera *Clostridium*, *Lactobacillus*, *Fusobactrium*, *Bacteroides*, *Bifidobacterium*, *Peptostreptococus*, and *Streptococcus* have been identified (Rehman et al. 2007). This bacterial activity lowers the detergent properties of bile acids in the emulsification of fat leading to growth depression in chickens (Knarreborg et al. 2002a). A normal poultry gut microbiota is described as being dominated by *Firmicutes* and *Bacteroidetes*,

while intestinal dysbiosis is associated with elevated *Enterobacteriaceae* (Kogut 2013). Many *Firmicutes* (*Faecalibacterium*, *Butyrivibrio*, *Megasphaera*, *Subdoligranulum*, *Oscillibacter*, *Anaerostipes*, and *Anaerotruncus*) have been shown to express enzymes required for butyrate production, the preferred energy source of epithelial cells. *Bacteroidetes* have been shown to express enzymes for propionate production, polysaccharide degradation and oligosaccharide transport pathways (Polansky et al. 2016). Spore-forming butyrate producers (*Naerostipes*, *Anaerotruncus*, and *Subdoligranulum*) have been suggested as potential targets for probiotic strain development (Polansky et al. 2016).

Metagenomic sequencing of the chicken caecal content has provided new information on gut microbial community function. Over two hundred different non-starch polysaccharide degrading enzymes, several fermentation pathways leading to short chain fatty acids and a dozen uptake hydrogenases have been identified (Sergeant et al. 2014). The uptake hydrogenases have been shown to be associated with several of the most abundant genera found within the caeca (*Campylobacter*, *Helicobacter* and *Megamonas*). It is speculative that the high abundance of these particular genera may be explained by their potential to remove hydrogen, hence benefiting other members of the microbiota and improving energy utilisation from the feed.

One of the greatest determinants of the host's gut microbiota, as well as health and performance, is the diet. It has been proposed that the dominant and non-dominant caecal microbiota are shaped differently, with the high-abundant microbiota shaped by diet and the diverse but low-abundant microbiota shaped by environment (Ludvigsen et al. 2016). The gut microbiota is influenced by dietary ingredients (Hubener et al. 2002, Rehman et al. 2007), nutrient levels of fat, protein and carbohydrate (Danicke et al. 1999, Knarreborg et al. 2002b, Drew et al. 2004, Wilkie et al. 2005), physical structure, such as particle size and processing technique (Apajalahti et al. 2001, Engberg et al. 2002, Bjerrum et al. 2005), use of in-feed antimicrobials (antibiotic and coccidiostats) (Dumonceaux et al. 2006, Johansen et al. 2007, Wise and Siragusa 2007, Torok et al. 2011) and use of exogenous feed enzymes (Choct et al. 2006, Torok et al. 2008). In particular, the cell wall and other fibre components of feedstuffs affect microbial communities as they are not digestible by the host and serve as the primary substrate for microbial growth (Rehman et al. 2007). Intestinal microbiota also influenced by host derived substrates, such as mucin and bile acids (Rehman et al. 2007).

Correlation between gut microbial community structure and the energy harvesting efficiency of broiler chickens has been previously demonstrated, although no definitive bacterial species or metabolic activities were identified (Torok et al. 2008, Torok et al. 2011, Rinttilä and Apajalahti 2013). The association of broiler chicken gut microbiota and bird performance, as measured by feed conversion ratio (FCR), were investigated in three Australian feeding trials which differed in diet composition, broiler breed, bird age and geographic locations. The results of the gut microbial profiling of birds from these trials revealed eight common performance related operational taxonomic units within both the ilea and caeca (Torok et al. 2011). Targeted cloning and sequencing of these operational taxonomic units revealed that they potentially represented 26 bacterial species or phylotypes, which clustered phylogenetically into seven groups related to *Lactobacillus* spp., *Ruminococcaceae*, *Clostridiales*, *Gammaproteobacteria*, *Bacteroidales*, *Clostridiales*/*Lachnospiraceae*, and unclassified bacteria/clostridia. Quantitative PCR assays have also been used to investigate the relationship between broiler feed efficiency and abundance of specific bacteria (*Lactobacillus salivarius*, *Lactobacillus crispatus*, *Lactobacillus aviarius*, *Eschericia coli)* and total eubacteria. Diet, environment (litter), and/or sex of birds all significantly influenced abundance of these bacteria. However, lactobacilli and total eubacteria were significantly decreased in birds that were more feed efficient across trials. *E. coli* was not consistently linked with either improved or decreased performance across trials (Torok et al. 2013).

Other studies investigating the relationships between broiler gut microbiota, microbial function and feed efficiency have identified caecal and faecal bacteria and genes associated with improved FCR (Singh et al 2014, Stanley et al. 2016). Stanley et al. (2016) identified significant differences in caecal bacteria associated with feed efficiency in three independent feeding trials. However, no common performance related bacteria could be identified among these trials despite sourcing birds from the same hatchery and breeder flock, rearing birds under the same environmental conditions and location, and feeding birds the same diet (Stanley et al. 2016). Furthermore, large variations in background microbiota among trials was observed. *Firmicutes* and *Bacteroidetes* have been shown to be less abundant and *Proteobacteria* more abundance in more feed efficient birds, with 33 faecal genera showing significant differences

between high and low FCR groups (Singh et al. 2014). In contrast, transcription of pro-inflammatory cytokines in broilers have been shown to be negatively correlated with the abundance of *Firmicutes* and positively correlated with the abundance of *Proteobacteria* (Oakley and Kogut 2016). Although, inflammation may negatively impact host energy metabolism, no measure of feed efficiency was made in this latter trial. Gut microbial genes associated with sulphur assimilation, flagellum and flagella motility have also been shown to be over represented in more feed efficient birds (Singh et al. 2014).

Despite several trials now having investigated linkages between poultry gut microbiota and feed efficiency, identifying consistent performance related bacteria has been elusive. The majority of poultry gut microbial community work published to date is based on genomics (16S rRNA characterisation), which identifies species but cannot discriminate between strains or identify gene functions. The microbiome (microbial genes) rather than the microbiota alone (bacterial community structure) may be more important in feed utilisation and performance. The vast metabolic activity of the poultry gut microbiome is not yet fully understood. Investigation of expressed metabolic pathways in major caecal microbiota has shown that similarities in protein expression tend to cluster bacterial genera into defined groups: *Firmicutes* and *Actinobacteria*; Bacteroidetes; and *Proteobacteria* (excluding *E. coli* which clustered with *Firmicutes*). Some *Firmicutes* (*Megamonas* and *Cetipeda*) have been shown to cluster with *Bacteroidetes* when considering proteins expressed (Polansky et al. 2016).

It has become evident that not only the bacterial species within the gut, but their metabolic activities are important. Turnbaugh et al. (2009) showed that a core set of genes encoded by the gut microbiome, rather than the bacterial composition may be more important in energy harvest in humans. They found that obese individuals had a greater proportion of genes for digesting fat, protein and carbohydrates which might make them better at extracting and storing energy from food. However, the gut microbiota was also found to differ in relative abundance of the *Bacteroidetes* and *Firmicutes* in genetically predisposed obese mice versus lean mice, indicating particular bacterial groups may also have increased capacity for energy harvest (Turnbaugh et al. 2006).

Characterisation of the gut microbiota and protein expression of the main colonisers following transplantation of hen caecal content into newly hatched chicks has shown that *Flexispira*, *Campylobacter*, *Mucispirillum*, *Nitratiruptor*, *Ornithinibacillus*, *Helicobacter*, *Megamonas*, *Wolinella*, *Solibacillus*, and *Caldicellulosiruptor* are capable of efficient colonisation, while *Fusobacterium*, *Methanobrevibacter*, *Paraprevotella*, and *Rikenella* are difficult to transfer from donors to recipients (Polansky et al. 2016). Furthermore, recipients of caecal inoculation showed higher abundance of certain genera than the donors, indicating that altering of the gut microbiota by the addition of microbes is not straightforward (Polansky et al. 2016). Interestingly, *Flexispira*, *Campylobacter*, *Helicobacter*, and *Wolinella,* all belonging to the order *Campylobacterales*, were shown to process carbohydrate-independent metabolism. There is potential for *Campylobacterales* to overgrow in the newly hatched chick as carbohydrate supply is limited.

Genomics have contributed significantly to our knowledge of the chicken gut microbial community composition. Yet our knowledge of the gut microbial metabolic functions and influence on animal health, welfare and performance is far from complete. Metaproteomics are slowly filling the gaps in our knowledge, with important microbial functions being attributed to whole genera and phyla rather than species. Previously, efforts have largely focussed on identifying particular microbial species, as desirable biomarkers of superior performance and gut health. It has become apparent that the intestinal microbiota is complex and difficult to manipulate precisely. Flocks' performance have been studied in various environments in order to try and characterise intestinal microbiota related to improve feed efficiency. Although there is increasing evidence for a connection between feed efficiency and gut microbiota in broilers, the relationship is still not yet fully understood. Greater focus should be placed on characterising microbial function to better understand the microbiota's role in energy utilisation. Understanding the dynamics of the gut microbial community, along with gene functions, is necessary to further establish strategies to improve feed efficiency and growth rates in the absence of in-feed antibiotics, avoid intestinal diseases and proliferation of food borne pathogens and identify better feed additives and nutrient levels that influence beneficial microbial activity.

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L35 Bacterial bile salt hydrolase: a gut microbiome target for enhanced poultry production

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Keywords: antibiotic growth promoters, alternatives to antibiotics, bile salt hydrolase, lipid metabolism, gut microbiome

Summary

To effectively mitigate antimicrobial resistance in the agricultural ecosystem, there is an increasing pressure to reduce and eliminate the use of in-feed antibiotics for growth promotion and disease prevention in poultry. However, limiting antibiotics use could compromise animal production efficiency and health. Thus, there is an urgent need to develop effective alternatives to antibiotic growth promoters (AGPs). Recent microbiota studies in poultry have shown the growth-promoting effect of AGPs was highly correlated with the reduced activity of bile salt hydrolase (BSH), a gut bacterial enzyme that has negative impact on host fat digestion; consistent with this finding, the population of *Lactobacillus* species, the major intestinal BSH-producer, was significantly reduced in response to AGP use. Therefore, BSH is a key mechanistic microbiome target for developing novel alternatives to AGPs, such as BSH inhibitors for enhanced feed efficiency and growth performance in poultry. Recently, we have identified a unique BSH enzyme from a chicken *L. salivarius* strain, developed an efficient high-throughput screening system to discover BSH inhibitors, and performed a series of functional, structural, and broiler studies to test our hypothesis. Our findings have provided compelling evidence that bacterial BSH in the intestine is a promising target for developing alternatives to AGPs.

Main text

Use of antibiotics clearly serves as a selective driving force to enrich antimicrobial resistance (AMR) genes and promote the emergence of resistant pathogens. Thus, reducing or eliminating the use of in-feed antibiotics in healthy animals has been a worldwide trend to effectively mitigate AMR and protect food safety. In particular, for more than 60 years, poultry industry has manipulated gut microbiota to increase feed efficiency and body weight gain through the routine use of low-dose antibiotics as feed additives, called antibiotic growth promoters (AGPs). Use of AGPs has been associated with the emergence of antibiotic-resistant human pathogens of animal origins. The European Union has banned AGPs since 2006. US FDA recently also implemented a new policy to recommend a voluntary withdrawal of medically important antibiotics from routing animal production practices by December 2016. However, AGP bans would have a negative impact on poultry production. Thus, ending the use of AGPs creates challenges for the poultry feed and feed additive industries. Developing effective alternatives to AGPs is urgently needed to maintain current poultry production level without threatening public health.

Although various products, such as prebiotics, probiotics, and organic acids, have been used to alter the intestinal microbiota for enhancing poultry growth performance, very limited data is available to clearly justify the choice of specific bacterial species or products for growth promotion in poultry; not surprisingly, results were inconsistent from independent studies. Examination of microbiota in response to AGP treatment would provide insights into the modes of action of AGPs and facilitate the develop-

ment of more effective microbiota-based strategies for growth promotion.

With the aid of culture-independent molecular approaches, investigations of the effect of AGPs on intestinal microbiota have been initiated in different food animals, including poultry and swine (Lin, 2014). These microbiome studies have shed light on the mechanism of mode of action of AGPs and on the development of novel alternatives to AGPs. To examine the response of gut microbiota to AGP treatment in chickens, we simulated environmental conditions used in the poultry industry and obtained the growth-relevant, high-quality fecal samples for microbiota analysis (Lin *et al.*, 2013). The fecal samples were subjected to analysis using different culture-independent approaches (phospholipid fatty acid analysis and 16S rDNA clone library analysis). The AGP treatment influenced the diversity of ileum microbiota in the chickens primarily in the Firmicutes division. In particular, *Lactobacillus* spp populations in each AGP-treated chicken were significantly lower than those found in the ileum of control chickens, which is consistent with the findings from other independent chicken studies (Lin *et al.*, 2013; Lin, 2014). This study and other published information strongly suggest that certain lactobacilli populations in the intestine, such as *L. salivarius*, have negative impact on chicken body weight gain, likely mediated through production of bile salt hydrolase (BSH), an intestinal bacteria-produced enzyme that exerts negative impact on host fat digestion and utilization.

The BSH enzyme produced by gut bacteria catalyzes deconjugation of conjugated bile acids by hydrolyzing the amide bond and producing free amino acids and unconjugated bile acids; this is an essential gateway reaction in the metabolism of bile acids in the small intestine (Begley *et al.*, 2006; Joyce *et al.*, 2014). The bile acids have dual digestive and signaling roles in the host; therefore, it has been recognized that intestinal BSH plays an important role in host lipid metabolism and energy harvest. *L. salivarius* NRRL B-30514, a strain isolated from chicken intestine, displayed potent BSH ability to hydrolyze conjugated bile salts (Wang *et al.*, 2012). A unique and potent BSH gene was identified and characterized from this *L. salivarius* strain (Wang *et al.*, 2012). The identified BSH displayed potent hydrolysis activity towards various conjugated bile salts. Different compounds that are used as dietary supplements in animal feeds were randomly selected for testing their inhibitory effects on the activity of the recombinant BSH using a standard *in vitro* BSH assay. Several dietary compounds, such as $CuCl_2$, $CuSO_4$, and $ZnSO_4$ displayed potent inhibitory effect on the rBSH. The inhibitory effect of copper and zinc on the rBSH is of particular interest. Recently, copper and/or zinc have been used at high concentrations (up to 250 ppm for copper and 3000 ppm for zinc) to aid in feed efficiency and growth promotion in poultry. To date, there is a lack a scientific evidence to explain why copper and zinc function as growth promoters at elevated concentrations. Our BSH study (Wang *et al.*, 2012) strongly suggest that the elevated concentrations of copper and/or zinc in feed exert inhibitory effect on the activity of intestinal BSH, consequently leading to enhanced lipid metabolism and host energy harvest. This finding strongly supports our hypothesis that BSH inhibitors may serve as promising alternatives to AGPs. Given the potential problems with long-term use of high doses of copper or zinc in animal feed, such as copper/zinc toxicosis and environmental contamination, novel BSH inhibitors with low toxicity and minimal environmental impacts should be identified.

Subsequently, by taking advantage of the unique feature of the *L. salivarius* BSH enzyme (Wang *et al.*, 2012), an efficient high-throughput screening system was successfully developed and used to discover BSH inhibitors (Smith *et al.*, 2014). Five compounds, caffeic acid phenethyl este, riboflavin, epicatechin monogallate, gossypetin, and carnosic acid, have been validated for their inhibitory on the *L. salivarius* BSH and are potential alternatives to AGPs for promoting poultry growth. Unlike many BSH from other bacteria that have narrow substrate spectrum, the *L. salivarius* BSH displayed potent hydrolysis activity towards both glycoconjugated and tauroconjugated bile salts. The broad substrate specificity nature of this BSH makes it an ideal candidate for screening desired BSH inhibitors. This speculation is further supported by our recent study showing the identified BSH inhibitors also exhibited potent inhibitory effects on a phylogenetically distant BSH from *L. acidophilus* (Lin *et al.*, 2014).

At present, structural basis of BSH function is still largely unknown, which has hampered development of BSH-based strategies for improving poultry production. Clearly, structural studies on BSH also will directly facilitate future translational research, such as using molecular docking to develop BSH inhibitors-based alternatives to AGP for growth promotion in poultry. As an initial step towards structure-function analysis of BSH, the C-terminal His-tagged BSH from *L. salivarius* NRRL B-30514 was crystallized recently (Xu *et al*., 2016). The 1.90 Å crystal structure of the *L. salivarius* BSH was determined

by molecular replacement using the starting model of *Clostridium Perfringens* BSH. It revealed this BSH as a member of the N-terminal nucleophile hydrolase superfamily. Crystals of *apo*-BSH belonged to space group $P2_12_12$, with unit cell parameters of 90.79, 87.35, 86.76 Å (PDB entry 5hke). Two BSH molecules packed perfectly as a dimer in one asymmetric unit. Comparative structural analysis of the *L. salivarius* BSH also identified potential residues contributing to catalysis and substrate specificity. Notably, unlike the binding pocket in other BSH that shows an open entrance with shallow bottom, a panel of unique residues in the *L. salivarius* BSH make this BSH enzyme display narrow entrance of the binding pocket and the increased inner capacity of the binding pocket, which may enable substrate to sit deeply in the pocket with different conformation and lead to the different enzyme-substrate interaction (or broad spectrum of specificity). Future in-depth structural analysis of the lsBSH (e.g. in complex with specific substrate) in conjunction with comprehensive amino acid substitution mutagenesis would help us discover critical residues in catalysis and help us discover novel BSH inhibitors.

In addition to discovering more novel BSH inhibitors, comprehensive animal trials are essential to further evaluate and select desired BSH inhibitors for use as alternatives to AGPs. Recently, the *in vivo* efficacy of riboflavin, an identified BSH inhibitor (Smith *et al*, 2014) was evaluated in a preliminary chicken study. Briefly, 200 one-day-old Hubbard broiler chicks were randomly allotted to 20 floor pens (10 chicks per pen) and assigned into two treatment groups (10 pens per group) that received a basal diet (control) or a basal diet supplemented with riboflavin (20 mg/kg of diet). At 21 days of age, average body weight per bird in riboflavin-treated group (0.4966 kg) is significantly higher (P=0.005) than that in control group (0.4605 kg). The Gain/Feed ratio per bird in riboflavin-treated group (0.6546) is also significantly higher (P=0.003) than that in control group (0.5925). Although riboflavin could have multiple modes of action on host physiology, this chicken study supported our hypothesis, and provided strong rationale for us to continue to comprehensively evaluate novel BSH inhibitors as non-antibiotic AGPs in conjunction with intestinal bile profile measurement.

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L36 Genomic selection and its application to poultry breeding

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Keywords: genomic Selection, genomic prediction, training population, breeding plans.

Summary

This paper gives a brief summary of the use of genomic information in poultry selection programs. Genomic information yields considerable increases in genetic gains, especially for traits that are difficult to improve by classical means. Efficient methods for predicting genomic breeding values are available if all animals are genotyped and efficient methods for combining information from genotyped and non-genotyped individuals are also available. Extension of these models to situations with very many genotyped individuals is still under development. Effective genomic prediction requires constant update of the training population that have both genotypes and phenotypes and suggestions for finding optimum strategies are provided. Estimation of genetic parameters in the age of genomics is a challenge. Classical methods will yield biased results but new methods and models accounting for selection on genomic breeding values and selective genotyping are under development and initial results are promising.

Introduction

In chicken the first draft of the full genome sequence became available in 2004 (Hillier et al., 2004) and at the same time millions of single nucleotide polymorphisms (SNPs) covering the whole genome were identified. This immediately led to initiatives in order to capitalize on this wealth of information. One of the most promising approaches are the use of genomic selection (GS) as proposed by (Meuwissen et al., 2001). GS is an alternative to marker assisted selection (MAS). In MAS selection is on either quantitative trait loci (QTL), i.e. identified genes with large effects on the traits of interest, or on genetic markers that are closely linked to QTL. Many genes with effects on commercially important traits have been found but in general they only account for a small proportion of total genetic variance in a selection line. This is because many complex traits such as growth or feed efficiency are influenced by a large number of QTL where each gene generally has a small effect. Accurate identification of such genes with small effects requires mapping experiments that usually are much larger (costly) than normally available in the animal sciences.

The GS approach does not rely on identified QTL's or only on markers closely linked to specific QTL. Instead a dense set of markers covering the whole genome is used. In this way each QTL is linked to at least one marker. Utilization of, maybe millions of markers, requires development of models that use regularization techniques in order to avoid bias and overfitting the data.

The whole process of utilizing genomic information in a breeding program is usually termed genomic selection (GS). This includes phenotyping and genotyping of potential parents, selection and mating based on genomic information. To perform the selection on genomic information, genomic breeding values (GEBV) needs to be estimated (predicted). This latter statistical process is called genomic prediction (GP) although in literature the two concepts are often confused.

Genomic prediction

Selection decisions need to be based on predictions of additive genetic merit. To accurately predict genetic merit we need to combine information from phenotypes with information from genotypes and from relatives. Such breeding values including genomic information are usually termed GEVB. Since the true additive breeding values is an unknown random number we use prediction methods to perform this evaluation. There exists a range of different methods to conduct this prediction process. The most common and simple one includes computing a relationship matrix (G) based on genomic information (VanRaden, 2008) and uses this relationship matrix as variance-covariance structure to obtain the best linear unbiased prediction (BLUP) of breeding values. An assumption of this model is that each marker explains the same proportion of total genetic variance. Since a relationship matrix is applied the method is easy to implement using standard software for genetic evaluation that used pedigree information. The only change is that pedigree relationships (A) are replaced with genomic relationships (G), (Calus et al., 2014).

A number of alternative prediction methods, based on Bayesian derivations, have been proposed. The advantage of these models is a relaxation of the unrealistic assumption that all genetic markers affect the trait of interest in the same way. These methods termed BayesA, BayesB etc. assumes that different markers have different effects on the traits of interest and in the extreme selects a small subset of markers with large effects on the traits and a large remainder that have no or very small effects. Simulation studies have shown that such models have a better fit to the data and also yield more accurate GEBVs. However, this is only true if the genetic architecture of the traits is such that it is influenced by relatively few genes with large effects (Calus, 2010). Practical experiences have shown that for the complex traits included in commercial breeding programs the differences between prediction methods tend to be small, because such traits generally are influenced by very many genes.

A major advantage of genomic prediction is that young individuals without own phenotypes can be evaluated. In classical pedigree BLUP, the prediction of breeding values such an evaluation would be the parent average which means that all members of the same sib groups would have the same EBV. Genomic prediction allows us to distinguish between such selection candidates because the genomic markers provide information on the Mendelian sampling process within the sib families. This is especially advantageous in breeding programs for layers where there is no information available on males before they have records on offspring performances (Wolc et al., 2016).

Single step genomic prediction

In most poultry breeding programs a large number of selection candidates is hatched and tested in every selection round. The cost of genotyping is high compared to the cost of each individual and, therefore, only a subset of potential parents is usually genotyped in each round of selection. This leads to a need for models that can combine all available information from both genotyped and non-genotyped birds. Such methods, usually termed single step methods, has been developed independently by (Legarra et al., 2009) and (Christensen and Lund, 2010)). The method relies on the derivation of a combined relationship matrix that utilize both pedigree and marker information. Application of single step genomic prediction in general leads to increased accuracy of predicted breeding values for both genotyped and non-genotyped individuals. Examples are (Christensen et al., 2012) in pigs and (Chen et al., 2011) in broilers.

An alternative derivation of the single step prediction model based on Bayesian principles were presented by (Fernando et al., 2014). If the variance components are known this model is equivalent to the Legarra-Christensen model mentioned above. However relaxation of assumptions on specific relations between different variance components in the model shows considerable improvements in the accuracy of predicting breeding values for both genotyped and non-genotyped birds compared with the Legarra-Christensen model.

Large number of birds genotyped

In poultry breeding programs a large number of selection candidates are commonly available in each round of selection. Depending on the strategy for genotyping this also often involves large numbers of animals genotyped. This may lead to computational problems in GP because this process involves computing inverses of the genomic relationship matrix (G). This computation becomes difficult or impossible if several 100K animals are genotyped so that a genomic relationship matrix of the same dimensions must be inverted. Furthermore, if the number of animals genotyped exceeds the number of markers, the resulting genomic relationship matrix is singular and thus cannot be inverted. To overcome these problems (Misztal et al.,) proposed an approximation to the inverse that relied on selecting a subset of animals where all genomic relationships were taken into account and then apply an approximate recursion for computing the inverse related to the remaining animals. This procedure was called APY because initial implementations relied on dividing animals into proven and young animals. Later investigations have shown that this division can be done in several ways (T. Ostersen personal communication). Later (Pocrnic et al., 2016) in a simulation study showed that an optimum choice of base population could be based on the number of eigenvalues in the genomic relationship matrix corresponding to a large proportion of total variance in the GRM. They further claim that this is related to the effective number of chromosome segments in the population. However, Fernando et al., (2016, in press), have shown that the number of animals with linear independent genotypes can at most be equal to the number of linear independent markers used in the genomic relationship matrix. This derivation leads to models where it is only needed to estimate a number of parameters equal to the number of linear independent markers. GEBVs for all other animals can be derived as linear combinations of this base set of parameters. This parametrization leads to exact computation in a form that is only linearly dependent on the number of animals genotyped. The earlier proposed APY algorithm should therefore be replaced with the new modified algorithm.

Optimal reference populations

In order to conduct genomic prediction a reference population of animals that have both phenotypes and genotypes available is needed in order to train the model. In early papers on genomic prediction it was assumed that first the models were trained no phenotypes were needed in following generations. However, this initial expectation has not been possible in practice. It is clear that accuracy of GEBVs decrease as the distance between the animals used for training the model and animals to be predicted increases (Habier et al., 2007; Wolc et al., 2011). This is because the linkage disequilibrium between markers and underlying QTL is reduced in every generation even though the reduction in accuracy is lower than for the classical animal model based on pedigree relationships only. Therefore common practice now is to update the reference population in every round of selection. Optimum strategies for updating the reference population depend on many factors including, cost of genotyping, phenotyping strategy, selection intensity in males and in females, mating ratio, and genotyping strategy in terms of genotyping all animals or only subsets of birds. For economic reasons only a proportion of all selection candidates are genotyped. And this subset is usually chosen either at random or only the best potential parents are genotyped with the decision of who to genotype based on early phenotypes. Alemu et al. (2016) investigated the effects of selective versus random genotyping, sex ratio of birds genotyped, and proportion of all birds genotyped in each update of the training population using a comprehensive stochastic simulation model. The results showed clear interactions between all the factors investigated. Choice of optimum strategy for updating the training population, therefore, will depend on the specific circumstances for each breeding program. However, in general it is advantageous to genotype a large number of birds in every selection round and a combination of selective and random genotyping will lead to the greatest long term selection response.

Updating genetic parameters

Selection programs in poultry generate fast genetic progress. (Hill, 2014). This leads to rapid changes in gene frequencies and, therefore, genetic parameters in terms of heritability and (co)variance components need to be updated with regular intervals. Estimating the genetic parameters under selection based on animal models using pedigree relationships is well established and yield unbiased estimates of genetic variance and associated parameters (Sorensen and Kennedy, 1984). The condition for these methods to yield unbiased estimates is that all information leading to selection decisions are included in the data used for analysis. However this condition is violated by using pedigree based animal models for the analysis of data resulting from one or more generations of selection on genomic information. Such attempts have clearly shown that the resulting estimates of variance components pedigree based animal models are unreliable (Wang et al., 2016).

In typical data from genomic selection programs estimation problems is further complicated by selective genotyping. It has therefore been attempted to utilize the Legarra-Christensen single step models described in a previous section to estimate the relevant variance components. However, such a model also yields biased results primarily because the model is not able to properly take account of selective genotyping. This has been shown both in real data and verified in simulated data (Wang et al., 2016).

The model of (Fernando et al., 2014) has been extended to allow for unknown variance components and a relaxation of initial requirements of certain relations between genomic variance and variance due to imputation of unknown genotypes for ungenotyped individuals. These extensions lead to estimates of genetic variances that in simulation studies are close to true values and accuracy of predicting breeding values is considerable improved. Further research is needed to properly estimate variance components in the age of genomics.

Conclusions

The use of genomic information has led to considerable increases in the genetic gains obtained in poultry breeding programs. This requires the use of a dense set of DNA-markers covering the whole genome. Methods for prediction of genomic breeding values are well developed but challenges still exist in being able to incorporate very large number of genotyped animals in the prediction models. New and exact methods for this have been developed but need practical implementation. To ensure good prediction ability the training population of genotyped and phenotyped animals needs to be updated in every generation or selection round. The optimum strategy for this updating depends on many interacting factors and need to be well adapted to the conditions in each breeding program. Large increases in prediction accuracy can be obtained by genotyping relatively many individuals in a mixture of selective genotyping the based on initial phenotypic performance and random genotyping to ensure to have all genetic variation covered. Re-estimation of genetic parameters in terms of heritability and genetic variances is a challenge in the age of genomics. Use of classical pedigree based methods will be biased but new methods accounting for the use of genomic information provide optimism for

better methods in near future.

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L37 Genetic architecture of abdominal fat content under divergent selection in chickens

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Keywords: broiler, adipose tissue, abdominal fat content, divergent selection, bioinformatics

Summary

Abdominal fat content is a complex trait influenced by multifactorial genetic and environmental elements. A better understanding of the molecular and genetic mechanisms of excessive fat deposition could help further improve the efficiency and sustainability of the modern broiler production system. We started out by constructing chicken resource populations, and various platforms for molecular cellular biology, functional genomics, genome sequencing and genome engineering. Especially, with the help of the Northeast Agricultural University High and Low broiler lines (NEAUHL) divergently selected for abdominal fat content beginning in 1996, we studied the pattern of growth and development of abdominal fat tissue, genetic variations in candidate genes and their functional importance, quantitative trait loci mapping through familial linkage and genome-wide association studies, gene expression profiling in both adipose and liver tissues, and epigenetic regulation as well. Currently, we are working on phenome database construction, integrating data analysis and mining on large-volume genome sequencing and functional genomics information, and attempting to better understand the underlying genetic and genomic changes accompanying the selection response, in order to find genome-assisted solutions and methods for effective molecular selection on fat deposition in broilers.

Main text

Introduction

Modern broiler production system is the most efficient among all livestock species, mainly through intensive selection for fast growth rate and feed efficiency in the past half century (Siegel, 2014). However, it concomitantly brings about the excessive deposition of adipose tissue, decreasing feed efficiency and carcass quality, causing health and welfare problems, and lowering down also the profitability. Therefore, to further improve the efficiency and sustainability of broiler production system, breeding broiler lines that are more efficient and of low fat percentage is needed, which is one of the key questions awaiting to be answered, and requiring a better understanding of the molecular and genetic mechanisms underlying the growth and development of adipose tissue in chickens.

In the past several decades, researchers have examined the effectiveness of several different breeding methods, from direct selection on abdominal fat weight/percentage, to indirect selection on the feed conversion ratio, blood plasma very low-density lipoprotein (VLDL) concentration or glucose level (reviewed in Baéza and Le Bihan-Duval, 2013), to select lines with lower body fat. In the last 20 years or so, we focused on the study of genetic principles of body fat traits, and molecular genetics of the growth and development of adipose tissue in chickens. Important genes and noncoding RNAs, microRNA and long noncoding RNA (lncRNA), were screened out at a whole-genome level, based on their roles in physiological and biochemical pathways, tissue gene expression profiling, and genome-wide association analysis. In addition, their functional and regulatory mechanisms were explored at the cell, tissue, individual and population level.

However, abdominal fat content is a complex trait, influenced by multiple genetic, nutritional and environmental factors (Baéza and Le Bihan-Duval, 2013; Fouad and El-Senousey, 2014), which asks for novel and innovative analysis methods and tools. Fortunately, the recent fast advancement of genome sequencing and engineering techniques provides new opportunities in disentangling the complex interplay between genetic and environmental factors. However, the analysis and interpretation of the large-scale genomics data to dissect the genetic architecture of complex traits and to propose new breeding theory poses great challenges to both academia and industry, which calls for the training of next-generation students, poultry geneticists and breeders,

to be well equipped with new skill sets and knowledge.

Resource populations

Our group has constructed two chicken resource populations. Since 1996, the Northeast Agricultural University broiler lines (NEAUHLF) were divergently selected for the abdominal fat content, by using AFP (abdominal fat weight/BW) and plasma VLDL concentration as selection criteria (Figure 1). The base generation (G0) came from the same grandsire line originating from the Arbor Acres broiler, which was then divided into two lines according to their VLDL concentrations at 7 weeks (wk) of age. At each generation, birds from each line were raised in two hatches, with free access to feed and water. Plasma VLDL concentrations were measured for all male birds at 7 wk, and AFP of the male birds in the first hatch was measured after slaughter at 7 wk. Sibling birds from the families with lower (lean line) or higher (fat line) AFP than the average value of the population were selected as candidates for breeding, considering plasma VLDL concentration and the bodyweight of male birds in the second hatch and egg production of female birds.

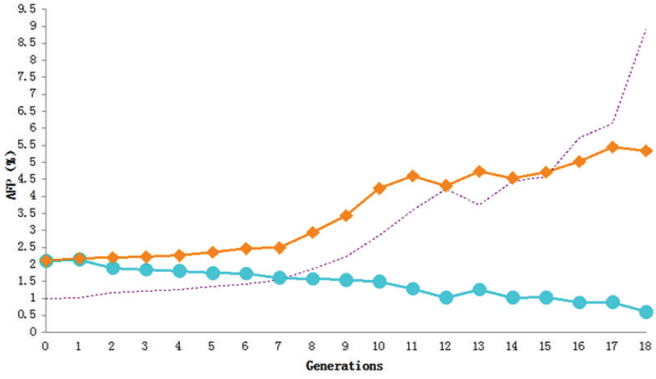

Figure 1. Dynamic selection responses of abdominal fat content in NEAUHL lines.

At generation 18, ~8.8 fold difference exists between the two broiler lines. Blue, low fat line; Brown, high fat line; Dotted line, fold-difference between two lines.

in both hatches (Wang et al., 2007; Gu et al., 2011). The second resource population (NEAURP), was built mainly for the purpose of mapping QTLs, using F2-design by crossing male broilers from the high fat line with a local breed, Bai'er Yellow chicken.

We collaborated with our colleagues in China Agricultural University, and used their resource population established by crossing White Plymouth Rock and Silkie chickens using also F2-design (CAUF2), and worked on candidate genes' association analysis.

Genetics of abdominal fat content in broilers

With the extensive use of NEAUHL broiler lines, we studied the pattern of growth and development of abdominal fat tissue, genetic variations and their functional importance for candidate genes, quantitative trait loci mapping of adipose fat content through familial linkage and genome-wide association studies, gene expression profiling in both adipose and liver tissues, and epigenetic regulation as well (details in Table 1). Recently, we took a systematic approach of mining public functional genomics data, and generated a reference catalog of 11, 180 chicken lncRNAs from public RNA-seq data from 12 different tissues and 8 different embryonic stages. Over the years, since we have collected a large amount of phenotypic and genotypic records, the NEAUHLF Chicken Phenome Database (NCPD) was created as the coordination center for data management and analysis (see Poster, Min Li et al.).

Genome sequencing and integrative genomics

To further elucidate the underlying molecular and genetic mechanisms for the growth and development of adipose tissue, we undertook an integrative genomics approach, by the collection, curate and compilation of genome sequencing and functional genomics data simultaneously. Our results showed that after 18 generations' intensive selection for abdominal fat content in NEAUHL broiler lines, a large number of genetic varia-

tions still exist, which can be utilized as the raw genetic material for the subsequent selection experiment (Hill, 2016). Furthermore, dynamic changing pattern of allele frequency spectra were found, which could partially explain the continuing selection responses over generations, and a regulatory gene network for lipid metabolism and adipogenesis was discovered to be involved in the striking difference between the high and low fat lines (Du et al., unpublished).

Conclusions

Genetic architecture of abdominal fat content in chickens is complex, however, during the past several decades, efforts from international community, and those working in model animals as well, have contributed in sketching out the genetic blueprint at an unprecedented level. With the fast advancement of genome sequencing and engineering, and the integration of molecular, physiological and nutritional information, the design principle of complex traits will definitely be understood in a great detail, which then can be used in improving further the efficiency and sustainability of chicken production system.

Acknowledgements

We greatly appreciate the help and contributions from NEAU poultry group members, and the funding supports from National 863 project of China, China Agriculture Research System, National Natural Science Foundation, and Programs for Innovation Research Team in University of Heilongjiang Province and Northeast Agricultural University.

Table 1. Genetics of abdominal fat content in NEAUHL broiler lines (representative works)

Main results	References
I. Adipose tissue growth and development	
We characterized the tissue growth pattern between the high and low fat lines, compared the fat content for abdominal adipose tissue, liver and (intra)muscle, computed genetic parameters for liver fat content, stimulated preadiopcyte differentiation by oleate in vitro, and established preadipoctye cell lines (Wang et al., unpublished).	Leng et al., 2016; Liang et al., 2015; Shang et al., 2014; Guo et al., 2011;
II. QTL mapping and GWAS	
QTLs mapped on chromosomes 1, 3, 5, 7, and fine mapping dissected out RB1 gene. GWAS using SNPchip array performed, as well as selective sweep, CNV and epistatic analyses. Large-scale genome-sequencing based association analysis performed (Du et al., unpublished).	Zhang et al., 2014; Li et al., 2013; Zhang et al., 2012; Liu et al., 2007;
III. Transcriptome profiling	
We profiled gene, noncoding RNA (microRNA and lncRNA) expression for two tissues (fat and liver) and two cell types (hepatocyte and adipocyte) at different developmental stages both in vivo and in vitro. For instance, liver tissues from a local Chinese breed (Bai'er) and fat line broilers, preadipocyte microRNA expression profiling, liver tissues from NEAUHL, and adipose tissue (genes in lipid metabolism, signal transduction, energy metabolism, tumorigenesis and immunity were differentially expressed)	Wang et al., 2015a;Wang et al., 2015b;He et al., 2014; Wang et al., 2007
IV. Proteomics	
Similarly, protein expression profiles for two tissues (fat and liver) and two cell types (hepatocyte and adipocyte) were also done, using different techniques (2D- DIGE, iTRAQ)	Wang et al., 2009
V. Candidate genes (molecular markers) and functional analyses	
51 candidate genes, including different types of FABP, UCP, Spot14, ApoB and IGFBP2 genes, had been assayed in NEAURP, CAUF2 population, and NEAUHLF population, and associations between genotype and phenotype had been investigated. Biomarkers were developed, e.g. 16 serum biochemical parameters. Functional analyses of important genes were also performed, for instance, PPAR γ, a key regulator of chicken preadipocyte differentiation; KLF2 inhibits adipogenesis, at least in part, through inhibition of PPAR γ and C/EBP α expression; BMP4 mRNA and protein expression levels in abdominal fat tissue, and serum BMP4 of fat males were lower;	Cheng et al., 2016; Dong et al., 2015; Duan et al., 2015; Zhang et al., 2014; Wang et al., 2008
VI. Epigenetics	
Methylation analyses on candidate genes (PPAR γ and C/EBP α), and genome-wide methylation sequencing analysis were performed for adipose tissue.	Gao et al., 2014; Sun et al., 2014

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L38 What is the risk of a new worldwide *Salmonella* pandemic in the 21st century?

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Summary

Infections caused by the broad-host range *Salmonella* serotypes Enteritidis and Typhimurium have caused a human pandemic worldwide. Major reasons have been the specific predilection of strains from the serotype Enteritidis for chicken eggs, often consumed raw, and multidrug resistance enabling the serotype Typhimurium to spread in the poultry and pig sector, leading to meat contamination. Typically, food poisoning *Salmonella* strains colonize their host animals asymptomatically, without causing visible changes in the contaminated food products, and thus not noticeable by farmers or consumers. Intensification and globalization of the poultry industry are risk factors for *Salmonella* infection and spread, and it is hypothesized that global warming will only contribute to the *Salmonella* problem. As such, the risk of a new pandemic strongly depends on whether a global coordinated approach will be developed for monitoring and control of *Salmonella* in poultry. Control should rely on an integrated strategy of multiple control measures at all levels of the production chain. If not, we may possibly face strains that combine the traits that allowed *Salmonella* Enteritidis and Typhimurium spread worldwide, i.e. multi-resistant egg-contaminating *Salmonella* strains.

Are the current *Salmonella* pandemics fading away?

Worldwide, the annual number of *Salmonella* infections in humans is high. These infections are caused by the so called non-host specific or broad-host range *Salmonella* serotypes, i.e. serotypes that can colonize the gut of multiple animal species, including humans. Human food-poisoning by *Salmonella* is caused by the consumption of contaminated animal-derived products, and poultry products (eggs, meat) are the most important source (EFSA, 2015). The number of *Salmonella* positive poultry flocks differs according to the region where the flocks are raised, and is highly influenced by regulatory authorities that can install control programs. In the EU and US, good monitoring systems are in place and accurate data are reported on the *Salmonella* flock prevalence. On other continents, monitoring is highly dependent on local authorities, and is often dependent on whether or not poultry products are exported to countries with a strictly regulated monitoring and control program.

Salmonella Enteritidis is a serotype that is of particular importance because it can spread to the reproductive tract of layers and contaminate eggs. A worldwide egg-associated salmonellosis pandemic has started in the '70s and is currently fading away in many countries, thanks to huge efforts of policy makers and the poultry industry. In other parts of the world, this decline is not evident yet. This pandemic has been specifically caused by the serotype Enteritidis. Due to its preferential association with hen eggs, combined with the way people tend to store (room temperature), handle and eat (undercooked) eggs, *Salmonella* Enteritidis has had and still has a major impact on human health. While *Salmonella* Enteritidis contamination levels have decreased in recent years in many countries in both humans and chickens (e.g. EU), this serotype is still the major chicken product-derived food poisoning problem worldwide. In addition to *Salmonella* serotype Enteritidis, also the serotype Typhimurium has caused and is causing concerns. *Salmonella* Typhimurium infections are often caused by the consumption of contaminated porcine and poultry meat. In addition to the human infections caused by the 2 predominant serotypes, Enteritidis and Typhimurium, also other serotypes can cause human gastroenteritis. These are mainly derived from meat sources, and the nature of the serotypes depends on the geographical location, and changes over time. Serotypes such as Hadar, Infantis, Paratyphi B, Heidelberg, Minnesota and many others can be derived from poultry meat, while serotypes other than Enteritidis are not commonly trans-

mitted through eggs. The Enteritidis pandemic is thus not fading away, although in specific parts of the world it has been shown that using monitoring and control methods (e.g. vaccination, biosecurity), it is possible to severely reduce chicken and as a consequence, human contamination. For *Salmonella* Typhimurium a similar situation occurs. As such, there is a strong decline in several parts of the world, but it remains unclear unclear and likely not yet established in other parts, and therefore remains a global concern. The success of eliminating Enteritidis and Typhimurium is thus limited, when looking on a worldwide scale.

Are intensive animal production and globalization driving forces for *Salmonella* transmission in poultry?

The last 50 years, the number of chicken farms that house animals in an intensive system has increased worldwide, and most regions worldwide have changed or are changing from extensive small-scale production to specialized intensive farming. Although in some countries the total number of farms is stagnating or even decreasing, the farm density can be high and the number of chickens per farm has raised. Chicken farm modernization and intensive production obviously has major benefits in producing high amounts of affordable protein, but a drawback is the high risk of introducing bacterial (and viral) pathogens that can easily spread within the flocks and be transmitted between flocks. Typically, the risk of disease outbreaks is highest in regions with a high density of poultry production. Although not easy to investigate, some studies indicate that intensification of poultry production increased the risk for infection with avian influenza (Van Boeckel et al., 2012a) and *Campylobacter* (Brena et al., 2016). As intensive poultry farming will further increase worldwide in the future because of the increase in global human population, an important issue will be controlling infections in areas with exponential increases in intensively-raised poultry farms. Not much data exist on the effect of farm density and intensification on *Salmonella*. In the EU, there is a clear trend that countries with low density chicken production have lower flock prevalences, although the correlation is not absolute. Trade in poultry and poultry products between countries and regions and thus globalization of poultry production is an additional risk of spreading *Salmonella*. It has even been suggested that the introduction of new breeding lines in the UK has introduced *Salmonella* Enteritidis in the early ‘80s (Ward et al., 2000). Intensification and globalization are integral parts of the future system to feed the world and will only gain importance in the future, and are clear risk factors for the spread of pathogens. When new strains or serotypes would gain importance in the future, it will be almost inevitable that, without intervention, these will spread regionally or even worldwide. This means that systems need to be put in place worldwide to monitor the *Salmonella* status on farms, trace origins of infections and control the pathogens in the whole production cycle.

Asymptomatic carriage in animals is essential for transmission to humans

While host-specific *Salmonella* serotypes, such as Gallinarum in chickens, cause systemic disease with high mortalities, food-poisoning serotypes do not cause symptoms in the animals. Exceptionally, it is possible that disease can occur in young birds that are infected with high doses. However, most infected chickens usually carry the pathogen in the gut without being ill, and can even carry the pathogen in internal organs, including the reproductive tract, without symptoms (Gantois et al., 2009). This explains the success of this pathogen to cause a pandemic. First, the bacterium is not affecting animal performance so this is not considered as problematic by the farmer, who is not spending efforts to eliminate the infection. Secondly, the bacterium can go unnoticed from farm to fork, and no visible or sensory change are noticeable in/on contaminated meat and eggs. In eggs for example, *Salmonella* Enteritidis can survive without proliferating, and as such is not noticeable by the consumers (Gantois et al., 2009). Even more problematic is the persistent nature of the infection, either by persistent colonization of the gut or organs, or the continuous shedding/uptake after survival in a farm environment. It has been shown that an infection with low doses results in long-lasting persistence, while high doses results in faster clearance, likely due to immune stimulation (Van Immerseel et al., 2004). Unfortunately, it is likely that birds are infected with low doses of the bacterium, thus enabling persistent infection.

Antibiotic resistant *Salmonella* strains are an emerging global problem

Antimicrobial resistance is a global concern in both veterinary and human medicine. Not only the

transmission of antimicrobial resistant bacteria to humans, but also the spread of resistance genes between bacterial species using transmissible DNA vectors is an important issue. In the EU, efforts are made to decrease the usage of antimicrobial agents in animal production, aiming to reduce the development of antimicrobial resistance. This is driven by consumer demands, and of course by the emergence of bacterial infections in humans that are difficult, or even impossible to treat. For animals, the commonly present antimicrobial resistance has also raised problems in therapeutic efficacy, and is even a driver for colonization by these bacteria as antibiotic usage selects for resistant bacteria. Specifically, the colonization and subsequent spread of antimicrobial resistant *Salmonella* strains can be stimulated when the antibiotic usage is high. While in certain regions of the world one becomes aware of this important problem, in other regions antibiotics are still routinely used as a preventive strategy, and thus often used without any diagnosis or antimicrobial susceptibility testing of the targeted bacteria. The global expansion of specific *Salmonella* Typhimurium strains (for example DT104 strains) can be explained partially by antimicrobial resistant strains that contain a gene cluster making the strains resistant to ampicillin, chloramphenicol, sulfonamides, streptomycin and trimethoprim (ACSSuT type) (Leekitcharoenphon et al., 2016). Variants of this resistance gene cluster were found in strains form other serotypes as well. Of particular concern is the emergence of fluoroquinonolone resistant *Salmonella* strains as these antibiotics are used to treat life-threatening *Salmonella* infections in adult humans (Wasyl et al., 2015), and the emergence of ESBL-type *Salmonella* strains, resistant to cephalosporins (Silva et al., 2013). The use of quinolones and cephalosporins in poultry is likely to be the cause of the emergence of these strains. Although specific countries (e.g. in EU) have action plans to reduce antibiotic usage in poultry, the future with regard to the emergence of these strains on a global scale is frightening. Indeed, it has been estimated that the worldwide antimicrobial consumption by livestock will rise by 67% by 2030 and nearly double in Brazil, Russia, India, China and South Africa, driven by the growth in consumer demand for livestock products in middle-income countries and shift to large-scale intensive farming (Van Boeckel et al., 2015). Antimicrobial resistant *Salmonella* will thus become a very significant health risk in certain regions of the world, potentially leading to high mortality in both animal and human populations due to the inability to treat infections of such strains effectively.

Specific virulence traits can be a cause of a future pandemic

The virulence of *Salmonella* is an important aspect in both host colonization and spread between hosts. It is well known that specific serotypes and strains within a serotype can differ in the ability to colonize the gut and the spread to internal organs, including the reproductive tract (Gantois et al., 2008), and the spread within an animal population. *Salmonella* carries a variety of virulence genes and pathogenicity islands, and genetic differences between strains (additional genes, point mutations …) clearly play a role whether or not a strain will regionally or globally emerge. In addition, a strain that colonizes and spreads in poultry is not necessarily important for human food-poisoning. The behavior of *Salmonella* in or on the food vehicle, such as in eggs, is important as well. Indeed, strains from *Salmonella* serotype Enteritidis (and to a lesser extent Typhimurium) can survive in egg white while strains belonging to other serogroups do not display this characteristic, although they are able to colonize the live bird (De Vylder et al., 2013). Survival in egg white and colonization of the reproductive tract is a key virulence trait that enabled Enteritidis to cause the pandemic that is still going on now. Although many genes are identified as potential causes of the predilection of *Salmonella* Enteritidis for eggs, it is still unclear why this particular serotype is associated with eggs while others are not. In the future, it could be that specific strains, possessing certain virulence traits of yet unknown function, become increasingly important. However, the experience obtained while dealing with the current pandemic will likely result in a quicker and more efficient response in the future, i.e. in tracing origins, monitoring, and vaccine development, among others.

Global warming as potential future problem

The average temperature has globally increased with about 1°C the last 100 years and sea level is increasing about 2 mm each year. It is estimated that by 2100 the global temperature will increase by between 1.8 and 4°C (Patz et al., 2008), while changed weather conditions will lead to periods of droughts and flooding. A clear correlation has been described between ambient temperature and *Salmonella* preva-

lence in humans (Lake et al., 2009; Akil et al., 2014), and is attributed to increased *Salmonella* proliferation in the food chain and influences on people's behavior (eating more raw products, more risky cooking practices). Although not yet studied, it can be hypothesized that the risk of environmental proliferation of *Salmonella* in more humid and warm environments is also increased and that this will have consequences for the spread of *Salmonella* to animals. As an example, when *Salmonella* contaminated manure is used as fertilizer, it seems obvious that these bacteria proliferate more easily and environmental contamination is higher when temperatures are higher, not only leading to raw plant product contamination, but possibly also to poultry feed ingredient contamination. Additionally, flooding has been identified as a risk factor for avian influenza (Van Boeckel et al., 2012), but it can as well be a risk factor for spread of *Salmonella* in the environment. This is especially the case in developing countries, where intensive poultry farming is distributed in regions of high human population density and with good access to large cities (Van Boeckel et al., 2012), and as these cities are often located near rivers or the coast, climate change and flooding will increase the risk of *Salmonella* spread to the farm environment and between farms. Another consequence of global warming will be the increase in the number of live vectors when the temperature in certain regions increases (e.g. insects), further contributing to the spread of *Salmonella*. However, at the moment, it is too early to fully assess the effect of global warming on *Salmonella* prevalence in poultry, but it is believed that the environmental conditions associated with global warming can increase the likelihood of infection spread.

Future perspectives

Globally, there is still a huge difference between countries that have installed monitoring programs for *Salmonella* in animals, and countries that are not monitoring for *Salmonella*, and thus have no data on the prevalence, sources and trends. In addition, control programs for *Salmonella* have been very effective in controlling *Salmonella* in the food chain in specific countries, while in other countries the use of control measures against *Salmonella* is poorly developed. *Salmonella* control should comprise an approach that encompasses different methods at different steps of the production chain, and should target the live animals (breeders, hatchery, and broilers/layers) but also include measures at the cutting plants and slaughterhouses, and at retail. During primary production, control tools such as vaccination and feed additives should always be accompanied by a strict biosecurity plan. The 'best-case' scenario is thus that monitoring and control programs should be installed worldwide, analogous to the system used in for example the EU, as this has proven to be efficient in reducing the *Salmonella* prevalence in chickens and consequently in humans. This would require a global coordination and governmental support, and clearly in many countries there are more severe diseases than food poisoning by *Salmonella*, making the decision to spend efforts and money in *Salmonella* control likely less urgent. Examples are tuberculosis, HIV, malaria and many more. Without a global *Salmonella* control strategy however, there will always be the risk of introduction and spread of *Salmonella* from highly contaminated regions to other regions, and these highly contaminated regions will form a reservoir for emerging strains or serotypes with specific, potentially dangerous characteristics. On the other hand, the 'worst-case scenario' would be the emergence of a strain with the perfect combination of traits to cause a new pandemic. Fortunately, when looking at the past global *Salmonella* problems with Enteritidis and Typhimurium, the egg-contaminating serotype Enteritidis does not easily take up foreign DNA so that antimicrobial resistance in this serotype is low, and the serotype Typhimurium, that easily becomes antibiotic resistant and even multiresistant, did not develop a predilection for eggs. Facing a multi-resistant egg-contaminating *Salmonella* strain, that is easily transmitted because of the intensification and globalization of the poultry industry, combined with increased environmental survival and spread because of global warming, and taking into account that certain world regions will not rapidly develop monitoring and control programs and thus act as a continuous reservoir, could have devastating consequences in the future.

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L39 Food−borne diseases of poultry in China

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Abstract

Food safety represents a primary public health issue throughout the world, including the major threats posed by food-borne bacterial diseases. In this review, we summarize the prevalence of food-borne bacterial zoonoses, including salmonellosis, campylobacteriosis, and infections caused by *Listeria monocytogenes* in China. Countermeasures against these bacterial diseases are summarized, including the need for improved food safety regulations and standards, strengthened prevention and control strategies for animal infectious diseases, and the establishment and improvement of early warning and control systems for food-borne diseases. Industry management and corporate self-management in food production and trading businesses, consumer education, and active international collaboration are also discussed.

Keywords: food-borne, bacterial zoonoses, *Salmonella*, *Campylobacter jejuni*, *Listeria monocytogenes*, prevalence, countermeasures

Food safety represents a primary public health issue throughout the world, including the major threats posed by food-borne diseases. The World Health Organization (WHO) defines food-borne illnesses as "diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food," including those caused by biological contamination and chemical residues. It has been reported that 1.8 million people died from diarrhoeal diseases in 2005 (http://www.who.int/mediacentre/factsheets/fs237/en/), which were largely attributed to the contamination of food and drinking water.

Prevalence of Food-borne Bacterial Zoonoses in China

Incomplete statistics in China show that of all the food poisoning cases between 1992 and 2001, 50.9% were classified as bacterial food poisoning, 28.6% as chemical food poisoning, 11.9% were attributed to unknown reasons and 9.6% were caused by other reasons (http://www.chinacdc.net.cn). The major food-borne zoonotic pathogens include *Salmonella* spp., *Campylobacter jejuni* (*C. jejuni*), *Vibrio parahaemolyticus* (*V. parahaemolyticus*) and *Listeria monocytogenes* (*L. monocytogenes*).

Salmonellosis

Prevalence of human salmonellosis in China

The Chinese Centre for Disease Control and Prevention (China CDC) reported in 2007 that as the leading cause of food poisoning, *Salmonella* is accountable for 13.8% of all food poisoning cases (http://www.chinacdc.net.cn). Wang *et al.*[1] reviewed 2447 papers from journals published in China between 1994 and 2005, and reported that among 181 *Salmonella* food-borne disease events, serogroup D accounted for 45.30% of these, followed by serogroup B (22.65%), serogroup C (7.73%), serogroup E (5.52%) and serogroup F (1.10%). Of the salmonellosis events for which the serotype was determined, *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) caused the most events (28.73%) followed by *S.* Typhimurium (14.36%), *S.* Dublin (5.52%), *S.* Blegdam (4.42%), *S.* Typhi (3.31%), *S.* Derby (2.21%), *S.* Saintpaul (2.21%), and *S.* Newport (2.21%). All other serotypes caused very small percentages of the total reported cases.

Wang *et al.*[2] demonstrated that the *Salmonella* isolates recovered from meat samples and human cases in the Jiangsu Province of China were commonly resistant to multiple antimicrobials, and genes conferring antimicrobial resistance in these isolates were often carried on integrons and plasmids that could be transmitted through conjugation. These mobile DNA elements might play an important role in the

transmission and dissemination of antimicrobial resistance among *Salmonella* strains. Molecular tracing was also conducted for 354 isolates of *Salmonella* spp. from 10 provinces in China, of which, 237 isolates were from chicken, 81 isolates were from animal food products and 36 isolates were from the intestines of people whom worked in the catering industry. Pulsed-field gel electrophoresis (PFGE) indicated that some of these human strains were similar to the food-borne strains, with a similarity value of 0.86 – 1, indicating that human salmonellosis is associated with *Salmonella* contamination of food products.

Prevalence of Salmonella contamination in food

Yang *et al.* reported that approximately 54% (276) of chicken, 31% (28) of pork, 17% (13) of beef and 20% (16) of lamb samples were positive for *Salmonella* from a total of 764 retail meat products, including 515 chicken, 91 pork, 78 beef and 80 lamb samples collected from Shaanxi Province in China during 2007 – 2008 [3]. Among 24 serovars identified, *S.* Enteritidis (31.5%) was the most common, followed by *S.* Typhimurium (13.4%), *S.* Shubra (10.0%), *S.* Indiana (9.7%), *S.* Derby (9.5%) and *S.* Djugu (7.0%). This study indicated that *Salmonella* contamination was common in retail meats, and that the isolates of this species were phenotypically and genetically diverse. More recently, Zhu et al reported prevalence and quantification of *Salmonella* contamination in raw chicken carcasses at the retail in China [4] .

Using the microbiological risk assessment model developed by WHO/Food and Agriculture Organization (FAO) or the Food Safety and Inspection Service/Food and Drug Administration of the USA, Fan *et al.* analysed the relationship between food-borne diseases and contamination of *Salmonella* spp. in catering foods [5,6].

Prevalence of Salmonella in animals

Lu *et al.* collected 311 *Salmonella* isolates from a chicken hatchery, chicken farms, and chicken slaughterhouses in China in 2008 [7]. Two *Salmonella* serotypes were detected, of which, 133 (42.8%) were identified as *S.* Indiana and 178 (57.2%) as *S.* Enteritidis. The highest positive rate of *S.* Indiana was detected in the slaughterhouses (71.4%), followed by the chicken farms (54.9%) and the chicken hatchery (4.2%). More than 80% of the *S.* Indiana isolates were highly resistant to ampicillin (97.7%), amoxicillin/clavulanic acid (87.9%), cephalothin (87.9%), ceftiofur (85.7%), chloramphenicol (84.9%), florfenicol (90.9%), tetracycline (97.7%), doxycycline (98.5%), kanamycin (90.2%), and gentamicin (92.5%). About 60% of isolates were resistant to enrofloxacin (65.4%), norfloxacin (78.9%), and ciprofloxacin (59.4%). Common PFGE patterns were found in most isolates from the chicken hatchery, chicken farms, and slaughterhouses, suggesting that many multidrug-resistant strains of *S.* Indiana were prevalent in the three sources. More recently, Cui *et al* further studies prevalence and antimicrobial resistance of *Salmonella* isolated from an integrated broiler chicken supply chain in Qingdao, China[8].

Campylobacteriosis

Prevalence of C. jejuni in diarrhoea patients

Epidemiological surveillance of C. jejuni in diarrhoea patients was conducted in China from 2005 to 2006[9] and of 3061 diarrhoea patients, 148 (4.84%) were positive for this species. Notably, the isolation rate of *C. jejuni* from outpatients (6.30%, 138/2191) was significantly higher than that from ward patients (1.15%, 10/870) (P<0.01). Stool specimens from patients with diarrhoea or gastroenteritis were cultured for *Campylobacter* spp. in central Taiwan, China [10]. Of 6,540 patients with diarrhoea or gastroenteritis, 162 *Campylobacter* isolates were identified, yielding an isolation rate of 2.5% from the entire population studied.

A wide range of phenotypic and genotypic typing systems have been developed and used for the epidemiological typing of *Campylobacter* spp [9, 11, 12].

Prevalence of C. jejuni in food

A total of 95 chicken samples that consisted of 34 whole chickens, 32 organs, and 29 chicken parts, collected from traditional retail markets and supermarkets in Taipei, were examined for the occurrence of enteropathogenic *Campylobacter* spp. [13]. The results showed that there were markedly different isolation rates of *Campylobacter* spp. between supermarket and retail market ($P < 0.05$). Enteropathogenic

Campylobacter (*C. jejuni* and *C. coli*) were found on 68% of whole chickens, 100% of chicken parts, and 100% of organs from retail markets. In supermarkets, the isolation rates of these *Campylobacter* spp. from whole chickens, chicken parts, and organs were 42%, 53%, and 60%, respectively. Yang *et al*. developed a PCR assay for the quantitative detection of *C. jejuni* in naturally contaminated poultry, milk and environmental samples without the need for an enrichment step [14]. More recently, Huang *et al* reported a quantitative survey of *Campylobacter* on retail raw chicken in Yangzhou, China[15].

According to risk assessment results for *C. jejuni* in chicken consumed by Chinese residents, it is possible to establish feasible measures, in terms of standard management practices, to reduce the risk of infection [6,15,16].

Prevalence of C. jejuni in animals

A total of 2609 faecal samples were collected from adult chickens aged ⩾6 months in the middle, southern and eastern parts of China [9]. Of 30 poultry flocks tested, 26 (86.67%) were *C. jejuni*-positive. Among 3132 chickens, 583 (18.61%) were positive for *C. jejuni* and the prevalence of infection varied from 0 to 73.33% between different flocks. The *C. jejuni*-positive rate of grandparent chickens (28.71%, 244/850) was significantly higher than that of parent (13.00%, 52/400) and progeny commercial chickens (18.03%, 245/1359) (P<0.05). Variation in *C. jejuni* prevalence was also observed between different breeds, such as Ai-jiao (73.33%), Nong-da (51.30%), Luo-man (33.90%), Xin Pu-dong (33.33%), and Guan-xi (37.33%).

The prevalence and antimicrobial resistance of *Campylobacter* spp. in broiler chickens were determined in Shandong Province, China[17]. In total, 275 *Campylobacter* isolates were obtained from 767 broiler cecal samples, including 208 *C. jejuni*, 53 *C. coli*, and 14 unidentified *Campylobacter* isolates. The vast majority of the *Campylobacter* isolates were classified as multidrug resistant.

A wide array of interventions has been developed to reducing the carriage of *C. jejuni* in livestock and poultry. Vaccination of poultry against *C. jejuni* is potentially the most effective measure, and remains a major strategic goal. A prophylactic strategy using chitosan-DNA intranasal immunization to induce specific immune responses has now been described and might facilitate disease control [18].

Infections caused by L. monocytogenes

WHO reports indicate that 4% – 8% of aquatic food products, 5% – 10% of milk, 30% of meat products and 15% of poultry products are estimated to be contaminated with *L. monocytogenes* (http://www.who.int/mediacentre/factsheets/fs237/en/). Monitoring and source tracking of *L. monocytogenes* have important public significance. To gain a better understanding of the prevalence of *Listeria* in Chinese food products, Chen *et al.* [19] reviewed the relevant studies published in China from 2000 to 2007. The average recovery rate of *Listeria* spp. was 3.7% (0.1% – 7.7%) in all food categories for 13 provinces, with raw meat being the leading source of infection. *L. innocua* (28.9%, 271/937) and *L. monocytogenes* (25.3%, 237/937) were more commonly isolated from all food types. Subtyping analysis in three laboratories in different provinces revealed that the majority of the *L. monocytogenes* isolates belonged to lineage II (67.1%), followed by lineage I (31.6%), which included the pathogenic serovars 1/2a, 1/2b, and 4b isolates. Lineage III isolates comprising the low-pathogenic serovar 4a were rare.

Zhou et al. further monitored the *L. monocytogenes* in the food products of retail markets and environmental samples [20]. The prevalence of *L. monocytogenes* (4.83%) in raw meat products was significantly higher than that in other raw food products. Amongst 844 RTE food samples, 21 samples were positive for *L. monocytogenes*. RTE packaged food products from two supermarkets had a prevalence ranging from 0.00 – 25.00%. The prevalence of *L. monocytogenes* in meat products of freshly slaughtered hogs was 0.95%, significantly lower than that observed in raw meat products from retail markets. Ten isolates were recovered from 645 water samples, which were collected after the hands of shopkeepers or waiters were washed.

PFGE analysis showed that *L. monocytogenes* in the same market had a high similarity (94%). The genetic similarity of *L. monocytogenes* between RTE market and CSF samples was 86.8% indicating that the infection was likely to be derived from the market sources. These isolates were further characterized into molecular subtypes based on DNA sequencing of the 597 bp 39-terminal region of the virulence gene *act*A [21].

Strategies for the Prevention and Control of Food-borne Bacterial Zoonoses

We firmly believe that with the promulgation and implementation of "Food Safety Law of the People's Republic of China" (2015 Revision) [22], it can have great significance for implementing food risk prevention and control measures and achieving food safety objective and further protecting public health.

Improving food safety regulations and standards
Strengthening the prevention and control of animal infectious diseases
Establishment and improvement of early warning and control systems for food-borne diseases
Enhanced rapid diagnosis and source-tracing technology for food-borne diseases
Establishment of a pro-active surveillance network for food-borne diseases
Risk assessment of pathogens in food
Strengthen industry management and corporate self-management in food production and trading business
Consumer education
Active international collaboration

Acknowledgements

This work was supported by the grant 31230070 from National Natural Science Foundation of China, the grant 2014BAD13B02 from National Key Technology R&D Program and the grant from the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

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L40 Research progress on healthy environment and housing systems for laying hens in China

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Keywords: laying hen, housing system, slightly acidic electrolyzed water, environment, welfare

Summary

Along with the economy, egg production in China has experienced tremendous development and expansion during the past thirty years. The industry has rapidly changed its production structure toward to large-scale production since the beginning of the new century. Stair-step cage system and stacked cage system are the two primary production systems. Both of them can provide a good hygiene during production. However, the poor leak-proof and heat-insulating properties of the layer buildings can easily cause bird diseases in China. Providing enough space for the physical exercise and natural behavior expression of hens will improve their abilities to resist diseases. To improve the hygiene of indoor environment and the disease resistance of birds, a new housing system named large cage aviary units (LCAU) is developed and spraying slightly acidic electrolyzed water (SAEW) is used for disinfection. Pilot-scale experiments on LCAU were conducted for laying hens and layer breeders. Peak egg production (90 plus percent) lasted about 6 months with an average egg laying rate of 91.4% (23-46 weeks), with egg laying rate increasing with the stocking density of 10-15 birds/m^2. For layer breeders, the average fertilization rate reached more than 94% during the period of peak egg production. The results suggest that LCAU is a proper alternative system for healthy egg production, especially for family farms. Spraying SAEW has been proven to be an alternative and environmentally friendly approach for reducing the microbial populations in layer houses. The airborne bacterial reduction by spraying SAEW is influenced by available chlorine concentration of SAEW, spraying volume, size of sprayed aerosols, and ventilation. Spraying with medium size aerosols (count median diameter=60-90 μm) is recommended.

Introduction

Over the past 30- years, egg production has experienced tremendous development and expansion along with the overall economy of China. However, the poor leak-proof and heat-insulating properties of the layer buildings are still common in China. There is big temperature differences between winter and summer time in North China. Sudden fluctuation of temperature usually causes bird diseases and affects birds' health. "Health" is a state in which the animal and the environment are in a normal dynamic balance. If this balance is broken, animals will easily get diseases. The decrease of body defense function for animals under modern high-intensive production is an important internal cause for broken balance. And the housing environment contaminated with a high population of pathogenic microorganisms, and the transmission of pathogenic microorganisms is an important external trigger of broken balance. For healthy egg production, both of these two aspects need to be considered, a good health condition of the animal and a good hygiene of the environment.

Increasing the locomotion of birds will contribute to enhancing their health. Currently, stair-step cage system and stacked cage system are the two primary production systems in China. These traditional cage systems cannot provide enough space for normal behavior expression and physical exercise of hens, which further increases their susceptibility to diseases. As the alternative systems, aviaries and multi-tier systems can fulfill the needs of behavior expression [1] and welfare [2]. These systems are featured with litter provision on the ground, fulfilling birds' motivation to dust bathing and foraging. However, the use of litter, usually with manure together, causes some problems in these systems. High ammonia and dust concentrations are detrimental for the respiratory tracts of both human and birds [3-5]. It is reported the dust concentration in conventional cages ranges from 0.035-1.7 mg/m^3, while in the aviary housing sys-

tem it ranges from 0.17-14 mg/m^3 [4]. Ammonia concentration in litter-based systems is 1.9-47.4 ppm which is much higher than 0.40-13.5 ppm in cage systems [6]. De Reu et al. demonstrated that litter-based systems had 10 times more airborne bacteria in the environment and 20-30 times more bacteria on egg shells compared with cage systems [7]. Direct contact with feces and dust for birds is at the risk of disease infection for pathogenic *Escherichia coli* [8, 9]. Occurrence of bacterial disease for laying hens housed in litter-based systems is significantly higher than in cage systems (P<0.001), accounting for a 72.9% and 65% of mortality in different systems respectively [10]. Studies also reported that birds in aviaries have higher incidence of bumble foot [11], more injuries on the soles of the feet [12].

Environmental disinfection is commonly used for disease-prevention in animal housing [13-16]. Disinfection can help to lower the potential for disease infection and transmission in animals by reducing the population of pathogenic microorganisms on the surfaces or in the air. Numerous chemical disinfectants such as benzalkonium chloride, formaldehyde and glutaraldehyde are commonly used for disinfection against bacterial infections in animal buildings [17]. However, utilization of chemical disinfectants has limited potential due to their toxic, corrosive and/or volatile problems [18], and resistance of pathogens to chemical disinfectants has been reported [19, 20]. Slightly acidic electrolyzed water (SAEW) has been considered healthy and environmentally friendly because no hazardous chemicals are added in its production, it causes less corrosion of surfaces and it minimizes the potential for damage to animal and human health. Due to these reasons, SAEW is an alternative disinfectant for decontamination in animal houses.

This paper will cover (1) a new housing system named large cage aviary units (LCAU), aiming to improve the hygiene of indoor environment and the disease resistance of birds; (2) an alternative disinfection approach by spraying SAEW, aiming to decrease the possibilities of disease infection for birds.

Development of LCAU system

Detrimental impacts on indoor environment for the litter use in aviaries have violated the original intention for the protection of five freedoms [21]. Stair-step cage system and stacked cage system can provide a good hygiene during production but not enough space for normal behavior expression and physical exercise. A housing system that combines the pros of both conventional cages and aviaries was developed. This new system is named LCAU. It frees the hens from imprisonment, enables them to express natural behaviors and provides them sufficient space for activities, resulting in the improvement on defense function of layers. LCAU is featured for big colonies with multi-tier raised netting floor and manure belt under the wire-mesh floor. Birds can access to three-dimensional space inside the colonies without direct contact with feces.

Several investigations were conducted to optimize design parameters of LCAU before the pilot-scale experiments, including:

(1) Effect of stocking density on the production performance of laying hens;

(2) Effect of nest design on the egg distribution on egg belt and floor eggs for laying hens;

(3) Selection for perches of laying hens: perch size, arrangement;

(4) Effect of group size on the performance of layer breeders.

Results showed that the egg laying rate increased with the stocking density of 10-15 birds/m^2. As shown in Figure 1, the egg laying rate during peak egg production was more than 96% and the average egg laying rates of 23-46 weeks were 89.1%, 91.1% and 93.8% for the stocking density of 10, 13.5 and 15 birds/m^2, respectively. Transforming group-nests (150 cm width) into smaller ones (37 cm and 48 cm width) had a positive impact on homogenizing eggs distribution and decreasing floor eggs. Eggs distribution was significantly influenced by separation (P<0.01) and the proportion of mislaid eggs was decreased by 3.8% in 37 cm width (P<0.05) and by 4.6% in 48 cm width (P<0.05) respectively. All of perch size, arrangement and material had impact on perch usage and behavior expression. Perches of 45mm were preferred than 32mm, 20mm and 25mm, indicated by use time, stability and the number of roosting hens at night. Perches in vertical rows were used less by birds in higher place but more at lower area compared to perches of stair step (Table 1). As expected, perches in lower place were used frequently in daytime and perches in higher place were used more at night. For layer breeders, the average fertilization rate reached 95.1% in groups of 110 birds (G110) and 94.2% in groups of 220 (G220) at a ratio of 1 ♂:10 ♀ during peak period, respectively (Figure 2). The fertilization rate in G110 was higher than that in G220. No outbreak of aberrant behaviors during production happened for both laying hens and layer breeders.

Table 1 The accumulative spent time at different perch in two perch layout (Mean ± SE; unit, min)

	h1	h2	h3	h4
Stair-step	124.0±3.1^{A}	58.7±2.8^{B}	38.0±6.1^{C}	35.7±3.5^{C}
Vertical rows	156.7±8.4^{A}	38.0±3.5^{B}	15.3±0.3^{C}	3.0±1.0^{C}
P-value	0.021	0.010	0.020	0.001

h1 to h4: different height of perches, 40cm, 70cm, 100cm, 130cm from the floor respectively. *P*-value: probability values less than 0.05 were considered as significant. $^{A, B, C}$: Different superscripts of capital in a row indicated significant differences among different height ($P<0.01$).

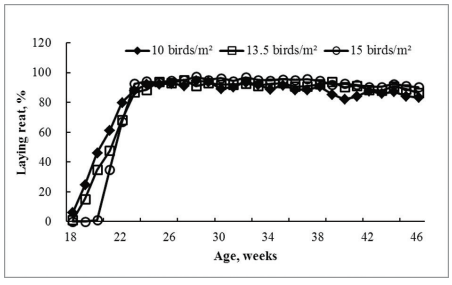


Figure1 Egg laying rates for different stocking densities

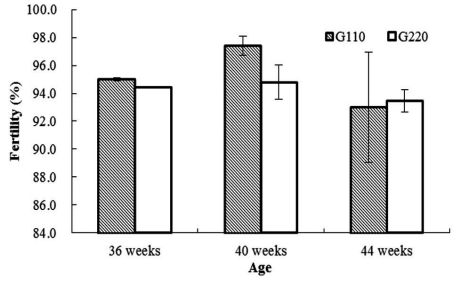
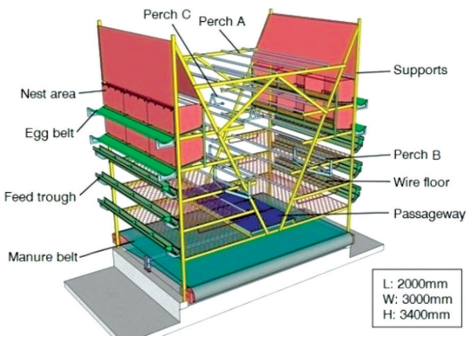


Figure2 Fertilization rates for different group sizes

As shown in Figure 3, the system is designed to increase the efficiency of house space utilization by using big colonies with multi-tier raised netting floor instead of conventional cages. Sufficient space for activities and natural behaviors is provided and different proper functional areas are allocated. Each unit, measuring 2 m L×3 m W×3.4 m H, can accommodate more than 108 birds. Animal behaviors, like nesting and perching are critical for concern to improve animal welfare. This system provides perches, nest boxes, egg belt, drinkers, feeders inside the colonies and manure-collection belt under the wire-mesh floor. Wire-mesh floor is used instead of litter floor to ensure the indoor air quality. Manure belts are equipped under the wire-mesh floor, taking feces out of the house every day. Above the wire-mesh floor, two-tier platforms with nests on the top are symmetrically set in the system. Nests are on the top of the platforms to provide private place for egg laying and egg belts are outside of the nests for egg collection. Observations from several studies revealed that hens prefer lower areas in daytime in multi-tier systems, so feed troughs and nipple drinkers are equipped at the level of platforms. The platforms have a slope of 7 degrees to avoid hens laying egg on them. Perches are equipped between the two symmetrical sides. Five highest perches are set over the nests for roosting during night. Perch arrangement is based on the criteria that the angle of up-down path is no more than 45 degrees and the horizontal distance should not exceed 50 cm.



(a)

(b)

Figure3 Schematic figure of the system (a) and view of the pilot−scale application (b) application (b)

Spraying SAEW in animal houses

Animal housing environment is often contaminated with pathogenic microorganisms. To reduce the risks of diseases caused by pathogenic microorganism in the indoor environment and the disease transmission between barns, disinfection to the environment is recommended. Spraying slightly acidic electrolyzed water (SAEW) has been proven to be an alternative and environmentally friendly approach for reducing the microbial populations in layer houses.

Generation and disinfecting mechanism

Electrolyzed water (EW) is produced by electrolysis of a dilute salt solution in an electrolytic cell. By subjecting the electrodes to direct current voltages, negatively charged ions such as chloride and hydroxide move to the anode to give up electrons and become available chlorine, including chlorine gas (Cl_2), hypochlorite ion (ClO^-) and hypochlorous (HClO) [22]. The level of available chlorine and the proportion of available chlorine in SAEW have been considered to be the main factors affecting its antimicrobial activity. EW with a pH of 5.0-6.5 is defined as SAEW [23-25]. The antimicrobial activity of SAEW relies on its available chlorine, primarily in HClO, which has strong antimicrobial activity than ClO^- and Cl_2. The highest proportion of HClO of EW was found at around pH 4-5. It has been reported to possess high antimicrobial activity against a broad spectrum of microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Salmonella spp.*, *Bacillus subtilis*, *Bacillus cereus*, *Listeria monocytogenes*, mold and yeast, *Vibrio vulnificus* and *Vibrio parahaemolyticus*, porcine reproductive and respiratory syndrome virus and pseudorabies virus.

Generation system of SAEW

One common system is to generate SAEW by electrolysis of a dilute HCl solution in a non-membrane electrolytic cell, then the produced highly concentrated HClO is diluted with tap water [26]. The SAEW generated in this system usually has a low available choline concentration (ACC, 5 to 50 mg/L, preferably 20 to 30 mg/L), but most of available chlorine is present as HClO. This system has been widely employed to generate SAEW with a low ACC for decontamination in the food industry. Another generation system is to generate SAEW by electrolyzing dilute NaCl and HCl solutions in a non-membrane electrolytic cell [27]. In this system, NaCl provides most of Cl^{-1} to generate available chlorine, and HCl is mainly to adjust the pH to 5.0-6.5. HCl solution can also be added after electrolyzing a dilute NaCl solution to adjust the pH. Using this system, SAEW with a large range of ACC was generated, from 0.5 mg/L-400 mg/L. Significant amounts of organic matter which can significantly reduce the bacterial activity of SAEW is usually attached in the animal houses. Therefore, SAEW with high ACC generated using this system is usually used for reducing microbial populations in animal houses.

Spraying SAEW in laying hen houses

We have pioneered SAEW application as a sanitizer in the egg industry, a large number of studies have been done on spray disinfection using SAEW. Spraying or flushing using SAEW has been proven to high-effectively reduce the microbial concentrations on the structural surfaces and equipment surfaces in the animal buildings [23-25]. Zang et al. reported that cleaning with tap water and spraying SAEW can inactivate *Salmonella Enteritidis* on the surface of plastic poultry transport cages [28]. The inactivation activity increased with increasing cleaning time, treatment time, and ACC of the SAEW. Spraying SAEW was also suggested to be a sanitizing solution for shell eggs [26, 29]. It is reported that SAEW has an equivalent or higher efficiency in reducing E. coli O157:H7, S. aureus, S. enteritidis and indigenous microbiota present on eggshells compared to chlorine dioxide and NaClO solution, and has similar bactericidal activities with AEW at the same ACC (60-100 mg/L).

Spraying SAEW or NEW is suggested to be an efficient approach to reduce airborne bacterial contamination in layer poultry houses [16, 30]. The ability of spraying SAEW to reduce airborne bacterial contamination in a laying-hen house showed a dose-dependent relationship with the ACC of SAEW and spraying volume [16, 31]. When comparing the change in airborne microbial populations after spraying SAEW or water in a layer chamber, Zheng et al. demonstrated that airborne culturable bacteria were reduced more by the bactericidal effect of SAEW than by the reduction in airborne particular matter [32]. Available chlorine loss caused by spraying is greatly dependent on the size of sprayed aerosols. The size of sprayed

aerosols can also influence the gas-liquid contact, which is important to the probability of exposure of airborne microbes to sprayed SAEW or NEW aerosols. Smaller sprayed aerosols may promote the gas-liquid contact but cause greater loss of available chlorine during spraying. Zhao et al. reported that an initial available chlorine loss of 11.7-13.2% when spraying SAEW with an aerosol count median diameter of 80 μm [33]. Our recent study evaluated the reduction efficiency of airborne culturable bacteria by spraying SAEW with different aerosol count median diameters in a controlled environment chamber. Spraying with medium size aerosols (count median diameter = 60-90 μm) is recommended for disinfection in animal houses. Spraying SAEW was also reported to be useful for scrubbing air exhausted from a poultry house by reducing ammonia and culturable bacteria [34].

Conclusions

LCAU is an alternative system for both laying hens and layer breeders which combines the pros of both conventional cages and aviaries. This system can provide birds with sufficient space for activities and also avoid direct contact to manure for birds. Birds can eat and drink ad lib and had good production performance in this system. Access to nests and perches is the primary enrichments at present. For further improvement, claw shortening, scratching pad, access to enclosed-outdoor or solar-house for dust-bathing will be equipped. Researches have revealed that SAEW is a novel disinfection agent for reducing microbial presence in laying hen housing environment, which is highly effective, healthy, and environmentally friendly.

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L41 Environmental challenges and opportunities with cage-free hen housing systems

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Abstract

The vast majority of egg production in the world has been done using conventional cage systems. In the United States, cage production system accounts for approximately 90% of the total flock. Continued advancements in hen genetics, nutrition, disease prevention, flock management, and product handling have made eggs one of the wholesome, safe and affordable animal proteins for the growing population. In the past decade, increasing concerns about farm animal welfare have led to shifts toward alternative egg production, either through local/national legislations or voluntary decisions, notably in the European Union and the United States. Most recently in the United States, an increasing number of food distributors, restaurants, grocers and travel service sectors have pledged to source only cage-free eggs by 2050. With the amount of eggs being used by these sectors, it will require nearly 60% of the total U.S. laying-hen flock (or 167 million hens) as cage-free hens by 2050 to meet the pledged commitments. The current U.S. national non-organic cage-free hens account for 5.4% (9.9% including organic eggs) of the total flock. Cage-free egg production has a number of environmental challenges, such as indoor air quality (ammonia, particulate matters, and airborne bacteria concentrations), air emissions, uniform distribution of thermal conditions throughout the barn during cold weather, and proper environmental design and operation to minimize floor eggs. Research and innovation are eminently needed to improve environmental conditions and sustainability of cage-free hen-housing systems.

Keywords: alternative hen housing, animal welfare, environmental impact, sustainable eggs

Statement of the situation

Global animal agriculture has made remarkable advancements during the past half of a century in terms of productivity, product quality and diversity, and operational efficiency. A recent life cycle analysis study revealed that from 1960 to 210 the environmental footprints (greenhouse gas, acidifying and eutrophying emissions) per unit of egg output decreased by 65 to 71% for the U.S. egg industry (Pelletier et al., 2014). To date the vast majority of commercial table egg production has been done using conventional cages with each cage holding 6-8 hens. For the United States (with approximately 308 million table egg laying hens), conventional cage egg production accounts for approximately 95% of the total non-organic flock.

In recent years, animal welfare-driven legislations in certain parts of the world have led to shift of the egg production pendulum. By January 1, 2012, conventional cages for egg production were banned in the EU. January 1, 2015 marked the implementation of the California [USA] Proposition 2, passed in 2008, which *"requires that calves raised for veal, egg-laying hens and pregnant pigs be confined only in ways that allow these animals to lie down, stand up, fully extend their limbs and turn around freely."* As a result, shell eggs sold in California must be produced by laying hens provided a minimum space allocation of 748 cm^2 (116 in^2) per hen. The most recent development in the United States is the growing pledges of shifting toward cage-free eggs. As of April 29, 2016, 38 grocery chains, 38 restaurant chains, 6 food distributors, and 7 hospitality and travel firms have pledged to source only cage-free eggs by 2025; and the list seems to grow by the day. At the current rate of demand for eggs, these pledges will require an estimated of more than 45 billion cage-free eggs per year, which translates to 167 million cage-free hens by 2025. Compared to the current non-organic cage-free flocks of 16.4 million hens and another 13.4 organic cage-free hens in the USA, these pledges will require additional 137 million hens to meet the commitments over the next 9 years and beyond (USDA NASS data, compiled by the Egg Industry Center, 2016).

A sustainable egg supply must be built on a set of foundations that not only include animal welfare, but also environment impact, food safety, food affordability, worker health and ergonomics. It was based on this principle that the Coalition for Sustainable Egg Supply (CSES) Project was recently completed in the United States-a result of public-private partnership among foodservice sectors, academia, government agency, and NGO's. The study systematically evaluated three types of hen housing systems-conventional cage, enriched colony,

and cage-free aviary, all with manure belts (expect that the aviary house also had litter floor) (http://www2.sustainableeggcoalition.org/). It demonstrated the trade-offs of each system with regards to the interdependent foundational components.

Figures 1 and 2 depict the schematic representation of a typical manure-belt conventional cage house and a cage-free aviary hen house. The conventional cage house has a capacity of 200,000 hens, whereas the cage-free aviary hen house has the capacity of 50,000 hens.

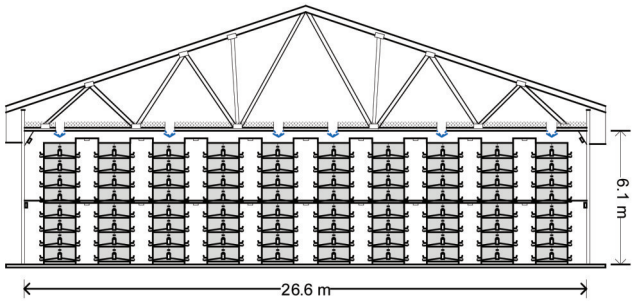

Figure 1. Schematic representation of a typical conventional cage manure−belt hen house.

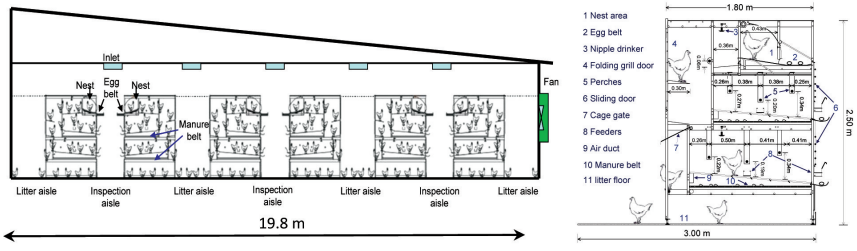

Figure 2. Schematic representation of a cage−free aviary hen house, where hens have access to the litter floor and the colony areas (where the feeders, drinkers, perches and nestboxes are located).

Environmental challenges with cage-free egg production systems

Housing style and manure management schemes have profound impacts on the indoor environment and emissions to the atmosphere (Xin *et al.*, 2011). For instance, use of manure belts to regularly remove manure out of the henhouses significantly reduces indoor ammonia levels and ammonia emissions to the atmosphere as compared to indoor manure storage used in high-rise houses. Even when emissions from on-farm storage are included, ammonia emission from a manure-belt housing system is still only about one-third of that from its high-rise house counterpart. When indoor ammonia is under control without the need for extra ventilation, minimum ventilation rate in the wintertime is used to control indoor relative humidity. This lower ventilation rate helps maintain the ideal house temperature while minimizing the need for supplemental heat. In fact, the CSES study revealed that even under the cold winter climatic conditions in the Midwest USA, an enriched colony henhouse with a lower stocking density of 748 cm^2 (116 in^2) (*vs.* the typical stocking density of 432 cm^2 or 67 in^2/hen) was able to maintain the desired house temperature without supplemental heat (Zhao *et al.*, 2015). The reason is that as the number of hens decreases in the house, so does the amount of moisture production, which in turn reduces the ventilation rate required for humidity control. The lower ventilation rate means less cold outside air introduced into the house, thus conducive to maintaining the desired house temperature. However, if the minimum ventilation is used to control ammonia (e.g., <25 ppm), additional fresh cold air needs brought into the barn, which will likely result in lower-than-desired house temperature. Although the sub-optimal house temperature will not adversely affect the well-being of the hens, it will cause increased feed intake and diversion of more feed energy to thermoregulation as opposed to egg production. Consequently, feed efficiency is reduced. This is one of the primary reasons that since 2012, 100% of the new henhouses in the USA are using manure belts, even though the cost of construction is 50% higher than high-rise houses.

Studies have shown that single- or multi-level (aviary) cage-free houses have much higher concentrations and emissions of ammonia, particulate matters (PM_{10}, $PM_{2.5}$) (Green et al., 2009; Hayes et al., 2013; Zhao et

al., 2015; Shepherd et al., 2015), and airborne bacteria (Zhao et al., 2016) than manure-belt cage or enriched colony houses. The higher pollutant levels and emissions of the cage-free houses arise from the accumulation of manure on the floor and activities of the hens (scratching, dustbathing, wing-flapping, etc.) on the litter floor which is generally quite dry (moisture content of 10-15%). Specifically, PM levels and emissions of the cage-free houses were found to be 6-8 times higher than those in the cage or colony houses. Due to the active nature of the birds, feed-to-egg conversion for these hens is less efficient, e.g., 2.12 for aviary cage-free hens *vs*. 2.02 for conventional cage hens and 1.99 for enriched colony hens (Karcher et al., 2015). Because 70-80% of the total carbon footprint for the poultry production chain stems from feed production (Pelletier, 2008; Pelletier et al; 2014), less efficient feed conversion translates to greater carbon footprint per unit egg output.

The much lower stocking density in the cage-free houses makes it necessary to provide supplemental heat during cold weather. Typically a 50,000-hen house in the Midwest USA will use a supplemental heat design capacity of 220 – 293 kW (750,000 – 1,000,000 BTU/hr). Distributing the supplemental heat uniformly throughout the house with a limited number of heaters can be challenging. For instance, placement of supplemental space heaters along the sidewall has proven to be rather inefficient and cause undesirable heat distribution in that areas in front of the heaters become warm while other parts of the house remain below the desired temperature.

To reduce the land footprint for construction, some producers are now building or considering multi-story cage-free houses. Such structural arrangements require more elaborate design, installation and operation of the ventilation system to ensure proper air distribution throughout the house in each story.

Opportunities with cage−free egg production systems

Working with cage-free henhouses undoubtedly requires a new set of management skills. Multi-disciplinary collaboration is imperative to systematically assess and improve production conditions in cage-free henhouses. From the standpoints of environment control and enhancement, there are a number of specific areas that need immediate research, as outlined below.

a)Suppressing the generation of ammonia, PM or dust and airborne bacteria simultaneously. Some of these aspects work against each other. For instance, increasing litter moisture content will reduce the generation of PM or dust, but will likely stimulate ammonia volatilization; and vice versa. An ongoing research at Iowa State University is tackling this challenge by applying electrolyzed water (EW) at lower pH value to the aviary henhouse litter. The same system (using regular water) can help alleviate heat stress in summertime. Our previous work has demonstrated appreciable effect of applying EW on reducing dust and airborne bacteria in cage-free henhouse setting (Zheng et al., 2014). However, with application of water to the litter facilitating ammonia volatilization, it is important to balance the trade-off of PM reduction at the expense of ammonia elevation. One possible way to accomplish this is to adjust the pH value of EW, applying litter amendment (Li et al., 2008), and/or through dietary manipulation (Li et al., 2012).

b)Exploring practical means to periodically remove manure on the house floor. This periodical removal of manure is particularly important when the hens defecate more on the floor than on the manure belts, hence causing the manure to accumulate faster than normal.

c)Improving distribution of supplemental heat throughout the barn. This may be accomplished by taking advantage of the air recirculation system used for manure-drying. The heat will be distributed through the manure-drying ducts from separate heating rooms to various parts of the house.

d)Evaluating behavioral and production responses of laying hens to different lighting regimens (e.g., intensity, spectrum, photoperiod, and placement) to ensure that hens will lay eggs at the designated places, i.e., nest areas or at least inside the colonies (i.e., minimizing or eliminating litter floor eggs) (Long et al., 2015, ab; Ma et al., 2015). Similar lighting studies should be performed to reduce pecking and cannibalism under cage-free setting.

e)Investigating alternative housing designs and configurations to reduce exposed litter surface area and thus dust and ammonia generation. For instance, designated dustbathing areas may be devised to reduce the overall exposed litter surface area. Slat flooring coupled with regular manure removal is another possibility. Other promising techniques such as electrostatic ionization/precipitation may also be incorporated to improve the production environment.

Closure

A growing number of food distributors, restaurants and grocers in the Unites States have recently pledged to source only cage-free eggs by 2025. If implemented as pledged, the amount of cage-free egg production by 2025 will reach nearly 60% of the national total table eggs. The socio-economic ramifications of such a shift

remain to be determined. However, one thing is certain: Research and innovation are urgently needed to provide the industry with the necessary information and skills regarding efficient operation and management of the cage-free flocks, improved indoor air quality and air emissions, reduced labor and minimizing the risk of food safety as well as improving the well-being of animals and workers. A holistic approach should be used in addressing these interdependent sustainable egg-supply foundations.

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L42 Egg quality in the genomic era

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Keywords: GWAS, eggshell, cuticle

Summary

Great progress has been made in identifying the proteins and genes associated with internal and external egg quality. Moreover, technical advances and reduced cost in SNP microarray approaches to Genome Wide Association Studies have introduced another powerful tool for selection programs. Egg production and egg quality have been under intense selection by breeding companies. Marked improvements in hen production and egg quality have occurred by co-selection for a number of important phenotypic characteristics reflecting both external and internal quality, with the stated goal of enhanced persistency for a "long-life" layer which remains healthy over a production cycle of 100 weeks / 500 eggs. Shell strength and eggshell quality are essential for the prevention of pathogen contamination of the nutritious table egg. Bacterial contamination is a major concern for unfertilized eggs that are meant for human consumption, and there is increasing recognition that cuticle coverage and thickness (quality) is very important to resist bacterial penetration. Selection strategies across hen ages must maintain the egg's innate antimicrobial activity as well as the physical barriers that prevent bacterial entry.

Introduction

The avian egg is a reproductive structure that has been shaped through evolution to resist physical and microbial challenges from the external environment, while satisfying the nutritional and metabolic needs of the developing embryo (Hincke *et al.,* 2012). Flaws in chicken eggshell fabrication are a human health and food safety issue; the nutritious table egg is an important source of protein, minerals and vitamins for the world's population. In this sense, the reproducible nature of egg formation is of critical concern to human society.

Commercial breeders pursue breeding strategies with the common objective to exploit the genetic potential of their stock to produce a maximum of saleable high quality products at minimum cost in a given production system. For layers, these objectives include low hen mortality and high adaptability to different environments; achieving the maximum number of saleable eggs per hen housed, low feed cost per egg (food conversion) and at the same time optimizing the external and internal egg quality (Preisinger and Flock, 2000). Marked improvements have been documented by breeders over the past century (Preisinger and Flock, 2000; Nys *et al.,* 2008; Rossi *et al.,* 2013; Bain *et al.,* 2016). Birds come into lay at earlier age and at peak a greater percentage of the flock is producing. With these and other improvements, there has been a steady increase in the total egg mass from each bird over this time. Concomitantly, egg quality parameters such as shell strength and albumen (Haugh units) have improved significantly, while maintaining important criteria such as % yolk and egg weight relatively unchanged.

Traditional quantitative genetic approaches have been responsible for clear progress in laying hen performance and in egg quality traits, which are under the control of numerous genes (Van Sambeek, 2010; Hyline, 2016. Great progress in understanding the origin of genetic variability has been made in recent years: full genome sequencing of Gallus gallus and other avian species, databases with large scale EST resources, cataloguing of markers of genetic variability such as short tandem DNA repeats (microsatellites) and single nucleotide polymorphisms (SNP's), as well as the corresponding arrays for Genome Wide Association Studies (GWAS).

Egg Formation

Formation of the egg follows a well-defined pattern of assembly as it travels the length of the oviduct

during its daily cycle of fabrication. Following ovulation, the yolk travels through specialized regions to acquire specific egg compartments. Every layer of the avian egg, from the vitelline membrane surrounding the egg yolk to the outermost cuticle, provides antimicrobial activity and serves as a protective barrier (Réhault-Godbert *et al.,* 2011). Egg and eggshell formation requires exquisite physiological control. Efficient eggshell mineralization is dependent upon the timing and kinetics of dietary calcium uptake and redistribution into appropriate compartments for the subsequent mineralization processes that occur in medullary bone and the uterus (Bar, 2009). Genetic factors that impact calcium metabolism affect shell calcification, and maintenance of bone health over the production cycle is of critical importance (Bain *et al.,* 2016). Provision of ions to the uterine fluid requires synergy between ion flux pathways; the ion pumps and transporters responsible for coordinated ion movement across the uterine cell wall are potential targets for selection (Jonchere *et al.,* 2012; Brionne *et al.,* 2014). Genetic variation in members of the sodium channel (SCNN1) family and other ion transporters is associated with eggshell quality traits (Fan *et al.,* 2013; Duan *et al.,* 2015). GWAS studies have identified chromosomal regions that are implicated in egg albumen quality (Honkatukia *et al.,* 2013), and in age-related variation in egg quality parameters such as eggshell strength, shell thickness and eggshell weight (Sun *et al.,* 2015a); egg weight (Yi *et al.,* 2015); yolk and ovary weight (Sun *et al.,* 2015b).

Eggshell Membranes

The membranes that line the interior of the calcified shell form a bacteriostatic meshwork with associated antimicrobial proteins such as lysozyme (Hincke *et al.* 2000), ovotransferrin (Gautron *et al.* 2001) and ovocalyxin-36 (Cordeiro *et al.,* 2013, Gautron *et al.,* 2007), in addition to ovodefensin (gallin) and avian beta-defensins (Rose-Martel et al., 2015). Both Gram-positive and Gram-negative bacteria are sensitive to most beta-defensins (summarized by Cuperus *et al.,* 2013). The genes encoding gallin are highly expressed in magnum; recombinant and synthetic forms of gallin inhibit the growth of *E. coli* and *Staphylococcus aureus* (Gong *et al.,* 2010; Hervé *et al.*, 2014; Whenham *et al.,* 2015).

Mineralized eggshell

The calcified shell is a composite bioceramic that strongly resists physical stresses; the intact shell represents a solid barrier to bacterial entry to the egg interior (Jones and Musgrove, 2005). Superior shell quality is an important characteristic, since cracked and damaged eggshell result in substantial economic loss to the egg industry and are a food safety risk (Bain *et al.,* 2006). Mineralization on the surface of the outer eggshell membranes is associated with an amorphous calcium carbonate phase (Rodriguez-Navarro *et al.,* 2015). The bases of the mammillary cones are continuous with the palisade region, which is organized into columns of elongated calcite crystals (crystallites). The outermost layer of the palisade layer consists of large calcite crystals that absorb external impacts and contain thin inter-crystalline organic layers which hinder inter-crystalline crack propagation (Nys *et al.,* 2004). Shell strength in the palisade region is correlated with variations in crystal orientation (Rodriguez-Navarro *et al.,* 2002).

Variation in nutritional, genetic or physiological factors (i.e. age) affect shell strength. The well-known reduction in shell proportion in eggs from aged hens is partially explains the 50% diminution in shell breaking strength. Elle coïncide avec une modification des proportions relatives de protéines de la matrice de la coquille (Panheleux et al., 2000) et de la texture cristallographique de celle-ci. La mue rétablie la solidité de la coquille et inverse les variations précédemment observées pour la matrice de la coquille et sa texture (Ahmed et al., 2005). However, this weakness coincides with a change in the relative proportions of matrix proteins in the shell (Panheleux *et al.*, 2000) and alterations in crystallographic texture.Par ailleurs, des déficits d'expression de l'ostéopontine dans des sites particulier de l'épithélium de l'utérus semblent être associés à des défauts de structure de la coquille (Arazi et al., 2009). The precursors of the eggshell matrix are present in the acellular uterine fluid, from which they become incorporated into the calcifying shell. Transcriptomic and proteomic studies have identified protein constituents of the eggshell that regulate mineralization and eggshell quality (Hincke *et al.,* 2012); these are potential targets for marker assisted selection to improve eggshell strength to prevent bacterial contamination (Dunn *et al.,* 2012). Association studies between polymorphisms of genes encoding shell matrix proteins and shell quality have revealed osteopontin alleles associated with the hardness of the shell, those of ovocleidin-116 related to elasticity and thickness of the shell and ovocalyxin-32 correlated to the thickness

of the mammillary layer (Dunn *et al*., 2008). Proteomic studies have recently revealed the complexity of the eggshell matrix. Quantitative changes in levels of matrix proteins in uterine fluid occur during shell mineralization, and in eggshell, with their functional correlates, have identified candidate regulators of eggshell texture (Hincke, 2013; Sun *et al.,* 2013; Rose-Martel *et al.,* 2015; Marie *et al.,* 2015a; Marie *et al.,* 2015b). Differential uterine expression of ovocleidin-17 was observed in hens laying eggs with weak versus strong eggshell (Zhang *et al.,* 2014)

Cuticle

The cuticle is the outermost layer of the avian egg and plugs the entrance to eggshell pores (Ruiz and Lunam, 2000). Egg washing is mandatory in many jurisdictions, such as Europe, and is often thought to remove the cuticle or degrade its quality. Cuticle quality (thickness, completeness of coverage) is heritable and is associated with resistance to bacterial penetration (Bain *et al.,* 2013). A significant association was revealed between cuticle quality and the alleles of several eggshell / cuticle protein genes, indicating that genetic selection should be possible to increase cuticle deposition in commercial poultry (Bain *et al.,* 2013).

The major cuticle protein (ovocalyxin-32) exhibits a high degree of genetic heterogeneity due to a large number of nonsynonymous SNPs which are predicted to alter the amino acid sequence of the protein (Fulton *et al*., 2012). There is considerable variation within commercial lines, and selection pressure for certain variants of the ovocalyxin-32 gene during breeding programs has been inferred. Recombinant *Gallus gallus* OCX-32 possesses antimicrobial activity (Xing *et al.,* 2007), and differential antimicrobial activity of the naturally occurring ovocalyxin-32 protein variants is under active investigation.

Conclusions

More knowledge about linkage between genetic markers and phenotypic traits is being reported at an increasing pace. The current goal of increasing the duration of the laying period and maintaining older hens with little reduction in shell quality and with associated control of egg size brings new challenges to the breeding industry. Moreover, it is now recognized that cuticle quality and coverage is a key feature to reduce salmonella penetration; this introduces a new trait (eggshell cuticle) that must be assessed by the breeder. New methods to assess eggshell quality (i.e. dynamic shell stiffness (K dyn), acoustic resonance frequency analysis acoustic resonance crack detection) have provided better tools to monitor the complex phenotype of shell strength. A challenge will be to develop high throughput phenotypic measurements that are related to new or previously untested properties of the egg and eggshell. Moreover, studies have been performed with a variety of experimental crosses, which may not be comparable to commercial lines, and therefore direct extrapolation may not yield anticipated results.

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L43 The current status and future of egg processing in China

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Keywords: egg production, egg processing, customer orientation, marketing

Summary

As the biggest egg production country, only 1% of eggs are processed (in the form of liquid and dried) in China, well below the average 30% in western countries. Shell egg is widely used (manually cracking) in food service and food industry, posing a high risk to the food safety. However, the market in metropolis like Beijing and Shanghai, the customers are educated continually and liquid egg is highly welcomed. The cost is the biggest challenge to persuade customers to replace shell egg, as the price of egg remains considerably low in the most time of a year thanks to the overcapacity of layer industry. The new Food Safety law took effect in 2015, in which a new strict measure requiring food producers must record incoming food raw materials indicating such information as name, specification, quantity, production date or batch number, shelf-life, purchase date, and supplier name and contact information. As one of the most important raw material in food, egg products will see an optimistic and fast growth in the near future.

Main text

Egg production in China

To explain egg supply chain (from farm to table) in China and current status and challenges of egg industry

Egg processing in China and around world

To present egg products in US, Japan and EU and egg processing (traditional and modern) in China

The trend of egg products

To share the emerging egg products concept in the market.

DQY's practice to promote egg products

To lay out DQY's strategy and practice to educate consumers and promote egg products

L44 Duck production in China

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Abstract: Both meat-type duck industry and laying duck industry, offering 7 million tons of duck meat and 4 million tons of duck eggs, respectively, are characteristic poultry industries in China. Chinese duck industry is in the stage of transformation and upgrading, traditional raising models in waters are being eliminated gradually, on the contrary, raising models such as indoor mesh bed model and thick cushion grass model which effectively protect waters and efficiently reduce emission have been developed into main raising models rapidly. Chinese duck species are quite diverse and preliminarily established into various excellent reproduction and breeding systems meeting multiple consumptions of duck food. Chinese scholars first estimated and evaluated multiple nutrient requirement data and established scientific and practical feeding standard of Chinese duck which increases the scientific and technical content of duck feed significantly, enhances feed availability apparently, and promotes the development of duck industry efficiently and healthily.

Keywords: China，duck, meat and egg production, raising model , breeder , feeding standard

Duck industry is an important component of Chinese rural economy

Duck production has been increasing annually by more than 5%. Chinese gross duck production in 2011 reached a peak, approximate 3.793 billion ducks[(1)(2)] and 7.69 million tons of duck meat in total accounting for one third of gross poultry meat production[(3)], according to survey data of 21 provinces, regions, and municipalities by *National Waterfowl Industry System* since 2009. However, from 2012 to 2013, meat-type duck production shrank severely by over 25%, especially for the reason that the incidents of human infections with the emerging avian influenza H7N9 negatively impacted the consumption of duck meat and eggs. The statistics of meat-type duck industry and laying duck industry from 2014 to 2015 were showed in Table 1. The total productions of meat-type ducks and spent laying ducks in 2014 were 3.106 billion and 0.158 billion, respectively. The total productions of meat-type ducks and spent laying ducks in 2015 were 2.862 billion and 0.224 billion, respectively. The total values of out-put of duck industry in 2014 and 2015 were 118.8 billion RMB (18.5 billion dollar)[(4)] and 133 billion RMB (21 billion dollar), respectively. In China，duck eggs are highly popular and well received by consumers. The total production of duck eggs in 2015 was up to 4.22 million tons, accounting for 15% to 20% of total poultry egg production. The production of duck's down, also an important byproduct of meat-type duck industry, is proximate 35000 tons of down valued at over 10 billion per year. Chinese duck industries related to meat and eggs mostly concentrate in East China, South China, and parts of the Southwest. The productions of Meat-type ducks and laying ducks in such provinces as Shandong, Sichuan, Jiangsu, Anhui, Zhejiang, Hunan, Hubei, Jiangxi, Guangxi, Fujian, Henan, Guangdong, Henan, and Chongqing account for over 90 percent of national total production.

Table 1 Economic Data of Duck Industries in 21 Provinces or Regions.

Breeds		Annual duck production ($*10^4$)	Annual meat production ($*10^4$Tons)	Annual production of Eggs ($*10^4$Tons)	Annual value of production ($*10^8$ RMB)
2014	Meat-type ducks	310589	698.8	—	828.9
	Laying ducks	15833	21.4	329.88	359.4
	In total	326422	720.2	329.88	1188.3
2015	Meat-type ducks	286234	644.0	—	842.9
	Laying ducks	22406	30.2	422.32	486.9
	In total	308640	675.2	422.32	1329.8

Chinese diversified consumption of duck meat and eggs effectively and practically guides the breeding of special duck lines

The numbers of consumers of duck meat and eggs are estimated to be more than 800 million. Consumption characteristics of duck meat in different regions are extremely different from each other. Beijing, Guangdong, Guangxi, and Yunnan mainly consume roast ducks or barbecued ducks, particularly, Beijing roast duck has been becoming an important food symbol of Beijing. Jiangsu mainly consumes salted ducks also known as *xianshui ya*. Hubei, Hunan, Jiangxi, Anhui, Sichuan, and Chongqing mainly consume Halogen ducks, Sauce ducks, and Dried salted ducks. Zhejiang mainly consumes old duck boiled soup and Halogen ducks. Different types of duck food must be supported by corresponding duck breeds owning different production performance[5].

At present, the major meat-type ducks in china are local Peking ducks, Peking ducks imported from Cherry Valley Corporation, and local Sheldrake ducks. Local Peking ducks, imported Peking ducks, and other local breeds account for 10% to 15%, 65% to 70%, and about 20% of market share, respectively. The share of meat-type ducks bred by Maple Leaf Farm or Orvia is relatively rare. Muscovy ducks and mule ducks of Kelimer Group mostly being raised in Fujian province as well as its surrounding areas have a larger market share, nearly 100 million ducks per year. The production of *Muscovey ducks* and mule ducks in Chinese Taiwan province are 10 million ducks per year.

There are two types of local Peking ducks in China, one of them is fat-type duck suitable for Peking roast duck as well as Guangdong roast duck whose subcutaneous fat is thicker and sebum rate is higher. Another type is called lean - type duck suitable for Salted duck, Dried salted duck, and Halogen duck whose sebum rate is higher and both breast muscle percentage and leg muscle percentage are higher. For instance, Chinese meat-type Peking ducks of commercial generation have the same production performances, feed-weight ratio is 1.90:1, breast muscle percentage is 15.5%, leg muscle percentage is 12.5%, and body weight can reach 3.2kg at 38 days of age, as Peking ducks bred by Cherry Valley Corporation. China also has such local breeds for meat as *huabian duck*, *linwu duck*, and *jianhong sheldrake,* whose meat qualities are higher but growth periods are longer, and which are suitable for salted duck, Halogen duck, Sauce ducks, or Dried salted duck well received by consumers.

Salted duck eggs and preserved duck eggs, as the main consumption forms of duck eggs, generally are the important parts of breakfast in most cities or regions in China. Preserved eggs congee is homely food for local residents in Guangdong province and Guangxi province. There are a lot of distinctive Laying duck breeds in China, such excellent breeds, whose egg production of 500 days of age are 280 to 320 eggs and feed-egg ratio is 2.65-2.85:1, as *shaoxing duck, jinding duck, shanma duck, youxian Sheldrake duck*, and *jingjiang duck*[6,7]. *Shaoxing* ducks have many obvious features after 20-year systematic breeding, such as high yield, early mature, and green shell. In multi-line hybrid breeding system of *shaoxing* ducks, the age at first egg is 108 days of age, egg production of 72 weeks are 327 eggs, average egg weight is 69.8 g, feed-egg ratio is 2.65:1, green shell percentage is 98%, and annual duck stocks are over 80 million ducks. *Jinding duck* with high cold resistance is larger and suitable to be raised in northern China. Adult female body weight is 1.67 kg, adult male body weight is 1.60 kg, egg production of 500 days are over 280 eggs, average egg weight is 72 g, and feed-egg is 2.85:1. *Shanma* ducks mainly being raised in Fujian province and Zhejiang province are the smallest laying duck breed in China. Adult female body weight is 1.35 kg, adult male body weight is 1.32 kg, egg production of 500 days are over 300 eggs, and feed-egg is 2.80:1[8]. At present, duck egg consumers generally prefer green-shell eggs, fortunately, *shaoxing duck*, *jinding duck*, and *shanma duck* primarily lay green-shell eggs.

Rapid transformation of raising models of meat−type duck and laying duck

Traditionally, scattered and small-scale raising models with lower industrialization degree were always equipped with simple facilities and extensive management pattern. Furthermore, those duck farms were generally built along rivers, lakes, and pools, and wastes probably triggering diseases were always drained into waters directly.

Over the recent decade, with the rapid development of industrialization and increasing awareness of environmental protection, traditional raising models have been transformed rapidly into indoor raising models far away from waters[4]. Indoor mesh bed model and thick cushion grass model are well received. Duck mesh bed with fermentation bed model and duck cage-raising model are undergoing rapid develop-

ment, the purpose of which is to transform wastes into organic fertilizers in the aerobic fermentation conditions and therefore to reduce environmental pollutions [9]. Under the encouragement of local government policies as well as the regulation of market, traditional management and raising model have been developed into intensive production pattern, like *corporation + poultry feeding base (or cooperation) + farmers*, which facilitates the progress of breeding industry in the direction of standardization, environmental protection.

Figure 1. Indoor mesh bed model

Figure 2 Thick Cushion Grass Model

The progress of duck nutrition and feed formulation supports the healthy development of duck industry.

Both meat-type ducks and laying ducks consume more than 35 million tons of feed annually in China [10], thus, it is of utmost importance for enhancing the feed availability to establish feeding standard suitable for Chinese duck industry. We established Chinese *Nutrient requirementds of meat-type duck* on the basis of data relevant to duck nutrition and feed science accumulated over last decade. This feeding standard divided whole growth period into three phases, that is to say, brooding period (from 0 to 2 weeks of age), growth period (from 3 weeks of age to 5 weeks of age), and fattening period (6 week of age). The whole growth period of breeding duck was divided into brooding period, growth period, incubation peri-

od, early laying period, peak laying period, and late laying period. Each physiological stage was recommended essential nutrient requirements, respectively, such as Energy, Crude protein, Lysine, Threonine, Tryptophan, Arginine, Isoleucine, Calcium, Phosphorus, Sodium, Copper, Iron, Zinc, Manganese, Selenium, Vitamin A, Vitamin D, Vitamin E, Nicotinic Acid, Riboflavin, Biotin, Choline, and so forth[11]. Meanwhile, metabolic energy as well as other nutrient contents of 40 kinds of commonly used feedstuff were measured and provided in that feeding standard. The feeding standard, based on its practicality and scientificity, has been applied in several nationwide large-scale feed enterprises producing more than 10 million tons of duck feed.

Conclusions

Both meat-type duck industry and laying duck industry are the important components of rural economy, offering consumers 7 million tons of duck meat of high quality and 4 million tons of duck eggs. Chinese duck industry is in the stage of transformation and upgrading, traditional raising models in waters have been transformed rapidly into such raising models as indoor mesh bed model and thick cushion grass model which effectively protect waters and efficiently reduce emission. China has diverse duck species, therefore, it is necessary to strengthen research of duck breeding technology, and to establish reproduction and breeding system meeting multiple consumptions of duck food. China established scientific and practical *duck nutrient requirements of meat-type duck* which needs to be revised further and be perfected along with the development of breeding and duck industry.

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L45 Meat duck nutrition－formulation considerations across genetic and feedstuff resources

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Keywords: duck, energy, protein, compositional yield, genetics

Summary

Over the past few decades, tremendous improvements have been made in Pekin duck breeding, whilst feed ingredient pricing and customer demands for carcass composition continue to vary worldwide. Shifts in genetics, feed resources, and market demands may have direct and indirect effects on the end product, yield, and profit of meat duck production. Therefore, the question remains as to how formulation consideration should be done across genetic and feedstuff resources to meet market needs. Recent research has shown that dietary energy, crude protein, and amino acids concentrations not only can affect the growth performance of Pekin ducks, but also influence carcass composition by altering breast meat, skin, fat, and feather yield. It is noteworthy that different responses to dietary nutrient modification exist amongst different duck strains, and thus evaluation of specific yield and composition response in each population requires further attention. Nevertheless, producers should be more conscious of the effectiveness of nutritional modification, which is capable of manipulating output within strain and producing a variety of products to satisfy specific markets. In many markets wherein additional value from the end customer can be captured, this nutritional modification should gather more attention and should be implemented for full valuation of duck production systems. In addition, as the duck market demands different ending BW and composition, modeling of nutrient responses based on end-product desired offers a more dynamic applicability than static requirements to ensure the optimization of global duck production.

Introduction

The global meat duck production is a rapidly growing industry, with total production rose from 2.9 million tonnes in 2000 to nearly 4.4 million tonnes in 2013 at a growth rate of 3.2% per year. As the leading producer of meat ducks, Asia shared 83.8% of the global output as of 2013; while mainland China represented approximately 80% of the regional production and continue to contribute more than two thirds of the global total [1]. During the same period, international trade of fresh and frozen duck meat increased dramatically from 107,000 tonnes to 206,000 tonnes [1].

In commercial meat duck production today, Pekin duck (*Anas Domestrics*) is undoubtedly the predominant breed (followed by Muscovy and Mule duck), primarily due to its rapid growth rates. Over the past few decades, tremendous improvements have been made in Pekin duck breeding through modern genetic selection, which has been mainly driven by the demand for faster growth rate, higher breast meat yield, and better conversion ratio. Currently, a typical Pekin duck can reach market weight at about 3.1 kg at 5 weeks, with a feed conversion ratio of approximately 1.7:1. Breast meat yield, which is considered the most important selection trait in certain markets, averages 20% of carcass weight. From 1980 to 2009, the live weight of Maple Leaf Farms (MLF) ducks, the largest producer of White Pekin ducks in North America, has improved from 2.8 to 3.01 kg, with an increase in carcass yield from 71 to 74% and a decrease in feed conversion ratio from 3.1 to 1.7. Additionally, boneless skinless breast yield almost doubled from 11.5 to 19.3% and carcass skin and fat has reduced from 33 to 20% during the same period, while market age reduced from 50 to 37 d [2]. Similar improvement has been seen in Cherry Valley ducks, another major commercial Pekin duck, with an increase in growth efficiency of 2% annually and breast meat yield from 16 to 19% from 1995 to 2007 [3].

Despite the advancement in genetic selection, optimal duck production can only be achieved by a thorough consideration of all factors of the production chain; and one such vital factor is the feed. Similar to broilers, in duck production, feed accounts for the primary cost, often exceeds 65 to 70% of the overall production costs. With the ever changing global market and trading situations, it is not surprising that different feedstuff resources and financial constrains exist in different geographic regions of the world. As of 2008, feed cost to produce a 2.3 body weight broiler varied from $0.81 in the U.S. to $1.18 in Central EU, with Brazil ($0.92) and South-

east Asia ($0.99) in between. Among all feedstuffs, energy and protein accounted for the biggest difference in cost between regions. Similar trends remain to be true today and can also be applied to duck production. On the other hand, feeding programs have been changing continuously with the change in market demand. Historically, a two-phase feeding program is often used in meat duck production, with a starter diet fed from hatch to 14 d and subsequently a grower diet fed till market age; while in the past decade, there is a trend to reduce days to market, resulting in a reduced duration of which the grower diet is fed. Shifts in genetics, feed resources, and market demands may have direct and indirect effect on the end product, yield, and profit of meat duck production. Therefore, the question remains as to how formulation consideration should be done across genetic and feedstuff resources to deliver a higher valued product to the end customer.

Nutritional manipulation within strain

Protein and energy are the most predominant dietary components in commercial duck feed, not only because of their high costs, but also their significant influence on growth performance, carcass quality, and health of poultry. Today, considerable variation within the industry exists as to how much protein and energy is fed. For instance, metabolizable energy (ME) used in the grower diet can range from 2750 to 3200 kcal/kg (11.5 to 13.4 MJ/kg), while dietary Lysine can range from 1 to 1.2%. In fact, the answer to the often asked question, how much should be fed, essentially depends on the varied demands for end product and composition. Despite the increasingly popular demand for duck meat in many restaurants and families worldwide, preferences with regard to the duck composition and method of preparation may differ widely depending on regions of the world, tradition, and cuisine preference. While the western countries predominantly use the duck breast meat and prefer minimal fat content, the famous Peking roast duck, on the other hand, prefers higher subcutaneous fat content to create the crispy fleshy texture. Yet other Chinese cuisines demand otherwise; for instance, the equally popular Nanjing roast salted duck utilizes smaller ducks (approximately 2 kg) with more lean meat. Meanwhile, in many parts of Asia, whole duck product is consumed at a much higher proportion than that in the west, and duck parts other than the breast are also in high demand. These include duck neck, feet, tongue, intestine, and even blood; and thus breast meat is, to some extent, a byproduct for many Asian consumers. Therefore, there is not a single standard for duck carcass characteristics and thus a better understanding of specific market requirements is needed.

Regardless, nutritional approaches can be used to produce a very different bird composition within strain simply by manipulating dietary ME, protein, and/or amino acid profile. A recent study from our lab explored the effects of dietary crude protein and energy concentrations in manipulating composition of White Pekin ducks [4]. When 14 to 35 d MLF ducks were fed 3 dietary ME (11.8, 12.8, and 13.8 MJ/kg) with 3 crude protein (CP) concentrations (15, 17, and 19%) in a 3 x 3 factorial arrangement, significant interactions were found between dietary ME and CP on BW gain, feed intake, and breast meat weight on d 35, where the best BW gain and feed conversion ratio was obtained when ducks were fed 13.8 MJ/kg ME and 19% CP. Carcass traits were also affected by dietary energy and protein profile, where the highest breast meat weight (426 g) was observed in ducks fed 12.8 MJ/kg ME with 19% CP. Increasing dietary CP concentration from 15 to 19% showed a significant main effect of increasing breast meat yield from 18 to 20% (which is close to the improvement from certain genetic selection), with a decrease of breast skin and fat yield from 6.78 to 6.18%. Conversely, increasing ME from 11.8 to 13.8 MJ/kg increased dressing percentage, breast skin and fat yield (from 6.2 to 6.9%), while decreased breast meat yield (from 19.4 to 18.4%). Consistently, previous report suggested that increasing dietary AME from 10.9 to 13.0 MJ/kg (with 18% CP) in Pekin ducks from d 14 to 42 would increase BW gain while decrease feed intake and feed conversion ratio; and abdominal fat was also increased (0.86 vs 1.65%) with increasing dietary AME [5]. Another study evaluating the effects of graded dietary crude protein concentration (17 to 23%) in Pekin ducks also revealed that breast muscle thickness and Pectoralis muscle yield significantly increased with increasing dietary crude protein [6]. Evidently, growth performance and carcass traits can be manipulated through modifying dietary protein and energy in Pekin ducks even within stain and genetic lines, and to an extend that is comparable with certain breeding program outcomes. Subsequent modeling of amino acid and energy inputs and corresponding outputs of growth and carcass characteristics warrants further attention. In many markets wherein additional value from the end customer can be captured, this nutritional modification should gather more attention and should be implemented for full valuation of duck production systems.

Response differences between pekin duck strains

Although performance and carcass characteristics can be altered via nutritional manipulation as discussed above, we must also appreciate that there are certain significant differences in the birds' response to nutrition-

al modification depending on their origin, which may partly attributed to their compositional difference. When fed the same starter and grower diet, the BW at 35 d of MLF ducks was significantly higher than that of Cherry Valley ducks by approximately 10%, along with a lower FCR (1.52 vs 1.72 for MLF and Cherry Valley ducks, respectively). Although carcass yields were similar, breast meat yield (16.3 vs. 12.0%) was significantly higher while abdominal fat and breast skin yield were lower in MLF ducks compared to Cherry Valley ducks [7].

Consequently, discrepancy has been noticed in nutrient requirement and birds' response to dietary modification between MLF and Cherry Valley ducks. In Cherry Valley ducks, higher energy increased abdominal fat, but did not affect breast and leg meat [5], unlike what was observed in MLF ducks. The AME requirement for Cherry Valley ducks from 2 to 6 week was estimated to be 3,008 and 3,030 kcal/kg for optimal weight gain and feed conversion rate, which is lower than the estimation for MLF ducks [4]. When MLF ducks were fed from 15 to 35 d, in addition to improved BW gain and FCR, increasing dietary Thr concentration from 0.64 to 0.72% also significantly increased carcass and breast meat yield while reduced breast skin yield (and presumably fat content) [8]. The requirement of Thr was estimated to be 0.73 and 0.80% for optimum breast meat and carcass weight, respectively. On the contrary, although BW gain and FCR were improved with increasing dietary Thr concentration from 0.5 to 0.82% in Cherry Valley ducks at 21 d, there were no statistical differences on BW gain and feed intake between the 0.66 and 0.74% Thr groups [9], indicating that MLF ducks may be more responsive to dietary Thr manipulation than Cherry Valley ducks. Unfortunately, no information is available on the effect of Thr concentration in Cherry Valley ducks at market age, thus comparison of carcass traits with MLF ducks in response to Thr modification cannot be performed. Methionine, as the first limiting amino acid in corn-soybean based diet of poultry, plays important roles in bird's growth, egg production, and feather production. When MLF ducks were fed graded concentrations of Met (0.35 to 0.75%) from 15 to 35 d, both growth performance (BW, BW gain, and feed conversion ratio) and yield (carcass and breast meat yield, breast skin and subcutaneous fat yield, and feather coverage) showed significant quadratic response to increasing dietary Met. Higher dietary Met concentration improved carcass and breast meat yield while reduced breast skin and subcutaneous fat. The optimal Met requirement was estimated to be 0.47, 0.41, and 0.48% for BW, breast meat yield, and feather length [10]. As for Cherry valley ducks, when graded Met concentrations were fed (0.20 to 0.58%) from 21 to 49 d, increasing Met also increased BW gain and breast meat yield, and decreased abdominal fat significantly [11]. However, the optimal Met requirement estimated (0.38%) for maximum weight gain and breast meat yield is much lower than that for MLF ducks (≥ 0.41%).

Clearly, there is intrinsic difference in amino acid and energy requirements between MLF ducks versus Cherry Valley ducks, partly due to the compositional differences between them. Therefore, nutritional manipulation that is effective in one may not be as effective in the other, and thus further exploration of their respective optimal nutritional manipulation to satisfy specific market is warranted. Notably, nutrient responses beyond growth and compositional yield are not as easily established, yet influence flock productivity and well-being. Feather growth is one such response, which has great importance in ensuring the duck's well-being and at the same time is economically valuable as a raw ingredient in various feather products. Traditionally, feather yield is often measured by subjective visual evaluation. While the length of the fourth primary wing feather is a more objective measurement, it may not be directly related to feather coverage. In the study of Zeng et al. [10], infrared thermal image approach was first used to evaluate feather coverage in response to dietary Met concentration using a FLIR infrared camera (Model T420, FLIR Systems, Boston, MA), which was proven to be an objective and accurate method in assessing feather quality. Because many amino acids, especially sulfur amino acids, are constituent components in feather, modification in dietary amino acid profile may have direct influences on feather production. In this study, feather growth as measured by infrared thermal image increased linearly as dietary Met increased from 0.30 to 0.68%. Relatedness to down feather growth and down feather-fill power (an economically desirable trait) with infrared thermography remains to be determined, but this emphasizes the need to better understand our measurements in duck research and further explore accurate approaches to evaluate such traits.

On the other hand, in today's global markets where ingredient pricing and product deliverables is varied, the development of a cost-return model for feed inputs and duck product outputs is greatly needed. Historically, nutrient requirement is defined as the minimum amount of the nutrient required to avoid any signs of nutritional deficiency, which inevitably has led to the imposition of "margins of safety". In today's ingredient pricing environment, we likely can no longer afford this vague notion for unspecified margins of safety without a quantifiable performance or yield response to nutrient input variation. Therefore, a better way to discuss nutrient requirement, or rather, "nutrient response", is to include considerations of marginal cost of nutrient input versus marginal returns of product [12]. As the duck market demands different ending BW and composition, mod-

eling of nutrient responses based on end-product desired offers a more dynamic applicability than static requirements in tabular form in the effort of optimization of the end product [13].

The global meat duck production is expected to grow continuously with genetic, nutritional, and management advances. Current duck genetic selection programs have brought significant improvements in economically important traits of duck production and will continue to do so. In the meantime, producers need to be more mindful of the effectiveness of nutritional modification, which can manipulate output within strain and produce a variety of products to satisfy specific market. At the same time, it must be noted that significant differences in response to dietary nutrient profile exist between different ducks, and thus evaluation of specific yield and composition response in each population requires further attention. Nevertheless, this nutritional modification should be implemented in the current duck production systems to embrace a pragmatic balance of breeding, nutrition, and management, and ultimately bring profitable advancement to the global meat duck industry.

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L46 Intestinal epithelial barrier in poultry: function and nutritional modulation

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Abstract

The intestinal epithelial barrier is the most critical element of maintaining an intact intestinal barrier and made up of a layer of columnar epithelial cells and intercellular junctional complexes including tight junctions, adherens junctions and desmosomes. Tight junctions(TJ), which are formed by proteins including occludin, claudins, junctional adhesion molecule and zonula occludens(ZO), are primarily responsible for the permeability of the paracellular pathway. In poultry, only claudin-1, claudin-2, claudin-3, claudin-5, claudin-16, ZO-1, ZO -2 and occludin are reported so far. The intestinal barrier function in poultry is evaluated by measuring intestinal permeability, plasma LPS concentrations and bacterial translocation. Few studies have shown the developmental profile of intestinal barrier function and tight junction protein occludin, claudin-3, claudin-5, claudin-16 and ZO-2 in the intestinal epithelium of chicks in embryonic phase or/and the early post-hatch period. Several feed additives, including nutrients (i.e. Zn), probiotics, prebiotics, functional polysaccharide, epidermal growth factor and enzymes, were shown to regulate intestinal barrier function by modifying expression and localization of TJ proteins, and in some cases prevents or reverses the adverse effects of pathogens and heat stress in poultry.

Keywords: poultry, intestinal epithelial barrier, tight junction protein, nutritional regulation

Introduction

The animal intestine has the roles of absorbing nutrients and also acting as a barrier to prevent pathogens and toxins from entering into the body and potentially causing disease. Maintaining the integrity of the intestinal barrier is fundamental to the proper functioning of the epithelial cells and to preventing the entry of pathogenic bacteria. Injured intestinal barrier is characterized by increased intestinal permeability, which allows luminal antigenic agents (e.g., bacteria, toxins, and feed-associated antigens) to "leak" across the epithelium to sub-epithelial tissues, resulting in inflammation, malabsorption, diarrhea, and potentially systemic disease[1]。 Under environmental, nutritional and pathophysiological stress conditions, animals including poultry subject to barrier impairment[2]. In this review, the components of intestinal epithelial barrier, the primary measures of determining intestinal epithelial barrier function, and the development and maturation of the intestinal epithelial barrier in poultry were summarized, and some nutritional solutions to modulate the intestinal epithelial barrier in poultry were discussed.

The intestinal epithelial barrier

The intestinal barrier is a complex structure made up of four main components: the intestinal epithelial, chemical, immunological and microbiological barriers[3]. The intestinal epithelium forms the largest and most important barrier between internal and external environments of animals. The following sections describe the role of the intestinal epithelial barrier in maintaining intestinal barrier function.The intestinal epithelial barrier is made up of a layer of columnar epithelial cells that forms the first line of defense between the intestinal lumen and inner milieu. The intestinal epithelial cells are mainly absorptive enterocytes (over 80%) but also include entero-endocrine, goblet, and Paneth cells[4]. The epithelium allows the absorption of nutrients while providing a physical barrier to the permeation of pro-inflammatory molecules, such as pathogens, toxins, and antigens, from the luminal environment into the mucosal tissues and circulatory system. The epithelial selective permeability includes two pathways: the transcellular and the paracellular pathway. The transcellular pathway is involved in the absorption and transport of nutrients, including sugars, amino acids, peptides, fatty acids, minerals, and vitamins. As the cell membrane is impermeable, this process is predominantly mediated by specific transporters or channels

located on the apical and basolateral membranes. The paracellular pathway is associated with transport in the intercellular space between the adjacent epithelial cells. These epithelial cells are tightly bound together by intercellular junctional complexes that regulate the paracellular permeability and are crucial for the integrity of the epithelial barrier。 These junctions allow the passage of fluids, electrolytes, and small macromolecules, but inhibit passage of larger molecules.

The junctional complexes consist of the tight junctions, gap junctions, adherens junctions, and desmosomes [7].Tight junctions are the most apical and are primarily responsible for controlling permeability of the paracellular pathway [5]. Adherens junctions are located beneath the tight junctions and are involved in cell-cell adhesion and intracellular signaling [6]. Both tight junctions and adherens junctions (together known as the apical junctional complex) are associated to the actin cytoskeleton [6-8]. Desmosomes and gap junctions are involved in cell-cell adhesion and intracellular communication [9], respectively. The cytoskeleton is an intricate structure of protein filaments that extends throughout the cytosol that is essential for maintaining the structure of all eukaryotic cells. Disruption of the cytoskeleton is linked to the loss of intestinal barrier integrity.

Tight junctions are formed by protein dimers that span the space between adjacent cell membranes (see Fig. 2). There are over 50 proteins with well recognized roles in tight junction formation. These proteins comprise four integral transmembrane proteins (e.g., occludin, claudins, junctional adhesion molecules (JAM), and tricellulin), and cytosolic scaffold proteins, such as zonula occludens (ZO) proteins. The extracellular domains of the transmembrane proteins form the selective barrier by hemophilic and heterophilic interactions with the adjacent cells. The intracellular domains of these transmembrane proteins interact with ZO proteins [10], which in turn anchor the transmembrane proteins to the perijunctional actomyosin ring [11]。 . The interaction of TJ proteins with the actin cytoskeleton is vital to the maintenance of TJ structure and function. In addition, the interaction of the TJ complex with the actomyosin ring permits the cytoskeletal regulation of TJ barrier integrity. Comprehensive reviews on the complex molecular structure of TJ are available [see, e.g., (12)].

Occludin was the first integral membrane TJ protein identified in 1993. Occludin can be specifically visualized at the tight junctions in the epithelia by confocal immunofluorescence microscopy, immunoelectron microscopy and freeze-fracture immuno-replica electron microscopy. The function of occludin is not yet fully understood, but numerous studies using animals and cell cultures indicate that it is required for TJ assembly and barrier integrity in the intestinal epithelia. Occludin has been linked to the regulation of intermembrane diffusion and paracellular diffusion of small molecules. The claudin proteins are considered to be the structural backbone of TJ. Claudins consist of at least 24 members in humans and mice, and each isoform shows a unique expression pattern in tissues and cell lines. In contrast to their structural similarities, claudins perform different functions and can be roughly divided into two types: those involved in barrier formation (decreasing paracellular permeability) and those playing a role in channel pores (increasing paracellular permeability) In the intestines, claudin-1, -3, -4, -5, -8, -9, -11, and -14 can be categorized as barrier-forming claudins, while claudin-2, -7, -12, and -15 are pore-forming claudins [for review, see (12)]. Several plaque proteins have been identified, including the zonula occludens (ZO) proteins, ZO-1, ZO-2, and ZO-3. ZO-1 interacts with the claudin proteins (36), other ZO proteins to form dimers (37), and JAM-A (38). Plaque proteins potentially play a central role in TJ regulation, because they can cause reorganization of the cytoskeleton. To our knowledge, only claudin-1、claudin-2、claudin-3、claudin-5, claudin-16, ZO-1, ZO -2 and occludin are reported in poultry so far[13-16]。

TJ are not static barriers but highly dynamic structures that are constantly being remodeled due to interactions with external stimuli, such as food residues and pathogenic and commensal bacteria[4]. Regulation of the assembly, disassembly, and maintenance of TJ structure is influenced by various physiological and pathological stimuli. Signaling pathways involved in TJ regulation, and interactions between transmembrane proteins and the actomyosin ring are controlled by several signaling proteins, including protein kinase C (PKC), mitogen- activated protein kinases (MAPK), myosin light chain kinase (MLCK), and the Rho family of small GTPases[17].

Assessing of the intestinal epithelial barrier function in poultry

The intestinal epithelial barrier function in animals including poultry is evaluated by measuring intestinal permeability, plasma LPS concentrations and bacterial translocation.

Intestinal permeability

Intestinal permeability is defined as the non-mediated diffusion of large (i.e., molecular weight >150 Da), normally restricted molecules from the intestinal lumen to the blood. The primary means of determining intestinal permeability in humans or animals is by measuring the passage of high molecular weight probes across the gastrointestinal tract barrier[20-22]. In humans, this involves ingestion of a solution containing nontoxic, non-metabolizable substances (such as sucrose, lactulose, sucralose and)[23, 24] and assessing their excretion in the urine. The appearance of probes in the urine indicates loss of barrier function in the gastrointestinal tract. But the process is inapplicable to poultry because separation of urine and feces is difficult in poultry. In animal models including poultry, intestinal permeability is frequently determined by infusing fluorescent probes, such as fluoroisothiocyanate (FITC)-dextrans, into the intestinal area of interest and measuring plasma concentrations over time[26]. Such probes come in various molecular weights ranging from several hundred to several million. Thus, these probes provide not only an index of intestinal permeability, but also provide an idea of how large the opening in the intestinal epithelium may be. Other commonly used probes, used in a similar manner in animal models, are [^{51}Cr]-EDTA [25]and horseradish peroxidase [27].The Ex vivo Ussing chamber is the most sensitive to test the intestinal permeability by measuring transepithelial electrical resistance (TER) and paracellular flux of probes to monitor intestinal permeability in most animals[2]. TER is considered to be the most sensitive measure of mucosal barrier function, since it reflects the opening of the tight junctions between epithelial cells and the paracellular permeability of the intestinal mucosa

Bacterial translocation

The disruption in barrier functions was associated with viral and bacterial translocation across the epithelial monolayers. Bacterial translocation is defined as the passage of viable bacteria from the intestinal tract through the epithelial mucosa into extra-intestinal organs. Impaired mucosal surfaces can increase vulnerability of the intestinal epithelium with an augmented risk of bacterial and viral penetration, or bacterial overgrowth in the in the intestine [28].

Plasma LPS concentrations

Another index of intestinal barrier dysfunction is the plasma lipopolysaccharide (LPS) concentrations. LPS is a highly pathogenic component of the walls of gram negative bacteria and is found in the intestinal tract in high concentrations. Its presence in the portal blood of animal models indicates passage from the intestinal lumen to the circulation [29]. Increased LPS concentrations in the systemic circulation likely indicate severe intestinal barrier dysfunction, in that its high permeability has overwhelmed the ability of the liver to clear it from the blood [28, 30].

Development and maturation of the intestinal epithelial barrier

Many reports have described the development of the junctional complex in the epithelium of animals during the embryonic phase or/and the early post-hatch period. In poultry, limited studies have shown the developmental profile of intestinal epithelial barrier function and tight junction proteins in the intestinal epithelium of chicks in embryonic phase or/and the early post-hatch period, indicating that developmental patterns of different intestines and tight junction proteins are not coincident.

Okamoto and Ishimura (1978) reported that incomplete tight junctions in the duodenal epithelium of chick embryos were already present after 6-7 days of incubation in the apical portion of the lateral plasma membrane, and the formation of the tight junction in chick duodenum might be complete by day 18 of incubation, suggesting that tight junctions begin to form in the gastrointestinal tract at an early developmental stage, thereafter, tight junctions develop rapidly and form complicated networks[31]. Kimura et al. (1996) revealed that the structure of the tight junction was already apparent in intestinal samples from chick embryos aged 13 days or more[32].

Kawasaki et al. (1998) [14. determined the developmental expression of occludin in the gastrointestinal tract of 3- to 21-day-old chick embryos. Occludin mRNA was first detected by RT-PCR in the chick embryo on day 3 of incubation, by northern blot analysis on day 4, and by western blot analysis on day 5, suggesting that synthesis of occludin begins in the chick embryo at a very early stage of development. In addition, the immune-histochemical study revealed that occludin began to be weakly expressed only

along the apical surface of the gastrointestinal epithelium of the 4-day-old chick embryo. As the embryo developed, the immunoreactivity gradually became stronger and formed more complex networks near the apical surface, which indicated that the developmental expression of occludin in the gastrointestinal tract is closely correlated with the morphological as well as functional development of the tight junction.

The expression patterns of intestinal tight junctional proteins during embryogenesis and post-hatch period are not all alike. Earlier work on expression of claudins in developing intestine revealed that transcripts for claudins -1 and -3 are present in the epithelial lining of 5- to 8-day old chick embryos [15, 32, 33]. OZDEN et al .（2010）explored the developmental patterns of claudin-3, -5, and -16 proteins in the epithelium of embryonic chick intestine from 9 days prior to hatching through the early post-hatch period [13]. These claudin proteins either changed their cellular localization or first appeared around the time of hatching. suggesting that in addition to their known barrier and fence functions within tight junctions, these claudins may have additional roles in the differentiation and/or physiological function of chick intestine[13]. Conversely, transcript levels of ZO-2 decrease from 18 to 20 days and reach an even lower level by 2 days post-hatch. It may be significant that ZO-2 transcript levels are high 2 days before the increase of claudin transcripts and prior to the movement of claudin -3 and -5 proteins to the epithelial periphery. However, Roberts et al. (2005) thought that the intestinal epithelial barrier function is not fully developed in chicks until d 11 of age for the jejunum and later than d 14 of age for the ileum.

Nutritional strategies to modulate the intestinal epithelial barrier in poultry

Numerous studies have shown that dietary factors and nutrients can regulate intestinal barrier structure and function in humans and animals [33-36], and some of these could be developed as preventive and therapeutic tools for defective barrier-associated diseases[34,36]. In contrast, limited studies in poultry have reported that dietary factors and nutrients, such as minerals, probiotics, prebiotics, and dietary enzymes, participate in intestinal epithelial barrier regulation.

Zn (Zinc)

The importance of Zn to intestinal development and function has been demonstrated in many studies, such as increased intestinal crypt-cell production, reduced duration of mitosis[37], and improved epithelial cell restitution[38], and maintaining the structure and function of the intestine barrier [39]. Zn as supplementation in diet reduced gut lesion scores, and reduced intestinal permeability and increased expression of ZO-1 and occludin in mammals[36-39.]. Zn deprivation induced a decrease of TER and altered tight and adherens junctions [40]。 In poultry, several studies have demonstrated the beneficial effects of supplemental Zn on the intestinal mucosal barriers. Zhang *et al.* (2012) reported that Zn (as $ZnSO_4$) up-regulated occludin and claudin-1 mRNA expression in the ileum and tended to reduce plasma endotoxin levels of chickens challenged with *Salmonella Typhimurium*, suggesting that regulation of occludin and claudin-1expression by Zn may be involved in ameliorating increased intestinal permeability induced by *Salmonella Typhimurium* challenge.[41]。 However, Hu *et al.* (2013) showed that supplemental ZnO or ZnSO4 did not affect ileal and colonic barrier function and intestinal microflora in broiler chickens, but supplementing 60 mg of Zn/kg as ZnO-MMT(zinc oxide-montmorillonite hybrid) increased colonic TER values, and reduced colonic probe mannitol permeability as well as ileal or colonic inulin permeability of chickens[42].

Probiotics

In human and animals, some probiotics have been shown to promote intestinal barrier integrity and to prevent, and even reverse, the adverse effects of pathogens and stress on intestinal barrier function both in vitro and in vivo[43-45]In poultry, several reports have showed probiotics could decrease intestinal barrier dysfunction induced by pathogens or stress.

The transmission electron microscopy confirmed that, compared to treatments with *Saccharomyces boulardii* and *Bacillus subtilis* B10, the tight junctions of jejunum and ileum of broilers were comparatively loose in the control group, and *Saccharomyces boulardii* and *Bacillus subtilis* B10 also improved the epithelial tight junctions through increasing occludin, cloudin2, and cloudin3 mRNA expression levels in broiler intestine[18]. The increased ocludin, claudin2, and claudin 3 gene expression might be due to a direct response to probiotics or a secondary response to induced inflammatory cytokine secretions of

IL-6 and TNF-α[18]. The addition of a microbial feed additive (L. salivarius and L. reuteri) to broiler diets increased glucose stimulated short-circuit current in both jejunum and colon in Ussing chamber, but the conductivity of jejunal and colonic tissues remained unaffected by the dietary inclusion of Lactobacillus sp. , which support the concept that this microbial additive improves intestinal nutrient absorption and enhances the maintenance and function of the epithelial barrier[43]. *L. fermentum* 1.2029 was able to ameliorate the severity of necrotic enteritis lesions and inflammation and improve epithelial barrier through increasing claudin-1 and occludin levels in necrotic enteritis -infected chickens [44].

In recent years, some reports have indicated that heat stress negatively affects intestinal mucosa and microbiota [45-47]. Heat stress also decreased jejunal TER，increased jejunal paracellular permeability of FITC-dextrans, and downregulated jejunal protein levels of occludin and ZO-1 in broilers[48]. Supplemental probiotic mixture containing Bacillus licheniformis, Bacillus subtilis and Lactobacillus plantarum increased jejunal protein level of occludin in broilers, revealing that dietary addition of the probiotic mixture was effective in partially ameliorating intestinal barrier dysfunction induced by heat stress in broilers[48].

Prebiotics

Prebiotics were defined as non-digestible food (feed) ingredients that beneficially affect the host by selectively stimulating the growth and/or activities of one or a limited number of bacteria in the gut, thereby improving host health. Cello-oligosaccharide is a functional oligosaccharide obtained from plant cellulose. As compared with heat stress group feeding basal diet, supplemental cello-oligosaccharide increased jejunal villus height and villus height to crypt depth ratio, as well as decreased jejunal paracellular permeability of fluorescein isothiocyanate dextran in broiler chickens, which demonstrated that cello-oligosaccharide supplementation partially ameliorated the adverse effects caused by heat stress in broilers through improving intestinal microflora, morphology and barrier integrity [49].

Functional polysaccharides

β-1,3/1,6-glucans from *Saccharomyces cerevisiae* have beneficial effects on both the innate and acquired immune systems in either non-challenged or challenged settings [51-55], and clearance of several important pathogens such as *Salmonella, Escherichia coli* and coccidiosis in broiler chickens [51,53,55]. In intestinal epithelial barrier**,** dietary β-1,3/1,6-glucan supplementation can reduce intestinal mucosal barrier impairment of broiler chickens challenged with *Salmonella* Typhimurium, and the partial mechanism might be related to the increased mRNA expression of claudin-1 and occludin, and increased goblet cell numbers and sIgA level in the jejunum of broiler chickens [56].

Soluble NSP from plantain banana was able to block adhesion of various enteric gut pathogens to the human intestinal epithelial cell or cell-line Caco2 [57-59], and inhibit invasion of Escherichia coli into human intestinal epithelial cells [60. and translocation across specialised microfold (M)-cells of the follicle associated epithelium cultured in vitro [61,62]. In chickens, In vivo dietary supplementation with plantain NSP reduced invasion by S.Typhimurium, as reflected by viable bacterial counts from splenic tissue, and in vitro plantain NSP inhibited adhesion of S.Typhimurium to a porcine epithelial cell-line and to primary chick caecal crypts. Adherence inhibition was shown to be mediated via an effect on the epithelial cells and Ussing chamber experiments with ex-vivo human ileal mucosa showed that this effect was associated with increased short circuit current but no change in electrical resistance [63]。

Epidermal growth factor (EGF)

EGF is a small amino acid peptide with a broad range of bioactivities on the intestinal epithelium, including the stimulation of cellular proliferation, differentiation, and intestinal maturation. Previous studies have shown that EGF administration plays a protective role in a variety of intestinal insults by either reducing injury [65]or accelerating repair [66,67]. In chickens, EGF reduced jejunal *C. jejuni* colonization and alleviated the dissemination of *C. jejuni* to the liver and spleen. In his in vitro study, the pretreatment with EGF abolished the *C. jejuni*-induced intestinal epithelial abnormalities, such as disruption of tight junctional claudin-4, increasing of transepithelial permeability and the translocation of noninvasive *Escherichia coli* C25. These findings highlight EGF’s ability to protect against pathogen-induced barrier defects[68]。

Enzyme

C. perfringens challenge increased intestinal lesion score and also resulted in increased plasma endotoxin [69,70]., passive transcellular permeability[62], while dietary addition of xylanase[71. and enzyme complex containing xylanase, glucanase and mannanase as major components[72. could alleviate the alteration caused by *C. perfringens* infection, indicating that dietary enzyme supplementation could benefit for gut barrier integrity of *C. perfringens* challenged chickens.

Lysozyme as a natural antimicrobial protein occurs in a number of animal secretions and is considered an important component of the innate immune system. The addition of exogenous lysozyme significantly reduced the concentration of Clostridium perfringens in the ileum and the intestinal lesion scores, and inhibited the overgrowth of E. coli and Lactobacillus in the ileum and intestinal bacteria translocation to the spleen of chickens challenged with Clostridium perfringens, suggesting that exogenous lysozyme could decrease Clostridium perfringens colonization and improve intestinal barrier function of chickens [73].

Other dietary components such as glutamine[52,53,54,55]. threonine[59. , fatty acids[64], and flavonoids[65. are also known to regulate intestinal epithelial barrier, but no reports were found in poultry. More nutritional approaches for improving intestinal barrier function and the underlying molecular mechanisms are needed to be investigated.

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L47 Energy intake and redistribution in stress−challenged laying hens

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Keywords: energy, stress, corticosterone

Summary

The regulating role of corticosterone, the stress hormone, in energy ingestion and distribution has been discussed. Corticosterone stimulates energy intake and a preference of high-fat diet by upregulating NPY expression via AMPK pathway. Corticosterone suppresses follicular development is energy dependent, by decreasing availability of circulating yolk precursor and the prevention of yolk deposition in follicles. Energy status is also involved in the rejuvenation in molt hens.

Main text

Stress is a common problem disrupting reproduction of laying hens. Glucocorticoids (GCs), as the final effectors of the hypothalamic-pituitary-adrenal axis, participate in the control of whole body homeostasis and the arousal of stress responses. GCs transmit information about environmental conditions to the hypothalamic-pituitary-gonadal (HPG) axis, which ultimately influences the egg production. Energy intake and distribution between organs or tissues influence the laying performances.

Corticosterone stimulates the appetite of chickens

Glucocorticoids (GCs) play a permissive role in the regulation of energy balance. GCs induce a dose-dependent increase in food intake in poultry (El-Lethey et al., 2001; Liu et al., 2014). An increase in plasma corticosterone (CORT) was observed after 24 h of food deprivation in chickens (Geris et al., 1999). CORT responses to fasting can vary among individuals and depend on energy reserves and experience (Richards, 2003). Some hypothalamic neurons were associated with the regulation of GCs on appetite (Liu et al., 2014). Two primary populations of hypothalamic neurons regulate nutritional status signals and influence energy homeostasis in birds. One population of neurons suppresses appetite through the release of proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) while the other group of neurons stimulates appetite through the release of neuropeptide Y (NPY) and agouti-related peptide (AgRP). Our previous study indicated that GCs promotes appetite by up-regulating NPY expression in chicks (Liu et al., 2014). Several features of high-fat foods, such as palatable flavor and reduced satiating effects, are thought to contribute to the overeating response (Chang et al., 2008). Recently, our study proved that when chicks were challenged with CORT, the chicks prefer to consume a high-fat diet (HFD) rather than a low-fat diet, compared with controls. The result indicates that CORT-challenge caused a high-fat diet preference. This result suggests that GC stimulates chicks to ingest more energy to copy with the environmental changes.

We further investigated that underlying pathway that GC passes the signals. AMP-activated protein kinase (AMPK) acts as a sensor of cellular energy charges and is activated by rising AMP levels coupled with falling ATP levels. Hypothalamic AMPK signaling is involved in the control of food intake (Minokoshi et al., 2004). Once activated, AMPK phosphorylates acetyl-CoA carboxylase (ACC) and switches on energy-producing pathways at the expense of energy-depleting processes (Kahn et al., 2005). Fasting increases hypothalamic AMPKα phosphorylation and mRNA levels in chicks (Song et al., 2012). In contrast, intracerebroventricular (ICV) injection of compound C, an AMPK inhibitor, induced anorexia in chicks (Kim et al., 2004; Liu et al., 2014). Liu et al. (2014) found the stimulatory effects of GCs on food intake and NPY gene expression were significantly attenuated by the blockade of AMPK signaling. Our recently study indicated that high-fat diet up-regulated the hypothalamic phosphorylated AMPKα and NPY expression during exogenous CORT administration. Activating AMPK with activator further enhanced the CORT-induced high-fat diet consumption and concurrently up-regulated NPY mRNA levels. Therefore, CORT challenge caused a HFD preference by enhancing the AMPK pathway in the hypothalamus.

Corticosterone regulation of ovarian follicular development is dependent on the energy status of laying hens

The onset of breeding involves the activation of the HPG axis. The secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus stimulates the release of the pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn activates the gonadal development and the release of sex steroids including estradiol (E2) and testosterone. The development of ovarian follicles is accompanied by the deposition of a large amount of yolk. During a laying cycle, follicular development is matched with a supply of yolk precursors, which are mainly synthesized in the liver and secreted in the form of yolk-targeted very-low density lipoprotein (VLDLy).

GCs trigger physiological and behavioral adjustments that shift energy investment away from reproduction and redirect it toward survival. An acute infusion of CORT resulted in a pause in laying and a severely reduced ovarian weight (Williams et al., 1985). Chronic and repeated exposure to CORT during the rearing phrase suppressed reproductive performance, resulting in a delay of first egg laid and a reduction of egg production (Shini et al., 2009). In immature chickens, GCs stimulated energy intake (Lin et al., 2004) and enhanced hepatic lipogenesis and fat deposition in adipose tissues, indicating the redistribution of energy stores in CORT-challenged chickens (Cai et al., 2009, 2011; Wang et al., 2010). Our study showed that CORT decreased the laying performance by suppressing follicular development in energy deficit state, rather than in energy sufficient state (Wang et al. 2013). In CORT-treated hens, the suppressed follicular development was associated with the reduced availability of yolk precursor (low levels of VLDLy and vitellogenin). CORT decreased the expression of apo-lipoprotein B and apo-lipoprotein VLDL-II in the liver. A drop in VLDL receptor content and an increase in the expressions of tight junction proteins occludin and claudin1 were also observed in hierarchical follicles. The results suggest CORT-suppressed follicular development is energy dependent. The decreased apolipoprotein synthesis and VLDLy secretion by liver are responsible for the decreased availability of circulating yolk precursor and the upregulation of occludin and claudin expression further prevent yolk deposition into oocytes. Exogenous GCs perturbed the reproduction of laying hens in an energy dependent-manner.

Increased hepatic yolk precursor synthesis, secretion and facilitated uptake by follicles are involved in the rejuvenation of reproductive performance of molted hens (Gallus gallus domesticus)

Molt is a special period that is naturally initiated at the end of the lay cycle in birds. As a natural behavior initiated at the end of a lay cycle in birds, molt is implicated in the regression of the reproductive system in birds followed by a rejuvenation of egg-laying potential. In commercial laying hens, induced molting has been used as a practical procedure for a second or third cycle of egg production. In jungle fowl, the wild ancestor of the domestic chicken, brooding of eggs is accompanied by weight loss and spontaneous anorexia, reflecting a change in the regulation of energy balance during incubation (Sherry et al., 1980). Molting is a process that requires an investment of substantial amounts of energy and nutrients and is usually temporally separated from reproduction, another energy-demanding process.

A general increase in reproductive performance following a forced molt has been referred to as rejuvenation, which may be mediated by an increase in tissue sensitivity or efficiency (Rake and Thaxton, 1979). Dramatic changes take place in the liver and the reproductive systems before and after molt. During a laying cycle, follicular development was matched with the supply of yolk precursors, which were mainly synthesized in the liver (Kuksis, 1992). Studies have shown that during one year of egg production, the lipid content in the liver increased with age and showed a dramatic decrease after molt (Garlich et al., 1984). Approximately one-fourth of the decrease in body weight was directly attributed to the loss of the liver, the ovary and the oviduct weights (Garlich et al., 1984; Kuksis, 1992). We therefore investigated the gene expression in the liver and the ovary before, during and after molting to evaluate the simultaneous changes in the functions of the liver and the ovary during the rejuvenation process. The results indicate that enhanced hepatic yolk precursor synthesis and secretion contribute to increased postmolt laying performance.

The regression of the ovary induced by the forced molt was a consequence of a reduction in LH secretion (Etches et al., 1984). The loss of support provided by ovarian steroid resulted in regression of the oviduct and molt, and the administration of exogenous sex steroids inhibited molting in domestic fowl (Berry, 2003). Our study indicated that molt enhanced the sensitivity of sex hormones in F1 follicles. Augmented gene expression in the ovary was involved in the rejuvenation of the reproductive performance of molted hens. These results suggest that facilitated yolk-precursor uptake by follicles is involved in the rejuvenation of the reproductive performance of molted hens.

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L48 The potential value of glucose oxidase in antibiotics−free feed

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Abstract

In decade, the feed enzyme industry grew fast. The deeper research that focus on feed enzyme, the more functional enzyme has been found, then the application of feed enzyme has been expanded as well. It's developing to the third generation, which has a special physiological function, from the first generation, which using for assisting digestion, and the second generation, which using for elimination anti-nutritional factors. Therefore this paper focus on glucose oxidase, thoroughly summarizeits function, mechanism, physical and chemical properties, and application effect, to provide a better support for research and innovation.

Keywords: glucose oxidase, antibiotics-free feed, third generation of feed enzyme, mechanism

Traditionally, there are two main purposes for application of enzyme in feed and cultivation. First, it can be a supplement of digestive enzymes, then directly improving digestion and utilization of dietary nutrients. Secondly, it can eliminate anti-nutritional factors in feed, then indirectly improving digestion of dietary nutrients. The successfulapplicationof feed enzyme in past was based on research and understanding. And the typical examples of two main purposes are exogenous digestive enzymes, such as protease, and nonstarch polysaccharidase enzymes, such as xylanase. But ever since feed enzyme development, it needs breakthrough and expansion. Glucose oxidase as the representative of the third generation of feed enzyme, it can sterilize and inhibit bacteria, then improved microecosystem and physiology of gastrointestinal tract through non-pharmacological mechanism. Therefore it can improve production performance of animal and has great potential on application, then open up a new field in feed enzyme.

The application of glucose oxidase in the third field of feed enzyme

Glucose oxidase (GOD, β-D-glucose oxidoreductase) is oxido-reductase that catalyses the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone. This enzyme is produced by certain species of fungi and insects and displays antibacterial activity when oxygen and glucose are present.

It's neither directly assist digestion, not eliminate anti-nutritional factors, even consume dietary nutrients (this consumption is negligible).But since glucose oxidase is an aerobic dehydrogenase, it can consume oxygen in the gastrointestinal tract, and then prevent the propagation of aerobic bacteria. And the hydrogen peroxide which from the glucose oxidation can sterilize bacteria as well. Therefore, glucose oxidase has many functions, such as eliminate of intestinal pathogens living environment, maintain the balance of intestinal flora, protect the intestinal epithelial cells intact, and reduce gastrointestinal pH. Thus we can define the purpose of elimination of intestinal pathogens living environment, which different from improving digestion of dietary nutrients and elimination anti- nutritional factors as the third field of feed enzyme. Although the second generation of feed enzyme (such as xylanase, β-glucanase, α-galactosidase, β-mannanase), has the function to improve the environment of the gastrointestinal tract, but its main role is to improve digestion and utilization of nutrients.

With the trend of feed safety the action of prohibit antibiotics and the so-called antibiotic -free cultivation is not a topic of discussion, but a practical application. Although academia and industry made a lot of discussion in this field, however based on our national conditions it still has some problems. Perhaps the single measure is far away from solution. While glucose oxidase can eliminate intestinal pathogens living environment, maintain the balance of intestinal flora, protect the intestinal epithelial cells intact, and reduce gastrointestinal pH, based on the above glucose oxidase can provide non-antibiotics realistic foundation and make "antibiotic -free" cultivation possible, especially with other means.

Because the effects of glucose oxidase are diverse, so it should also be evaluated in various forms.

The improvement of animal performance and feed efficiency is anticipated, but among the functions of glucose oxidase, the most anticipated is unconventional animal production performance, which we propose in the evaluation of the value of feed enzymes. The generalized indicators of animal performance should include others; even those could not be quantified, such as outlook, health status, uniformity, proportion of slaughter, survival rate. Perhaps those indicators could better reflect the effect of glucose oxidase. (Dingyuan Feng and Jianjun Zuo, 2009).

Glucose oxidase has the following characteristics: 1. the gastrointestinal of animals do not secrete; 2. It does not hydrolyze or decompose anti-nutritional factors; 3. It consume dietary nutrients; 4. It sterilize and inhibit bacteria through non- pharmacological mechanism; 5. It produces organic acids and has an acidifying agent effect.

People found the glucose oxidase as early as 1904. However, due to the lack of understanding of its commercial value, glucose oxidase did not arouse people's enough attention. Until 1928, Muller first discovered glucose oxidase from the cell-free extract of Aspergillus Niger, and studied its catalytic mechanism, then officially named as glucose oxidase, which was classified as dehydrogenases（Li *et al*，1993）. China started to study the purification process of glucose oxidase since 1986. In 1998, glucose oxidase began to be produced and it was identified as one of feed enzyme can be used by the Ministry of Agriculture in 1999. Glucose oxidase, which extracted from Penicillium notatum and Aspergillus Niger, has been included inChinese Ministry of Agriculture <feed additive varieties catalog (2013)>. Glucose oxidase widely exists in animals, plants and microorganisms. Bacteria and fungi are the main microorganisms that produce glucose oxidase. However Penicillium and Aspergillus Niger are general used to produce glucose oxidase in industry.

High purity glucose oxidase is pale yellow powder, soluble in water, insoluble in ether, chloroform, butanol, pyridine, glycerol, ethylene glycol and other organic solvents.50% acetone and 60% methanol can make it precipitate.Its molecular size is between 150 kD and 152 kD. Glucose oxidase is stable in pH 4.0 ~ 8.0 and functioning with temperature between 30℃ to 60℃ .General products of glucose oxidase contain catalase enzyme and its maximum absorption wavelength is 377 ~ 455 nm.Glucose oxidase has no fluorescence when exposed to ultraviolet light, but after treated by heat, acid or alkali, it will emerge green fluorescence. Solid glucose oxidase preparation can keep stable for at least 2 years at 0℃ and 8 years at -15℃ .Glucose oxidase is stable in pH 4.0 ~ 8.0, and optimum pH is 5,without the existence of glucose and other protective agent, it will be rapid deactivation under the environment of pH higher than 8 or lower than 3.The activity ofglucose oxidase is not inhibited by ethylenediamine tetraacetic acid, potassium cyanide and sodium fluoride, but inhibited by mercuric chloride, silver chloride, 4-chloromercuribenzoic acid and phenylhydrazine. Subtilisin (pH6.0), trypsin (pH6.8) and pepsin (pH4.5) cannot disassociation it.

Mechanism of glucose oxidase

Glucose oxidases catalyze oxygenolysisof glucose, and cause two results: First consumption of oxygen in the atmosphere. Second is to produce gluconic acid. These two results play significant roles in animal digestive environment.

Glucose oxidase is able to consume O_2, and thencatalyze oxygenolysisof glucose.Catalase can decompose hydrogen peroxide to water and oxygen. Subsequently, water combines with glucolactone to product gluconic acid.

In the absence of catalase,Catalyzed reaction product of glucose oxidase is gluconic acid and hydrogen peroxide. In the presence of catalase, gluconic acid is produced.With the existence of ethanol and catalase, gluconic acid, acetaldehyde and water will be generated. Enzymatic reaction of glucose oxidaseis little affected by the concentration of substrate.When glucose concentration is 5% to 20%, the reaction rate is almost unchanged. By polarimetry, the initial product of GOD reaction is a 6-gluconolactone, and then6- gluconolactone spontaneous hydrolyzes to gluconate by non-enzymatic reaction. According to the reaction conditions, the forms of catalytic reaction of glucose oxidase as followed:

1. in the absence of catalase:

$C_6H_{12}O_6$ (glucose) $+O_2 \rightarrow C_6H_{12}O_7$ (gluconic acid) $+H_2O_2$,β-D-glucose$+O_2 \rightarrow$

δ-D-gluconolactone$+H_2O_2$

2. in the presence of catalase:

$C_6H_{12}O_6$(glucose)+1/2O_2→$C_6H_{12}O_7$ (gluconic acid)+H_2O_2;

3. with the existence of ethanol and catalase:

$C_6H_{12}O_6$ (glucose) +C_2H_5OH+O_2→$C_6H_{12}O_7$(gluconic acid)+CH_3CHO+H_2O_2

Glucose oxidase only acts on D-glucose, and is completely inactive on L-glucose. Na^+、Ca^{2+}、Mg^{2+}、Zn^{2+}、Mn^{2+}could activate the enzyme with varying degrees.

Functions of feeding glucose oxidase

Inhibiting the growth of enteric pathogens, maintaining the ecological balance of intestinal flora

Ingut,glucose oxidase catalyzes the glucose to produce gluconic acid and hydrogen peroxide; this reaction can consume oxygen, lead to anaerobic environment for anaerobic bacteria proliferation such as bifidobacteria. Meanwhile proliferation of beneficial bacterium form microflora competitive advantageresult in inhibition of the growth of enteric pathogens. When hydrogen peroxide accumulated to a certain concentration, growth and reproduction of E. coli, Salmonella, Pasteurella, Staphylococcus aureus, andVibrio is inhibited directly. Its mechanism is completely different from antibiotics, does not produce bacterial resistance or drug residues.Generated gluconate can reduce the gastrointestinal pH, manufacturing acidic environment for growth of lactic acid bacteria. It is benefit for controlling infection, eliminating corruption substances, and increasing macrophage activity, thereby enhancing immunity.

The anaerobic environment formed by glucose oxidase has a better effect on the treatment of persistent physiological diarrhea of livestock, especially when seasons alternate from spring to summer and autumn to winter each year.

Protection of intestinal epithelial cell integrity and reduction of gastrointestinal pH

Maintaining the integrity of intestinal epithelial cells can prevent the invasion of pathogens. Under stress, the organism of poultry experiences a series of oxidation, producing a large amount of "free radicals". Especially when the generation of free radicals exceeds the body's own ability to clear, intestinal epithelial cells will be destroyed. However, in the presence of catalase, glucose oxidase can directly use of oxygen free radicals to protect integrity of the intestinal epithelial cells.

Glucose oxidase catalyzes glucose to play the role of an acidifying agent in the intestine, creating an acidic environment. What's more, the pH of the stomach is reduced and pepsin is activated, promoting absorption of minerals and vitamins A, D. Besides, the acidic intestinal environment may reduce the propagation of harmful bacteria to prevent diarrhea. Glucose oxidase can simultaneously improve the palatability of feed. When animals are anorexia, indigestion and diarrhea, adding glucose oxidase can gradually return to normal intake. After glucose oxidase into the gastrointestinal tract, the glucose acid continuously product so that the pH value of the gastrointestinal tract decreased. And glucose acid has the similar function with the glucose oxidase.

Reducing the content of mycotoxins in intestine, relieving drug poisoning and ensuring the quality of feed

In high temperature season, the toxin produced by mildewed feed may causes hepatomegaly, thymic atrophy and macrophages being poisoned, leading to immune suppression. Supplementation of glucose oxidase can control these symptoms, relieve the harm of mycotoxins in feed and improve immunity of animals. On the one hand, glucose oxidase can not only inhibit the proliferation of Aspergillus flavus, Rhizopus, Penicillium and so on, but also have a good preventive effect on aflatoxin B1. On the other hand, glucose oxidase produces a certain amount of hydrogen peroxide during the reaction. Especially when the accumulated concentration of hydrogen peroxide reaches a certain level, oxidized mycotoxins inactivated because of oxidation characteristics, to reduce the content of mycotoxin in gastrointestinal tract (Hu et al., 2014). The reason why adding glucose oxidase to feed is great beneficial to silage processing is that it can consume oxygen of silage to reduce oxygen level, which is conducive to the proliferation of anaerobic lactic acid bacteria and speed up the fermentation process so as to quickly produce large amounts of lactic acid, and the pH value of the silage drops quickly at the same time, inhibiting the propagation of harmful bacteria to avoid abnormal fermentation, and at last, the quality of silage is guaranteed. In addition, adding glucose oxidase in fat can consume oxygen to resist growth of microbial and prevent rancidity of fat.

The control effect of glucose oxidase on parasitic diseases such as coccidiosis

Coccidiosis is a common multiple of parasitic disease and seriously harmful to poultry. Currently, the drug is still mainly prevention in domestic, but coccidiosis is extremely easy to produce drug resistance so that existing coccidian drugs has been difficult to control coccidiosis. The priority for promoting poultry production is safety, so how to make the method of coccidiosis prevention effective and economic is the most important. In the presence of catalase, glucose oxidase can directly use of oxygen free radicals to reduce the oxidation effect of oxygen free radicals on intestine and protect the integrity of the intestinal epithelial cells, which makes the opportunity of coccidium invade intestinal parasitic site greatly reduce, so as to achieve the purpose of defense coccidium. Liang (2004) selected 10,000 of Jinshui silky fowl which were randomly divided into control group and glucose oxidase group and added diclazuril in water from 15 days to 60 days in the control group, and drink samwei coccidiostats 3d after the occurrence of coccidiosis. In the glucose oxidase group, add 0.2% glucose oxidase at the age from 1 to 15 days and 0.12%~0.16% from 16 to 60 days, and diclazuril 1~3d if there is coccidiosis. The results showed that onset of coccidiosis in glucose oxidase group delayed (at age of 32 days), while the control group was 21 days. Besides, glucose oxidase group has milder symptoms, shorter course of disease, no relapse, and greatly lower coccidium mortality (0.12%) than the control group (4.2%), the difference was extremely significant. What's more, the results indicated that glucose oxidase has an inhibited effect on invasion of coccidium in the gut. Glucose oxidase can not only match with antibiotics and other antimicrobial drugs to improve efficacy by synergism, but also replace antibiotics and anti-coccidiosis drugs at some extent.

The application of glucose oxidase in breeding industry

The application of glucose oxidase in poultry feed

Previous studies showed that glucose oxidase showed positive effects on improving the growth performance of poultry. Among them, Li Jing (2009) added 0.2% glucose oxidase preparation (GOD), AA broiler survival rate was significantly higher than that in control group, survival rate improves 3.5 percentage points, and the weight gain rate is increased by 6.23%, feed weight ratio lower than that of the control group. Pang Jiaman(2013) study showed that glucose oxidase had a significant effect on growing weight, feed weight ratio and nutrient metabolism rate among 36~70 day old yellow feather broiler, and 380g/t was the most appropriate addition amount. Zhang Xiaoyun(2007) found that the addition of glucose oxidase in laying hens could promote the proliferation, inhibit the proliferation and increase the serum albumin level. Research showed that addition of GOD in fed diets had effects on serum biochemical indexes of laying hens, GOD add group could significantly increase the content of total protein and albumin, and increase the synthesis of protein. Among them, the group whit 0.4% addition improved the most obvious ($P<0.05$); The addition of GOD could increase the content of globulin, calcium, phosphorus and alkaline phosphatase, reduce total cholesterol and triglyceride levels and improve the activity of alanine aminotransferase and aspartate aminotransferase while the difference didn't reach significant level; The group whit 0.4% GOD addition significantly decreased creatine kinase activity ($P<0.05$) and enhanced the body resistance. Glucose oxidase canreplace growth promoting agent by relieving the harm of mycotoxins in feed, and improving animal immunity.Wen Qi (2003) reported, the used of God in the parent stock had obvious effect to prevent Escherichia coli caused diarrhea under normal circumstances, the addition rate was 0.2% in the beginning and 0.1% after one week. In addition, the feather of the experimental group was neater and shinier than that of the control group, and the padding was also dryer, when Li Yan (2004) used glucose oxidase in broiler diets, which indicated the positive effect to improve the culture environment by adding glucose oxidase.

Beside, research shows that GOD can be a substitution of coccidiostats. Liang (2004) selected 10,000 of Jinshui silky fowl which were randomly divided into control group and glucose oxidase group and added Diclazuril in water from 15 days to 60 days in the control group, and drink samwei coccidiostats 3d after the occurrence of coccidiosis. In the glucose oxidase group, add 0.2% glucose oxidase at the age from 1 to 15 days and 0.12%~0.16% from 16 to 60 days, and Diclazuril 1~3d if there is coccidiosis. The results showed that onset of Coccidiosis in glucose oxidase group delayed (at age of 32 days), while the control group was 21 days. Besides, glucose oxidase group has milder symptoms, shorter course of dis-

ease, no relapse, and greatly lower Coccidium mortality (0.12%) than the control group (4.2%), the difference was extremely significant.

The application of glucose oxidase in swine feed

Biage (2006) investigated the effect of GA on in vitro growth response and metabolism of swine cecal microflora and on animal growth performance, intestinal wall morphology, and intestinal microflora. During a 24h in vitro cecal fermentation, total gas production and maximum rate of gas production were increased by GA (linear, $P < 0.001$). Ammonia in cecal liquor was reduced by GA after 4, 8, and 24 h of fermentation (quadratic, $P < 0.01$). After 24 h of fermentation, total short-chain fatty acids, acetic acid, propionic acid, n-butyric acid, acetic to propionic acid ratio, and acetic + butyric to propionic acid ratio were linearly increased by GA ($P < 0.001$). In the in vivo study, 48 piglets were divided into 4 groups and housed in individual cages for 6 wk. Piglets received a basal diet with a) no addition (control) or with GA addition at b) 3,000 ppm, c) 6,000 ppm, or d) 12,000 ppm. After 6 weeks, 4 animals per treatment were killed, and samples of intestinal content and mucosa were collected. Compared with control, GA tended to increase average daily gain (+13 and +14% for GA at 3,000 and 6,000 ppm, respectively; P of the model = 0.11; quadratic, $P < 0.05$). Daily feed consumption and gain to feed ratio were not influenced by GA. Intestinal counts of clostridia, enterobacteriaceae, and lactic acid bacteria were not affected by GA. Gluconic acid tended to increase total short-chain fatty acids in the jejunum (+174, +87, and + 74% for GA at 3,000, 6,000, and 12,000 ppm, respectively; P of the model = 0.07; quadratic, $P = 0.07$). Morphological evaluation of intestinal mucosa from jejunum, ileum, and cecum did not show any significant differences among treatments.Biage's study showed that feeding GA influences the composition and activity of the intestinal microflora and may improve growth performance of piglets after weaning. Jiuxian Yang (2011) focus on the effects of glucose oxidase (GOD) in performances of weanling piglets, and gastrointestinal tract health.In the experiment 160 pigs were randomly divided into 4 treatments, with each treatment represented by 5 replicates of 8 pigs. The pigs in group Ⅰ fed basal diets, did not give GOD and biomycin, group Ⅱ fed basal diets with the addition of 100 mg/kg biomycin, groups Ⅲ and Ⅳ received basal diets with the addition of 0.1%, 0.2%GOD, respectively. The experimental period was lasted for 4 weeks. The pigs in group Ⅳ obtained a higher yield of average day gain than that in group Ⅰ ($P<0.05$). The F:G was tendency decreased ($0.05<P<0.15$) in group Ⅳ when compared with group. Piglets supplied with GOD increased height of duodenum villus and ratio of VH/CD ($P<0.05$), but decreased pH of stomach and duodenum ($P<0.05$) when compared with not supplied GOD and biomycin piglets. Digestibility of dry matter and crude protein of dietary were higher in piglets added with GOD when compared with not supplied with GOD groups. The results showed that GOD increased piglets ADG and CRF, and then help the weanling pigs increase their production, through improved piglets gastrointestinal structure and environment.Ji Yin (2012) selected 66 sanyuan hybrid piglet with which average weight were 17kg, then randomly divided into control group and glucose oxidase group,in the glucose oxidase group was added 0.5% glucose oxidase (45U/g), trial lasted 40 days. The results shows that compared with the control group, the ADG of glucose oxidase group was significantly increased 7.03% ($P<0.05$), and the FCR of glucose oxidase group was significantly decreased 2.75% ($P<0.05$).

Tang Haiou et al. (2013) researched the effects of diets supplemented with different doses of glucose oxidase on the growth performance and economic efficiency of farming. The results showed that, average daily gain and feed intake of groups of 100 g/t and 200g/t glucose oxidase added were significantly higher than those of control treatment ($P<0.05$). Compared with those of control treatment, average daily gain and feed intake of group of 400 g/t glucose oxidase added were significantly lower ($P <0.05$). Feed conversion ratio of all test treatments significantly decreased ($P <0.05$). Group that added 400 g/t glucose oxidase had lower diarrhea ratio than that of control treatment ($P<0.05$). The benefit of the groups of 100 g/t and 200g / t glucose oxidase added were significantly higher than that of control treatment ($P <0.05$). The results indicated that the average daily gain，feed intake，feed conversion ratio of piglets and economic efficiency could significantly improve by supplementing GOD.

Glucose oxidase consumes oxygen in the process of reaction. Due to most enteropathogens are aerobic bacteria, and most beneficial microorganisms are anaerobic or facultative anaerobes. Therefore, glucose oxidase in the gut consumed oxygen helps the proliferation of beneficial bacteria and inhibits harmful bacteria, to maintain the balance of intestinal flora and ensure animal health. Gluconate can arrive in

the large intestine, and stimulate the growth of lactic acid bacteria. The properties of gluconate are similar to prebiotic. Gluconate is rarely absorbed in the small intestine. However, gluconate can be utilized by the located microflora to generate butyric acid, when it reaches the end of the gut. Butyric acid is a kind of short chain fatty acid (SCFA), which can be rapid absorbed by large intestine mucosa, providing energy for intestinal epithelial. Butyric acid is the most effective SCFA in Stimulating intestinal epithelial cell proliferation, promoting sodium and water absorption efficiency. Furthermore numerous reports confirmed that butyrate can inhibit intestinal cancer. In addition to the function of acidifier, gluconic acid may play a significant role though butyric acid, its fermentation product in the back of intestinal segment.

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L49 Vitamin and minerals removals from poultry feed

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Abstract

Vitamin and minerals play an unquestionable role in poultry health. Commercial poultry industry adds vitamin and mineral mix in excess of recommended amounts to avoid growth retardations. Some researchers argue that such practices are both unnecessary and costly. Thus "Vitamin Mineral Removal (VMR)" at finishing periods is recommended to save cost. On the other hand the reports from research centers are not conclusive. Earlier literature (1990s) indicated that VMR has no ill effect on production and is economically feasible, there are many unanswered questions and evidence that cast doubt over the "usefulness" and "profitability" of VMR. The focus and urge for better environment, welfare and meat (and egg) quality and enrichment in new millennium has also to be taken into consideration to make a judgment about usefulness of such practices. Also, the term "profitability" needs to be fully understood and clarified before a final decision is made as to whether VMR justifies its cost saving. It is thus essential to have a thorough knowledge and understanding of different aspects of research reports on VMR before advising and/or lunching such practices.

Keywords: vitamin, trace mineral withdrawal, broiler, layer

Introduction

Vitamins and minerals have important biological functions and must be given in adequate amounts. NRC (1994) have developed standards for vitamin and mineral requirements in animals that give the minimum levels that are necessary for optimum growth, productivity, egg production, egg quality, hatchability, etc. However, environmental variation, stress, vitamin and mineral interactions and oxidation losses are not considered in the determination of requirements. On the other hand rapid growth, high production and metabolic disorders may increase the need for supplemental vitamins and minerals requirements (Coelho' and McNaughton 1973). Ziaie et al., (2008) argued that NRC (1994) for most minerals were based on research published between 1952 and 1983. The authors argued that modern broilers differ considerably from older strains in both performance and bone conformation.

According to Deyhim and Titer, (1993) Corn-soybean meal based rations, formulated to satisfy the energy and amino acid needs of the commercial broiler, are typically deficient in several vitamins and trace minerals. However, in practice, food manufacturers and producers use two to ten times more vitamin and mineral supplementation than the stated requirements specified by NRC (1994) (with the exception of a few nutrients) to ensure vitamin and mineral deficiencies does not occur. An example of the dietary composition made according to NRC (1994), breeding company recommendation, and others suggested vitamin and mineral supplement need to be added to the diet (Table 1).

Table 1. Mineral and vitamin requirements suggested by the NRC (1994), Ross−308 nutritional guide and those provided by experimental diets (Alaedin et al., 2013)

Experimental diets(kg)	Vitamins(mg)										Minerals(mg)				
	A	K	B_1	B_2	B_6	B_{12}	Biotin	Folic[a]	Niacin	P.A[b]	Cu	Fe	Mn	Se	Zn
NRC,94	1 500	0.5	1.8	3.0	3.5	0.007	0.12	0.50	25.0	25.0	8.0	80.0	60.00	0.15	40.0
Ross 308	9 000	3.0	2.0	6.0	4.0	0.016	0.20	1.75	55.0	13.0	16.0	40.0	120.00	0.30	100.0
Diet 1	12 000	4.0	6.2	9.5	20.9	0.030	0.34	2.65	61.7	22.1	16.5	86.7	107.70	0.35	98.2
Diet 2	6 000	2.0	4.7	7.4	14.6	0.015	0.24	1.65	41.8	14.6	11.5	83.7	62.68	0.20	63.2
Diet 3	-	-	3.2	1.5	4.2	0.010	0.14	0.65	21.8	7.14	6.5	80.8	17.70	0.05	28.3

[a]Folic: Folic acid, [b]P.A: Pantothenic acid diet 1, 2 and 3 contained 5%, 2.5% and no VM supplement respectively

Addition of vitamin and mineral supplements make up only 0.02% – 0.05% of the diet and 1.5% of food cost (REF). However, vitamin and mineral removal from the diet can save money if there is no significant deleterious economic loss as a result of vitamin and mineral removal, since 25% of the feed consumed takes place during the last 7 days before processing.

Vitamins and mineral removal impact on performances

Under commercial growing conditions, using practical feedstuffs, it may be difficult to produce vitamin and/or trace mineral deficiencies in birds during the finishing period when diet were adequately supplemented during the growing period (Skinner et al., 1992). Mairoka et al., (2002) emphasized using a practical diet while supplemental vitamin and mineral are withdrawn from the diet is not an issue since corn and soybean meal provide a portion of the vitamin and mineral requirements. Further, fat-soluble vitamins are stored in the fatty tissue of the body and can be remobilized when a deficiency occurs (Mairoka et al., 2002). Jafar et al., (2005) indicated that with the exception of trace minerals like manganese and zinc, other requirements are met by corn and soybean meal used in poultry rations. It has also to be emphasized that the deficiency of minerals and vitamins requires long periods to demonstrate clinical signs (Lesson and Summers, 2008).

On the other hand commercial poultry industry adds MV mix in excess of recommended amounts to overcome any (unforeseen) shortcoming that affects growth retardations. However, vitamin and mineral removal from the diet can save money if there is no significant deleterious economic loss as a result of vitamin and mineral removal.

Waldroup et al., (1968) was first to report that the presence or absence of a commercial VM (vitamin and mineral) mix in a corn-soybean meal diet had no significant effect on body weight gains, feed utilization or the incidence of toe and hock deformities of broilers 0 to 28 days of age. Thomas and Wining (1971) concluded that supplementation with vitamins and trace minerals does not appear to be necessary provided that broilers are not kept on withdrawal feeds for longer than 10 days.

According to Skinner et al., (1992) under unstressful environmental condition vitamin and mineral removal has no harm effect on poultry performances. They postulated that if Ca and P levels could be reduced during 42 to 49 day finisher period without adversely affecting performances, production costs could be markedly reduced. Their findings showed weight gains, feed consumption, feed conversion ratio, and mortality did not differ when calcium and phosphorous were removed from the finisher. They also indicated that calcium and phosphorous withdrawal from the finisher diet had no effect on the broiler' s length and width, dressing percentage, and tibia ash.

Deyhim and Tetter (1993) indicated that trace mineral removal alone was without consequences, while birds fed diets lacking vitamin exhibited reduce weight gain and feed efficiency. Khajali et al., (2006) results showed that VM mix withdrawal from finisher diet did not impair either weight gain or feed conversion efficiency from day 42 to 56. Feed intake, however, was significantly increased when both mixes were omitted from the diet. These authors reported that dietary treatments had no effect on carcass yield or proportion of breast and thigh meat and abdominal fat deposition.

Moravej et al., (2012) compared the effect of vitamin withdrawal in two rearing (floor vs cages) systems. They presumed that caged chicken require more dietary vitamins than those on floor because of free access to coprophagy. Moravej et al., (2012) hypothesized that the excreta plus litter would provide additional V and TM intake. Floor-raised broilers can get fecal vitamins. The results of their study indicated that in the battery cage system it is possible to reduce the dietary vitamin premix during the finisher period, but withdrawal can negatively affect performance, ALP activity in blood and mechanical parameters of bone of broiler chickens. On the other hand, in the floor system it is possible to withdraw vitamin supplements in broiler finisher diets withdrawal can negatively affect performance.

Stress and environmental challenges

Dayhim and Titter (1993) assessed the effect of VMR (28-42 days) when birds were heat distressed (24-35 *c). The V withdrawal during days 28-49 depressed bird growth rate and feed efficiency. They indicated under the reported conditions, significant economic loss can occur when supplemental VM are withdrawn from the diet. Vitamin withdrawal consequences were exacerbated in the presence of Trace mineral. TM withdrawal in the presence of vitamin did not impact performance or immunological criteria. Khajali et al., (2006) reported packed cell volume (PCV) significantly decreased as a consequence

of removal of V, Trace mineral and VTM. Neither heterophil:lymphocyte ratio, haemagglutination inhibition nor total antibody titre measured by ELISA were influenced by removal of V or TM supplements. They concluded that 14- d withdrawal of V or TM mix did not influence the immune competence of broilers and that V and/or TM supplements can be satisfactory removed from finisher diets for 7 days. Siahpour et al., (2008) reported that per Kg body weight, the percentages of bursa of fabricius, spleen, and abdominal fat carcass were not influenced by VM withdrawal.

Impact of VMR on meat quality and Content

Chicken meat is a good source of mineral and vitamins. Although VM removal might have no adverse effect on performance and/or benefit profitability, it may result in meat being devoid of vitamin and minerals. The absence of mineral and vitamins in meat might also lower the shelf life of meat during storage and freezing. Patel et al., (1997) reported that dietary levels of choline, thiamin, pantothenic acid and B6, or riboflavin have a negligible effect on broiler performance and the meat nutrient content, but riboflavin removal from the diet reduced riboflavin content of both breast and thigh muscle. Zapata et al., (1998) reported withdrawal of minerals and vitamin from final diets of broilers does not affect mineral contents in the meat. However, the content of calcium in meats was adversely affected by the withdrawal of mineral and vitamin. The authors noted that broilers mineral meat was not affected by sex while Ca, Na, Fe and Zn content were higher and P, Mg, and K were lower in the dark meat compared to light meat.

VMR and environmental pollution.

Intensive farming has resulted in environmental pollution. As such, attempts are made to reduce excessive excretion of minerals into litter. However, if the presumed claim that 2 to 10 times minerals and vitamin is used, then it is clear that higher amounts of minerals are excreted in litter. Mineral removal could be a way to reduce mineral excretion. Ziaie et al., (2008) reported decreasing dietary Ca and P had no significant overall effect on water intake, excreta dry matter content, Ca and P retention or metabolisability of DM. According to Wang et al., (2008) reduction of "trace mineral premixes" in broiler diets could reduce mineral excretion to the environment. Levels of Fe, Cu, Mn and Zn in broiler excreta decreased when mineral premix levels were reduced.

Profitability issue of VMR

The cornerstone of VM removal advocates is profitability. Yet, rarely this was assessed properly. Coelho and McNaughton (1995) dealt with several interesting and important criteria measurements of "body weight uniformity" "carcass grading" and "profitability" in their report on vitamin supplementation levels. They expressed that bird uniformity is becoming more important since it increases processing efficiency and improves the consistency of the final processed product. Measuring individual body weights (coefficient of variations) at 42 and 51 days of age to determine body weight uniformity, they showed that high (5%) levels of vitamin supplementation improved body weight uniformity.

The authors expressed that total skin tears and scratches were also important since both carcass grading and consumer preference may be impacted. Past research indicated that inadequate vitamin supplementation leads to weak tissue and poor skin integrity and strength with biotin, pantothenic acid, or vitamin A deficiencies. They showed that vitamin withdrawal significantly increased the number of skin tears and scratches in all stress regimes.

Although bird performance tends to be measured in terms of weight gain, feed efficiency, and mortality, according to Coelho and McNaughton (1995) maximum performance may not always be the most profitable strategy. They argued that net income per processed bird is the most accurate index of flock performance. Thus, profitability and return on investment should be calculated by considering live performance, processing factors, actual feed costs, and average industry grow out and processing costs. They showed that vitamin supplementation significantly improved profitability.

Processing cost is one criteria not considered in experiments, except that of Coelho and McNaughton (1995). Most experiments are carried under control research conditions with great care, while what happens in farm, during transport and processing plant is stressful. Any lack of nutrient supplementation could have a devastating effect on the final product and thus profitability.

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L50 Poultry immunogenetics: protecting health and food security, expressing genetic potential

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Keywords: genetics, immunity, disease, food safety

Summary

Poultry meat and eggs will serve a major role in addressing the nutritional demands of the future in developed and, especially, in developing countries. Improving poultry health by increasing genetic resistance to disease is essential to meet the increasing emphasis on animal welfare, food safety, environmental impacts, and production efficiency. To fully utilize their maximum genetic potential to produce wholesome and economical products, the health of poultry must be optimal. Genetic selection for improved response against pathogens is an important part of a comprehensive poultry health protection program. Knowledge of the genomic location or identity of genetic elements that control the many facets of host response to pathogens is an essential foundation for enhancing disease resistance. Deeper understanding of the function of these genetic elements also facilitates the development of other strategies to protect health, such as improved vaccines and natural immunomodulators. This paper introduces immunogenetic approaches to enhancing disease resistance and selectively reviews research on genomic resistance of chickens to bacterial and viral pathogens, focusing on recent studies in the author's laboratory.

Impacts of disease and of genetic selection

Infectious diseases have a significant negative impact on the poultry industry. Ten to 15% of the potential profit in commercial poultry production has been estimated to be lost because of disease (Biggs, 1982). The impact is even greater on the livelihood of the rural poor in developing countries, where up to 25% of monthly income may be lost due to poultry disease (Rist et al., 2015). Zoonotic pathogens threaten human health and undermine consumer confidence in the safety of poultry products, thus reducing market demand. Trade barriers can be invoked to prevent movement of poultry and their products, thereby limiting export markets. Because of consumer preferences, retailer specifications and government regulations, the use of antibiotics in poultry production is decreasing. Protection of poultry against pathogens adds the expense of vaccination programs for those few pathogens for which vaccines currently exist, and can require expensive changes in management practices such as heightened biosecurity. Disease can cause mortality or condemnation of birds, leave them susceptible to secondary infections, or reduce their efficiency because fighting infections diverts critical resources away from growth and production (Siegel et al., 2008). The biological impact of a microbial burden reduces growth and reproductive performance (Klasing and Korver, 1997). Reduction in performance and efficiency occurs even in typical production environments in which microbes are present but are not causing clinical disease.

After successful, long-term genetic selection for traits such as growth or reproduction, as has been done for commercial poultry, populations sometimes appear to be more at risk for immunological disorders (reviewed in Rauw *et al.* 1998). These undesirable consequences of commercial selection arise from selective sweeps of the genomic regions flanking the genes influencing commercial traits and increases in frequencies of deleterious recessive alleles (Hocking, 2014). Thus, genetic improvement of immune response is an essential goal for sustainable poultry production, to enhance immune response and reduce pathogen burden, thereby enhancing vaccine response, disease resistance, animal health and food safety (Figure 1).

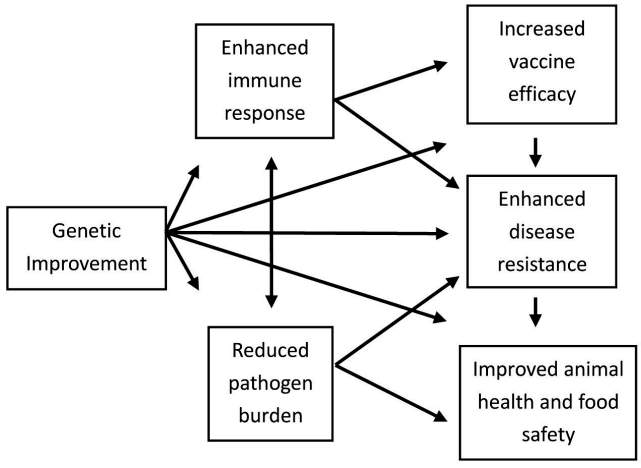

Figure 1. Positive impacts of genetic improvement in immune response.

Immunogenetics for enhanced disease resistance

Genetic selection for improved response against pathogens is an important part of a comprehensive poultry health protection program, which may also include strong biosecurity, vaccines, immunomodulators and judicious use of antibiotics. Breeding for disease resistance can be approached in multiple ways. Direct selection for resistance and immunity can be done after measuring the response of breeding candidates or their relatives (Lamont et al., 2003). Direct responses to pathogen challenges to define disease resistance in the live bird are, however, very expensive, welfare-compromising, environmentally risky and labor-intensive phenotypes to collect. Thus, alternative tests such as molecular biomarkers are needed (Cheng et al. 2013; Lamont et al., 2014). Genetic markers typically are not themselves the causative genetic variants but are in linkage disequilibrium with causal variants, such that increasing the frequency of the desired marker variant in the population also increases the frequency of the beneficial allele for the resistance trait. Many molecular markers for immunogenetic traits have already been identified through comparative or functional genomics studies, testing of biological and positional candidate genes, and through genome-wide association studies, amply illustrating the feasibility of identifying useful markers (reviewed in Abasht et al., 2006; Cheng and Lamont, 2013; Lamont, 2010; Lamont et al., 2014).

Most immunity and disease-resistance phenotypes result from the interaction of many different cell types, signaling and effector molecules and, in the case of infection, dynamic interaction between the pathogen and the host. Understandably, then, the genetic architecture of disease resistance is complex, highly polygenic, and determined by variation in both structure and expression of many host genes. To obtain a comprehensive picture of the immunogenetics of poultry, therefore, integration of approaches that combine genome-wide and gene-specific studies of both structural and expression-level variation is required (Cheng et al. 2013).

Approaches to elucidate genetics of disease resistance

To illustrate the varied approaches that can be taken to enhance immunogenetics and elucidate the genomics of disease resistance in poultry, this section gives brief, selective summaries of some recent studies conducted in the author's and collaborators' laboratories on genetics of pathogen resistance in chickens, with a focus on only those studies that used high-throughput genomic analyses.

The use of genome-wide association studies (GWAS) provides an approach that does not bias the findings toward pre-selected target genes, but allows the interrogation of the entire genome for associations with the tested traits. This approach is especially useful to reveal previously unknown associations of host response with pathogen infection. Using a high-density panel to genotype single-nucleotide polymorphisms (SNPs), many novel loci were identified by GWAS for the response of heterophils, one of the first cellular responders to infection, to infection with *Salmonella enteritidis*. Many previously identi-

fied associations were also confirmed (Redmond et al., 2011).

Analysis of RNA sequence (RNAseq) from birds infected with Avian pathogenic *E. coli* (APEC) revealed genes and pathways that were differentially expressed (DE) between resistant (low lesion score) and susceptible (high lesion score) birds. In the bone marrow of susceptible birds, lymphocyte differentiation, proliferation, and maturation were impaired, while innate and adaptive immune responses, including dendritic cells, monocytes and killer cell activity, TLR- and NOD-like receptor signaling, T helper cell and cytokine activity, were enhanced (Sun et al., 2015a). In the bursa, the DE genes were associated with signal transduction, immune response, cell growth and cell death pathways (Sun et al. 2015b).

The RNAseq of lungs of birds infected with avian influenza virus (AIV) revealed differences in expression between inbred lines of chickens that are relatively resistant or susceptible to the virus. These differences suggested the involvement of hemoglobin family genes, oxygen transport and circulation, and the cell adhesion molecule signaling pathway in resistance to AIV infection (Wang et al., 2014).

The unique anatomy of the immune system of birds affords the opportunity to investigate the functional genomic response of novel tissues and organs to pathogen infection. The Harderian gland is a compact lymphoid tissue in the orbit, closely associated with the eye. It is ideally situated to respond to pathogens that infect the eye, such as Newcastle Disease virus (NDV) and to eye-applied and spray vaccines. In chickens inoculated via the eyes and nares with NDV, RNAseq demonstrated differences in the Harderian gland transcriptome between inbred lines that had differential responses to clearance of the virus. Between infected versus non-infected birds of the susceptible (Leghorn) line, many immune-related genes were differentially expressed, including Mx, IFN-beta, and IL8, suggesting their involvement in NDV response (Herrmann et al., unpublished data).

microRNAs (miRNAs) are a relatively newly identified category of RNAs. A systematic analysis of the miRNA transcriptome of tumors from tissues of Marek's disease virus-infected birds versus the same tissue type from non-infected chickens revealed many known and several uncharacterized miRNAs. Target genes for the differentially expressed miRNAs were predicted, and some were experimentally verified in reporter gene assays. These miRNAs are strong candidates for involvement in MDV-induced lymphomagenesis (Lian et al., 2012).

In addition to variations in DNA structure and RNA expression, phenotypic traits can be influenced by epigenetic mechanisms of the host. Methylation of DNA is one of the primary epigenetic modifications. The spleen methylome of broilers that were APEC-infected compared to non-infected controls, and integration of those data with gene expression data, showed differences in several cytokine genes, and gene network analysis suggested involvement of pathways involving inflammatory response, organismal injury and abnormalities, cell signaling and molecular transport, and cell cycle regulation (Xu et al., 2014). The observation that immune-related GO terms were significantly enriched from genes within the differentially methylated regions between relatively disease-resistant (Fayoumi) and disease-susceptible (Leghorn) inbred lines implicates DNA methylation as a genetic regulatory mechanism modulating immune response differences between these lines (Li et al., 2015).

In summary, there is evidence of genetic control of response of poultry to disease pathogens at many different levels. Poultry populations may possess natural variation in both structure and expression of genes and other genetic mechanisms that influence immune response and disease resistance. Successful breeding for improved disease resistance requires deep phenotyping for relevant traits and may benefit from both genomic selection and selection on variation in targeted candidate genes, as well as the incorporation of new gene-editing technologies.

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L51 From quantitative trait loci (QTL) discovery to nutrigenetics

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Keywords: nutrition, genetics, nutrigenetics, poultry

Summary

The study of the interactions between genetics and nutrition has gained new impetus with the development of high-throughput genomic approaches allowing for a comprehensive approach of the variability of gene sequences or activity. The object of nutrigenetics is to identify the genetic determinants (sequence polymorphisms) of the variability of individual responses to nutrition.

A quantitative trait locus (QTL) is a chromosomal region involved in the control of quantitative phenotypic traits. Based on the knowledge of the chicken genome sequence, numerous positional candidate genes can be proposed within a QTL region, and further evaluation is needed to test the link between polymorphism of those genes, their activity and the expression of the phenotype.

As a proof of principle that such an approach is relevant to nutrigenetics, we identified a polymorphism in the gene encoding the enzyme b-carotene 15,15'-monooxygenase 1 (BCMO1), which affected the response to dietary intake of b-carotene in term of plasma, liver and duodenal concentrations of lutein.

Another project aims to identify the genetic determinants of digestive ability through the development and characterization of two experimental lines of chickens divergently selected for this trait. Recently, chromosomal regions controlling this trait have been identified, and further studies of sequence polymorphisms and gene expression (functional genomics) data in extreme animals issued from a cross population are underway.

Eventually, the tools of nutrigenetics will predict the potential for adaptation of genotypes to various feeds. Nutrigenomics, allowing a better understanding of the mechanisms by which nutrition affects gene activity, is expected to propose new nutritional strategies to guide phenotypes and new selection strategies to improve birds' ability to adapt to different diets. The combination of both approaches should permit to identify genes controlling those phenotypes.

Main text

Interactions between genetics and nutrition are old concerns which can now be studied in more details, thanks to the development of high-throughput genomic approaches. The object of nutrigenetics is to identify the genetic determinants (sequence polymorphisms) of the variability of individual responses to nutrition. While this opens great promises in term of personalized nutrition for humans, its potential application in animal nutrition and selection deserves some thoughts. Broilers have been intensively selected for growth rate and carcass conformation in a relatively standard environment including standard diets mainly based on corn and soybean. There is ample evidence that those animals do not express their full genetic potential for growth when fed alternative feedstuffs, even if their nutritional requirements are matched. Although genetic selection does not result in complete homogeneity of the animals on the market, some alleles are more frequent in selected populations and sometimes even fixed (Hayes et al, 2009). Identification of those alleles which are associated with the ability of birds to cope with particular feedstuffs would permit to choose the most appropriate strain or candidate to selection for a given objective, taking into account the context of available feedstuffs. Besides, this could also allow the breeders to develop specifically adapted strains on demand. The present paper will attempt to illustrate the feasibility of identifying such alleles and the potential application for the selection of broilers.

Polymorphism at the BCMO1 locus identifies a nutrigenetic control of carotenoid metabolism

Experimentally selected broiler lines with High and Low growth rate have been used as a model to

identify the mechanisms controlling growth, body composition and meat quality traits. After several studies comparing the lines, a cross population was constructed to identify the QTL that governed the corresponding traits. At the time, only a limited number of polymorphic microsatellite markers, around 130, could be used. Nevertheless, many QTL were identified and among them, one that controlled meat yellowness was the strongest (Nadaf et al, 2007). Following further studies implying finer mapping and bioinformatics, the BCMO-1 gene was identified as a double positional and functional candidate gene for the QTL (Le Bihan-Duval et al, 2011). An allelic polymorphism at the BCMO1 locus, within the promoter region, conferred a differential transcriptional activity to the gene in the breast muscle which was negatively correlated with the intensity of breast meat yellow color and its content in carotenoid pigments. A genetic test based on HRM of a PCR product was developed to genotype the chickens at this locus, and was also validated in unrelated broiler lines, and patented. It permitted to produce and select birds homozygous for one allele or the other, and exhibiting a high or a low BCMO-1 gene activity (High BCMO vs Low BCMO), within the same genetic background, for use in further studies (Jlali et al, 2012). Supplementation with β-carotene was provided to dissect the mechanisms underlying this genetic difference. Genotype by diet interactions were observed for retinol accumulation which increased following β-carotene supplementation in the duodenum of chickens with the High BCMO allele, and for xanthophyll's and vitamin E accumulation which decreased in duodenum, liver and plasma of chickens with the Low BCMO allele (Jlali et al, 2014). Carotenoid metabolism is therefore under a nutrigenetic control at the BCMO1 locus, which could be identified following observations made during a QTL analysis.

Towards the identification of alleles controlling broiler adaptation to various feedstuffs

With the objective of developing more sustainable poultry production schemes, the availability of broilers which could adapt to variable feedstuffs is a priority. The between-birds variability in digestibility had been shown to depend on the wheat varieties (Carré et al, 2002, Choct et al, 2009) and the demonstration that chicken digestive ability was an heritable trait was first made by Mignon-Grasteau et al (2004), by comparing birds fed a wheat based diets incorporating Rialto wheat, a hard and viscous variety selected for its low digestibility values. They also demonstrated the possibility of divergently selecting broilers on this trait and created an experimental model of good and bad digesters, which they subsequently used for further characterization of the underlying physiological and genetic mechanisms of adaptation to feed. These two lines differ for their response to dietary variations such as the incorporation of different wheat varieties (Péron et al, 2006) and to the incorporation of xylanase or antibiotics (Garcia et al, 2007). They also show marked differences in the relative development of their digestive organs – the good digesters showing a more developed gastric compartment (gizzard and proventriculus) and a lesser developed intestine (Péron et al, 2006, Garcia et al, 2007, Rougière et al, 2009, de Verdal et al, 2010), and a slower transit (Rougière and Carré, 2010). Broilers with enhanced digestive abilities also perform better on a more classical corn soybean diet, although the difference is less pronounced (Mignon-Grasteau et al, 2010; 5-10 % instead of 30-40 % in AMEn with wheat based diet). To evaluate their robustness against pathogens, the experimental lines were subjected to challenge by *E. coli*. While lesion scores did not differ between the two lines, mortality and bacterial loads were lower in the good digesters than in the bad digesters (Calenge et al, 2014).

A reference population resulting from the cross between the two lines was constructed and used for the identification of QTLs controlling numerous traits of importance for broiler production. The F2 population was genotyped with 3000 informative SNPs markers (Tran et al, 2014). A strong QTL controlling digestive use of starch and of dry matter was identified on chromosome 20 (Tran et al, 2014). Further QTL controlling feed intake, feed efficiency, growth, body composition, anatomy and excretion traits were also described (Mignon-Grasteau et al, 2015-a). Co-localisation of QTL controlling feed efficiency and development of the gastro-intestinal tract suggest a functional or even causal relationship between these traits. Functional candidates could be proposed and discussed for several of these traits. In their most recent paper, Mignon-Grasteau et al (2015-b) used extreme animals from the crossed population to examine the relationship between digestive ability and microbiota composition through the determination of cecal content of targeted bacterial species by quantitative PCR. This study demonstrated a link between host genetics, microbiota composition and feed efficiency or digestive efficiency. Lactobacillus and *E. coli* were more frequent in the less efficient birds. The digestive use of dry matter was posi-

tively correlated to the levels of *L. crispatus*, *C. leptum* and *C. coccoides*. Moreover, QTL controlling *C. leptum* and Lactobacillus were identified. The most significant QTL controlling *C. leptum* was on chromosome 6 in a region which includes gene involved in inflammatory responses of the gut and the motility of the digestive tract.

The next objective is to validate genetic markers which could be used for selection in commercial populations. The identification of the genes that control the traits would be the ultimate goal for both fundamental and applied purposes. Some of the underlying physiological mechanisms, have also been unravelled, which opens perspective for nutritionists.

Conclusions and perspectives

Our recent data obtained using experimental lines of chickens show that some responses to diet are genetically determined and therefore, that chickens can be selected on this criterion. In the case of carotenoids in the diet, an allelic variation in a single gene has been identified as a key determinant of the response. The same allele as detected in the experimental cross can be tracked in commercial populations and used for selection purposes. In the case of digestive ability for various feedstuffs, the feasibility of selection has been demonstrated and correlated responses and some of the underlying physiological mechanism have been described. A number of genomic regions controlling this trait and related traits of interest have been identified. Further studies are underway to identify the genes controlling those traits and their alleles. This will provide new tools for selecting birds with an increased adaptability for more various feedstuffs, which in a longer term could improve the sustainability of broiler production schemes.

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L52 Unravelling the related genes underlying morphological traits in chickens

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Keywords: gene mapping, morphological trait, chicken

Summary

Chicken is a well studied model organism of avian species, due to its abundant genetic and morphological diversity, easily accessible genomic resources, and the manipulability of the embryos. These characteristics of chicken have facilitated its research and produced important implications in agriculture and fundamental biology. Researches on gene mapping in domestic animals have established a framework towards a better understanding of genetics and developmental biology, and provided a plenty of markers that could be applied for marker-assisted breeding.

Main text

During the process of evolution, chickens have accumulated many well-marked morphological traits such as rose comb, silky-feather, black skin, etc. The morphological variations is a primary resource for the study of evolution, development, differentiation, and gene interaction. Almost all the attracted morphological traits are derived from the ectoderm which are adaptions to natural selection or results of artificial improvement of livestock under domestication.

The traditional strategy for gene mapping of the morphological traits including several steps: i) construction of a resource group; ii) get the markers information for each samples; iii) genome wide association study (GWAS) and linkage analysis; iv) fine mapping using more markers in target regions; and v) functional analysis. With current genomic technologies, parts of the causative genes for these variations have been identified by the efforts of geneticists in recent years (Table1). From these variations with known genetic causes, we can extrapolate that both complex genomic structural variations and single nucleotide variants could lead to apparent phenotypic differences.

Herein, we introduced several cases about mapping of monogenic characters in chickens in our lab (Fig. 1) and discussed the importance of researching on structural variations.

Rose−comb

Rose-comb is a classical comb variant that drastically altered comb morphology in chickens (Fig. 1A). Using a F2 resource population that derived from a cross between the White Plymouth Rock × Chinese Silkie (Gao et al., 2006), the Rose locus was mapped to GGA7 through linkage analysis. Based on the suppression of recombination observed in Rose-comb heterozygotes and the loss of heterozygosity observed in Rose-comb homozygotes between a 7.6-Mb interval, we implied that Rose-comb might be associated with an inversion. Using PCR analysis, we confirmed the breakpoints of this inversion which showed complete association with the Rose-comb phenotype. The inversion causes the relocalization and ectopic expression of the MNR2, and in this way, contributes to the Rose-comb in chickens (Imsland et al., 2012).

Silky−feather

Silky-feather is a variant that altered the structure of feather by inhibiting hooklet development (Fig. 1B). It was first mapped to a 380-kb region on GGA3 by linkage analysis in the White Plymouth Rock × Chinese Silkie F2 resource population (Gao et al., 2006). IBD mapping narrowed the region to 18.9 kb. Within the critical interval, a C to G transversion at GGA3: 70,486,623 bp showed completely association with Silky-feather locus. Through in vivo and in vitro experiments, we demonstrated that the silky-feather cis-regulatory mutation decreased the mRNA expression of PDSS2 by reducing promoter activity (Feng et al., 2014).

Table 1 Gene mapping in domestic chickens

Gene	Phenotype	Mode of inheritance	Reference
BCDO2	Yellow skin	Autosomal recessive	(Eriksson et al., 2008)
EDN3	Dermal Hyperpigmentation	Autosomal dominant	(Dorshorst B, 2011)
SLCO1B3	Blue eggshell	Autosomal dominant	(Wang et al., 2013)
FMO3	Fishy taint	Autosomal recessive	(Honkatukia et al., 2005)
MNR2	Rose-comb	Autosomal dominant	(Imsland et al., 2012)
SOX5	Pea-comb	Autosomal dominant	(Wright et al., 2009)
EOMES	Duplex-comb	Autosomal incomplete dominance	(Dorshorst et al., 2015)
HOXC8	Crest	Autosomal incomplete dominance	(Wang et al., 2012)
PDSS2	Silky	Autosomal recessive	(Feng et al., 2014)
BMP12/GDP7	Naked neck	Autosomal incomplete dominance	(Pitel F, 2000)
FGF20	Featherless	Autosomal recessive	(Wells et al., 2012)
KRT75	Frizzle	Autosomal incomplete dominance	(Ng et al., 2012)
SLC45A2	Silver	Z-linked	(Gunnarsson et al., 2007)
TYR	Recessive white	Z-linked recessive	(Tobita-Teramoto et al., 2000)
EDNRB2	Mottled	Autosomal recessive	(Kinoshita et al., 2014)
MLPH	Lavender	Autosomal recessive	(Bed'hom et al., 2012)
MC1R	Extended black	Autosomal recessive	(Kerje et al., 2003)
PMEL	Dominant white	Autosomal dominant	(Kerje et al., 2004)
SOX10	Dark brown	Autosomal recessive	(Gunnarsson et al., 2011)
CDKN2B	Sex-linked barring	Z-linked	(Hellstrom et al., 2010)
SLC45A2	Sex- linked imperfect albinism	Z-linked recessive	(Gunnarsson et al., 2007)
TYR	Autosomal albino	Autosomal recessive	(Tobita-Teramoto et al., 2000)

Crest

Feather-crested head is a trait characterised by tufts of elongated feathers atop the head of chicken (Fig. 1C). It was first confirmed to be associated with a C cluster of HOX gene family in the non-recombination homologous region. Through RACE, a 2227-bp HOXC8 transcript was obtained after splicing with upstream and downstream fragment in cranial skin tissue where crest formed. Expression analysis of skin tissues from Crested and non-Crested chickens revealed an ectopic HOXC8 expression in cranial skin during embryonic development. Using the same population describe in mapping Silky-feather and Rose-comb loci, we performed linkage analysis and whole genome association study analysis, and showed that Crest was located on the E22C19W28 linkage group and completely associated with the HOXC-cluster on this chromosome. This further validated the discovery that HOXC8 gene is correlated with chicken Crest phenotype (Wang et al., 2012).

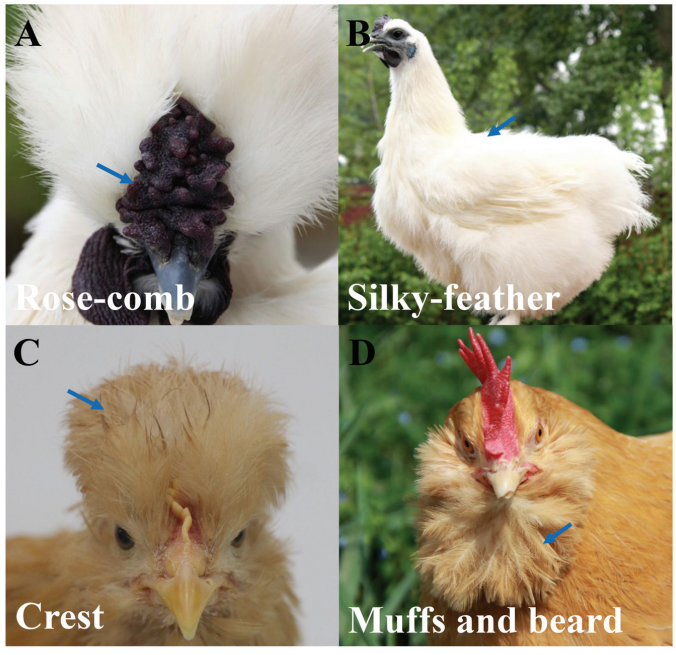

Fig.1 (A) Rose−comb, (B) Silky−feather, (C) Crest, and (D) Muffs and beard in chickens.

Conclusion

Muffs and beard

Muffs and beard (Mb) is elongated feathers that gather from both sides of the face (muffs) and below the beak (beard) (Fig. 1D). To dissect the genetic basis of Mb, a F2 intercross population between Huiyang Bearded and High Quality of Chicken Line A consisting of 584 birds were bred as our mapping population (Sheng et al., 2013). Through genome-wide association, linkage, and IBD analysis, we identified a critical region on GGA27 which showed complete association with Mb. Fine-mapping demonstrated that Mb in chickens is caused by a complex structural variation (SV) on GGA27 involving three >14-kb-genomic-region duplicating and tandem inserting into the downstream of the first copy number variation (CNV) region. Successively expression tests of genes located in these regions during the critical periods of Mb morphogenesis were demonstrated in both embryos and adults. And the results showed continuous ectopic high expression of HOXB8 in facial skin indicating a new role for HOXB8 in directing feather-regional development (Guo et al., 2016).

Discussion

Due to the rapid development of the genomic technology, an overlooked significant type of variation: genomic structural variants emerge in front of us. It happens a lot less times in genome than single-nucleotide variants (SNVs) but accounts for quite a lot of phenotypic variants (Eaaswarkhanth et al., 2014). Also, this rule applies for the morphological variants of chicken too. Besides the traits we have deciphered, there are many morphological traits finally be confirmed to have association with SVs. Both the Pea-comb (Wright et al., 2009) and Duplex-comb (Dorshorst et al., 2015) are caused by large-scale genomic SVs that induce changes in expression of conserved genes. Dermal hyperpigmentation in chicken is associated with a complex genomic rearrangement which involves the EDN3 (Dorshorst B, 2011). Also, small genomic SVs have a conspicuous consequence such as the Frizzle feather mutation which is a 69 bp in-frame deletion in a conserved region of KRT75 (Ng et al., 2012).

Though several hypothese have given possible ways (Hastings et al., 2009; Zhang et al., 2009), the accurate mechanism for (why? When? How?) SV formation remains largely unknown. However, the cur-

rent studies have verified the functional significance of SV. We believe that the genomic structural variation is a motivation for biological evolution and a reason for biological diversification in domestic animals, and the variations are reserved consciously or unconsciously during the natural and artificial selection.

Overall, the cases here illustrate the value of utilizing morphological mutations in domestic animal to dissect the genetic basis of morphological traits, and further, providing novel insights into the contribution of SV on the formation of traits and the likely roles of genes in development and differentiation.

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L53 Diagnosis and control of avian tumor viruses in poultry: a review

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Keywords: avian tumor viruses, diagnosis, control, Marek's disease, avian leukosis, reticuloendotheliosis

Summary

Virus-induced neoplastic diseases of poultry, namely Marek's disease (MD), induced by a herpesvirus, and avian leukosis and reticuloendotheliosis induced by retroviruses can cause significant economic losses from tumor mortality as well as poor performance. Successful control of MD is and has been achieved through use of effective conventional vaccines. For now and the foreseeable future, use of vaccines represents the principal strategy for the prevention and control of MD. However, effective biosecurity measures and genetic resistance are critical adjuncts to vaccination in any successful strategy to control MD. On the other hand, there is no doubt that extensive use of vaccines over the last few decades has contributed to the increase in the tendency of MD virus (MDV) to evolve to greater virulence. This increase in MDV virulence is a critical factor that is considered in developing various strategies for control of the disease, as it tends to make earlier vaccines obsolete. Recent advances in MD research, namely revealing sequence of MDV genome and the development of new molecular approaches to manipulate MDV genome are currently being used to identify and characterize function of important viral genes and to develop more effective recombinant vaccines. In contrast to MD, there is no commercial vaccine available for control of retrovirus virus infection in poultry, namely avian leukosis virus (ALV) and reticuloendotheliosis virus (REV). Eradication programs adopted by primary chicken breeders are the most effective means for controlling ALV infection in chickens. Eradication programs similar to those used to control ALV have not yet been adopted by commercial chicken and turkey breeders for control of REV infection.

This review is primarily aimed at immunopathogenesis, diagnosis and control of avian tumor viruses and the tumors they induce, namely MD, avian leukosis and reticuloendotheliosis[2, 31, 32, 35].

Main text

Marek's disease

Marek's disease (MD), a T-cell lymphoma of primarily chickens is caused by a highly cell-associated alphaherpesvirus termed MD virus (MDV)[35]. The disease is and has been controlled since early 1970s by use of conventional vaccines. During the last four decades, research on MD has resulted not only in improved conventional vaccines, but also in improved methods of vaccination, namely in-ovo vaccination[36]. Good biosecurity practices and host genetic resistance are also recognized as important factors in implementing any strategy for control of MD. However, despite widespread use of vaccines and development of new methods of vaccination, economic losses from mortality of layers and breeders and condemnation of broilers continue to occur[35]. During last decade, MD has been diagnosed in commercial turkey flocks in Germany, France, Israel and Ukraine[8, 9, 18, 19, 35], suggesting that the host range of MDV has apparently expanded to include turkeys. Factors that lead to MD outbreaks in commercial turkeys are poorly understood and need to be determined.

The fact that MDV continues to mutate to greater virulence, reducing the effectiveness of many existing vaccines[35] is a major concern to the poultry industry. Clearly, definitive diagnosis of MD and the ability to differentiate the disease from other virus-induced lymphomas are considered critical factors in the successful control of MD[3, 39]. Obviously, an important challenge regarding control of MD in the future is developing new strategies to control losses caused by new emerging MDV pathotypes. Development of a new generation (recombinant) vaccines against Marek's disease named the Meq-deleted MDV vaccines has recently been shown to be potentially superior to currently available commercial vac-

cines[22, 24, 25, 26, 27, 30, 35]. Development of vaccines that can interfere with replication and shedding of MDV, and understanding role of host genes involved in resistant to MD will undoubtedly improve our ability to implement a better strategy for control of MD in the future.

Avian leukosis

The leukosis/sarcoma (L/S) group of diseases designates a variety of transmissible tumors of chickens caused by L/S group of avian retrovirus. Under natural conditions, avian leukosis, caused by a virus termed avian leukosis virus (ALV), is the most common form of L/S group of diseases seen in field flocks[31]. Although ALV is capable of inducing a variety of neoplastic conditions in chickens, lymphoid leukosis (LL), a B-cell lymphoma affecting primarily the bursa of Fabricius and visceral organs is the most common form of leukosis that arise from infection with ALV. However, with the recognition of subgroup J ALV (ALV-J) infection in the early 1990's, myelocytomatosis, has emerged as a tumor condition that is frequently detected in ALV-J- infected meat-type chickens; however, ALV-J-induced myelocytomatosis has also been recently reported in commercial layers[31]. Most recently, histiocytic sarcomatosis has been noted in persistently viremic, but not in immunotolerized meat-type chickens infected with ALV-J[33]. Like other retroviruses, ALV mutates at a high rate and can recombine with endogenous (subgroup E ALV) elements resulting in new recombinant ALVs[31]. These endogenous subgroup E ALV elements not only contribute to recombination, but also can interfere with diagnosis and control of ALV infection, as well as the enhancement of spontaneous LL in certain genetic lines of chickens[4, 31].

Recombination can also occur between members of two different subgroups of exogenous ALV. Recent laboratory observations provided evidence for recombination between subgroup A and J ALV (ALV-A/J), a recombinant ALV with the envelope of subgroup A and long terminal repeat (LTR) of subgroup J; this recombinant ALV resulted from passing ALV-J in cells expressing subgroup A envelope[29]. Recombination between members of two subgroups of ALV can also occur under field conditions, resulting in the emergence of a natural recombinant virus. Recently, an ALV-B/J, a recombinant ALV with envelope of subgroup B and LTR of subgroup J was isolated from commercial layers affected with myelocytomatosis[17].

Natural infection with ALV has been known to cause significant economic losses in commercial layers and breeder flocks due to mortality and lower productivity. As a potential contaminant of live-virus vaccines of poultry, ALV can also cause significant losses if contaminated vaccines were used in susceptible flocks. Most recently, a recombinant subgroup A ALV containing envelope of ALV-A and LTR of ALV-E was isolated from commercial Marek's disease vaccines[14, 37].

To date, because no commercial vaccines are available for control of ALV infection, eradication of virus infection at the primary breeder level remains to be the principal method for controlling ALV infection in chickens[31]. The new advancements in knowledge regarding molecular characteristics of ALV genome, development of highly specific reagents such as monoclonal antibodies and other technologies such as cloning of viral genes have contributed significantly to improved diagnosis and control of ALV. Clearly, diagnosis and control of re-emerging recombinant ALV and the tumors they induce in chickens represent new challenges that must be addressed in order to reduce losses from future outbreaks with previously unrecognized subgroups of ALV.

Reticuloendotheliosis

Reticuloendotheliosis virus (REV) is an avian oncornavirus that is structurally and antigenically unrelated to the leukosis-sarcoma group of viruses[31, 32]. Recent classification has placed REV within the family *Retroviridae*, subfamily O*rthoretrovirinae*, genus G*ammaretrovirus*[32]. All REV isolates are antigenically related to each other. However, using monoclonal antibodies[7], REV isolates can be classed into three different subtypes, 1, 2 and 3[5]. REV infects chickens, turkeys, ducks, geese, pheasants, quail, prairie chickens, and probably many other avian species[1, 2, 6, 11, 12, 13, 28, 32, 34, 40, 41]. Based on virus or antibody detection assays, REV infection has been shown to be common, but not ubiquitous in many countries[2, 32, 40, 41]. Despite the fact that REV infection is common in most countries, the incidence of REV-associated clinical disease in commercial poultry varies from sporadic to negligible. The most common clinical diseases induced by REV are chronic lymphomas (reticular cell tumor, B- or T-cell lymphoma) and an immunosuppressive runting disease[32, 38, 42, 43]. Although losses in REV affected flocks can be significant

due to tumor mortality and immunosuppression, the principal economic concerns of REV infection are as contaminants of live virus vaccines of poultry or as a barrier to export of breeding stock to certain countries.

Because REV can be transmitted vertically from dams to offspring, embryos and tissue cultures prepared from such embryos may harbor REV; therefore these embryos or cells could serve as a source of REV contamination of poultry and other vaccines. Accidental contamination of live virus poultry vaccines such Marek's disease virus (MDV) and fowlpox virus (FPV) with REV[15, 16, 20, 23] is an important source of virus infection and can cause runting disease as well as lymphomas in vaccinated chickens resulting in significant economic consequences.

Further, the fact that partial or complete insertion of REV genome in large DNA viruses such as FPV and MDV can occur[10, 20, 21, 23, 32] also poses a problem. This phenomenon of REV genomic insertion is important, as these REV insertions may change the biological properties of the recipient virus and because infectious clones of REV packaged in other viruses may provide a novel mechanism for transmission of REV.

Contamination of vaccines, partial or complete insertion of REV genome in other large DNA viruses and developing new control methods represent important challenges that must be addressed in order to develop effective strategies for control of REV infection in poultry.

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L54 Viruses, vaccines and feathers

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Keywords: feathers, viruses, vaccines, chickens, turkeys

Summary

Feathers are unique in the bird anatomy. We review now the feather use in the diagnosis of avian viruses and of monitoring live vaccine uptake following vaccination. The use of feathers is beneficial because they are easy for sampling, no need for bleeding, bird killing and necropsy, and the possibility of continuous flock monitoring without economic loss. Feather pulps in young feathers resemble the blood content, while in mature feathers, the biological substances and viruses dry on the feather shafts, turning them into "archives" of past events. Unlike in plasma or tissues, the deposited viruses are stable for prolonged time periods.

Our findings demonstrated the feather use in diagnosis and research concerning several avian viruses: Marek's disease (MDV), chicken anemia (CAV), the retrovirus leukosis, subgroup J (ALV-J), as single and multiple infections with MDV. We also described for the first time detection of the infectious laryngotracheitis virus (ILTV) and turkey meningoencephalitis virus (TMEV) in chicken and turkeys feathers, respectively. Our studies pioneered for the first time the presence and spread of CAV by feathers, and CAV visualization by immunohistochemistry in feather follicle epithelium cells, similarly to MDV. An additional novelty of our studies is monitoring vaccine virus uptake following vaccination using feathers, allowing the evaluation of the vaccination uptake of four live vaccines, MDV, CAV, TMEV and ILTV.

The lecture will also present the stability of DNA viruses in feather shafts, the dissemination and mucosal infectivity of feather homogenates from MDV and CAV-experimentally infected chickens, the interaction of MDV and CAV at the feather level, and the evaluation of environmental dissemination of pathogens.

Past and present advantages in diagnosis and research using feather shafts will be reviewed and novel findings will be presented and implicated for future application for the poultry industry.

Main text

The feathers reflect the chicken well being and their integrity is a major concern both in layers and broilers. A vigorous feathering is necessary in broilers for covering and for external protection, while in layers they are also related to feeding and laying efficacy. Feathering is a multifactorial process that is determined genetically, hormonally, by the environmental conditions and the nutritional status. However, viral and bacterial pathogens might also influence the feather development, by either a pre-hatch or a post-hatch replication in the feather follicles or tissues.

Avian viruses replicate in epithelial cells of the feather follicles or in lymphocytes and myelocytes that circulate throughout the body, reach the feather calamus cavity and accumulate as the feather pulp, which then represent the systemic blood content. The use of feathers as the sample for diagnosis is very advantageous; feathers are easy to collect, bleeding or necropsy can be avoided, and their examination is suitable for environmental monitoring of avian pathogens in poultry houses and the surrounding grounds (Davidson et al., 2003). Efficient detection is feasible only if the pathogen is stable on the feather and a sensitive assay is performed.

The present review focuses on 3 avian DNA viruses, Marek's disease (MDV) (Schat and Nair, 2013), chicken anemia (CAV) (Schat and van Santen, 2013) and Infectious Laryngotracheitis (ILTV) (Garcia et al., 2013), the turkey flavivirus, Turkey Meningoencephalitis Virus (TMEV) (Guy, 2013) the retrovirus avian leukosis (ALV) (Nair and Fadly, 2013) and the respiratory viruses avian influenza virus, H5N1 (AIV) (Swayne et al., 2013) and Newcastle disease virus (NDV) (Miller and Koch, 2013). We demonstrated the presence of the avian viruses in feather tips and revealed the feather significance for diagno-

sis of commercial chickens. Feathers and feather dust can carry viruses and contribute to the horizontally virus-infections. While most studies on avian pathogen detection in feathers were concerned with experimental infections, we described initially experimental infection studies, and then the knowledge gained in the lab was applied to the field to commercial chicken flocks, where feathers of naturally-infected chickens were approached to reflect "real-life" situation. The flocks were submitted to our laboratory for differential diagnosis of clinical lesions, unnormal growth, immunosuppression, and more.

Feathers and Marek's disease virus

MDV causes tumors, immunosuppression and neurological symptoms. The virus replicates in the feather follicle epithelium (FFE) and spreads horizontally in poultry house with dust and dander. MDV was the most studied avian virus regarding replication, presence and spreading pattern in feathers (Couteaudier and Denesvre, 2014, Couteaudier et al., 2016). Early studies of Calnek and Hitchner commencing in 1969 described the FFE as the only anatomical site where MDV productive replication occurs. Further, the infectivity of cell free virus in feather dust was demonstrated, and its blockage by commercial air filters was demonstreted. The MDV prominent trait to be produced and accumulated on the feather shafts and pulp and to remain infective on dry feathers was utilized by us and by others to demonstrate the kinetics of MDV shedding and to develop the molecular diagnosis of commercial flocks (Davidson and Shkoda, 2005, Davidson, 2009). Whole viruses were separated from the feather tip extracts by Pulsed Field Gel Electrophoresis (Borenshtein and Davidson, 2002) and the infectivity of the feather tip extracts for mucosal surface of the eyes and airways was demonstrated experimentally (Davidson and Borenshtain, 2003)**.** That approach was novel, as the intraperitoneal injection using cell-associated virus was routinely used in MDV-infection trials. Also, the MDV vaccination efficacy and uptake (Davidson et al., unpublished) could be estimated according to the vaccine virus presence in the feathers (Ralapanawe et al., 2016).

With the development of the real-time amplification technology, several assays to identify and quantify virulent and vaccine viruses of the 3 serotypes in FFE were reported targeting the meq gene (Abdul-Careem et al., 2006) the pp38 gene (Gimeno et al., 2012, Haq et al., 2012, Renz et al, 2013, Baigenet et al., 2016), and the 132 bp BamH1-H tandem repeats (Davidson et al., 2013).

Feathers and circoviruses

Circoviruses, unenveloped small single-stranded DNA viruses spread horizontally and vertically. The presence of two circoviruses in feathers was studied, CAV and the psitaccine beak and feather disease virus (PBFDV). CAV infections are manifested with visible or invisible, clinical signs, having multi-potent efficacy to infect and deplete stem cells of the hematopoietic and lymphocytes cell lineages in the bone marrow. CAV detection in the feather tips was first time reported (Davidson et al., 2008), initially in experimental infection trials, and applied then for CAV diagnosis. Similarly to MDV, feather tip extracts were showed to be infective by mucosal routes.

As for CAV, PBFDV was detected by PCR in feathers and applied for the environmental monitoring of the cage floor using feathers (Bendheim et al., 2006). In BFDV-infected psittacine species circovirus infection causes various feather lesions, slow growth, down-loss, sheath enlargement, and discoloration.

While MDV presence in feather shafts is the outcome of FFE being the only site where MDV is replicated productively (Schat and Nair, 2013), no such information was available for CAV. Histological sections of FFE of CAV-infected chicks provided the first evidence for CAV-specific histological changes in feather shafts. It seems that the viral presence is not due only to blood constituents, but is associated with specific histological changes of squamous epithelium focal necrosis, cellular ballooning and vacuoles (Davidson et al., 2008). Experimental evidence indicated of a mutual interference in the horizontal spread of both MDV and CAV in a dually-virus infected SPF chicks (Davidson et al., unpublished).

Use of feathers for virus detection in single and in dual MDV and CAV infection

Both MDV and CAV are ubiquitous viruses worldwide, and commercial flocks carry both viruses at various viral quantities. Two groups were examined: (a) experimentally dually-infected SPF chicks and (b) commercial chickens of different types and ages, (Davidson et al., 2013). Multiple viral infections represent the actual acquirement of infection from environmental sources. Feather tips and visceral organs

(pooled liver and spleen) of the same birds were examined to cover the variability rainbow of infections of commercial flocks, in terms of infection time, flock uniformity, virus load, bird susceptibility, management conditions, multiple-pathogen infections, state of immunity and/or immunosuppression. The parallel examination of feathers and visceral organs was performed on 95 chickens for CAV and 80 chickens for MDV. The linear correlation of C_T values obtained by rtPCR on feather tip and organ DNA from the same birds for both viruses had a high statistical significance ($P<0.001$). These comparative analyses show that feather shafts can be used as sampling organs to detect MDV and CAV. These findings provided a solid foundation for the use of feathers for MDV and CAV diagnosis and confirmed a previous comparison for MDV detection only, performed on feather pulps and blood (Cortes et al., 2011).

The severity of disease caused by both viruses is proportional to the viral loads. We developed a quantitative multiplex real-time PCR assay to determine the dual presence of MDV and CAV to monitor the authentic conditions in commercial poultry houses and to evaluate simultaneously the infection level of the clinical samples (Davidson et al., 2013). In contrast to previous studies, in which quantitative real-time PCR assays were developed to detect each virus in separate, the multiplex real-time PCR for MDV and CAV has an extensive dynamic range capacity for use in both experimental infected and commercial chickens. The detection limit of the MDV and CAV qRT-PCRs were comparable for both types of chickens and estimated as about 3,000 – 10,000 viral copies. It seems that while in clinical cases, the MDV and CAV viral copies are abundant, in sub-clinical and/or immunosuppression manifestations, the MDV and CAV infection is characterized by the presence of lower viral copies.

The stability of DNA viruses on feathers

As previous studies determined the prolonged stability of MDV in poultry house dust, we questioned now the possibility to detect MDV and CAV on feathers on poultry house floors or nearby (Davidson et al., 2003). Feather tips of feathers from MDV of CAV-infected chickens were cut and pooled, then distributed in groups of ten tips and kept in open microcentrifuge tubes for various periods of times. Three series of tubes were prepared for incubation at different temperature and humidity conditions: room temperature (22-25^{0}C at medium humidity), cold room (4^{0}C with high humidity) and warm room (37^{0}C with low humidity) all resembling relevant climatic situation. At each time point (0, 1, 4, 7, 13, 25 and 32 days) DNA was purified from each tube and amplified, for both MDV and CAV. MDV was amplified over at least 32 days at the 3 conditions, where

CAV was amplified for 32 days at 4^{0}C and RT, but not at 37^{0}C. Further investigation might be needed to clarify the inability to amplify CAV sequences from feathers incubated at warm and dry conditions.

Environmental monitoring of DNA viruses using feathers

The association of viruses with feathers, the high stability of DNA viruses in feather dust and dander, and our findings on MDV and CAV stability over time drove the use of feathers for monitoring the viral contamination of the poultry house environment (Davidson et al., 2003). Broiler flocks with uneven or retarded growth, haemorrhages, necrotic dermatitis, immunosupression were assayed for MDV and CAV using visceral organs and feathers of sick birds, as well as feathers from poultry house floors or nearby surrounding. It was shown that by PCR the evaluation of environmental contamination was feasible. The presence of ubiquitous viruses like MDV and CAV can also serve as a potential signal for insufficient biosecurity. Further, poultry house dust was described as an efficient source of DNA for MDV and ILTV amplification for assessing the infection status of commercial flocks (Walden-Brown et al., 2013, Roy et al., 2015). In addition, we utilized that approach to evaluate the presence of the PBFDV in the feathers that were shed by affected pssitacines, thus exemplifying a possible way to control the biosecurity in the surroundings of breeder birds (Bendheim et al., 2006).

Infectious Laryngotracheitis virus and feathers

Infectious laryngotracheitis (ILT) is a respiratory disease of poultry caused by an alphaherpesvirus, ILTV (Garcia et al., 2013). The disease severity varies from mild to acute with high mortality rates. The live vaccine is applied worldwide by drinking water, by the respiratory route, and by vent application. No system of direct evaluation of the efficacy of vaccination exists today, except antibody elicitation, which is an indirect indication and might reflect environment exposure. While ILTV infection status was

implicated by dust monitoring (Roy et al., 2015), we lately suggested an assay to evaluate the accuracy of the vaccination process in commercial flocks (Davidson et al., 2016a). The direct monitoring of vaccine uptake by virus detection in the feather shafts of vaccinated birds represents the systemic spread of live vaccine. Moreover, the continuous survey of the vaccine virus unveiled the different kinetics of viremia by the different vaccination routes; while after the vent vaccination the systemic viremia peaks during the first week afterwards, after two consecutive vaccine administration by drinking water with a 6 day-interval, the viremia peaks only after the second administration. A robust amplification was needed because the vaccine ILTV was present in the bird in minute quantities compared to the wild-type virus (Davidson et al., 2009). For the vaccine virus identification in feather shafts, a nested real-time PCR for the TK ILTV gene was developed. The sensitivity of detection of the nested rtPCR was greater by 1000 compared to conventional nested PCR and 10 times that real-time PCR (Davidson et al., 2008).

Turkey meningoencephalitis virus and feathers

The Turkey Meningoencephalitis Virus (TMEV), causes a neuroparalytic disease in adult turkeys leading to paresis, in-coordination, drooping wings and an economically-important mortality. The TMEV-induced disease is controlled by vaccination with a live attenuated virus. (Iancunescu et al., 1975). TMEV is an enveloped flavivirus, containing a single-stranded positive-sense RNA genome of approximately 11 kb in length. A new look at the flavivirus taxonomy became evident in light of the phylogenetic tree, confirming that BGAV and TMEV are similar viruses, and may represent the same virus under different names. Resulting from that novel finding, a new name *avian meningoencephalitis virus* (AMEV) was proposed (Fernandez-Pinero et al., 2014, Davidson, 2015).

The turkey brains are the TMEV target organ for virus replication, and accordingly they were demonstrated as the useful sampling site (Davidson et al., 2000). We explored for the first time the feather pulps of turkeys as a non-invasive target organ for TMEV molecular detection, which avoid invasive sampling and bird killing for obtaining the brain tissue. Similarly to chickens, the use of turkey feathers pulps would be advantageous for detection of turkey pathogenic viruses. Only two studies approached the possible detection of flaviviruses in feathers. In the first study the BGAV, considered be synonymous to TMEV, was detected in immature feathers of red-legged partridges (Liorente et al., 2015). In the second, the TMEV closely- related flavivirus, *West Nile virus*, was detected in the feathers of crows (Docherty et al., 2004, Shirafuji et al., 2008). We recently demonstrated that parallel TMEV diagnosis in brain and feather-pulp RNA were similarly useful for diagnosis, at least in experimentally-infected turkeys and in several cases of disease encountered in commercial flocks (Davidson et al., 2016b, submitted).

Avian retroviruses and feathers

Limited studies were dedicated to the presence of retroviruses in feathers. Unlike MDV that belongs to the herpesvirus family and is transmitted horizontally, retroviruses are transmitted both vertically and horizontally. While MDV is relatively stable in dry feather dust, retroviruses are unstable outside the bird and infection requires a direct contact with biological material. For that reason the transmission of retroviruses by air is not trivial and was considered relatively unimportant, although avian leukosis virus, subgroup A (ALV-A) was detected, and cultivated from the feather pulps and from FFE. The feather pulp was also used in an group-specific ELISA test The avian leukosis virus, subgroup J (ALV-J) spread horizontally and vertically to a greater extent than ALV-A. A breakthrough was made in 2002 when three separate and independent studies were published, all showing that ALV-J could be detected in the feather tips of infected chickens (Davidson and Borenshtain, 2002b; Sung et al., 2002; Zavala et al., 2002). The retrovirus detection targeted the proviral DNA form, as replication within chicken cells employs a rapid transition to DNA by a reverse transcriptase step. The retroviral genomes are then transmitted as DNA viruses, enabling their detection in the feather tips similarly to DNA viruses.

Avian respiratory viruses and feathers

The feature of high pathogenic AIV H5N1 and of velogenic NDV to replicate massively and spread systemically was the basis of exploring the feasibility of feathers to be utilized for diagnosis (Yamamoto et al., 2008, Nuradji et al., 2015), Newcastle disease virus (Roy et al., 1998, Lee et al., 2015). In experimentally-infected chickens and ducks the AIV H5N1 titers were higher than in swabs in immature feath-

ers, suggesting that feathers might be an alternative sample for diagnosis. In vNDV-infected chickens the viral antigens and RNA was also detected in the feather pulps, suggesting that feathers might contribute to the viral spread.

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L55 Avian metapneumovirus infection and control

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Introduction

Avian metapneumovirus (aMPV), a member of the family Paramyxoviridae, is associated with acute respiratory tract infection characterized by catarrhal inflammation of upper respiratory tract, clear or mucoid nasal and ocular discharge, foamy conjunctivitis, unilateral or bilateral swollen infraorbital sinuses, as well as reductions in egg production and quality in turkeys, chickens, ducks, and other avian species [(1)]. aMPV is an enveloped, nonsegmented, single-stranded, negative-sense RNA virus composed of a genome of approximately 13 kb in length, which organized as 3′-leader-N-P-M-F-M2-SH-G-L-trailer-5′ [(2)]. Four subgroups (A, B, C, and D) have been recognized for aMPV. After its finding in South Africa in 1978[(3)], different subgroups of aMPV, mainly A or B, were reported in Europe, Asia, and some other parts of the world in turkeys and chickens. Subgroup D was only detected in France[(4)]. Subgroup C aMPV, which was antigenically and genetically different from these three subgroups, was first found in turkeys in USA in 1996[(5)], farmed ducks in France[(6)], pheasants in South Korea[(7)], some wild birds (e.g., American coots, American crows, Canada geese, cattle egrets, and sparrows) in USA[(8)], and chickens and ducks in China[(9,10)]. Sequence analysis showed that the chicken aMPV/C strain JC is more closely related to hMPV strain BJ1816 (78.5%) isolated in China than to other aMPV/C isolates (75.5-77.8%) as compared to matrix gene sequences[(9)]. Both inactivated and live vaccines were used to control aMPV infections in Europe[(11-14)]. However, only live vaccine was used to control subgroup C aMPV (aMPV/C) infection in the USA, because killed vaccines have not conferred good protection against aMPV/C infection[(15)].

Here, in the case of aMPV/C strain JC infection, we documented pathogenesis, contribution of host signaling to viral replication, and prevention and control techniques for aMPV infection.

aMPV/C pathogenesis in chickens and BALB/c mice

Chicken infection

We determined the pathogenesis of the aMPV/C strain JC in specific-pathogen-free (SPF) chickens[(9)]. Some of the inoculated chickens showed symptoms such as nasal discharge, sinus swelling, and watery eyes at 3-7 days postinoculation (dpi). Histopathological findings were characterized by mild to severe inflammation in the tracheae and lungs, including disruption of the epithelial architecture, sloughing of epithelial cells, loss of ciliation, and infiltration of inflammatory cells. We documented hemorrhage, infiltration of lymphoid cells, and hyperplasia in the tracheal epithelium and propria lamina of the aMPV/C- infected chickens. Examination of the lungs showed peribronchial lymphoplasmocytic infiltrates, edematous thickening of the bronchial submucosa, and lymphoid cell hyperplasia. Diffuse mild expansion of the alveolar interstitium caused by mononuclear cell infiltrates and edema was also observed. Sloughed epithelial cells, heterophils, macrophages, and amorphous debris were visible in the bronchial lumens. Immunohistochemical staining revealed that viral antigen was detected in both morphologically normal and degenerated respiratory epithelial cells. In addition, mucus cells, basal cells, and luminal cellular debris that included sloughed epithelial cells and macrophages stained positive for aMPV antigen.

BALB/c mice infection

To further evaluate its pathogenesis, we used the aMPV/C strain JC to inoculate experimentally BALB/c mice and found that the aMPV/C can efficiently replicate and persist in the lungs of mice for at least 21 days with a peak viral load at day 6 postinoculation (16). Lung pathological changes were characterized by increased inflammatory cells. Immunochemical assay showed the presence of viral antigens

in the lungs and significant upregulation of pulmonary inflammatory cytokines and chemokines including MCP-1, MIP-1a, RANTES, IL-1b, IFN-g, and TNF-a were detected following inoculation. These results indicate for the first time that chicken aMPV/C may replicate in the lung of mice. Whether aMPV/C has potential as zoonotic pathogen, further investigation will be required.

Contribution of host signaling to aMPV/C replication

aMPV/C infection-mediated apoptotic responses

As demonstrated above, aMPV/C caused variable severe respiratory signs in local meat-type commercial chickens in China and can efficiently replicate and persist in the lungs of inoculated mice, leading to increased inflammatory responses. Regulation of apoptosis plays an important role in host defense and virus survival. For here, aMPV/C infection was further investigated for its effect on apoptotic responses in vivo and in vitro. TUNEL staining and electron microscopy observation demonstrated that aMPV/C could induce apoptosis in lung tissues of mice characterized by chromatin condensation, plasma membrane blebbing, cell shrinkage, and DNA fragmentation after inoculation. Lung tissues in the aMPV/C-inoculated mice also showed strong positive reaction of cleaved caspase-3 (CCasp3). In vitro experiments, we further demonstrated that aMPV/C-induced apoptosis required the activation of caspase-9 rather than caspase-8 in the cultured Vero cells. The activation of caspase-9 leads to the activation of caspase-3 as observed by an increase in the cleavage of the caspase substrate in the infected cells. Collectively, our data indicate that aMPV/C could induce apoptosis in vivo and in vitro via the caspase pathway, particularly involving caspase-9.

Autophagy promotes aMPV/C replication via the Unfolded Protein Response

An increasing number of research data have demonstrated that autophagy played an important role in the infection process of diverse pathogens. However, to date, it is unknown whether autophagy is induced in avian metapneumovirus (aMPV)-infected host cells, and if so, how this occurs was unclear. Here, we report by detecting the classical autophagic feature, including autophagsomes formation, GFP-LC3 dots and the conversation of LC3-I to LC3-II, that aMPV/C induces autophagy in the infected cells with enhancing the degradation of autophagic protein. Suppression of expression of ATG7 or LC3 proteins that mediates LC3 lipidation reduced aMPV/C replication, indicating the positive role of these proteins in aMPV/C replication. This induction of autophagy depended on the unfolded protein response (UPR), as the suppression of UPR signaling pathways affected aMPV/C replication. Our results delineate that autophagy promotes the replication of aMPV/C in host cells and the molecular pathway by which aMPV/C induces autophagic vacuoles, thus providing new insight into the molecular mechanisms of the aMPV-host interaction

Proteome analysis of aMPV/C-infected Vero cells

To further delineate the underlying mechanism of aMPV/C infection, we performed an iTRAQ-based proteomic study to quantitatively identify differentially expressed (DE) proteins in the aMPV/C-infected Vero cells. We identified 59 differentially expressed cellular proteins at 48 hpi, and 39 DE proteins at 72 hpi ($P<0.05$, quantitative ratio $\geqslant 1.5$). The Gene Ontology analysis indicated that the molecular function of DE proteins was involved in binding, nucleic acid binding and hydrolase activity. Ingenuity Pathway Analysis was also employed to investigate the canonical pathways related to aMPV/C infection. These DE proteins were involved in metabolic, Toll-like receptor signaling and Jak-STAT signaling pathway. Based on these data, it was indicated that aMPV might utilize extracellular signal transduction pathways for viral replication and infection. The study first analyze the protein profile of aMPV/C-infected cells by quantitative proteomics, and might provide new insights for better understanding the host response to aMPV/C infection.

Prevention and control of aMPV/C infection

Due to the absence of protection after inactivated aMPV/C vaccine, we used the aMPV/C strain JC to adapt Vero cells to develop an attenuated live aMPV/C vaccine. SPF chickens were vaccinated with one dose of the attenuated live virus and challenged with the virulent virus 3 weeks post-vaccination. No

clinical signs were seen post-vaccination. Upon challenge, no clinical signs were observed in vaccinated chickens but severe clinical signs, including unilateral or bilateral nasal discharge, sinus swelling, and watery eyes, were observed in non-vaccinated, challenged chickens. The IgG antibody levels in vaccinated chickens were not very high. None of the vaccinated chickens were found to shed virus after challenge in their choanal secretions whereas all of the non-vaccinated, challenged chickens shed the virus. The absence of clinical signs and virus shedding in vaccinated chickens as compared to that in non-vaccinated chickens suggests that this cell-adapted strain is a viable candidate for use as a live, attenuated vaccine in chickens.

To further develop a novel vaccine against aMPV/C infection, we developed a recombinant vaccine by expressing the Fusion (F) protein of aMPV/C on the surface of baculovirus under an immediate early ie1 promoter. It was showed that the F protein located on the plasma membrane of infected cells as observed by indirect fluorescence assay and its antigenicity was confirmed by Western blotting. The protective efficacy of the recombinant Bac-F experimental vaccine was further evaluated by challenging chickens inoculated intranasally with the virulent virus. Bac-F-immunized chickens conferred protection virulent aMPV/C challenge as evidenced by decreased viral load in the respiratory mucosa and no clinical sign as compared to control. Therefore, the recombinant Bac-F vaccine might serve as an effective vaccine candidate against aMPV/C infection for further development.

Conclusion

In conclusion, we for the first time isolated and characterized an increasingly prevalent aMPV/C strain from meat-type commercial chickens with severe respiratory signs in China and further found that the aMPV/C strain caused severe respiratory infection and pathological inflammatory lesions in chickens and BALB/c mice efficiently support aMPV/C replication, with being depressed, fever, and significant lung inflammation, as observed for hMPV in BALB/c mice. We also found that aMPV/C infection induced apoptotic response in the cultured cells via caspase-9/3 pathway and aMPV/C infection-mediated UPR-dependent autophagy contributed to viral replication. We also found that varieties of host cellular signals involve in aMPV/C infection by the iTRAQ-based proteomic study. For control of aMPV/C infection, we have adopted two different approaches to develop an attenuated live cell-adapted vaccine and a recombinant Bac-F vaccine with expression of F protein on the surface of baculovirus, and they conferred effective protections against aMPV/C challenge. This development of two vaccines will facilitate to effectively controlling aMPV/C infection.

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L56 Omics approaches to unravel meat quality determinism in poultry

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Lately, poultry industry is facing numerous meat quality issues that impacts on its competitiveness. Like in pig, poultry meat is very sensitive to postmortem pH variations that in case of extreme values lead to defects of appearance, conservation and processing yields. More recently, myodegenerative defects, such as white striping and wooden breast, have appeared that are clearly related to improvement in animal growth rate and muscle yield. Meat quality is under a complex control including genetics, rearing and slaughter factors. These factors influence the way muscle develop and as a consequence its chemical composition, structure and metabolism which are all involved in the determinism of meat technological and sensory quality. Understanding of the molecular pathways that control the quality of the meat is essential for an integrated management of the latter. Recent studies have used high-throughput omics approaches, such as genomics or metabolomics, to unravel the molecular mechanisms involved in the control of meat quality traits. They were developed either on specific experimental models, such as two lines selected for poor or high processing ability, or in commercial populations affected by myodegenerative defects. These studies aimed at determining genes or molecular pathways to be targeted either by genetic selection or rearing practices to decrease the incidence of meat quality defects in poultry production. A direct application for poultry production is the development of genetic or biological markers useful for selecting breeders with a high meat quality potential. The identification of pertinent biological markers, such as blood metabolites, can also help to build predictive models to optimize the quality of poultry meat in relation to several rearing or nutritional factors. This review aims to present recent advances in the understanding of biological control of meat quality obtained thanks to high throughput omics technologies and the development of dedicated animal genetic model.

L57 Molecular genetic study of intramuscular fat (IMF) deposition and its application in chicken breeding

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Keywords: chicken, IMF, genes, genomic selection, breeding

Summary

During the past decades, meat poultry breeding has been predominantly focused on accelerating growth rate, efficiency and the yields of breast and thigh meat. The impressive progress made in these traits, however, has been accompanied by deterioration of taste quality of the broiler meat and, in some markets, decreased acceptability of the meat by consumers. In contrast, Chinese local breeds, such as the Beijing-You chicken and others, are recognized for their superior meat quality and acceptability. They have been widely used in cross-breeding with other commercial chicken breeds in order to produce meat with improved sensorial characteristics. The variety of breeds have provided excellent models for examining the molecular genetics of meat quality traits.

Main text

The IMF content has an important influence on meat quality of chickens

Chicken fat mainly distributes in the abdominal cavity, subcutaneous adipose tissue, muscle bundles and muscle fibers (intramuscular fat, IMF). IMF refers to the lipids deposited in muscles, distributed in the epimysium, perimysium and endomysium. Many studies have shown that a certain amount of IMF deposition increase the sensual satisfaction of meat and enhance the flavor of meat, tenderness and juiciness (Fernandez et al., 1999a b; Suzuki et al., 2005).

Compared with subcutaneous fat and abdominal fat, IMF contains more phospholipids. In the breast muscle of 90-d-old Beijing-You (BJY) chickens, the phospholipid content is 67.12 mg/100g, while the triglyceride is 24.69 mg/100g (Qi, 2009). Zhao et al (2011) found that the breast muscle from 120-d-old BJY chickens, typical market age, has significantly higher contents of phospholipids and essential fatty acids and better muscle fiber characteristics than breast muscle from 42-d-old AA chickens.

Generally, compared with market-age fast-growing broilers, Chinese indigenous breeds used for meat production have higher IMF content and better fat distribution, while early maturing broilers deposited more abdominal fat, have poorer juiciness, and less flavor. The content of IMF can serve as an objective, though indirect, index for meat quality and flavor of chicken meat, and research on the regulatory mechanism of IMF deposition would be of great value for breeding of meat poultry.

Genetic mechanism of IMF deposition

Histological analysis with Oil-red O staining shows that embryonic day 17 to day 1 after hatching is an important period for deposition of IMF in breast (IMFbr). The relative amount of IMF is highest at d 1 and the amount decreases sharply from d 1 to 14 (Wang, 2015). Measurement of IMF content of BJY chickens showed significant accumulation of IMFbr and IMF in thigh (IMFth) after d 56. Accumulation of both IMFbr and IMFth from d 56 to 98 exceeded that from d 98 to 140; IMFth was 4 to 7 times that of IMFbr (Fu et al., 2014).

Candidate genes and pathways for IMF

Using proteomics techniques, the development of breast muscle and the molecular mechanism of IMF deposition were studied in slow-growing indigenous BJY and fast-growing Cobb chickens during late embryonic stages to the early post-hatch period. The study suggested that fatty acid degradation, fatty acid metabolism, and PPAR signaling pathway were involved in embryonic IMF deposition. ACADL, APOA1, HADHA and HADHB may be the key proteins in IMF deposition in the early development period (Wang, 2015).

Agilent cDNA microarray analyses were conducted to determine gene expression profiles of breast muscle sampled at 4 and 5 post-hatching developmental stages of BJY and AA chickens. Of the differentially ex-

pressed genes (DEGs) identified between different ages, 34 to 70 were related to lipid metabolism or muscle development processes in each breed. KEGG pathway analysis of DEGs in both BJY and AA chickens indicated that in addition to pathways affecting lipid metabolism (MAPK and PPAR signaling), cell junction-related pathways (tight junction, ECM-receptor interaction, focal adhesion, regulation of actin cytoskeleton), which play a prominent role in maintaining the integrity of tissues, could contribute to the IMF deposition (Cui et al., 2012). Of special interest, follicle-stimulating hormone (FSH), well-known as an effector in reproductive tissues, was found to associate with IMF in our study and was shown to stimulate both abdominal fat and IMF accumulation in chickens using *in vitro* and *in vivo* approaches (Cui et al, 2012; Cui et al., 2016).

The sex-linked dwarf (SLD) chicken, which has a 1773-bp deletion mutation in the 3′UTR of the growth hormone receptor (GHR), has an inferior growth rate, but shows increased fat content of muscle compared with the normal chicken. Gene expression profiling in thigh muscle of 7-wk-old dwarf (SLD) chickens was performed (Ye et al., 2014). Compared to normal chickens, the increased IMF deposition in skeletal muscle of the SLD chicken might relate to the changing of adipocytokine and insulin pathways and other downstream signaling pathways (TGF-β/SMAD3 and Wnt/catenin-β pathway).

Molecular markers for IMF

Correlations with selected molecular markers, using more than 2000 samples, showed that the fatty acid binding protein gene (*FABP*) was significantly associated with IMF content and abdominal fat weight. The mutation of A-FABP/H-FABP, G51C/G926A can be used as a molecular marker for IMF improvement (Ye et al., 2010).

Selection of chicken lines with higher IMF

Selection of higher IMF based on pedigree performance

The content of IMF has a low to moderate heritability (h^2 = 0.08 to 0.22) (Zerehdaran et al., 2004; Chen et al., 2008; Zhao et al., 2007) but can be selected for, both upward (High) and downward (Low), using conventional breeding approaches. In the high lines, IMF contents of G1, G2, G3 and G4 increased 4.3%, 8.9%, 10.7% and 17. 8% over G0 and CVs decreased from 29.6% in G0 to 21.7% in G4. The genetic selection program increased the IMF content in chickens at 90 d; muscle shear force was decreased and sexual maturity was advanced (Zhao et al., 2007).

Balanced selection of IMF and abdominal fat

Previous study has shown that selection for increased IMF was accompanied by unwanted increases in abdominal fat. A balanced selection program for increased IMF while negatively selecting for abdominal fat percentage (AFP), measured in full sibs, in Jingxing yellow chickens was effective. After five generation (G5), the IMF was increased by 11.4% and AFP decreased by 1.5% compared with the G0 generation. In a parallel control selection for increased IMF, without any constraint on AFP, IMF increased more (17.6%), as expected, but was accompanied by an 18.7% increase in AFP. The balanced selection program, as described, was effective in increasing IMF without undesirable increases in AFP (Jiang et al., submitted).

Evaluation of Genomic selection for IMF

At present, conventional selection, as exampled above, can be combined with marker-assisted selection (MAS) using information from a single or a few markers. With the development of relevant statistical methods of genomic predictions and the decreasing cost of high throughput genotyping platforms, genomic selection would be beneficial for quantitative traits that are difficult or expensive to measure *in vivo*.

With the aim of assessing the effectiveness of genomic predictions for the IMF trait in chickens, the training population consisted of 996 male BJY chickens generated from 250 families were genotyped with commercial 60K chicken SNP Beadchips. The genomic estimated breeding values (GEBV) were calculated for 150-160 males using genomic BLUP (GBLUP) for the experimental line and a control line, with conventional selection, was generated from the same base population. After selection for three generations (G3), the average IMF of the experimental line was 2.93%, about 10% greater progress than that achieved in the control line (2.66 %).

Genome-wide association studies (GWAS) were conducted using data from the 60 K SNP Beadchips applied to 367 individuals from the F2 population derived from BJY and a commercial fast-growing broiler line (Cobb-Vantress). Genes and variants related to IMF and ABF were systematically screened (Liu et al., 2013; Sun et al., 2013). The informative SNPs will be incorporated into the design of a customized SNP chip for use

in genomic selection of the fat traits of interest.

Prospects for the future

For the reasons stated earlier, IMF is an important economic character, related to meat quality in poultry. Genetic progress in IMF content can be achieved and it can be obtained without the correlated increase in ABF. Due to the difficulty in directly measuring of IMF *in vivo*, and the slow progress attained by conventional selection, genomic selection taking advantage of whole-genome information associated with IMF deposition, will likely accelerate progress. In the future, the customized low to moderate density SNP chip, under development for genomic selection, will be employed for breeding poultry with superior meat quality.

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L58 Meat quality of fast−growing broilers: problems and solutions

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Keywords: chicken meat, quality, abnormalities, appearance, tenderness

Summary

Nowadays most of the world's chicken meat production is merely based on intensive farming of few fast-growing hybrids reaching the slaughter weight in a very short time and having high meat yields. The shift from the sale in whole carcass to ready-to-eat and ready-to-cook products has increased importance of quality traits of both fresh meat and raw meat material used for the manufacture of products. This evolution has led to an extreme modification of the genetic background of modern hybrids which are currently used worldwide for the production of chicken meat. However, during the last decades, these evolutions have certainly favoured the occurrence of a high number of abnormalities that are increasing the meat downgrading rates for fresh market retailing and sometimes decreasing the nutritional, sensory and technology proprieties of raw meat materials used for further processing. Among these, the poultry industry faced the occurrence of visual defects such bruises or haemorrhages and muscle exhibiting abnormal colour (i.e. PSE- and DFD-like conditions) as well as toughening due to early deboning. Nowadays, however, the greater concern is towards occurrence of abnormalities characterized by a myodegeneration affecting breast fillets (white-striping, woody-breast and spaghetti-meat) which seems directly or indirectly induced by high growth rate and hypertrophy characterizing modern fast-growing broilers.

Introduction

The development in industrialization and specialization of broiler meat production chains that took place starting from the end of World War II, has led to a worldwide remarkable increase in both the efficiency and the chicken meat production. In the recent years, the lifestyle changes have also dramatically modified the way in which the poultry meat is marketed and consumed and therefore food technologies have become part of the poultry industry, and today much of the production is marketed in the form of cut-up and processed products (Table 1).

Table 1 Trend of world chicken meat production (FAO, 2016), progress of broiler performance and evolution of market segments and forms of chicken meat in the US (adapted from NCC, 2016).

Year	Production	Broiler performance					Market segments		Market forms		
	(.000 tons)	market age (d)	market weight (kg)	average daily gain (g/d)	feed to meat gain (g/g)	mortality (%)	retail grocery (%)	food-service (%)	whole (%)	cut-up parts (%)	further processed (%)
1945	-	84	1.37	16.36	4	10	-	-	-	-	-
1955	-	70	1.39	19.89	3	7	-	-	-	-	-
1965	9,365	63	1.58	25.06	2.4	6	-	-	78	19	3
1975	16,326	56	1.71	30.46	2.1	5	75	25	61	32	7
1985	27,293	49	1.90	38.79	2	5	71	29	29	53	18
1995	46,352	47	2.12	45.07	1.95	5	58	42	10	53	36
2005	70,259	48	2.44	50.75	1.95	4	55	45	11	43	46
2015	96,338	48	2.83	58.97	1.89	4.8	55	45	11	40	49

As a result, nowadays, most of the world's production is merely based on intensive farming of few fast-growing hybrids rapidly reaching the slaughter weight and having high meat yields. In addition, because of the consumers' preference for breast meat and as a consequence of the developing market of

cut-up and processed products, broilers are slaughtered at increased weights. Within this context, as a result of the shift in market form from whole carcass to ready-to-eat and ready-to-cook products, the importance of quality traits of both fresh meat and meat used as raw materials for processed products manufacture has remarkably increased. This evolution has led to extreme modifications in the modern hybrids which are currently selected and used worldwide to produce chicken meat.

Notwithstanding, the differences existing in meat quality among the most popular hybrids (i.e. Ross, Cobb and Hubbard) are very limited if compared to the ones observed among and within the medium- and slow-growing genotypes. Thus, the changes in meat quality traits existing in different fast-growing hybrids mainly arise by farming factors and, especially in recent years, by the pre-slaughter and slaughtering phases. In this regard, it is also well known that some features observed in fast-growing hybrids (i. e., muscle hypertrophy, accentuation of glycolytic metabolism of the muscles, poor thermoregulatory capacity, skeletal and vascular fragility, insufficient vascularisation), might directly or indirectly be induced by selection predisposing to the occurrence of meat abnormalities with increased incidence within the last 30 years (Petracci *et al*., 2015; Velleman, 2015).

This review is therefore intended to make a summary of the most important qualitative issues affecting the chicken meat of fast growing broilers reared in industrial farming and slaughtering conditions.

Main meat quality problems

Green muscle disease (GMD)

Deep pectoral disease, also known as Oregon disease was observed for the first time in fast-growing birds about 30 years ago (Siller, 1985). Nowadays, the occurrence of GMD abnormality is still a relevant quality issue in poultry since its incidence may vary between 0.02% and 1.9% (Kijowski *et al*., 2014). In detail, its incidence is higher in broiler chickens with a higher growth rate and of a great extent in roosters rather than in hens (Lien *et al*., 2011). GMD exhibit haemorrhagic appearance with a swollen reddish-brown lesion, typical of a early developing stage, that gradually becomes green in the old one. Thus, the presence of GMD may result in significant commercial complaints, if the whole carcass is sold to cut-up as well as processors units or butcher shops and economic losses for the poultry industry as a consequence of the trimming procedures resulting in breast meat downgrading (Petracci *et al.*, 2015). The occurrence of GMD certainly appears to be related to the tremendous development of the pectoral muscles observed in modern commercial hybrids coupled with the relatively low activity during growing period. Being confined within an inelastic compartment, the *Pectoralis minor* muscles are insufficiently enlarged if poorly exercised (Siller, 1985). In addition, according to their behaviour under organic farming conditions, this lack of movement observed in fast-growing chickens is mainly due to a genetic predisposition rather than to an intensive farming environment (Castellini and Mugnai, 2002). Recently, Lien *et al*. (2012) found that GMD appears to begin at approximately 26 and 36 days of age in males and female broilers, respectively. Within this context, an increased bird activity (flock nervousness, flightiness, struggle, and wing flapping) induced by several factors such as feed or water outages, lighting intensity and programs, human activity and excessive noises in and around chicken houses should be considered a trigger for the development of GMD in broilers. Lien *et al*. (2012) suggest to the use of creatine kinase levels as tools in selection programmes to mark the susceptibility of muscle for GMD.

Colour abnormalities

Among all the quality attributes, the appearance is one of the most critical for the selection of many food commodities, including poultry meat. One of the main components contributing to meat appearance is colour, which is the most important parameters for the selection of both deboned and skinless raw meat and exerts a relevant role in the final evaluation of many cooked products. Indeed, pink or red appearance in cooked poultry meat is generally associated with undercooking and is therefore highly undesirable. In addition, being associated with freezing prior to cooking, bone darkening is also considered a defect in fully cooked products (Fletcher, 2002).

Other visual defects are mainly associated with bruises and haemorrhages. Modern broilers are very prone to develop bruises and haemorrhages as a result of the grater body size and the increased blood vessel and skeleton fragilities. In detail, 90-95% of the bruises found in broilers occur during the last 12

hours prior to processing with breast, wings and legs being the most affected as a result of the manual catching of broilers (by the legs) through which operatives may carry up to five birds, each held by one leg, in each hand. In this condition, the potential for trauma is therefore considerable (Petracci *et al*., 2011). In addition to the *ante-mortem* steps involving manual handling of the broilers, the stunning phase is of significant importance. Although electrical stunning represents the most widely used system for poultry, under certain conditions, can result in meat defects such as broken keels and wings, engorged wing veins, and broken capillaries within the breast muscle as a consequence of hyper-contractions and subsequent haemorrhages caused by blood vessels rupture and damaged muscle fibres (Savenije *et al*., 2002). Animal welfare and product quality issues arising as a result of to the use of high current electrical stunning have led in Europe (Council Regulation n. 1099/2009) to the development of alternative techniques such as gas stunning. During gas stunning, the birds are exposed to gases which may induce either anaesthesia (carbon dioxide) or anoxia (argon and nitrogen). Gas stunning has a high potential for humane stunning or stun-to-kill, it requires sophisticated and expensive technical equipments with the main advantage being the moderate handling stress for the birds since they can remain in transport crates from the time they are carried from the farm to the slaughterhouse reducing both animal distress and subsequent carcass defects (broken bones, haemorrhages, etc.) (Petracci *et al*., 2011).

Raw meat colour differing from the expected pale tan to pink results in consumer rejection of the product itself. The main factors affecting poultry meat colour are: i) its myoglobin content; ii) the chemical state of the haem structure; and iii) the pH. The myoglobin content in chicken meat basically depends on genotype, muscle type and age at slaughter. However, since similar muscle characteristics and age at slaughter are found between the modern fast-growing hybrids, the differences were found only when white (i.e. breast) and dark (i.e. leg, wing) meats are compared. In detail, considering breast meat, the *post-mortem* muscle acidification is the major factor responsible for fresh meat colour variations in broilers. Muscle pH has been shown to be primarily related to the biochemical state of the muscle at slaughter and following *rigor mortis* development. In addition, besides a higher ultimate pH is associated with darker meat, lower pH values are associated with lighter meat. In the extremes, high and low pH meats are often characterized by being dark, firm, and dry (DFD-like) and pale, soft, and exudative (PSE-like), respectively. Both of them associated with poor functional properties or, at the very last, considered major contributing factors to product variation (Fletcher, 2002).

Especially PSE-like meat has been the major source of breast meat downgrading at least before the appearance of myodegeneration related abnormalities. The occurrence of PSE-like condition in chickens can be associated with both the rapid glycolytic process taking place during *post-mortem* time (pH lower than 6.0 just after few minutes after the death) and the extensive acidification leading to achievement of extremely low ultimate pH values (< 5.7-5.8) (Petracci *et al*., 2015). The genetic selection for increased growth-rate and breast yield has resulted in an altered inter-compartmental regulation of cations within the muscle cells of modern chicken hybrids, reflecting an adaptive response to an augmented metabolic demand (Sandercock *et al*., 2003). In addition, an increased stress level of broilers may exacerbate these issues and underlie additional quality defects such as PSE-meat. Indeed, it has been generally recognized that stressors during pre-slaughter phases (i.e. catching, transportation and lairage) exert detrimental effects on meat quality and exposure to high temperatures may exacerbate such negative effects. If compared to their genetic predecessors, fast-growing and heavier birds have been found to be more susceptible to heat stress because of their reduced thermoregulatory capacity. Moreover, acute heat stress has been demonstrated to increase superoxide free radical production in chicken skeletal muscle. Thus, this mechanism may be responsible for both the heat stress-induced muscle damage and the changes in muscle and meat quality observed in broilers (Petracci *et al*., 2015). On the other hand, genetic studies suggested that breast yield is highly positively correlated with ultimate pH. The ultimate pH of breast muscle is mainly determined by the glycolytic potential which is associated with glycogen stores at slaughter (Berri *et al*., 2007). Le Bihan-Duval *et al*. (2008) found a strong genetic correlation (very close to 1) between breast muscle glycolytic potential and pHu. Therefore, since these studies evidenced that it is possible to select broilers with a higher pHu without any adverse effect on growth rate and breast yield, this strategy can be effective in reducing the occurrence of PSE-like condition in broiler breast meat (Petracci *et al*., 2015).

On the other hand, the occurrence of DFD-like meat is favoured by depletion of glycogen stores during the *ante-mortem* period as a result of prolonged feed withdrawal and transportation. Glycogen deple-

tion determines an increase in muscle ultimate pH leading to a reduced protein denaturation that results in meat with higher water holding capacity and darker colour. Although not all these effects are detrimental for meat, they could undoubtedly contribute to a higher variability in quality. Moreover, according to Allen *et al*. (1997) darker broiler breast meat with higher pHu values exhibits a faster microbiological spoilage than the paler and low-pH ones. Furthermore, Delezie *et al*. (2007) also evidenced that stocking density in transport crates often overruled the effects of feed withdrawal and transport.

Tenderness

Texture is probably the single most critical quality parameter associated with consumers' ultimate satisfaction with poultry meat products. The two major factors contributing to poultry meat tenderness are the maturity of the connective tissue and the contractile state of myofibrillar proteins. As the modern broiler industry developed and began to dominate the chicken meat market, the issue of an age-related toughness (resulting from connective tissue cross-linking) has virtually disappeared (Fletcher, 2002). However, the market shift towards further processed products has pushed processors involved in the production of boneless breast meat towards not only quality and uniformity, but also efficiency. If the carcass is cut-up and the breast removed from the carcass prior to the completion of *rigor mortis*, muscles fibers will contract and shorten unimpeded by the normal skeleton restrains resulting in less tender meat. It was extensively argued that a minimum of 4 to 6 h ageing is required to allow the breast muscles to complete rigor development before cutting it up without any excessive toughening. Thus, since carcasses usually complete the water-immersed or air chilling at about 1.5 h and 2-3 h *post-mortem*, respectively, additional 2.5 to 4.5 h of refrigerated storage are needed before deboning (Petracci *et al*., 2011). Undoubtedly, because ageing is an expensive process with the majority of the costs being associated with the loss in meat yield and energy extensive research has been done to develop slaughter methods which allow to accelerate the onset and resolution of *rigor mortis* (Fletcher, 2002). Among them, electrical stimulation of carcasses immediately after death reducing the aging time and accelerating the energy depletion in rigor mortis development seems the most promising technique underling a reduced shortening-related toughness of early deboned meats. In addition, some of the electrical stimulation systems have the additional effect of inducing physical disruption within the muscle tissue, reducing therefore its integrity (Petracci *et al*., 2011).

Emerging abnormalities as related to myodegeneration

In recent years, a new group of muscle abnormalities characterized by myodegeneration has appeared. This group includes manifestation of white striations parallel to muscle fibres mainly occurring on the ventral surface of breast fillets (white striping, Kuttappan *et al*., 2009), myodegeneration of *anterior latissimus dorsi* (Zimmerman *et al*., 2012), woody breast condition (often associated with white striping) where muscles are visually hard, out bulging and pale (Sihvo *et al*., 2014) and poor cohesion of meat or "spaghetti meat" abnormality (tendency toward separation of muscle fiber bundles) (Sirri *et al*., 2016).

Histopathological evaluations of skeletal muscles affected by these abnormalities exhibited similar features including the presence of degenerative and atrophic fibres associated with loss in cross striations, variability in fibre size, floccular/vacuolar degeneration and lysis of fibres, mild mineralization, occasional regeneration (nuclear rowing and multinucleated cells), mononuclear cell infiltration, lipidosis, and interstitial inflammation and fibrosis. However, it has been currently shown that the incidence of degenerated fibers is very high also in breast muscle without any macroscopic lesions such as white striped and woody breast (Mazzoni *et al*., 2015; Soglia *et al*., 2016). In addition, it is interesting to note that the presence of similar histological lesions (i.e. hypercontraction, mononuclear cell infiltration, alterations in cell membrane integrity and the loss of myo-cellular constituents) in fast-growing chickens has been reported in several works since the 90s (Mahon, 1999). Therefore, the existence of a common myodegenerative process leading to the separate or combined emergence of all the muscle abnormalities may be hypothesised.

Nowadays, the occurrence of abnormalities affecting breast fillets (white-striping, woody-breast and spaghetti-meat) which is the most valuable part of broiler carcass, causes increased downgrading of meat because of the reduced visual appearance, sensory and technological quality (when used for further processing) of meat, especially for woody breast abnormality. Overall, the incidence rates of these abnor-

malities is alarmingly and seems no longer sustainable for the poultry industry.

These abnormalities appear only in fast-growing broiler hybrids, so it is therefore clear that direct and indirect promoting causes can be found in pectoral muscle hypertrophy and high growth rate. Until now, several studies have been conducted in order to identify the possible factors involved especially in the occurrence of white striping (Table 2). It is clear that the incidence is higher in flocks belonging to high-breast yield hybrids and increases with increasing growth rate and weight at slaughter. On the other hand, males seem more prone to develop especially woody-breast abnormality (Trocino *et al*., 2015). Otherwise, since an overall reduction in the occurrence of muscle abnormalities was observed only as an indirect consequence of reduced growth rate and/or slaughter weight, the dietary and prophylactic treatments seem not to be directly associated with the incidence of emerging abnormalities. As a consequence, no effective management and dietary solutions are available to mitigate the occurrence of these abnormalities without negatively affecting live production traits even if it was recently claimed that there is a strong non-genetic component for all the breast muscle myopathies (Bailey *et al*., 2015).

Table 2 Factors affecting occurrence of emerging abnormalities as related to myodegeneration.

Factor	Occurrence	References
Genotype	High > Standard breast-yield	Petracci et al. (2013), Lorenzi et al. (2014), Trocino et al. (2016)
Body weight at slaughter	High > Low	Lorenzi et al. (2014), Kindlein et al. (2015), Ferreira et al. (2016)
Growth rate	Fast > Slow	Kuttapan et al. (2012a, 2013)
Gender	Males > Females	Lorenzi et al. (2014)
	Males = Females	Trocino et al. (2015)
Crude protein level	High > Low[1]	Kuttapan et al. (2012a)
Vitamin E	No effect	Kuttapan et al. (2012b)
Selenium	No effect	Ferreira et al. (2016)
Lysine	High > Low[1]	Cemin et al. (2016)
L-arginine	No effect	Christensen et al. (2015)
Early dietary restriction	No effect	Trocino et al. (2015)
Full dietary restriction	Ad libitum > Feed restricted[1]	Livingston et al. (2016)
Coccidiosis control	Anticoccidial ≈ Vaccination	Unpublished data,
	Anticoccidial > Vaccination[1]	Dalle Zotte et al. (2015)

[1] *changes related to decrease of growth rate and/or body weight at slaughter.*

Conclusions

The evolution of the chicken meat market has increased the relative importance of quality parameters that affect the appearance (in terms of absence of visual defects and abnormal colours) and the sensory profile with special emphasis to tenderness. However, during the last decades, the genetic characteristics of current fast-growing hybrids have certainly favoured the occurrence of a high number of abnormalities that are increasing the meat downgrading rates for fresh market retailing and decreasing the nutritional, sensory and technology proprieties of raw meat materials used as raw material for further processing.

Therefore, it seems that poultry industry cannot longer postpone a closer view of problems related to the proper muscle growth and then of the quality of resulting meat when selecting genotypes for broiler production.

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L59 International development trends of layer industry and prospect for China

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Abstract

At present, the Chinese layer industry has entered the key period which accelerates the transformation and upgradation. The system summary for status and trends of international layer industry and prospects for China contributes to coordinate and crack the problems which exists in the laying hens industry development. The development of Chinese layer industry ranked the first in the world, not only in production capacity, but also in laying hens scale; the ecological construction performance has improved significantly in recent decades. Driven by consumers' egg demand, the international layer industry had been keeping developing and growing. Asia region accounted for the largest proportion in the international pattern of egg production. In recent years, it shows variety features such as development of large-scale farming, attaching importance to scientific and technological support or environmental protection, the development of the egg deep-processing, emphasizing on animal welfare of laying hens and the further expanding for layer industry scale in developing countries. Differ from international laying hens industry trends, Chinese layer industry has shown a series of characteristics which includes the speeding of industry consolidation, scale and standardization, the strengthening for role of market-oriented, the balancing between supply and demand of eggs, the domestic breeding, the promoting of waste utilization on account of ecological agriculture development and environmental policies. Combined with the development challenges which the domestic layer industry faced and the international trends, this paper proposes some policy recommendations, such as intensifying follow-up study on the welfare of laying hens and related systems, strengthening scientific and technological innovation, increasing the focus on the promotion of technical efforts, accelerating the process of ecological farming poultry industry, exploring new layer feed to avoid feed risk, strengthening co-ordinate planning for regional industrial, etc. in order to accelerate the process of healthy and susta inable development for laying hens industry.
Keywords: layer industry, international, China, trends, prospects.

Introduction

According to records, Chinese breeding hens could be traced back to primitive society, in order to meet the demand for animal food. Since the establishment of new China, especially after the reform and opening up, laying hens industry keeps developing and growing. In 1985, Chinese egg production surpassed the United States, and had been ranking the first in the world since then. Nowadays, the layer industry in China has entered a key period in order to accelerate the transformation and upgrading (Yang N, et al., 2014; Zhao Y, et al., 2014). It still faced a series of prominent challenges such as resource, technical, economic and environmental constraints, although it had met the demand needs of the consumer, led to the development of related industries, promoted the employment and income of famers (Zhao Y, et al., 2012). So, it is urgent to explore the international development trend of layer industry, comply with the law of industrial development, assist to crack problems that exist in development which based on clarifying the situation of domestic hens industrial development, in order to accelerate healthy and sustainable development of Chinese Layer Industry.

Achievements and contributions of Chinese layer industry

Keep increasing for overall production capacity

After the rapid development for more than thirty years since the reform and opening up, the comprehensive production capacity of Chinese layer industry has been steadily increasing. In 1985, China's egg production surpassed the United States and had been ranked as the first in world since then. In 2014 Chinese egg production is about 24.6 million tons (Figure 1), accounting for about 35% of the world; pos-

session of eggs per capita is about 17.98 kg, ranked the third in the world, which made a great contribution in meeting the nutritional needs of urban and rural residents.

According to the report from China Animal Husbandry Association, by of the end of April 2015, there is 24 ancestral farms and about 0.59 million units of herds. The parental breeding stock is maintained at around 20 million units. Commercial layers population is about 1.5 billion, of which there are about 1.2 billion laying hens, and all these indicators are in the primacy of the world. From Breeder localization trends, by 2014, the proportion of domestic Breeder in China has reached to 67%.

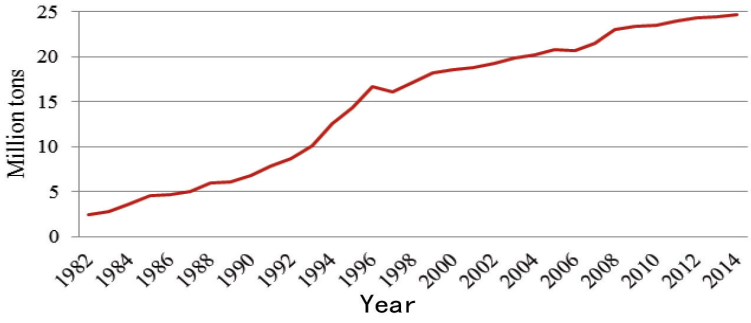

Fig.1 Chinese egg production trends from 1982 to 2014
Source: over the years of "China Statistical Yearbook"

The highlights of Scale breeding for hens

In the past decade, the scale of Chinese commercial layers population has been greatly improved. According to the survey data from national layer industry technology system, the extent of China's large-scale farming of laying hens has reached to 70%, and it develops along with the large-scale, standardized and intensive path(Gao G.et al.,2011).

Since 2004, the cost and value of Chinese laying hens has showed a rising trend, the total feed costs per hundred laying hens rose to 16407.30 yuan in 2014 from 8590.03 yuan in 2004, which made an increase of nearly doubled in the last decade; the output value per hundred hens rose to 18069.46 yuan from 9659.54 yuan, which made an increase of 0.87 times cumulatively; the net profit per hundred hens rose to 1662.16 yuan in 2014 from 1069.51 yuan in 2004. Among them, it reached the different highest points of the stage in 2004, 2007, 2011 and 2014, showing a basic three-year fluctuation cycle. It could also be seen from Figure 2 that the farming profits didn't keep sustainable growing, but showing a clear fluctuation. The inter-annual profit rate was fluctuated and lack of stability.

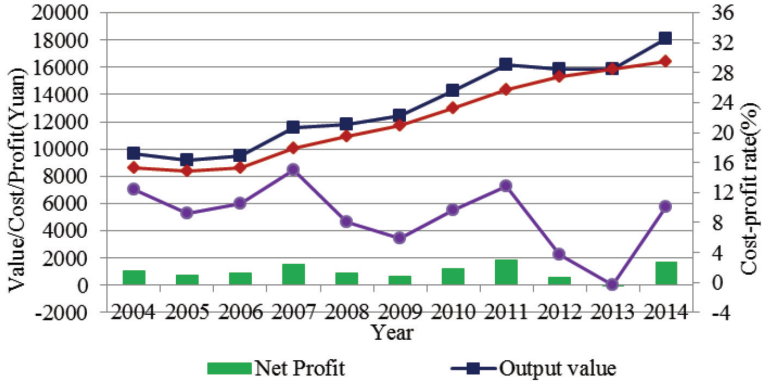

Fig.2 Changing Trend of cost−benefit for Chinese scale−breeding hens from 2004 to 2014
Source: over the years of "Compilations of national cost−benefit in agricultural"

Effect of ecological building gradually revealed

In recent years, Chinese egg industry pay more attention to adjustment of farming methods and pollution prevention. The application of biogas, aerobic and anaerobic composting technology and the spread of large tank fermentation technology reduced the damage of ecological environment owing to waste pollution of layers. Especially, the use of layer manure fertilizer technology integrates aquaculture and farming effectively and closely, which provides a reference and demonstration for solving the problems of coordinated development between China hens scale farming and environmental protection.

The overview of international layer industry development

Since 1961, the world's egg production has been rising, and the rapid development of Chinese layer industry has made tremendous contributions in driving the growth of world egg production after 1985. Owing to the impact of world financial crisis and avian influenza, the global egg production growth has been slowed down to some extent since 2008. But the average annual growth rate of world egg production also as high as up to 3% from 1961 to 2013(Yang D, et al., 2013).

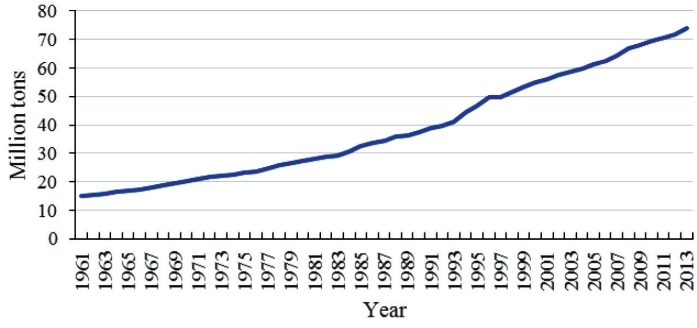

Fig.3 The world egg production from 1961 to 2011

Source: FAO

Asia accounted for the largest proportion in world's regional pattern of egg production, which exceeded Europe in 1988. The United States was the world's largest producer of eggs before 1985, but showed a clear downward trend afterwards. In addition, Africa and Oceania are not the main producing areas for eggs, but keep stable relatively.

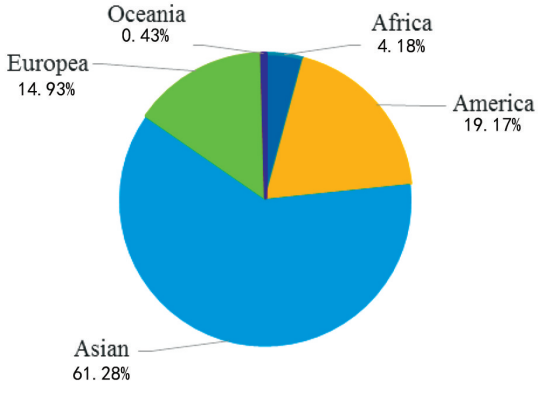

Fig.4 Changes of egg production for continents

Sources: FAO

As can be seen from Figure 5, Asia is the main egg producing area, which China ranks the first in the world, the United States ranks the second, India and Japan are the third and fourth respectively. Total egg production of China, India and Japan accounted for 49.31% of the world.

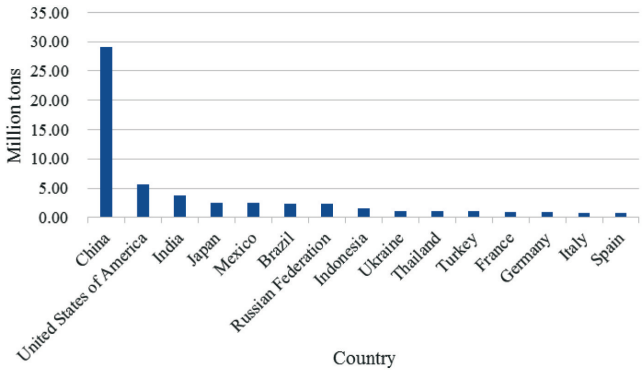

Fig.5 Egg production in main countries of World in 2013(former 15)
Sources: FAO

International trends for layer industry

Large-scale farming and its scale economy of benefits

By constraints of labor costs, land resources and other factors of production, the major developed countries abroad focus on large-scale and standardization of farms in layer industry, in order to achieve economies of scale by the development of capital-intensive, technology-intensive professional scale farms. In addition, large-scale development of laying hens also further promote the rational distribution of the whole industry chain, and finally realize the effective concentration in space.

Focus on scientific and technological support

Developed countries pay more attention to strong support of science and technology for laying hens industry, the development of molecular breeding techniques has led to the rapid development of layer breeding industry. In the United States, for example, contribution to the development of the layer industry from new varieties has accounted for more than 50%. The investment to automated farming machinery effectively solves the problem of high labor costs during artificial feeding in developed countries and achieves higher farming revenue.

Stress importance of ecological and environmental protection

Laying hens' environmental pollution has been highly stressed in developed countries, mandatory requirements are fixed legally or institutionally by many countries. The protection of ecological environment is achieved by taking a combination of crop planting and animal farming. At the same time, the subsidy and payment system for waste processing is improved in order to incentive the environmental behavior of farmers in economic perspective, which further guarantees the friendly ecological environment development.

Develop the egg deep-processing industry

The domestic processing and international trade are two very important aspects for new added egg production in the Netherlands and the United States. More than 30% of the merchandise is made into liquid egg or other egg products in the United States, and processed egg consumption will continue to rise in the future. The development direction of egg processing mainly reflects in the improvement of productivity, exploration of featured products and balanced nutrition.

In consumer market, per capita consumption of eggs has now been on the way down after years of rising for majority of consumers in developed countries. From the consumption structure, the pattern of egg consumption has been transformed; in form of products, processed egg consumption ratio in developed countries is increasing, including liquid egg, egg powder, ice eggs, special egg products; the proportion of ordinary egg consumption showed a downward trend as well, but due to the consumers' deepen understanding for nutritional value of eggs, consumer demand and capacity of high quality eggs are

rising, market of organic eggs, free-range eggs, shelled egg continues to expand, such consumption also leads the development direction for the world.

Emphasis on animal welfare of laying hens

On farming methods, with practice of European animal welfare legislation and the opposition to caged laying hens environment by domestic parties in America, the Netherlands, the United States and the South Korea have been facing changes of hens farming equipment and environmental upgradation. Since the European Union banned the use of conventional multi-layer cages in hens breeding in 2012, the majority of Dutch farmers reared laying hens in large cages. The United States plans to apply the progressive use of "loose cage systems" instead of conventional cages over the next 15 years. At the same time, they gradually enlarge the breeding space, and make an unambiguous identification of conventional cages or loose cages. In addition, consumers' concept about "animal welfare" and the potential market demand for animal welfare eggs is growing.

Further expand for scale of breeding hens in developing countries

With the rapid economic development and population growth in developing countries, egg production has been increasing continually, and its economic status improved significantly. Especially in China, India, Brazil and Mexico, egg production made a rapid increase. But the growth rate is relatively slow in developed countries, the proportion is also declined. Currently, loose caged pattern has become the main development direction in the United States, Australia, New Zealand and EU countries, which rises the costs of breeding hens significantly and narrowed the total amount of laying hens.

Prospects for China's Layer Industry

The trends for layer industry in China

After experiencing a long period of rapid development, the laying hens industry in China has now entered the stage to accelerate the transformation and upgrading. At present, macro environment of layer industry has undergone some major changes, and would promote the Chinese laying hens industry go into a new development stage.

Accelerating industry consolidation

With the changes of breeds, herd sizes, marketing tools, industry self-regulation, government policy, as well as medium and small-scale farmers' decline and new large-scale farms' operation, appropriate scale, family-based management, specialization of production, social services, product branding and so on will emerge after emerge gradually, so as to improve the ability to resist breeding risk in hens industry effectively (Yang N, 2015).

Speeding up scale and standardization

In the process of laying hens industry development in China, some factors such as industry gathering, policy support and economic benefits etc. gradually reduce the number of laying hens farms, the retail farers gradually withdraw out of the layer lines, the proportion of large-scale farms grows obviously. Large enterprises in vertical integration formula have developed rapidly, standardized production mode is adopted broadly. Especially, modern machinery and equipment's replacement of manpower and comprehensive application of new technology would be the new direction.

Strengthening the role of market-oriented

Since the reform and opening up, by experiencing the "enriching project" as the goal to improve family income and "vegetable basket project" which meet the animal protein needs of residents, development of layer industry has been in the transition to the stage of "food project" whose target is to produce safe eggs. It is more obvious that the development of laying hens would be subjected to market demand-oriented.

Fitting eggs' supply and demand

Egg consumption mainly based on household consumption in China. Currently, the income elasticity

of urban and rural residents continued to decline in egg consumption, market demand's increasing will mainly be driven by population growth and urbanization. In this context, the equilibrium between supply and demand is going to be approached (Liu H, et al.,2016).

Breeding localization gradually

In 2014, Office of the Ministry of Agriculture issued "Notice on the release of the first batch of list for core breeding farm of laying hens and layers promotion base of national seed multiplication" ([2014] No.31), five companies were selected to be core breeding farms, 10 enterprises were chosen as promotion bases of national seed multiplication. These enterprises will serve as the backbone of laying hens seed industry in China, and will continue to support the development of the industry.

With the implementation of "National hens' genetic improvement programs (2012-2020)", fine varieties of breeding hens which are suitable for local laying hens farmers would be selected from domestic excellent germplasm. Thus, the domestic market share for varieties of independent breeding hens would be further increased.

Waste recycling friendly

With the development of ecological agriculture, demand for organic fertilizer in agricultural production is growing. As major organic fertilizer, layer waste has become the main suppliers. Driven by market demand, utilization of organic fertilizer has become the focal point of expansion in layer industry.

At the same time, the conservancy and other related waste disposal machinery has been included within the scope of state subsidies, financial support on devices for farmers has been provided in order to encourage farmers. In addition, subsidies for organic fertilizer and other policies relating to waste disposal also started around the province level, and greatly promoted the harmless use of laying waste.

Recommendations for the layer industry development in China

Chinese layer industry has made remarkable achievements, at the same time it also faces many problems such as increasingly prominent contradictions between the resources constraints and the sustainable development, the rising production costs and the advancing quality or efficiency, the fluctuating of egg price and the increasing of farmer's income, the low level of breeding scale and standardization and the ascension of the degree, the low level of egg processing and the upgrading of industrial development, the transformation and upgrading of consumption and the difficult security control, the biological safety barrier and the severe disease prevention and control. Combined with the development trend of the international layer industry, the following suggestions are put forward.

Strengthen the follow-up study of laying hens welfare system

International standards for breeding hens are changing rapidly. As new internationally accepted standards, welfare farming is gradually expanding in worldwide. Although it is not long for Chinese layer industry getting into track of modern intensive caged hens, the welfare farming of laying hens should be taken seriously considering the importance of ecological environment, food safety, national health and other conditions. EU, US, Japan and other countries have been carrying out in-depth studies on the various methods for welfare farming of laying hens. From brewing to the implementation, it took 10 years for laying hen welfare system in EU, which still make a greater impact on other countries. Lessons should be learned from EU for China, and strategic planning should be done as early as possible.

Focus on promoting scientific and technological innovation

In the process of laying hens' industrialization, investment must be increased on supporting technology which complies with industrialization, marketization and modernization as much as possible. New technological revolution for breeding hens should be accelerated and the role of science and technology in the development of the layer industry should be highlighted. To this end we should make efforts in the following aspects: Firstly, initiating "seed project" to accelerate the process of breeding hens. Secondly, promoting the comprehensive and efficient breeding technology in breeding, focus on the farming techniques in scale and efficient factory. Thirdly, strengthening prevention and control for layer diseases, accelerating the development and the promotion of rapid diagnostic techniques, new vaccines and new

agents, strong support is urgently necessary need in this areas. Finally, strengthening research and applications for egg preservation and processing technology. Keeping eggs fresh, long-distance transport and new processing technologies should be focused in R&D, which may improve the quality of health processed eggs.

Accelerate the process of ecological farming

To enhance environmental awareness among farmers of laying hens, the harm of pollution in layer breeding may be popularized through radio, television, newspapers, Internet and other forms. Technical information to avoid pollution in layer raising can be issued to improve people's awareness of the environment, in particular to raising farmers. Meanwhile, scientific planning and rational distribution should be made by the agricultural sector. In the fed region, the development of layer farming should stay away from densely populated areas and environmentally sensitive areas, adhere to principles of balance between breeding and planting, and make strict control of breeding hens per unit of arable land.

Explore new layer fodder

In recent years, with the rapid development of China's poultry industry, soybean meal, corn and other imports showed a rising trend. In the state of shortages for global food supply, it should be an effective way to ensure the safety of layer industry which based on the domestic farming and production of layer feed. It suggested that the relevant departments choose domestic crop varieties and develop new domestic hens feed, as well as select standardized way of breeding hens which support new domestic fodder.

Stress overall planning for regional industry

In development of layer industry, dominant position of hens breeding in industrialization of agriculture should be recognized. Overall planning within the region should be made to promote industrial development. Firstly, proposing the development plan for layer industrial management with the actual situation. Secondly, functions for the various departments in layer industries should be further clarified so as to implement the integrated management. Finally, improving the system of contracts, market acts and regulations, such as developing realistic management regulations, making specifications and terms of the contract between farmers and leading enterprises, strengthening law enforcement and protecting the legitimate rights and interests in all sectors of the layer industry chain. For the healthy development of layer industry, it is very necessary for getting industrialization of layer into a legal and standardized track.

Acknowledgements

This work is supported by Special Project of Modern Agricultural Technology System-National Layers Industry Technology System (CARS- 41- K26) and China Postdoctoral Science Foundation (2015M571182).

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L60 Economics of poultry production: critical success factors

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Abstract

Production costs are an important indicator in evaluating the economics of poultry production. The production costs have large impact on the profitability of a farm and are an influencing factor in competitiveness between countries. Feed costs are the main component in the production costs (61%), followed by costs of day old chicks (15%), other variable costs (10%) and housing (9%). Based on Dutch data it was illustrated that there is a wide variation in the level of production costs between individual farms. Also between countries there is a substantial variation in production costs. Production costs can increase with higher feed prices (as in the year 2008, 2011 and 2013) and as a result of legislation or societal demands. An example of the latter is the production of alternative broilers, which increases the production costs by 31% for extensive indoor and 41% for free range systems.

Keywords: production costs, economics, broilers, competitiveness

Introduction

Production costs have a large impact on profitability on farm level and production costs are an influencing factor in competitiveness between countries (Porter, 2000). In this article different aspects of production costs at broiler farm level are analyzed.

Components in production costs

Production costs in broiler production give the total costs to produce a kilogram of live weight. In calculating the production costs the continuation of the company is the basic principle in which costs of investments are calculated on replacement value. The total costs are the sum of the variable costs and the fixed costs. The variable costs are the costs of the day old chick, feed and other variable costs. Other variable costs are all costs directly related to the number of broilers, such as costs of heating, electricity, animal health and catching. The fixed costs are the costs for housing, labor and general costs. The costs of housing relate to depreciation, interest and maintenance on the investment of the poultry house and complete inventory (feeder and drinking systems, ventilation, feed storage etc.) These total costs can be divided by the number of broilers housed (costs per broiler) or the total number of kg live weight (costs per kilogram live weight). Figure 1 give the share of all components in the total production costs in Europe for regular broilers based on 2013 prices. The share of feed costs is 61%, followed by 15% for day old chicks (doc), 10% for other variable costs and 9% for housing.

Feed costs

By far the main component in the total production costs is feed. When the feed costs of broiler breeders for the production of day old chicks is taken into account the share of the feed costs is even higher: 65%. Feed prices are fluctuating in time. In a year with high feed prices the share of feed costs in total production costs can even reach 70%. Figure 2 gives an overview of the development in price of broiler feed and layer feed in the Netherlands (in euro per 100 kg) from January 2006 to December 2015. Figure 2 shows the large fluctuations in recent years, with high prices in 2008, 2011 and 2013. In 2014 and 2015 prices were at a more moderate level. Figure 2 clearly illustrates that the development of the broiler feed price is similar to the price of layer feed, but at a 30% higher level. As broiler feed has a higher protein content the additional price mainly depends on the market price of soybean meal. Figure 2 also gives the development of the feed price of layer feed in the USA (US$ per 100 kg). In the Netherlands the price of layer feed the farmers are paying to the feed mill is available on a monthly basis. Every month the LEI research institute presents this price on their website (LEI Wageningen UR, 2016). In the

USA the layer feed price is calculated based on published prices of corn and soybeans in addition to the costs of other ingredients, milling and transport costs. This price is calculated by the Egg Industry Centre in IOWA (EIC, 2016). The feed price in both countries shows a similar development.

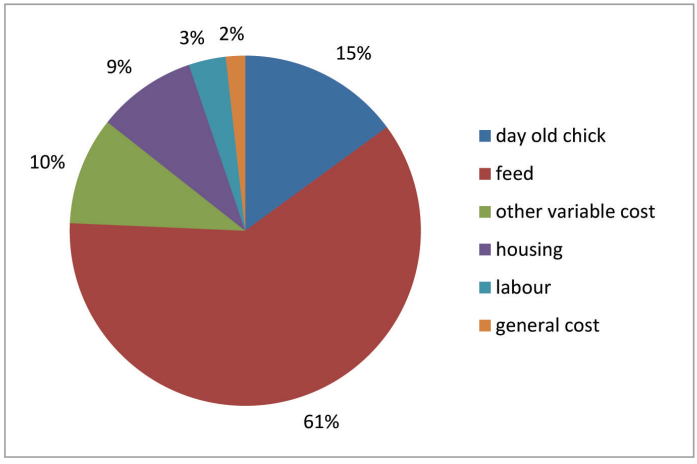

Figure 1. Share (in %) of components of production costs of broilers (EU prices year 2013).
Source: LEI Wageningen UR

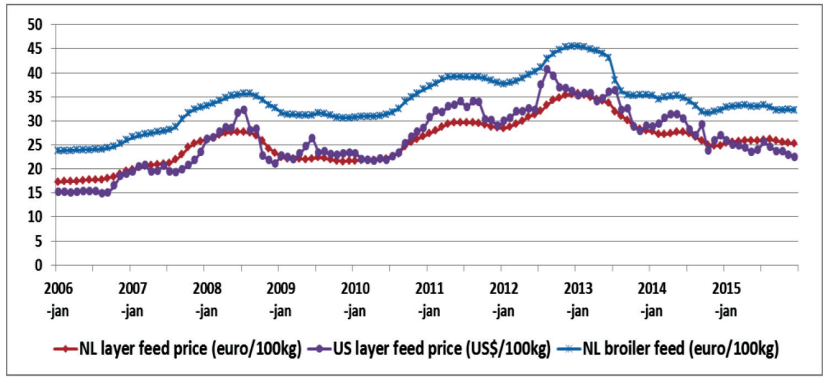

Figure 2. Development of the price of broiler feed and layer feed in the Netherlands (NL, euro per 100 kg) and the price of layer feed in the USA (US$ per 100 kg) in the period 2006–2015.
Source: LEI Wageningen UR and Egg Industry Center, Iowa.

Differences between broiler farms

LEI Wageningen UR is collecting data on a group of broiler farms, which are a random sample out of the total population. Of these farms data are collected of production results (live weight, feed intake, mortality), prices (feed, day old chicks, energy) and financial results (interest rate, investments). From the database we can derive the results of the farms. Figure 3 gives the total production costs per kg live weight of 27 broiler farms in the Netherlands. The average production costs for all flocks delivered in the same year were 0.98 euro per kg. The range is wide with some farms having production costs as high as 1.07 and other farms as low as 0.92. The impact of farm size (horizontal axes) is limited. Part of the variation in production costs can be explained by the difference in feed conversion. The differences are shown in figure 4. The average feed conversion was 1.65. However, there is wide range in level of feed conversion between farms. As feed costs are the main component of the total production costs these dif-

ferences in feed conversion can partly explain the differences in production costs.

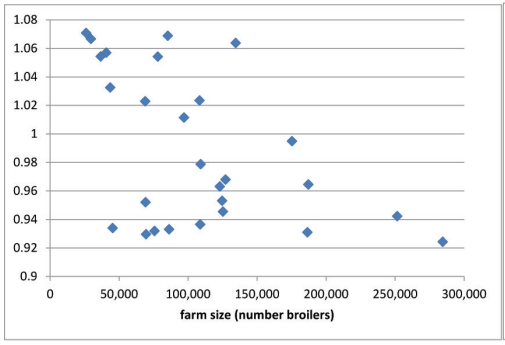

Figure 3. Production costs (E per kg live weight) on 27 farms in the Netherlands (data 2011)
Source: BINternet database of LEI Wageningen UR

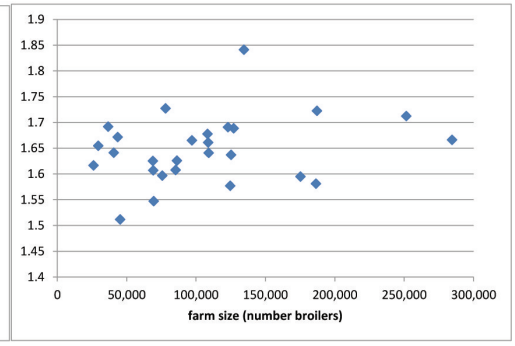

Figure 4. Feed conversion on 27 broiler farms in in the Netherlands (data 2011)

Countries

The production costs of broilers have been research for some EU countries and selected countries outside the EU. The calculated production costs at farm level were based on the situation in 2013. The calculation of the production costs was done with the same method and based on average production data (performance) and economic data collected in the selected countries. Figure 5 gives the results for the Netherlands, Germany, France, UK, Poland, USA, Brazil, Thailand and Ukraine. Within the EU Poland has the lowest production costs due to cheaper housing and lower labor cost. Outside Europe production costs are low in Brazil. In Brazil feed ingredients are available in large quantities (maize and soy bean). Besides low feed costs Brazil has the advantage of lower housing and labor costs. In almost all countries outside the EU producers have lower costs, because on many topics no legislation exists as in the EU. Examples are the use of antimicrobial growth promotors and meat-and-bone mail in broiler feed, and the absence of environmental legislation (van Horne and Bondt, 2014).

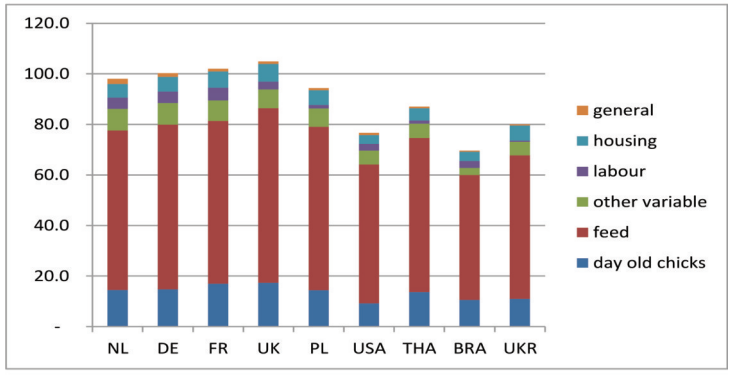

Figure 5. Production costs (in euro per kg live weight) in the Netherlands (NL), Germany (DE), France (FR), United Kingdom (UK), Poland (PL), United States (USA), Thailand (THA), Brazil (BRA) and Ukraine (UKR).
Source: Van Horne and Bondt, 2014

Alternative broiler production

Around the world the broiler sector commonly uses fast growing genotypes broilers to produce poultry meat. These broilers achieve the target weight of 2 to 2.5 kg in around 5 to 6 weeks. Alternative broil-

er production that uses slower growing genotypes is increasingly gaining attention in many EU countries. The poultry meat of slow growing broilers is a premium product, and farmers and processors receive a higher market price to compensate for the higher production costs. The conditions and names of the alternative broiler production in the EU are regulated by Regulation EC/543/2008. For extensive indoor (EI) and Free range (FR) the minimum age is 56 days, the maximum density indoor is 15 respectively 13 birds per m^2 poultry house. For free range the access to an outdoor run is minimum 1 m^2 per bird (van Horne and Bondt, 2014). Figure 6 give the production cost of regular broilers, extensive indoor and free range. Compared to the regular production the increase in costs for the extensive indoor system is 31% and for the free range system 41%. As figure 5 shows the main increase is for feed (higher feed conversion), followed by housing (as a result of the lower density) and other variable costs (extra heating cost). Broilers produced in these systems need a bonus from the market to compensate the farmer for the additional costs.

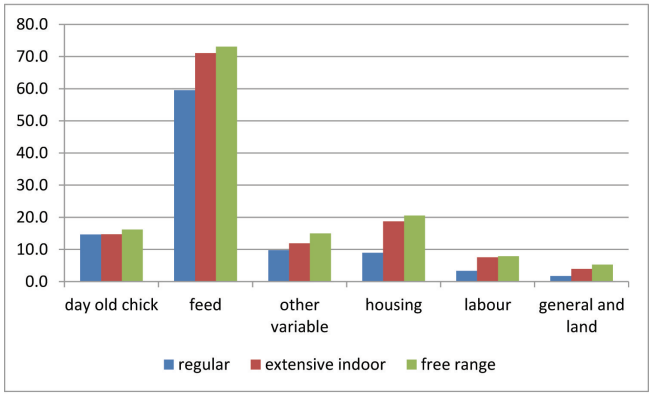

Figure 6. Costs components (in euro per kg live weight) for regular, extensive indoor and free range production based on the European regulation.

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L61 Challenges and opportunities in meeting agricultural workforce needs−a USDA vision

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Keywords:agriculture, education, food production, workforce development, USDA

Summary

The world's food systems and our ability to ensure global food security are being impacted by major challenges and risks, including climate change, diminishing land and water resources, changing incomes and diets, increasing urbanization, environmental degradation, and the need to ensure better health outcomes. We believe the solution to these pressing challenges lies in transformative discoveries, translation of discoveries into innovations and solutions delivered to the end users, and education of the pipeline of young people needed. From the educational perspective, there is need to incentivize young people to enter agricultural fields and to provide the rigorous education such that they have sustainable livelihoods. A recent study by Purdue University concluded that the United States will produce an average of 57,900 jobs per year between 2015 and 2020 for graduates with a bachelor's degree or higher in agriculture-related fields; however, there will only be an average of 35,400 new graduates in these fields. The Agricultural Science Workforce survey undertaken by the Coalition for a Sustainable Workforce concluded that the top-paying jobs would require graduate degrees, including PhDs. A 2014 study by the Science, Technology, Engineering, and Mathematics (STEM) Food and Ag Council indicates that during the next five years, the United States agricultural workforce in specialized areas is expected to grow by 4.9 percent, adding 33,100 net-new positions. All three studies indicate that there are significant shortages in specialized areas, including plant and animal breeding, crop and animal sciences, entomology and plant pathology, weed science, soil science, food science and engineering, natural resources engineering, and agribusiness. Additionally, with the aging farm population- the current average age of the American farmer is 58.3 years-there's a critical need to attract young people to produce food. This paper offers a few approaches that the U.S. Department of Agriculture (USDA) believes will ensure that a well-trained workforce is available and ready to undertake challenges associated with meeting the nutritional needs of a growing global population.

Introduction

There is significant rethinking in the United States in regards to higher education in agriculture and transformative changes are called for to better prepare graduates for the future (NRC, 2009a). Kunkel et al (1996) concluded that "the purpose of education in agriculture is to provide for the needs of society and industry in a changing world, to produce graduates with flexibility, diversity, perspective and values." Similarly, in *Agriculture and the Undergraduate* the National Research Council asked how do we educate students to meet the demands of the world, such as global competitiveness and hunger, inequities in food distribution, as well as environmental and health issues (NRC, 1992). Building on this vision are recent and urgent calls for action, including the *Report to the President on Agricultural Preparedness and the Agriculture Research Enterprise* (PCAST, 2012a); *Achieving the New Vison for Agriculture: New Models for Action* (WEF, 2013); *Building a Common Vision for Sustainable Food and Agriculture* (FAO, 2014); and a report from The Chicago Council on Global Affairs (Bereuter and Glickman, 2015) that challenges the U.S. to leverage the strength of its research infrastructure to introduce a major transdisciplinary initiative to train the next generation of agriculture, food, and nutrition leaders through research partnerships, work force development, and outreach services in developing countries.

According to the United Nations (UN, 2013), the world population is predicted to reach 9.6 billion by 2050, just 35 years from today. If this prediction is accurate, humankind's greatest challenge may be feeding this population. To feed a population of 9.6 billion, the Food and Agricultural Organization

(FAO) projects that agricultural production (food, feed and fiber) will need to increase by 70 percent (FAO, 2009). In order to achieve this global food security, we will need to improve agriculture and its distribution, reduce waste, ensure safe food for us and our ecosystems, as well as use our crops effectively and nutritiously, while providing livelihoods to farmers and rural communities (Economist, 2011). Furthermore, it is estimated that about one-third of the world population (about 1 billion people) go hungry every day, crops are used for bioenergy and other industrial purposes, and the future demand for food will increase the pressure on scarce environmental resources.

As we look toward the future, our "education vision" needs to include "what is good agriculture?" The World Economic Forum concludes that the world needs a new vision of agriculture (WEF, 2013). We believe the solution to these pressing challenges lies in education, inspiring our young people to enter agricultural fields and providing the rigorous education these disciplines demand.

Challenges

College graduates in the United States are severely lacking basic skills (job skills)-especially problem solving, decision making and the ability to prioritize tasks (Silingo, 2015). Similarly, the Collegiate Learning Assessment Plus (CLA, 2015) found that 40 percent of college seniors lack the reasoning skills needed in today's workplace. In addition, the skills of graduates depended on their college major. Math and science students scored significantly higher than those in other disciplines. Furthermore, the Association of American Colleges and Universities Survey (APLU, 2015) found that employers consistently rated students much lower than students judged themselves. For example, 26 percent of employers said graduates had critical thinking skills compared to 66 percent of the graduates (students). These findings confirm what companies have long complained about- that many college graduates are not ready for work and the global job market.

Exacerbating the problem, the capacity of existing U.S. students has been declining. American students are no longer ranked in the top 20 countries in math and science capability. Less than half of the ACT test takers are prepared to take college level math and science (ACT, 2014). Congress and federal agencies have been responding to the need to produce more STEM students. However, this may not translate into more students going into agricultural sciences and related sciences without additional incentive programs.

The number of students enrolled in production agriculture has been declining as well as the proportion of graduate students concentrating in agricultural sciences. Academic leaders in agricultural education are very concerned and industry leaders are spending increasing amounts of money to train new employees who have majored in other scientific disciplines to work in agricultural areas (AAAE, 2012). In general, U.S. students are not interested in pursuing careers in science, technology, engineering, and mathematics (STEM), which have major implications for sustaining American competiveness and economies in agriculture and other industries (NSB, 2007).

Education is one of the current Administration's priorities. In particular, President Obama emphasizes the need to "increase STEM literacy so that all students can learn deeply and think critically in science, math, engineering, and technology." He calls on the country to "address college completion and strengthen the higher education pipeline to ensure that more students succeed and complete their degree." The President also wants to "invest in community colleges to equip a greater share of young people and adults with high-demand skills and education for emerging industries."

Opportunities

To meet these challenges, a new generation of well-prepared, innovative scientists in the agricultural sciences and natural resources is necessary.

In 2015, an employment outlook report released by NIFA and Purdue University (Goecker et al., 2015) concluded that there is an average of 35,400 new United States graduates with a bachelor's or higher degree in agriculture-related fields, 22,500 short of the jobs available annually. Basically, there are not enough agricultural scientists to meet the demand. According to the report's projections, between 2015 and 2020, our nation expects to see 57,900 average annual openings for graduates with a bachelor's degree or higher in agriculture-related fields. The Agricultural Science Workforce survey concludes that the top-paying jobs would require graduate degrees, including PhDs (CSAW, 2013). Re-

cent analyses undertaken by the STEM Food and Ag Council indicate that during the next five years, the U.S. agricultural workforce in specialized areas is expected to grow by 4.9 percent, adding 33,100 net-new positions (SFAC, 2014). Taken together, these results indicate that most of the demand is in specialized areas, such as plant breeding/genetics, plant protection, plant sciences, and animal sciences (Buchanan, 2014). One must not forget that the average age of the American farmer is 58.5 years and there were 4.3 percent less farms in 2012 than in 2007 (NASS, 2012).

These reports show that there is an incredible opportunity for highly-skilled jobs in agriculture, which will address some of the world's most pressing challenges, such as developing solutions to feed 9 billion people by 2050.

A supportive infrastructure including academic institutions, purposeful and mission-oriented curricula, engaged students at all levels, sound education policies, and budgetary commitments will be the key ingredients in defining any renewed vision to redefine agricultural education that is supportive of our grand agricultural challenges. While multiple USDA agencies are engaged at various levels in providing both formal and non-formal teaching, learning and training opportunities, NIFA serves as the lead USDA agency with legislative authorities that support agricultural education in a broader sense (NIFA, 2014). These programs include funding to support education infrastructure at land-grant universities as well as ensuring that our education system is being responsive to the changing demographics across the country. In addition to 58 land-grant universities established through the Morrill Act of 1862, of notable mention are NIFA's programs that support educational activities in 19 historically black land-grants established via Morrill Act of 1890, as well as 34 American Indians colleges and universities authorized by Congress as land-grants in 1994. Education opportunities are also afforded to Hispanic populations through education programs targeted for numerous Hispanic serving institutions.

USDA believes that innovation is a key ingredient-and that we educate to innovate. Indeed, USDA's investment in partnership with national and global entities has led the way in sequencing genomes of crop plants, domestic animals and microorganisms. These genome sequences are of great value for agriculture productivity, bio-based materials manufacturing, industrial bioprocessing, and biodiversity conservation as well as for disease diagnosis, treatment and prevention (Hoffman and Furcht, 2014). USDA is committed in ensuring that the next generation of scientists is available and ready to take advantage of cutting-edge discoveries to be unearthed during this genomic exchange era. Finally, discipline-based gaps in agricultural sciences expertise-for example, plant breeding, animal breeding, integrative plant and animal sciences, food process engineering, and pest management-need to be met through targeted training grants.

What is the USDA doing?

The USDA's vision for education starts with helping students cross the K-20 continuum (kindergarten through college). We see these challenges and opportunities partitioned into four components, (1) coordinating education across USDA agencies, (2) NIFA's Beginning Farmer and Rancher Development Program, (3) NIFA's AFRI Education and Literacy Initiative, and (4) NIFA's 4-H and Positive Youth Development. Improving these components will help USDA strengthen the science literacy and other 21st century skills into a pipeline between secondary and higher education so that our students will be better positioned for the global marketplace.

Coordinating Education across USDA Agencies

USDA's Office of the Chief Scientist through its Science Council chartered an Education Coordinating Committee to improve coordination of all USDA education activities and to leverage resources to build effective partnerships across the U.S. federal enterprise. All seven USDA mission areas and the 12 agencies are represented in this education coordination effort. This committee developed the USDA's education portfolio around five common themes: (1) learning and engagement, (2) training and education, (3) internships, (4) capacity building, and (5) educational campaigns and outreach. In addition, the committee ensures synergy and best practices among these diverse agencies and their programs.

Each USDA agency contributes to the education portfolio and its activities. For example, the Agricultural Research Service (ARS), USDA's principal intramural scientific research agency, is involved in training the next generation of scientists through graduate assistantships, traineeships and mentoring.

The National Agricultural Statistics Service (NASS) supports graduate fellowships, internships and provides statistics for K-12 education. The Economic Research Service (ERS) provides education-related research and data. In addition, ERS supports distance learning through a program that puts agency scientists into the classrooms of minority-serving institutions using interactive real-time video seminars.

The Food Safety and Inspection Service (FSIS) and the Food and Nutrition Service (FNS) provide consumer education about food. FNS has a series of Web and printed content targeted at different age groups. FNS's "Serving Up MyPlate," is a new collection of classroom materials that helps elementary school teachers (grades 1-6) integrate nutrition education into math, science, English, language arts, and health (FNS, 2012).

The Animal and Plant Health Inspection Service (APHIS) develops and applies scientific methods that educate consumers, protect the health of American animal and plant resources, and sustain agricultural ecosystems. Rural Development (RD) focuses on teaching the cooperative business model to secondary education level students using instructor guides and lessons. Finally, the Forest Service (FS) is engaged in educating the nation about forests, natural resources, and other conservation issues. FS's "Natural Inquirer" is a middle-school science journal about America's forests and research that promotes active learning through the scientific process.

NIFA is involved in educating and training the next generation of agricultural employees. These include: (1) the beginning farmer and rancher initiative to address the dearth in farming skills among new farmers, (2) education and literacy initiative to help develop the K-20 pipeline in the setting of formal education, and (3) 4-H and youth development programs to help engage youth in the entire food, agriculture, natural resources and human science spectrum.

NIFA's Beginning Farmer and Rancher Development Program

NIFA's Beginning Farmer and Rancher Development Program (BFRDP) was launched in 2009 to support local and regional training, education and outreach, and technical assistance to address the critical needs of beginning farmers. Training is offered in a variety of topics, including (1) production and management strategies to enhance land stewardship by beginning farmers and ranchers, (2) business management and decision support strategies that enhance the financial viability of beginning farmers and ranchers, (3) marketing strategies that enhance the competitiveness of beginning farmers and ranchers, and (4) legal strategies that assist beginning farmers with farm or land acquisition and transfer. BFRDP complements several programs offered by other USDA agencies to support beginning farmers. These programs provide for voluntary participation, offer incentives, and focus on equity in beginning farmer opportunities for all communities.

NIFA's Education and Literacy Initiative

In 2012, the President's Council of Advisors on Science and Technology (PCAST, 2012a) identified a few challenges and offered recommendations to boost agricultural research enterprise in the United States. PCAST concluded that the U.S. Agriculture workforce challenges include (1) inadequate support for a well-trained workforce (primary concern); (2) the best students do not view agriculture as an attractive career option; (3) the industry has difficultly recruiting the technical employees for its research programs; (4) the talent pipeline begins well before college admission; (5) at the baccalaureate level, a comprehensive array of undergraduate programs relevant to agriculture and the food industry are needed and; (6) USDA, in collaboration with NSF, expand its national competitive fellowship program for graduate students and post-doctoral researchers.

In support of USDA's Goal of Education and Science Literacy as well as in responding to the PCAST's recommendations, NIFA recently launched a new Education and Literacy Initiative (ELI) offered through a competitive funding mechanism. USDA's guiding principal is that education must be more than learning facts. Students also must be offered the opportunity to be incorporated into and be involved in the discovery through delivery continuum, i.e., experiential learning in both the research (discovery) and Extension (delivery and engagement) domains. The goal of ELI, therefore, is to produce graduates with skills needed to address the new challenges of the 21st century in food, agricultural, natural resources, and human sciences.

This program has now evolved with a focus on immersive learning experiences in non-formal educa-

tion to help secondary school teachers identify and integrate successful lessons in their classes; enhance capacity of academic institutions to produce graduates with work-ready skills with special emphasis on research and Extension based experiential learning opportunities for undergraduates; and advance science by supporting graduate and postdoctoral education. The overarching theme that clearly echoes throughout USDA/NIFA education programs is that a robust workforce is essential if the United States is to face predictable and unpredictable challenges and opportunities in the food and agricultural sectors.

H and Positive Youth Development

Headquartered at USDA, 4-H is the nation's largest youth development organization, empowering millions of young people throughout the United States. 4-H reaches every corner of our nation—from urban neighborhoods to suburban schoolyards to rural farming communities. 4-H started as an agricultural-based youth organization and has today evolved into an education program that focuses on citizenship, healthy living, as well as science and technology programs.

Through the land-grant universities and their Extension System, and partnering with the USDA and NIFA, as well as county governments and communities-4-H helps shape youth in the United States like no other youth organization. The 4-H vision is to prepare young people to make a positive impact in their communities and the world. For example, a study conducted by the Tufts University (Lerner and Lerner, 2011) reported that participants in 4-H, compared to young people involved in other non-formal programs (i.e., Boys & Girls Clubs, Big Brothers/Big Sisters, YMCA, and scouting), had (1) better grades, (2) more wanted to pursue careers in STEM disciplines, and (3) planned and applied to college. Tufts research indicates that involvement in 4-H programs substantially increases life skills and youth development in a non-formal learning environment.

What is the way forward?

America's food and fiber producers operate in a global, technologically advanced, rapidly diversifying, and highly competitive business environment (Pardey et al., 2013). Therefore, USDA is constantly helping agricultural producers and industry meet the needs of the nation and of the world. In addition, with the continuous changes of agricultural policies and farming methods, it is crucial that agricultural education evolves with them, pushing towards innovations rather than accepting conventions (ASPB, 2013; NRC, 2009b).

Agriculture is much more than simply growing plants or raising animals. It has increasingly become a science and technology based complex, interdependent, and multifactorial enterprise. Governance of agricultural enterprise has to keep up with constantly changing social and regulatory oversight. Our workforce needs to be trained in "systems approach" as opposed to a silo-based training in a single subject matter expertise. Therefore, entities such as academia, industry, policy makers, funding agencies, and societal leaders-all have a distinct role to play towards a cohesive agriculture production system. Academia needs to be able to offer state of the art education and training that is clearly aligned with and supportive of the needs of production agriculture. Youth must be engaged much earlier through formal and non-formal education in activities that spark their interest in their joining and supporting both biophysical and social sciences aspects of production agriculture.

The evidence indicates an acute shortage and immediate need for significantly more agricultural graduates than currently being produced in the U.S. There may not be an easy or a single solution to accomplishing this challenge. However, it must be addressed to ensure that our future workforce and next generation of scientists is indeed fully trained and equipped with the skill set needed to make innovations and discoveries in meeting future food demands and solving societal challenges dependent upon the agricultural enterprise (PCAST, 2012b).

Community and technical colleges will be key players in that they work closely with local government, industry partners, workforce intermediaries, as well as community members to identify existing and emerging sectors workforce needs and prepare students accordingly. With 2014 enrollment levels of 12.8 million, the nation's 1,167 community colleges enroll 45 percent of all undergraduate students in the United States, 51 percent of minoritiy undergraduate students, as well as 36% of first generation college students, and are key to ensuring that the nation has the workforce it needs (AACC, 2014). We must ensure that community colleges are an integral part of the agricultural workforce pipeline. Several U.S.

universities have developed 2 + 2 articulation programs with the community colleges so that a student interested in higher education can jump to a four-year college as a part of the agriculture education pipe line continuum.

Indeed, all such considerations need funding. USDA is committed to championing the worldwide funding of agricultural education, Extension and research programs to increase productivity, minimize international trade distortions, improve rural education and job creation in developing countries, reduce food waste and find ways to meet the food needs of the world's chronically undernourished and malnourished population (Hofstrand, 2011). A recent review of USDA/NIFA competitive programs by the National Research Council (NRC, 2015) recommends that funding for NIFA's competitively awarded programs should be increased significantly. Such a support will further strengthen a diverse education portfolio within USDA and help establish partnerships with other federal agencies with common interests in supporting this education enterprise. USDA is playing a leading role in support of President Barak Obama's charge that "We must educate our children to compete in an age when knowledge is capital, and the marketplace is global."

Conclusions

Today, the world is facing major challenges. According to the United Nations (UN, 2013), the world population is predicted to reach 9.6 billion by 2050, just 35 years from today. If this prediction is accurate, humankind's greatest challenge may be feeding this population. To feed a population of 9.6 billion, FAO projects that agricultural production (food, feed and fiber) will need to increase by 70 percent (FAO, 2009). The world's food system is inundated by major challenges and risks, such as food security, agricultural sustainability, and economic opportunity.

We believe the solution to these pressing challenges lies in education, inspiring our young people to enter agricultural fields and providing the rigorous education these disciplines demand. USDA's agricultural education pipeline takes an overarching approach through the involvement in 4-H programs that substantially increases life skills and youth development in a non-formal learning environment; provides immersive learning experiences in non-formal educational programs for secondary school educators, enabling them to identify and replicate best practices to enhance student outcomes in the food, agricultural, natural resources, and human sciences; engages undergraduates through experiential learning opportunities so that they are better prepared to join the workforce; and lastly, train the next generation of scientists through pre-and-post doctorate fellowship experiences.

The World Economic Forum concludes that the world needs a new vision of agriculture (WEF, 2013). Vision is basically rethinking what is possible. Therefore, the USDA is rethinking its agricultural education mission to present a framework for making transformative changes in higher education in agriculture. As we look toward the future-we have reached the point in history where we must answer two questions-"What's for Dinner?" and "Will there be food for tomorrow?" These basic questions clearly show the importance of agriculture. Finally, echoing Freudenberger's (1994) question, "Is there any subject more critical?"

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L62 Diversified poultry production in India: an overview

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Summary

The diversified poultry farming systems in India includes Japanese quail, turkey, guinea fowl, duck farming. These are now considered as the viable livelihood option for small and marginal farmers. Japanese quail meat and eggs have become popular. Ducks account for 7% of poultry population and are mostly found in coastal states of the country and in states with more lakes and rivers like West Bengal, Orissa, Andhra Pradesh, Tamil Nadu, Kerala, Assam, Jammu and Kashmir and Tripura. Duck farming is still in primitive stage and indigenous ducks out number exotic ducts in spite of their inferior performance. Turkey, Guinea fowl and goose farming are still in their infant stage due to lack of awareness about quality nutrients. Emu and ostrich farming have recently been initiated for the production of leather / skin (for the production of various instruments), feather/plumage (for ornamental purpose), oil (high medicinal value) and cornea / tendon (for surgical transplantation).

Global poultry industry

The poultry market outlook over the next decade remains mostly positive, according to FAO projections that show production of all meats expanding by 24 percent. By 2024, an additional 26 million metric tons of poultry will be produced worldwide, accounting for more than half of additional total meat production by 2024, total poultry meat production is forecast to reach about 133 million metric tons. Poultry production will account for more than half of the world's additional total meat output over the next decade. Global meat production has risen by almost 20 percent over the last 10 years, a large part of which can be attributed to poultry. In 2015, the world is projected to produce nearly 112 million metric tons of poultry meat, but this figure is projected by the FAO to surpass pig meat production over the outlook period reaching almost 134 million metric tons by 2024. Poultry meat will capture more than half of the worlds share of additional meat produced by 2024, compared to the base period production in 2012-14. Avian influenza in many major egg-producing countries over the past year lowered short-term output, but egg production is still on track to hit the 100-million- metric ton mark in the next two decades.

Indian poultry industry

India being in the tropical region of the world, the prevailing macro-climatic conditions is mostly congenial to poultry production. Among the many subsectors of agriculture, livestock sector is gaining momentum in India and within the livestock sector, poultry occupies a premium position. The contribution of livestock sector to India's GDP is $ 47.33 billion during 2010-11 with the value of output from poultry sector being $ 8.26 billion. The organized sector of poultry industry is contributing 70 % of its total output. India ranked the 3rd largest egg producer and the 4th largest meat producer in the world. Egg and poultry are the cheapest source of animal protein next only to milk. Poultry production can be taken up in limited land area and the Indian climate also suits it. National Sample Survey Organisation, in its 68th round report showed that, as per capita income increased the demand for food has shifted towards proteins, fruits and vegetables. In urban areas, the demand for milk, egg and chicken meat increased from 80, 34 and 9 % to 84.9, 37.6 and 27 % respectively during the last two decades while the demand for fish and prawn, and mutton decreased from 27.1 and 12.3 % to 21 and 10 % respectively. FAO also predicted that 42 % of meat that will be consumed worldwide by 2020 will be chicken meat, overtaking pork and beef. According to the Indian Council of Agricultural Research vision 2025, an increase in per capita availability of one egg will generate 50,000 more jobs. Further increasing population, growing demand for convenient foods, awareness about inclusion of animal protein, rising per capita income are some of the factors propelling the growth surge of the industry.

Japanese quail production

Quail, popularly known as 'Kadai' (in Tamil) and 'Bater' (in Hindi) is a table delicacy since olden times. The domesticated subspecies, *Coturnix coturnix japonica*, is called Japanese quail but is also known by other names: Common quail, Eastern quail, Asiatic quail, Stubble quail, Pharaoh's quail, Red-throat quail, Japanese gray quail, Japanese migratory quail, King quail, and Japanese King quail.

The acceptance of Japanese quail is increased considerably in India and other parts of the world. Quail meat is an excellent source of pyridoxine, niacin, thiamin, riboflavin, pantothenic acid, minerals and essential fatty acids. Japanese quail eggs are also an excellent source of vitamins and thus they are named as "vitamin bomb". The Japanese quail egg contains more amounts of B-complex vitamins and also Vitamin-A, Vitamin-D and Vitamin-E when compared to chicken egg. Likewise, the quail egg contains more Iron, phosphorus and higher Calcium, Sulphur and Zinc. Because of high content of vitamin, minerals and quality of protein together with the nature of its fatty acid profile, quail egg are recommended for medicinal treatments like anaemia, diabetics, ulcers, asthma and tuberculosis by restoring the optimal metabolism in the body cells. Due to higher acceptance for its meat and egg, Japanese quail are reared exclusively for meat or egg purpose as that of chicken broiler and layer production and also for both meat and egg production (dual purpose).

History and domestication of Japanese quail farming

Quail belong to the Family Phasianoidea of Order Galliformes of the Class Aves of the Animal Kingdom, as that of chicken, pheasants and partridges. Species or subspecies of the genus *Coturnix* are native to all continents except the Americas. One of them, *Coturnix coturnix* or common quail are migratory birds of Asia, Africa and Europe. Several interbreeding subspecies are recognized and the more important being the European quail (*Coturnix coturnix coturnix*) and the Asiatic or Japanese quail (*Coturnix coturnix japonica)*. The oldest indication of quails in human culture comes from a hieroglyph from the Old Kingdom of Egypt (about 3000 BC). The common quail have been used as food since biblical times. One subspecies that commonly migrates between Europe and Asia was eventually domesticated in China. These birds were raised as pets and singing birds. The domesticated coturnix were brought at about eleventh century to Japan from China across the Korean bridge. In any event, coturnix were first domesticated in the Orient.

The first written records of domesticated quail in Japan date from the twelfth century. These birds were initially developed for song. It is claimed that a Japanese Emperor obtained relief from tuberculosis after eating quail meat, and this led to selection of domestic quail for meat and egg production in Japan in the latter part of the nineteenth century. By 1910, the Japanese quail in Japan were widely cultured for their meat and eggs. Between 1910 and 1941, the population of Japanese quail increased rapidly in Japan especially in the Tokyo, Mishima, Nagoya, Gifu and Toyohashi areas. This period also represented a time of imperial expansion in Japanese history and domesticated Japanese quail were established in Korea, China, Taiwan and Hong Kong, and later on spread to Southeast Asia. During the 60´s to 80´s the quail production was regarded as subsistence activity in Brazil, in the small backyard rearing system.

Global status of Japanese quail farming

The major meat production countries are Spain, France, China and the United States of America. Leading the production of quail eggs are China, Japan, Brazil and France. In the Latin America, Brazil leads the production, followed by Venezuela, Peru, Colombia and Bolivia. Global trends in the rearing of quail follow opposed directions when compared Brazil and the rest of the world. Worldwide there is a decrease in the use of quails in research as animal models, followed by a significant decline in peer reviewed articles. The current situation in Brazil is a growing demand for processed eggs (pickles), however, there is a limited supply in day-old chicks what will restrict the market. On the other hand, there is a need to develop breeding programs to obtain better defined strains to produce eggs of good size and in the case of quails for meat production, the reduction of the effects of inbreeding. Studies of interaction between factors responsible for changes in production characteristics are critical for the quail egg chain. The general trend in egg production is the automation in big farms, integrated with the egg processing in-

dustries. Advances have also been observed in the production technology of pickled eggs, always seeking to improve the quality of the product to the consumer.

Breeds / varieties/ species of Japanese quail

There are different breeds / varieties/ species of quails are available in world; some of the popular breeds / species are listed below:

❖Wild type(Pharaoh quail)❖ British range ❖Manchurian golden ❖English white ❖Tuxedo

Bobwhite Quail (Colinus virginianus) / Northern Bobwhite / Virginia Quail	Blue Scale Quail (Callipepla squamata)
Mountain Quail (Oreortyx pictus) / Codorniz de mountana/ Painted quail/ Plumed quail / San Pedro quail	Mearns Quail (Cytonyx Montezuma)
Gambel´s Quail (Callipepla gambelii) / Arizona / top−knot/ desert quail	California quail (C. californica) / Catalina quail/ Valley Quail
Asian Blue Quail or Button quail (Coturnix chinensis)	Blue Quail (Coturnix adansonii)
Brown Quail (Coturnix ypsilophora)/ swamp quail / swamp quail	Harlequin Quail (Coturnix delegorguei) / Montezuma Quail / Mearns´ Quail / Fool´s Quail
Himalayan Quail (Ophrysia superciliosa)	Japanese Quail (Coturnix japonica)
Jungle bush−quail (Perdicula asiatica)	Manipur bush−quail (Perdicula manipurensis)
New Zealand Quail (Coturnix novaezelandiae) / koreke quail	Rain Quail (Coturnix coromandelica)
Rock Bush−quail (Perdicula argoondah)	Snow Mountain Quail (Anurophasis monorthonyx)
Stubble Quail (Coturnix pectoralis)	Marbled Wood Quail, Odontophorus gujanensis
Spot−winged Wood Quail, Odontophorus capueira	Black−eared Wood Quail, Odontophorus melanotis
Rufous−fronted Wood Quail, Odontophorus erythrops	Black−fronted Wood Quail, Odontophorus atrifrons
Chestnut Wood Quail, Odontophorus hyperythrus	Dark−backed Wood Quail, Odontophorus melanonotus
Rufous−breasted Wood Quail, Odontophorus speciosus	Tacarcuna Wood Quail, Odontophorus dialeucos
Venezuelan Wood Quail, Odontophorus columbianus	Black−breasted Wood Quail, Odontophorus leucolaemus
Stripe−faced Wood Quail, Odontophorus balliviani	Starred Wood Quail, Odontophorus stellatus

Status of Japanese Quail farming in India

Japanese Quail was the only avian species chosen for unique research in one of the NASA space programme. The domesticated variety of Japanese quail was originally brought from California, USA and introduced in India by Central Avian Research Institute (CARI), Izatnagar, Uttar pradesh in the year 1974. And subsequently 1978 from West Germany and 1988 from Korea. The CARI has come out with following four strains

CARI UTTAM, CARRI BROWN, CARI Sun-heri, CARI UJJAWAL (White breasted quail), CARI SWETA (White feathered quail) and CARI PEARL (White egg shell line), Japanese quails was introduced in Tamil Nadu in the year 1983. The Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Chennai has also released three strains of Japanese quails namely Nandanam Japanese quail – I, Nandanam Japanese quail – II and Nandanam Japanese quail – III and Veterinary College and Research Institute, TANUVAS, Namakkal, has released meat-type Japanese quail hybrid strain, Namakkal quail – I, Namakkal gold quail for the benefit of farming community. Japanese quail farming is making rapid strides in South India, especially in Tamil Nadu, where there is a huge market particularly in cities.

Nandanam Quail I (1987)

❖First strain of Japanese quail released in South India

❖Dual purpose variety with average 6th week body weight of 137.6 g with hen housed egg production of 62.50 % and feed conversion efficiency of 3.83 and livability of 88 %

Nandanam Quail II (1993)

❖Improved strain of meat type Japanese quail

❖Heavier body weight of 180 g at 6th week of age with feed efficiency of 2.76 and livability of 88.20%

❖Popularly referred as "desi quail"

❖Very popular among farmers, entrepreneurs and unemployed youth as a basic stock for promising enterprise

Nandanam Japanese quail – III production standards (2004)

❖Much improved high yielding meat type quail

Egg weight	8-13 g
Chick weight	7-12 g
Market age	4 to 5 weeks of age
Market weight	Male - 200 g; Female-250 g
Feed consumption	490 g
FCR	2.70
Age at first egg	End of 6th week
Dressing percentage	72
Sex ratio	1:3
Egg number (7 to 47 weeks)	250 eggs
Feed consumption layer/breeder /day	30-35 grams
Average fertility (24 weeks)	75-80%
Average hatchability(24 weeks)	65-70%
livability	95%

Nandanam broiler breeder quail (2013)

❖Meat type Japanese quail were subjected to three different methods of individual phenotypic selection viz., high two week body weight (SWL), four week weight (FWL) and high four week body weight coupled with low relative body weight gain between 4-6 weeks of age (LWL). Selection was carried out for three generations

❖As a Japanese quail broiler breeder strain capable of producing higher number of hatching eggs for production of fast growing commercial meat type Japanese quail chicks.

Production performance of Nandanam gold quail

Age at maturity (days)	39.8
50 % egg production (days)	49.5
Average body weight (g)	261.8
Maximum egg production (%)	99.2
Economic viability (weeks)	52
Average egg weight (g)	13.2
Feed consumption during laying (g)	33
Feed consumption per dozen eggs (g)	416
FCR for 1 kg egg mass	2.63
Livability (6- 52 weeks) (%)	95
Cost of production of an one egg (Rs.)	1.03

Guru Ananad Dev Veterinary and Animal Science University(GADVASU), Luthiana, India

GADVASU has released thre strains namely Punjab-I, Punjab-II and Punjab-III quails

Central Poultry Development Organization (Northern Region)

Quail with body weight of 5th week-120 g

Central Poultry Development Organization (Western Region)

Broiler type quail with body weight of 5th week-180 g

Breeding Strategies

Breeding programme for Japanese quail layer line for small scale production

❖ Breeding Pens : 60 males + 240 females (shift mating)

- ❖ Replacement population size : 2000 day-old chicks
- ❖ Population size at 7th week : 60 sire families (12Females X 60 families = 720 females)
- ❖ Egg Recording Period : 24 weeks (7-30 weeks)
- ❖ Selection criteria : Egg Production up to 30 weeks
- ❖ Aids to selection : Sire family selection for egg production in females and sib selection in males

Breeding programme for Japanese quail broile line – A for small scale production

Producing broile dam line 180 grams at 4th week with 2.7 FCR and 95 per cent livability.

- ❖ Breeding Pens : 60 males + 240 females (shift mating)
- ❖ Replacement population size : 2500 day-old chicks
- ❖ Selection criteria : 4th week body weight
- ❖ Aids to selection : Individual selection

Breeding programme for Japanese quail broile line – B for small scale production

A broiler sire line with 4th week body weight of 200 grams, FCR of 2.5 and livability of 92% by implementing breeding programme as to that of Broiler line-A. In deep litter pen mating is followed in our Institute.

Colour variants of Japanese quail

Varieties : ❖Golden ❖White ❖White Breasted

Newer tools to improve hatchability performance

Name of the Authors	Species	Newer techniques	Results
Tarasewicz *et al.*,(2006)	Pharaoh hatching quail eggs	Exposure of variable magnetic field	❖Highest percentage of dead embryos in control group (11.36%), while the smallest was from treated (4.17%). ❖Hatchability was higher in exposure group (91.6%) than control group (86.3%).
Pandian *et.al.*, (2014)	Japanese quail	PEMF at 1 Hz frequency with 1500 nT intensity for 18, 12 and 6 hours respectively at 18^{0}C and 80 per cent relative humidity	❖Total and fertile hatchability performance showed highly significant (P< 0.01) difference between treatment and control groups ❖Significant differences (P<0.05) observed for mean per cent embryonic mortality, mean per cent dead in germ and dead in shell between treatment and control group
Santos *et al.*, (2011)	Japanese quail egg and meat line	Holes on vitelline membrane on germinal disc	No. of holes (per mm^2) on the vitelline membrane on the germinal disc area was higher in meat quails (2.89 ± 0.21)
			No. of holes (per mm^2) on the vitelline membrane on the germinal disc area was lower in egg line (2.15 ± 0.13)
Huddson *et al.*, (2014),	Japanese quail	Inner Perivitelline Layer (IPVL)	Number of IPVL holes(56.83 ± 15.99) was more in Japanese quail eggs
	Turkey		Number of IPVL holes (24.5±2.81) was more in Turkey

Management of Japanese quail

Housing for Japanese quails

Depending on the scale of production, there are two basic housing systems. Floor systems are used by small to medium-scale enterprises and normally utilize existing poultry houses or rooms. Commercial quail enterprises, where birds are kept intensively, largely use cage systems alone or in combination with floor system**.** Normally the units are stacked up to five or six deep, with an overall height of 185 cm.

Cage systems.

Brooder cages are meant for rearing chicks from day 1 to 14. Maintained in four tiers of 180 X 120 X

25 cm size with four compartments of 90 X 60 cm each (each tier can hold 400 chicks). Grower cages measures about 240 X 120 X 25 cm with four compartments of dimension 120 X 60 cm (each compartment can hold 60 birds). A 4 Feet x 8 feet x 1 foot high cage can house 250-300 layers. The flooring and all sides are made of ½-inch mesh welded wire. The quail has a tendency to fly upwards if the top of the cage is made of mesh wire, and this may cause head injuries. Cages can also be made smaller (2 feet x 4 feet x 1 foot) and stacked in four decks, with 3 to 4 inches between the decks.

Deep litter system

The floor is made of concrete and covered with litter material, 3-5 cm in summer and 5-8 cm in winter. Depending upon the season and bodyweight, 4 to 5 Japanese quails can be reared in one square foot area of floor space. Between batches of birds the litter is completely removed and thoroughly cleaned before being used again. Feeder, drinker and floor space requirements for quails are given below:

Up to 2 weeks of age, drinkers of 10 cm diameter, 1.5 cm high in sides and 500 ml capacity and feeders of 22 cm diameter and 2 cm are used. From 3 weeks of age drinkers of 15 cm diameter, 2.5 cm high and 1200 ml capacity and linear feeder (2.5 cm high, 45 cm long and 10 cm wide) are used.

Feeds and feeding management

Three types of Japanese quail feeds are available and are chick feed (0-2 weeks), grower/finisher feed (3-4 weeks) and layer/breeder feed (above 6 weeks). Broiler quail consumes 550-600 gm up to marketing and adult layer/ breeder quail consumes 35- 40 gm per day. Quail feed can be compounded using commonly available feed ingredients or quail can be fed with any available chicken feed. Feed should be given *ad libitum* and the feeder should not be filled more than 2/3rd of its capacity to prevent wastage.

Nutrient requirements for Japanese quail

Nutrients	Japanese quail chick diet (0-2 weeks)	Japanese quail grower diets (3-5 weeks)	Japanese quail breeder diets (>5 weeks)
Crude Protein (%), min	23.0	20.0	20.0
M.E. (kcal/kg), min	2850	2900	2800
Crude Fibre (%) Max	7.0	7.0	7.0
Calcium (%) Min	0.80	0.90	3.00
Lysine (%) Min	1.50	1.30	0.95
Methionine (%), Min	0.50	0.45	0.40
Linoleic acid (%)	1.0	1.5	1.5

Nutrient composition of Earthworm meal, Japanese quail hatchery waste and Shatavari rootl

Nutrients	Earthworm meal (Deepika, 2015)	Japanese quail hatchery waste (Sathishkumar and Prabakaran, 2008)	Asparagus racemosus (shatavari) root powder (Jothie, 2014)
Dry matter (%)	5.39	3.70	94.55
Crude protein (%)	29.49	36.24	4.98
Crude fibre (%)	1.25	0.92	15.03
Ether extract (%)	3.75	29.5	0.23
Total ash (%)	45.66	25.16	2.75
Calcium (%)	2.89	10.73	0.72
Phosphorus (%)	1.37	0.69	0.49
ME (kcal/ kg)	1870	2795.24	3237

Production performance

Name of the Authors	Species	Supplementation	Results
Pratheeba (2013)	Japanese quail	Nano- selenium at graded level	vImproved production performance, fertility and hatchability
Amritha (2014)	Japanese quail	green microalgae at 0.5, 1,2, and 4 %	v0.5 % supplemented group showed improved production performance upto marketing age
Mishra (2014)	Japanese quail	Dried blood and rumen content (DBRC)	vDietary inclusion of DBRC at 30 per cent reduced the cost of production vIncreased PUFA content in the meat (16.51%-21.51%) vDecreasing trends in instrumental colour of meat showed acceptable range
Jothie (2014)	Japanese quail	Asparagus racemosus (shatavari) root powder	vSupplemention upto 1.5 per cent level in Japanese quails without affecting the growth performance and carcass characteristics vAdvanced age at sexual maturity by 3 days, increased hatchability on total eggs set, did not influence on embryonic mortality, increased fertility and increased the relative weight of ovary and oviduct vIncreased serum oestrogen levels (305.87 ± 22.10) in treatment group
Deepika, 2015	Japanese quail	Fish meal was replaced with 55, 75 and 100 % earthworm meal	vFish meal replaced with 75 % earthworm meal showed better body weight gain in Japanese quail
Mohapatra, 2016	Japanese quail	Fish meal was replaced with 55, 75 and 100 % maggot meal	vFish meal replaced with 100 % maggot meal showed better body weight gain

Japanese quail biological experiment was conducted by Ezhilvalavan *et al.,* (2014) to study the effect of Omega -3-fatty acids rich fish oil on the fatty acids composition of Japanese quail egg. Supplementation of Omega -3-fatty acids rich fish oil at (1, 2 %) had no adverse effect on body weight, feed consumption, feed efficiency and Organoleptic properties of Japanese quail meat were recorded on a nine point hedonic scale with ascending ratings for the desired attributes of appearance, flavour, juiciness, tenderness and overall acceptability. Supplementation of Omega -3-fatty acids rich fish oil in Japanese quail ration had significant ($P<0.01$) increase of Omega -3- fatty acids composition such as linolenic acid, Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), total n-3 fatty acids concentrations. This study concluded that supplementation of Omega-3-fatty acids rich fish oil can be used in the Japanese quail feed to produce health conscious designer food.

Mean (± S.E.) fatty acids composition (%) in breast and thigh muscle of Japanese quail at fifth week of age as influenced by Omega−3−fatty acids sources in feed

Treatment Groups	Linolenic acid		EPA		DHA		Total n-3	
	Breast muscle	Thigh muscle	Breast muscle	Thigh muscle	Breast muscle	Thigh muscle	Breast muscle	Thigh muscle
T_1 - Control	0.63^a ± 0.05	0.50^a ±0.80	0.62^a ±0.24	0.30^a ±0.21	0.64^a ± 0.35	0.50^a ±0.04	01.89^a ± 0.20	01.30^a ±0.53
T_2 - FO 1 %	2.50^b ± 0.69	2.10^b ±0.42	3.00^b ±0.20	3.40^b ±0.21	6.50^b ± 0.65	5.20^b ±0.20	12.00^b ± 0.45	10.70^b ±0.21
T_3 - FO 2 %	3.10^b ± 0.43	3.50^b ±0.20	5.10^c ±0.85	4.80^b ±0.85	7.30^c ± 0.21	8.30^c ±0.42	15.50^c ± 0.35	16.60^c ±0.80

Mean values not sharing a common superscript column wise differ significantly. ($P< 0.01$)

FO-Fish oil

Management of commercial quail chicks (0 - 5 weeks of age)

The hatch weight of quail chick is only 8.5 gm. Hence more attention is required in management of quail chicks. Also most of the mortalities of young chicks are due to drowning in the waterer. It can be practically avoided by placing pebbles inside the drinker during first 7 days.

Management of laying/ breeding quails (> 6 weeks of age)

Japanese Quail start laying at 6th week of age. The quail used for breeding purpose should be reared under restricted feeding and lighting during the growing period to avoid early sexual maturity. Also, it is advisable to rear males and females separately from 3rd week onwards and are to be fed with separate diets. During laying, the quail layers are provided with 16 hours total light and hence a minimum of 4 hours extra artificial light is required for maximum egg production

Integration in quail farming

Integration is the association, co-ordination and or amalgamation of various Japanese quail production activities at the same level or different stages of production. A person or firm who integrates or co-ordinates these activities is called as an "integrator". Japanese quail production is a complex phenomenon as that of broiler production, involving various activities from parent stock production to selling of commercial quail for market.

In vertical integration, there will be association between various stages of production; such as hatchery, farm, feed manufacturing plant, marketing unit etc. The integrator will own a hatchery, having a feed mill also and supplying chicks and feed to contract growers or poultry farmers. Later, they will take back the adult quail and marketing them. This sort of vertical integration will increase and ensure profits; because even if they lose at one stage, they will regain at the other stages. The vertical integration may either forward, backward or both. In forward integration a farmer will start his own processing or marketing outlets to get better prices for his birds. In backward integration, the farmer will start his own hatchery and feed mill, to reduce the cost of inputs.The quail integration involves both horizontal and vertical integration; to reduce the overall cost of production. Moreover, the contract growers or farmers will be getting regular income, as commission for growing the birds. The farmers are growing the birds on contract basis. In the later case, the integrator is the owner of the birds and he is paying commission to the farmer / grower, for the work done by him.

In Tamil Nadu, few of the Japanese quail breeders have opted for integration (contract system) of Japanese quail production to expand their level of operation. The farmers have adopted all-in-all-out system. Average batch size is about 20,000.They market their quails at about 5 weeks of age. The mean rearing cost paid to the farmers upto INR 2.00 per quail that includes the cost for brooding, litter and labour. The integrators will be supplying day-old chicks, feed, medicines and also carrying out free technical service to the contract grower and taking back the birds and marketing them. The contract grower will be owning the poultry house and equipment. The recurring expenditure is very little. He will use his family labour, purchase litter and bears electricity and watering cost. Therefore, the capital investment needed is less for him and making sure profits as growing commission. The commission is based on all the economic traits like body weight, feed conversion ratio, livability and growing period, mainly based on livability and F.C.R. The top growers are also getting some bonus or premium. Therefore, integration is a way for sustainability and assured profits in Indian Japanese quail farming.

Turkey production

Turkeys are native to North America. They belong to the order Galliformes, along with chickens. There are two species of wild turkey: the North American Wild Turkey (*Meleagris gallopavo*) and the Central American Ocellated Turkey (*M. ocellata*). The modern domesticated turkey was developed from the North American Wild Turkey by the indigenous people of Mexico (Aztecs). Turkeys were taken to Europe by the Spanish, who found them as a favourite domesticated animal among the Aztecs. The Aztecs used turkeys as a source of protein (meat and eggs) and the feathers for decoration. The "breeds" of turkeys often referred to are actually varieties that originated from the North American wild turkey. The seven standard varieties recognized by the American Poultry association are

- ❖Bronze White
- ❖White Holland
- ❖Bourbon Red
- ❖Narragansett
- ❖Black
- ❖Beltsville Small White

The two varieties that are not recognized are Broad-Breasted Bronze, Broad-Breasted White. (also called Large White).The most commonly raised commercial variety is the Large White. The Broad-Breasted Bronze, similar in size and conformation, is less popular because of a preference for white feathering.

The major primary breeders of turkeys in the world are:

❖ British United Turkeys ❖Hybrid Turkeys ❖ Nicholas Turkey

They offer 10 parental strains Big 6, Big-9,BUT-8, BUT-9,BUT-10, Hybrid converter, Hybrid Grade maker, Hybrid XL, Nicholas 900 and Nicholas 300.

Breeding programme for Nandanam Turkey-1

❖To obtain 16th week body weight of 2.80 kg in Nandanam Turkey-I with FCR of 3.45 and livability of 85 % at 16 weeks of age

❖Present status : Random mating population

❖Breeding Pens : 20 breeding pens (sex ratio-1 : 4)(20 males and 80 females)

❖Aids to selection : Individual selection based on 16th week body weight

❖Replacement population size :600 day-old poults in 8 hatches

❖Selection pressure : 1 in 4 for females; 1 in 15 for males

Global turkey meat production (million tonnes)

Region	2000	2006	2007	2008	2009	2010	2011	2012
Africa	0.06	0.08	0.10	0.09	0.11	0.12	0.10	0.12
Americas	2.82	3.18	3.41	3.63	3.34	3.36	3.37	3.45
Asia	0.16	0.13	0.13	0.12	0.11	0.12	0.11	0.11
Europe	2.01	1.73	1.72	1.76	1.81	1.84	1.88	1.92
Oceania	0.03	0.02	0.04	0.03	0.02	0.02	0.02	0.02
WORLD	5.08	5.14	5.39	5.63	5.39	5.46	5.48	5.63

Source FAO

Top five leading turkey−producing countries (´000 tonnes)

Country	2007	2008	2009	2010	2011	2012	2013	2014
US	2,664	2,796	2,535	2,527	2,592	2,671	2,599	2,575
EU	1,790	1,835	1,795	1,946	1,950	2,010	1,985	1,975
Brazil	458	465	466	485	489	510	520	535
Canada	170	180	167	159	160	161	165	165
Russia	30	39	31	70	90	100	100	105
WORLD*	5,143	5,337	5,018	5,212	5,308	5,480	5,393	5,379

* Total for selected countries-USDA

Global turkey meat per capita consumption (kg)

	PCC	PCC	PCC	PCC
Turkey Meat	2000	2005	2010	2011
Israel	22.83	17.16	11.22	11.25
Hungary	9.71	9.84	8.59	8.38
USA	7.82	7.28	6.99	6.73
Ireland	1.02	6.76	5.55	5.61
France	7.73	6.15	5.33	5.23
Portugal	4.81	4.19	4.57	4.53
Tunisia	3.10	3.20	4.55	3.90
Canada	4.84	4.36	3.83	3.85
Germany	3.98	3.81	3.62	3.14
Austria	2.98	3.63	3.11	2.99

PCC - Per capita Consumption (kg)

Debeaking:

Debeaking controls feather picking and cannibalism. All confined birds and most range turkeys are debeaked. Debeak poults at 6-10 days of age.

Desnooding:

Desnooding removes the snood, a fleshy appendage located just behind the top of the base of the upper beak. Removing snoods of male poults reduces fighting, prevents head injuries and prevent or reduce the incidence of erysipelas. Male poults are usually desnooded at the hatchery on request of the grower.

Toe Clipping:

Removal of the toenails of turkeys is usually done at the hatchery, but toes of turkeys as old as 5 weeks can be clipped .Toe clipping can improve the grade of processed turkeys. Turkeys in large groups, especially when excited, often step on flock mates and cause scratches or skin tears on the backs and sides. Increasing flock sizes and densities aggravate the problem, especially when turkeys are reared in confinement.

Nutrient Standards for Turkey (ICAR,2013)

	0-6 weeks	6-12 weeks	12-18 weeks	18 weeks - prelaying	Breeder
CP (%)	24.00	22.00	18.00	15.00	15.00
ME(kcal/kg)	2800	2800	2650	2600	2650
Lysine (%)	1.55	1.20	1.05	0.72	0.60
Methionine (%)	0.55	0.45	0.35	0.25	0.20
Calcium (%)	1.20	1.00	0.80	0.60	2.25
Phosphorus (%)	0.55	0.50	0.38	0.30	0.35

Production performance of Nandanam Turkey – I

Hatch Weight (g)	44.39±0.16
8th Week body wt (kg)	1177.74±7.21
12th Week body wt klg)	1791.40±35.34
16th Week body wt (kg)	
Male	3465.20±26.44
Female	2584.44±30.49
24th Week body wt (kg)	
Male	6746.25±65.705
Female	3454.50±36.135
Livability(%) (upto 16 weeks)	89.16
HDEP(28-51 weeks) (%)	36.58
HHEP(28-51 weeks) (%)	35.19
Age at Sexual maturity (days)	177
Egg weight (28 weeks)	72.59±0.78
Total hatchability (%)	53.33
Fertile hatchability (%)	69.60

Production performance of Nandanam Turkey-II

❖Beltsville Small White birds evolved as Nandanam Turkey-II by the individual selection for 20 generations

❖Enhanced body weight gain, better egg production and improved hatching performance

❖Suitable for commercial farming

❖Annual egg production (28-44 weeks): 70

❖24th week body weight: Male- 3.7 kg; Female: 2.9 kg

❖Hatchability: 70 %

Methods to control mortality due to starve out condition

Young turkey poults are not capable of taking feed properly. Therefore, in poults, starve out deaths are more common. In order to avoid this, feed can be kept in coloured plastic plates. One or two trainer chicks (chicken) can be allowed along with the turkey poults, so that the poult starts taking the feed along with the chicks. Colored marbles or pebbles are placed in feeders and waterers to attract poults towards them. Since turkeys are fond of greens, some chopped green leaves can also be added to the feed

to improve the feed intake. The brooder diet has to contain amino acids like lysine and methionine in required quantity. To prevent the occurrence of paralysis in turkeys, calcium and phosphorus have to be added in the diet during the first eight weeks of age.

Marketing of turkeys

The marketing of turkeys has been confined largely to the festive season during the months of November, December and January. There has been a definite trend during the past few years to extend the marketing of turkeys over a longer period. Turkeys are ready for market when they are 16 to 24 weeks of age, since it is not economical to feed turkeys beyond this period. There is an increase in the quantity of feed required to produce a kilogram of turkey after about 20 weeks of age. The absolute gains in weight during the later stages of growth are large, but in terms of cost these gains are also the most expensive. The average body weight is 4-6 Kgs and the birds are generally lifted by the traders from the farm. There appears to be no retail market for the birds on day to day basis except during festive season like Christmas, Deepavali etc.The Indian turkey market remains primarily a live bird market. Although there is a growing trend in consumption of chilled and frozen turkey products in restaurants, most consumers prefer fresh turkey meat. Birds for home consumption are typically purchased live and slaughtered in poultry retail shops. Demand for frozen turkey products will continue to remain constrained by inadequate cold chain facilities. Luxury hotels account for most of the consumption of processed turkey products such as sausages, salami, etc.

Indian duck production system

Duck production system in India is characterized by nomadic, extensive and seasonal and it is still held in the hands of small and marginal agricultural labourers as well as weaker section of the society. Among other species of chicken production, duck production involves low input and low maintenance cost. The world duck population was 947.7 million in 2000 and increased to 1187.7 million in 2010. China stands first place in the world duck population of 789.6 million and contribute 66.5 per cent and India stands sixth place (26 million) and contribute 2.2 per cent. On the world stage, duck meat represents about 4 per cent. Asia is the main world region for producing duck meat, having a market share of 84.8 per cent. In India, migratory duck farming is mostly practiced. Duck flock size is generally estimated in dozens. The common unit size of 25-30 dozens can be maintained by a well trained team of two or three family members. Ducks are more suitable for integration with rice and fish farming. In duck- rice integration system, ducks are allowed in the paddy field. Duck's excreta become fertilizer for the paddy field. Moreover, stirring reaction caused by duck inhibits the growth of weeds. In duck-fish integration, duck's excreta fertilize the pond. In India the duck population is concentrated in Eastern, North eastern and Southern states. West Bengal has the highest duck population followed by Assam, Kerala, Tripura and Jharkhand. Duck eggs are the second largest source of table eggs in India. The intensive system of chicken production accounted for 94 per cent of total eggs produced in India, whereas duck and other eggs accounted for only 6 per cent in the year 2010-11. During 2007 and 2010-11 the total number of duck population was 12887 (000 no.) and 14572 (000 no.) and egg production was 1. 5 billion and 1.6 billion respectively. An indigenous duck produces 130-140 eggs per year. However, the annual production under the nomadic system of rearing averages 180-200 eggs.

World duck egg production scenario

China, USA, India, Mexico, Japan, Russia, Brazil, Indonesia, Ukraine and France have provided 65 % of the world's egg in 2011 and the first five countries were responsible for around 55 % of all production. Asia now accounts for around 59 % of the eggs produced worldwide and the Americas contribute more than 20 %, with 4 % coming from Africa and less than 0.5 % from Oceania. But Europe's share has fallen by almost half since 1990, down to 16.5 %. Asia includes China, India and Japan having a combined share of more than 46 %, although nearly 38 % of that contribution originates from China alone. The world egg production reached 69 million metric tons in 2011. The global demand for egg could reach to 71 million metric tons by 2015. The largest increases in egg production between 2011 and 2015 are likely to occur in Brazil, Turkey, India and China.

The top five duck population countries are China (66.5 %), Vietnam (5.8 %), Malaysia (4.1 %), Indo-

nesia (3.8 %) and Thailand (2.5 %), which account for 82.7 % of the duck population in the world. The world's top ten countries of duck chicken population is shown in Table No.1. The global duck production will approach 4.4 million tonnes in 2013 and 4.6 million tonnes in 2015. India occupied the third position in the world egg production scenario.

World duck and chicken population (Million) −2010

Duck			
S. No	Country	Million stock	% to world
1	China	789.6	66.5
2	Viet Nam	68.6	5.8
3	Malaysia	48.2	4.1
4	Indonesia	45.3	3.8
5	Thailand	29.2	2.5
6	India	26.0	2.2
7	Bangladesh	25.0	2.1
8	France	22.5	1.9
9	Myanmar	12.6	1.1
10	Philippines	10.3	0.9
World : 1187.7 million			

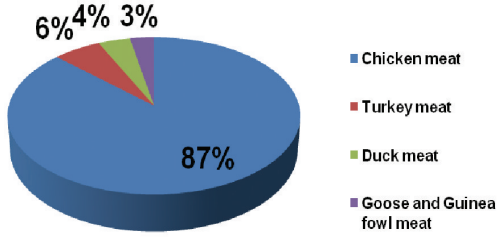

Fig. World poultry meat production by species

The current egg consumption in Asia is 175 eggs per person per year. The intensive system of chicken production accounted for 94 per cent of total eggs produced in India, whereas ducks and other eggs accounted for only 6 per cent in the year 2010-11. In India, during 2012-13, the total number of duck population, duck egg production and average yield per annum were 1,65,66,000 and 19,269 (laks no.) and 116.32 respectively. An indigenous duck produces 130-140 eggs per year. However, the annual production under the nomadic system of rearing averages 180-200 eggs. Ducks are the second largest source of table eggs in India. In India the duck population is concentrated in Eastern, North eastern and Southern states. West Bengal has the highest duck population followed by Kerala, Assam, Jharkhand, Tripura, Jammu and Kashmir and Bihar.

Global duck meat production (1000 tonnes)

Country	Year						
	2001	2002	2003	2004	2005	2010	2011
China	1965.9	2087.7	2230.5	2262.3	2350.1	2736.30	281.80
France	231.1	253.9	240.2	238.1	208.0	276.00	290.90
Malaysia	68.8	52.7	81.6	102.0	105.0	116.30	112.60
Vietnam	77.4	81.6	82.8	88.2	88.2	74.80	105.00
USA	56.3	52.9	50.8	58.0	85.1	52.60	54.43
Thailand	105.0	93.0	72.0	84.8	85.0	80.00	81.75
Hungary	45.5	66.8	64.7	65.0	68.0	52.43	59.25
India	57.2	59.8	62.4	65.0	65.0	37.40	39.00
Republic of Korea	45.0	56.0	46.0	46.0	48.0	65.0	69.00
World	3025.1	3181.9	3322.9	3386.8	3495.7	4031.5	4187.17

The world's production of poultry meat is approaching100 million metric tons for the first time in history. The number of ducks produced for meat globally was about 800 million in 1990, rising to 2 billion in 2000 and nearly 2.7 billion in 2010. On the world stage chicken production represents about 87 % compared with 6 per cent for turkey meat, 4 per cent for duck meat and less than 3 per cent for the combined category of geese with guinea fowl. USA, Germany, Brazil, France and Italy produced 78 per cent of the turkey meat. Likewise China, France, Malaysia, Myanmar and Vietnam produced 82.5 per cent of the duck meat. China, Egypt, Hungary, Poland and Italy produced 98 per cent of the geese and guinea

fowl meat. In the world poultry meat production scenario India occupies the fifth position. The world average consumption of poultry meat was 12.5 kg per person in 2011. According to the FAO, the world meat production will grow at an average rate of 1.8 per cent per year until 2020 and the world's chicken meat production will be 122.5 million metric tons by the year 2020.

Common breeds of ducks

Common ducks are believed to have originated from the wild Mallard (*Anas platyrhynchos*). Some of the better known breeds of common ducks include the Pekin, Aylesbury, Rouen, Call, Indian Runner, Khaki Campbell, Cayuga, Albio, Maya, and Tsaiya. Common ducks can interbreed, and produce fertile offspring. Eggs from common ducks require about 28 days hatching. Muscovy eggs require about 35 days to hatch. While Muscovies can be crossed with common ducks, their offspring are sterile.

Indigenous or non-descript types

In India, 90-95 percent of ducks are indigenous or non-descript types, which are hardy, with mediocre egg production and highly suitable for extensive system of rearing. The important Indian breeds are Sythet mete and Nageswari of Eastern region, Aarani ducks of Tamil Nadu, Chara and Chemballi of Kerala, desi breeds of West Bengal and other states. Apart from these, distinct local varieties have also been identified. Pati, Deo, Cinahanh and Raj Hanh are local indigenous breeds found in Assam.

Duck egg production in India (1999–2013)

Year	No. of layers(000 Nos)	Duck egg production (lakh Nos)	Average yield per annum (Nos)
1999-2000	11142	14194	117.00
2000-01	11480	14051	112.00
2001-02	11569	13759	108.00
2002-03	12329	14729	109.15
2003-04	12673	14900	106.77
2004-05	12897	14110	99.58
2005-06	13080	14512	101.93
2007-08	11911	14648	138.42
2008-09	12907	15760	122.00
2009-10	13427	15387	114.00
2010-11	14572	16044	105.00
2011-12	15180	16722	122.00
2012-13	16566	19269	116.32

Source: State / UT Animal Husbandry Departments, India

Per capita consumption of duck meat

Duck Meat	Per capita Consumption kg	Per capita Consumption kg	Per capita Consumption kg	Per capita Consumption kg
Countries	2000	2005	2010	2011
France	3.69	3.63	4.37	4.49
Malaysia	2.80	3.98	3.83	3.88
Hungary	1.83	3.67	3.06	3.69
Bulgaria	1.00	2.20	2.74	2.91
China, Taiwan	2.88	2.94	2.84	2.81
Myanmar	0.65	1.31	2.05	2.18
China, Mainland	1.42	1.59	1.98	2.03
South Korea	1.05	1.11	1.35	1.43
VietNam	0.88	0.87	0.85	1.18
Thailand	1.48	1.27	1.08	1.13

Duck rearing systems

Foraging / duck backyard / duck-rice integrated system / duck fish integrated system/ nomadic system

of rearing is followed in India.

Nutrient requirements of ducks (ICAR, 2013)

Nutrient	Starter	Grower	Rearer	Layer
	0-8	9-16	17-20	>20
ME (kcal/kg)	2800	22650	2500	2650
CP (%)	20.5	16.5	15.0	16.50
Lysine (%)	1.0	0.75	0.60	0.75
Methionine (%)	0.45	0.35	0.30	0.30
Calcium (%)	1	1	1	3.00
Available Phosphorus (%)	0.54	0.35	0.35	0.35

Duck egg marketing

A part of the requirements of Kerala state is met by the duck eggs produced from Tamilnadu, even though Kerala is a duck producing state. Considering the appearance of atomistically competitive seller and buyer groups, product differentiation and the degree of market intelligence, duck egg market in Tamil Nadu is considered as "pure competitive market". A total of five channels were identified through which the duck eggs were moved from producer (farmer) to the ultimate consumer as follows:

Channel Ⅰ: Producer-Trader-Wholesaler (consumption centre)-Retailer -Consumer
Channel Ⅱ: Producer-Trader-Retailer (consumption centre)-Consumer
Channel Ⅲ: Producer-Wholesaler-Secondary wholesaler (consumption centre) -Retailer-Consumer
Channel Ⅴ: Producer-Wholesaler-Retailer (consumption centre)-Consumer
Channel Ⅵ: Producer-Consumer

Of the five channels, the quantum of eggs transacted through first channel was high while the volume transacted through fifth channel was meager.

Duck meat marketing

Only live drakes and spent ducks are sold for meat purpose, unlike broiler chicken which was usually sold as live bird, dressed and/ or frozen meat. Though duck products are available in large quantity, their consumption was mainly in the adjacent state, Kerala which could be attributed to enormous availability of chicken products in Tamil Nadu. Three channels were identified in marketing of duck meat. They are as follows;

Channel I: Producer-Trader or Wholesaler-Retailer (Consumption centre)-Consumer Channel II : Producer-Retailer-Consumer

Channel III: Producer-Consumer

Of the above three channels, Channel I handled large quantum of duck meat while Channel III transacted a negligible or meager quantity.

Guinea fowls production

Global guinea fowls production

Guinea fowls originated from Central Africa. The family Numida has many subspecies with mutated colours of pearl,, white, lavender, blue and royal purple, porclain, slate buff dundotte chocolate and coral blue. Initially from Africa guinea fowls were exported to western countries for genetic improvement. In Africa it is known as poor man's pheasant. Today there is increasing demand for guinea fowl because of its ornamental nature due to attractive plumage and value as a table bird with gamy - flavored meat. They are currently being reared in many parts of the world. In countries such as France, Belgium, Canada and Australia, the bird is now produced commercially, while in most African countries which include Nigeria, Malawi and Zimbabwe, guinea fowl production is in its infancy. Helmeted guinea fowls (*Numida meleagris*) originated in Africa and are indigenous to West Africa. Guinea fowls were first domesticated by ancient Egyptians. These birds were imported to Indian sub-continent from Africa about 600 years back and are being maintained in small holder backyard system of production. In India, free-range flocks can be seen in Punjab, Uttar Pradesh, Assam, Orrisa and Madhya Pradesh.

Production of Goose and guinea fowl meat (tones)

Region	2000	2005	2009
Africa	55,566	55,340	55,152
Americas	1,797	1,901	1,956
Asia	17,66,560	19,48,533	23,42,631
Europe	86,989	98,998	75,744
Oceania	100	120	120
WORLD	19,11,012	21,04,892	24,75,604

Guinea fowl Production in India

India is inhabitated by over 60 million tribal people, constitutes about 7.5 % of total population. Tribal people prefer hardy birds, and the guinea fowl is the choice. Guinea fowl farming is profitable and sustainable enterprise in densely populated country like India where there is routine shortage of conventional poultry feed resources. Diversification would maximize the returns and also minimize the risks. In India guinea fowl is popular among marginal farmers and other vulnerable groups as small scale poultry enterprise. Guinea fowl population rank after chickens, quail, turkey and ducks. It is being raised in semi arid pockets of many northern states and in the south mainly in states of Andhra Pradesh and Tamil Nadu. Pearl guinea fowl is the common variety found in villages and referred as the local breed.

In India guinea fowl farming is popular as back yard poultry because it is an additional income generating activity other than main occupation, providing eggs and meat for family consumption. It is also regarded as an alternate way to alleviate poverty for socially and economically disadvantaged rural household. It is kept along with desi chicken as a companion under mixed farming systems. Country chickens are used to hatch guinea fowl egg and brood the day old keets by being foster mother. Guinea fowl has a unique ability to scavange in free range and is resistant to most of common diseases of chicken. Rearing chicken and guinea fowl together helps for taming guinea fowl. Strategic supplementary feeding, sexing, use of light control programmers for breeders and selection can lead to an increase in guinea fowl productivity in smallholder-farming sector.

Different varieties of guinea fowl in the world are Pearl,White, Lavender, Buff, Buff dundotte, Coral blue, Azure,Royal purple, Porcelain and Slate. In India three namely pearl, white and lavender are available.

Improved varieties in India

In India predominantly pearl variety of guinea fowl is seen. GUNCARI is an improved Guinea fowl variety developed at Central Avian Research Institute, Izat Nagar. Three GUNCARI varieties are Kadambari, Chitambari, and Swetambari. Which have pearl, lavender and white plumages respectively. TANUVAS has released Nandanam Guinea Fowl-I and it is popular among Indian farmers.

Breeding programme to improve Nandanam Guinea Fowl – I

Objective: To achieve a body weight of 800g at 12 weeks of age.

- Breeding Pens : 20 breeding pens (sex ratio-1 : 3) (20 males and 60 females)
- Aids to selection : Individual selection based on 12th week body weight alone
- Replacement population size :600 day-old keets
- Selection pressure : 1 in 5 for females; 1 in 15 for males

Systems of rearing

Extensive or Free range system

The stocking density in free range system is 500 to 600 birds per acre. In most of the countries, guinea fowls are reared along with desi chicken under mixed farming system in free ranges. Country chicken is used for hatching guinea fowl eggs and is a good foster mother in brooding young keets. Bamboo basket is used for protecting keets from predators.

Semi Intensive system

This system is a combination of extensive and intensive system of rearing and is suited for small domestic flocks.

Intensive system

Guinea fowl can be reared like commercial broilers under intensive system.

Deep litter system,

Cage system.

Deep litter system: Minimum space requirement for meat purpose guinea fowl is 1.5 sq feet per bird in deep litter system,0.75 sq feet per bird in cage system and for layers 2.5 sq feet per bird in deep litter system, 1 sq feet per bird in cage system of rearing has to be provided inside the pen. Guineas begin to fly at very early age and can be confined only in covered pens.

Cage systems

Cage rearing can be chosen to rear day old keet till they attain 8 weeks of age. This system is adopted when the availability of the land is limited.

Feeding management

Guinea fowl has a unique ability to utilize a wide range of flora and fauna. It consumes non-conventional feed that is not used in feeding chicken. Guinea fowls can digest lignin components of feed better than chicken. To reduce the concentrate requirement birds, can be fed with grasses such as subabul, stylo haemata and desmanthus. Guinea fowl relish on moderately tough leaves. Leaves of mango, guava, and jack fruit are not relished by the bird as they contain tannin.

Marketing of guinea fowl

Currently acceptance of guinea fowl and its products are increasing among consumers. There is quite good future potential for egg and meat of guinea fowl. Marketing should be diversified. Marketing economic avenues like commercial guinea fowl farming for egg production, meat production, guinea fowl feed production, keet production, hatching egg production and guinea fowl fast food preparation can be established. Either same farm producer can operate all diversified approaches of guinea fowl farming or encourage the potential entrepreneur to start the concerned avenues.

andanam Guinea Fowl−I production performance

Parameters	
Egg weight (g)	38-40
Weight of day old keet (g)	25-29
Age at first egg (weeks)	24-26
Egg production (eggs)	120-160
Male : female ratio	1 : 4
Fertility (%)	75-80
Hatchability (%)	70-80
Livability (%)	90
Weight at 4 weeks of age (g)	160-180
Weight at 8 weeks of age (g)	500-550
Weight at 12 weeks of age (g)	950-1000
Weight at 16 weeks of age (g)	1100-1200
Age at slaughter (weeks)	16
Marketable age(weeks)	16
Weight of adult male (above 24 weeks of age) (kg)	1.5
Weight of adult female (above 24 weeks of age) (kg)	1.4
Incubation period (days)	28

Nutrient requirements of guinea fowl

Nutrient	Pre brooder	Brooder	Grower	Layer/breeder above 25 weeks
	0-4	5-8	9-24	
ME (kcal/kg)	2850	2800	2750	2650
CP (%)	24.5	20.5	17.0	18.0
Lysine (%)	1.45	1.15	0.86	0.99
Methionine (%)	0.42	0.38	0.35	0.37
Calcium (%)	1.18	1.08	1.18	3.51
Available Phosphorus (%)	0.52	0.45	0.51	0.65

Only such chain of enterprise can encourage the organized guinea fowl marketing trend. Marketing capability is an individual talent which has to be exploited for getting good profit. In Tamil Nadu, right now guinea fowl farming is slowly getting established with very good demand potential. Major limitation of guinea fowl farming in Tamil Nadu is the lack of availability of elite germplasm, lack of consumer awareness, lack of technology dissemination on rearing guinea fowl and support from government organizations.

L63 Nutritional perspective of the chicken embryo during incubation

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Keywords: chicken embryo, egg nutrients, yolk sac membrane, intestine, incubation

Summary

The nutrition of the developing chick embryo represents a relatively unstudied area in animal research. A better understanding of how the chick embryo utilizes the egg nutrient resources may help achieve an optimal development of the embryo as well as the broiler. The appropriate delivery of YS nutrients to the embryo has a direct effect on embryonic development, hatchability, hatchling quality and subsequently on broiler chicken performance

During the 21 days of embryonic development, the chick embryo utilizes essential nutrients, from the egg yolk and albumin, for tissue growth, extra embryonic tissue development and for its energetic needs.

Crucial morphological and metabolic processes take place in the yolk sac membrane which affects egg nutrients utilization. From E10 to almost end of incubation the yolk sac membrane has the major role in carbohydrates metabolism, in digestion of nutrients and their transport to the developing embryo and in the synthesis of plasma proteins. Towards end of incubation the intestine starts to digest and absorb yolk content and amniotic fluids. Egg nutrients utilization by the chick embryo is affected by environmental factors in the hatchery, nutritional factors and genetic factors.

The importance of embryo nutrition during the incubation phase

The avian embryo is dependent upon the nutrient deposits in the fertile egg (Romanoff, 1960). These nutrient resources and their utilization by the chick embryo have a direct effect on embryonic development, hatchability, hatchlings quality and subsequently on chicken performance. Thus, the nutrition of the developing chick embryo affects not only hatchling weight and vitality but also the broiler's weight at market age. The importance of an adequate nutrition of the embryo during the 21 days of incubation increased in the last decades as broiler chickens reach market age earlier every year due to the intense artificial selection for body weight performed in the breeding companies for the past 6 decades. Consequently, the 21-day incubation period of the embryonic chick makes up approximately one-third of the broiler growing period. Therefore, data on embryo nutrient utilization, the developmental profiles and capacities of the physiological, cellular and molecular mechanisms that digest and absorb nutrients during incubation need to be investigate. In light of the increased metabolic rate recorded in today's commercial embryos, research related to the nutrition of the broiler embryo can clarify questions which have implications to both poultry broiler breeder nutrition and to hatchery management.

Some of the questions are: Do broiler breeder eggs have sufficient nutrients for fulfilling the genetic potential of the broiler embryo development?; What are the nutritional needs and limitations of the embryo today? What are the means by which the embryo consume the nutrients packed in the egg? What are the environmental factors in the incubator (oxygen, temp, humidity) that affect nutrients utilization? ; How to achieve an optimal utilization of nutrients by the chick embryo, thereby reducing embryonic mortality and improving hatchlings vitality and broiler weight. More questions are: What are the mechanisms by which nutrients are digested, absorbed and delivered from the egg compartment to the embryo? Do broiler breeder eggs have sufficient nutrients for normal embryo development, especially in light of the increased metabolic rate in today's commercial embryos? Can a nutritional deficiency be compensated by changing the broiler breeder diet?

Answering these questions will help us better understand the process of nutrients absorption, as well as clarify what are the nutritional needs of the developing embryo throughout incubation and at hatch for better hatchability and performance.

Nutrient resources available to the chicken embryo during incubation

The fertile laid egg provides a closed environment with the nutrient requirements needed for the devel-

oping embryo. Unlike mammals, where a continuous transport of nutrients takes place from the mother to the embryo through the placenta, the chick embryo is separated from the hen, and derives all of its nutrients during incubation from the contents of the egg that were deposited by the hen- the albumen and the yolk (Moran, 2007; Sheng and Foley, 2012). The ratio between these two egg compartments, their integrative relationship during incubation, and their nutrient composition, have a major role in the optimal development of the embryo. The albumen compartment represents about 65 to 75% of the egg's total content, and consists of approximately 88% water and 12% protein. The yolk compartment consists of approximately 50% water, 15% protein, less than 1% carbohydrates, and 33% fat, which is only found in the yolk (Romannof, 1960; Shenstone, 1968). The composition of the fertile egg is not fixed and is greatly dependent upon egg weight, genetic strain, and hen age (O'Sullivan et al., 1991; Vieira and Moran, 1998; Peebles et al 2000; Suk and Park 2001; Yadgary et al 2010).

Fat uptake by the embryo during incubation

The utilization of fat from the yolk sac (YS) by the chick embryo provides the main energy source for tissue development during the last week of incubation (Noble and Cocchi, 1990). In agreement with previous studies (Noble and Ogunyemi,1989; Noble and Cocchi, 1990), we found that only a small amount (less than 10%) of the yolk fat had been absorbed by the chick embryo by E13, whereas from E15 to E21 more than 50% of YS fat was utilized for the growing nutritional needs of embryo (Yadgary et al 2010; 2013). We also observed that between E15 and E19, long chain poly-unsaturated fatty acids (22:6, 20:4) were more rapidly utilized from the YS as compared to all other examined fatty acids (Yadgary et al 2014). To elucidate these results, we examined possible processes and mechanisms by which YS lipids are transferred to the embryo: the absorption and transport of lipoproteins and free fatty acids by apical membrane receptors and the lysosomal digestion of lipids and their emulsification by bile acids. Analysis by high throughput gene expression analyses revealed that a vast variety of lipoprotein receptors mediate lipoprotein entrance to yolk sac membrane (YSM) epithelial cells (Yadgary et al 2014). Among these receptors is VLDLR, which has a binding domain that recognizes VLDL and vitellogenin. VLDLR has been previously suggested to have a major role in YSM lipoprotein uptake (Hermann et al., 2000), however we showed that VLDLR had relatively low levels of expression in the YSM. On the other hand LRP2, a receptor for a range of lipoproteins (Kozyraki and Gofflot, 2007), had the highest expression levels among all lipoprotein receptors with an increase from E13 to E17 and a decrease from E17 to E21. Expression levels of several other lipoprotein receptors in YSM epithelia (LRP1, 5, 6, 12) imply that LRP2 does not have an exclusive role in YSM lipoproteins uptake.

To elucidate the differential utilization of yolk fatty acids during incubation, we examined the expression of genes responsible for absorption and transport of free fatty acids and found that different membrane fatty acids transporters were expressed in YSM epithelia, as well as cytosolic fatty acids binding proteins that also play a role in uptake of long chain poly-unsaturated fatty acids. Among them was FABP5 which exhibited a substantial up-regulation between E15 and E21 (Yadgary et al 2014). Inside YSM epithelial cells, lipoproteins are transferred to lysosomes for digestion (Murray et al., 1999; Powel et al., 2004; Bauer et al., 2013). The utilization of YS fat is affected, among other, by the digestive capacity of YSM lysosomes, nevertheless YSM lysosomal pathways have not been fully described yet. In our study we found that several lysosomal digestion related genes were among the 50 most highly expressed genes in YSM epithelial cells. Prosaposin, which facilitates the catabolism of glycosphingolipids in lysosomes, was the highest expressed gene from E15 to E21. In addition, many lysosomal proteases such as cathepsin A and B that can digest lipoproteins, were highly expressed in the YSM (Yadgary et al 2014).

These results provide support to the hypothesis that yolk lipoproteins are hydrolyzed in the lysosomes of YSM epithelial cells into free fatty acids, partial glycerides and glycerol. We attained further support for this hypothesis from the activity of the digestive enzyme lipase in the YSM (Yadgary et al 2013): Total YSM lipase activity relative to fat content (U per g YSM fat) increased from E15 to E21. Lipase activity in the YSM is probably associated with the lysosomal digestion of lipoproteins. Furthermore, we found that this digestion is apparently aided by bile acids that could serve as an emulsifier of lipids in the lysosomes of the YSM (Yadgary et al 2013 ;2014).

To our knowledge, our studies are the first to verify the hypothesis that bile acids are found in the YSM of the chick embryo. This may be the reason for the typical green color of the bile which is observed in the yolks of 18-19 E embryos. It had been previously suggested that the origin of YS bile

may be from the transfer of intestinal bile into the yolk sac through the yolk stalk (Surai and Speake, 1998 Speake and Teale, 2006), however our gene expression analyses point out for the first time that the epithelial cells of the YSM synthesize bile as well as enzymes involved in the conjugation of bile acids.

Lipids are transported from the epithelial cells of the YSM to the embryo as newly formed lipoproteins (Kanai et al., 1996; Murray et al., 1999; Powel et al., 2004). An integral part of these new lipoproteins are apolipoproteins that serve as structural proteins and cell surface receptors. In the current work, apolipoproteins genes (apoA1, A2 A4, B, C3,) were among the most highly abundant in the YSM. Our results reflect the intensive re-synthesis of lipoproteins in the YSM and indicate that both HDL and LDL are produced in the YSM and are then secreted to the embryonic circulation as previously suggested by Kanai et al. (1996).

Protein uptake by the embryo during incubation

The fertile egg yolk, before setting in the incubator, contains approximately 15% protein (Romannof, 1967; Shenstone, 1968). The uptake of protein from the yolk sac is difficult to evaluate because in agreement with previous studies. In agreement with Sugimoto et al 1989;1999) we observed that protein and water pass from the egg albumen compartment and from the amniotic sac to the yolk sac during incubation (Yadgary et al 2010). Nevertheless, we attempted to estimate the YSM's absorptive capacity of yolk protein by examining mechanisms of yolk protein break-down and peptide or amino acid uptake by the YSM.

Lipoproteins are made up of approximately 10% protein and therefore their uptake provides one transport mechanism for proteins through the YSM. However, other than endocytosis of lipoproteins, no study has elucidated the molecular and cellular mechanisms in the YSM that are involved in nutrient uptake by the epithelial cells. We therefore examined expression patterns of nutrient transporters and digestive enzymes such as the digestive enzyme APN (aminopeptidase N), the di- and tripeptide transporter PepT1, and the cationic amino acid transporter CAT1 (Yadgary et al 2011). The expression patterns of APN and PepT1 genes, which were up-regulated between E13 and E17 and down-regulated between E17 and E21, may be indicative of a change in the YSM's capacity to digest and transport yolk peptides during the second half of incubation. Expression pattern of the CAT1 transporter differed from that of APN and PepT1. This difference may be associated with the transporters' locations in the absorptive cells: CAT1 at the basolateral membrane, and APN and PepT1 at the brush-border membrane.

Carbohydrate metabolism in the embryo during incubation

Towards the end of incubation, the high demand for energy to support the dramatic physiological changes of the hatching process drives the embryo towards catabolism of glucose. Previous studies determined that during chick embryonic development, the liver performs most of the essential processes involved in carbohydrates metabolism and in the supply of glucose to muscle tissues (Christensen VL 2001; De Oliveira et al 2008). However based on our publications (Yadgary et al 2010; 2014), which examined the role of the YSM in the supply of carbohydrates to the chick embryo during incubation, it can be concluded that YSM has the major role in producing glycogen stores for the embryo during incubation period.

Our studies showed that the levels of glucose in the yolk increased between E11 and E19, and then decreased until E21. Only trace amounts of glycogen were found in the yolk of the fresh laid fertile egg (E0), whereas on E11 glycogen amount in the yolk was 25 mg, and then increased by ten-fold between E13 and E19. Between E19 to E21, glycogen levels decreased by 100 mg. Liver carbohydrates amount had a similar pattern compared to the YS, yet the liver had significantly lower levels of glycogen (20-50 folds lower) because of its substantially smaller size as compared to the YS (Yadgary et al 2012).

The increased levels of glucose and glycogen in the YS, which far exceeded the amount of carbohydrates in the yolk on E0, indicated that glucose is synthesized in the YS during embryonic development. To elucidate whether glucose is synthesized in the YS, gene expression of enzymes involved in gluconeogenesis and glycogenesis were characterized in the YSM (Yadgary and Uni 2013). Expression levels of genes coding for enzymes (FBP1, PEPCK, G6PC2, PEPCK-C) that are exclusive to the gluconeogenesis process indicated that glucose is synthesized in the YSM from amino acids and glycerol. In addition, the YSM tissue was clearly seen to express the key enzyme involved in glycogen synthesis—GYS2.

Thus, it can be postulated that during the last week of chick embryonic development, the YS serves as the main organ synthesizing glucose and storing it in the form of glycogen, with a quantity 20 times

greater than in the liver on E19. Between E19 and E21, YS glycogen is intensively broken down to glucose 6-phosphate which is probably further converted by G6PC2 to free glucose. The free glucose might be subsequently released into the blood to be used in the days prior to hatch and during the hatching process as an additional source of energy (to β-oxidation of fatty acids), and as the major source of energy in the form of carbohydrates. Our hypothesis is that the major part of glycogen-derived glucose during incubation is released from the YS into the blood for delivery to the embryonic tissues.

Digestion and absorption organs and cells in the chicken embryo

The transport of nutrients into and out of the digestive and absorptive cells is necessary for nutrient assimilation and growth of the embryo. Several proteases act to break down proteins and peptides into free amino acids and small peptides, which are then transported into cells by amino acid and peptide transporters. Similarly, carbohydrates are broken down by carbohydrates to yield monosaccharides, which are then transported into cells by monosaccharide transporters.

Current research have shown that during incubation the YSM and the embryonic intestine serve these functions (Speier et al 2012 ;Yadgary et al 2014): The digestive enzymes (APN, SI), amino acid and peptide transporters (B0AT, EAAT3, CAT1, PepT1), and monosaccharide transporters (SGLT1, GLUT5) showed a diverse array of developmental gene expression profiles in the YSM and in the neonatal small intestine. All genes had a tissue by embryonic day interaction. Although the YSM and embryonic small intestine are a continuous entity throughout development, their roles in nutritional assimilation differ significantly as they have different expression pattern along incubation

Towards the end of incubation both amnion and albumen are totally consumed by the embryo, but not all of the YS. From E19, the residual yolk begins to be internalized into the embryo's body cavity, and at hatch, it constitutes about 15 to 20% of the chick's body weight, providing the hatchling with immediate nourishment until exogenous feed is given in the brooder house. After hatch the yolk sac supplies nutrients not only to the blood circulation through the YSM, but also directly from the YSC to the small intestine through the yolk stalk (Esteban et al., 199l; Noy et al., 1996).

The yolk sac membrane (ysm)

Initially, the yolk of the fertile egg is surrounded by the vitelline membrane, which had been laid down during follicular maturation (Speake et al., 1998 a,b). In the initial phase of incubation, the vitelline membrane gradually disintegrates and the YSM, which develops rapidly from the hind gut of the embryo, replaces it. The advancing edge of the YSM contains a nonvascular region of simple columnar endodermal cells which increases in area and spreads over the surface of the yolk, concomitant with the formation of an outer supportive ectodermal layer (Mobbs and McMillan, 1981; Moran, 2007). The increase in endodermal surface area leads to the formation of villus-like folds into which mesodermal cells migrate, between the ectodermal and endodermal cells, and differentiate into blood vessels, blood cells and connective tissue (Romannof 1960; Sheng and Foley, 2012).

By E10 the entire yolk is surrounded and vascularized, and the endodermal epithelial cells (EEC) of the YSM have developed into columnar epithelial absorptive cells in close contact with the yolk (Noble and Cocchi, 1990). These endodermal epithelium cells function as the absorptive and digestive cells of the YSM and are the mediators for the transport of nutrients from the yolk content to the developing embryo (Nakazawa et al 2011). Nutrients are absorbed into the EEC from the yolk content , undergo different metabolic, digestive and re-assembly processes, and are then secreted to the blood circulation of the embryo (Mobbs and McMillan, 1981; Kanai et al., 1996).

In the second half of incubation, after all extraembryonic tissues had been fully developed and the YSM had totally surrounded the yolk, the chick embryo begins its rapid growth. The transition from early embryogenesis to the second phase of incubation is characterized by prompt utilization of nutrients by the embryo. Yadgary et al 2013 found that total area of the YSM and its absorptive area increased up to E17 and decreased until hatch. These changes may relate to the increasing demand for nutrients from the yolk but may also be related to the assimilation of the YS into the embryo's body cavity toward hatch.

Bauer et al (2012) who investigated the mechanism for acquisition of chick yolk sac function by receptor expression, lipoprotein secretion, and lipid droplet metabolism in endodermal epithelial cells (EECs) cells concluded that vascularization and gain in function of the developing yolk sac are coordinated processes and that the changes in gene expression during differentiation of EECs are critical for

yolk sac maturation. His findings suggest a differentiation process that orchestrates the vascularization of the developing YS with the induction of yolk uptake and lipoprotein secretion by EECs to ensure embryo nutrition.

The intestine

Form E 15-E16, the chick embryos start transition to intestinal absorption of nutrients. The uptake of nutrients is mediated by a variety of membrane- bound transporter proteins. Speier et al 2012 determined the expression profiles of nutrient transporters and digestive enzymes during incubation in the yolk sac membrane (YSM) and embryonic intestine of egg- laying (Leghorn) and meat- producing (Cobb) chickens. The examined transporters included the peptide transporter PepT1, the glutamate/aspartate (EAAT3), cationic (CAT- 1) and neutral (B0AT) amino acid transporters, and the fructose (GLUT5) and glucose (SGLT1) transporters. Digestive enzymes included aminopeptidase N (APN) and sucrase- isomaltase (SI). Expression of these genes were assessed by real- time PCR using the absolute quantification method embryonic day (E) 11, 13, 15, 17, 19, 20, and 21 and intestine at E15, 17, 19, 20, and 21 . The findings were that The PepT1 and APN gene expression in the YSM increased until E15 and then decreased until E21, whereas expression in the intestine increased from E15 to E21. The B0AT showed a similar pattern in both YSM and intestine with greatest expression in the YSM occurring at E17/E19.

The CAT1 and GLUT5 genes showed decreased expression in the YSM and increased expression in the intestine until E17/E19 and then a decrease until E21. Expression of SGLT1 and EAAT3 showed increased gene expression over time in both the intestine and YSM. Expression of SI showed little to no gene expression in the YSM, whereas the intestine exhibited consistently high levels of gene expression. In YSM and intestine, SI expression was greater in Leghorn than Cobb, whereas CAT1 and GLUT5 expression was greater in Cobb than Leghorn. Expression of the APN, CAT1, and SI genes was greater in embryos from young flocks than old flocks in YSM and intestine.

These results demonstrate that the YSM expresses many of the digestive enzymes and nutrient transporters typically associated with the intestine and that these genes show tissue- and development-specific patterns of expression.

The analysis of expression levels of these genes in neonatal intestine compared with YSM elucidate their role in embryonic chick nutrient assimilation and growth and show potential differences in growth rate and nutrient utilization of embryos from different genetic lines and laying flock ages.

Factors affecting yolk utilization

The transport of nutrients from the YS to the chick embryo is controlled by the embryonic demands for nutrients. These demands are dictated by the genetic potential of the embryo to build tissues. Yolk sac nutrient utilization by the chick embryo is also affected by environmental factors and nutritional factors.

Inadequate environmental conditions in the incubator (for example: above optimal temperature or CO_2 level) have deleterious effects on physiological processes and biochemical reactions in embryonic and extraembryonic tissues of the chick, thus reducing YS utilization and embryo development (Burnham et al., 2001, Piestun et al., 2009; maatjens et al 2014; Reijrink et al., 2010); affects glucose metabolism (Molenaar, et al 2010), decrease growth performance and increase incidence of ascites in broilers (Molenaar, et al 2011)

A variety of nutritional factors contribute to the dietary composition of the egg and thus influence utilization of nutrients from the egg. These include egg size, breeder age, maternal nutrition, and in-ovo nutrient supplementation. Eggs from young and old breeder hens differ in their composition- older hens lay larger eggs with a larger yolk to albumen ratio, compared to young breeder hens. Hence, eggs from breeder hens of different ages provide an excellent model to study the effect of egg composition on YS utilization. Several studies have investigated YS fat utilization by embryos from young and old breeder hens (Noble et al., 1986; O'Sullivan et al., 1991; Burnham et al., 2001; Yalcin et al., 2008), and some of which have associated higher late mortality of young hens' embryos to a reduced transfer of yolk lipids.

Embryos and chicks of different strains have a different genetic potential and body composition, and therefore may have different nutritional demands to build body tissues. However, the effect of the nutritional demands of the embryo on yolk utilization has hardly been studied, due to the difficulty to sepa-

rate this factor from other factors affecting YS utilization such as egg weight, egg content, incubation conditions, genetic strain, breeder hen diet and age.

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L64 Gut microbiome: a new target for managing human health

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Humans are superorganisms with two genomes that dictate phenotype, the genetically inherited human genome (25,000 genes) and the environmentally acquired human microbiome (over 1 million genes). The two genomes must work in harmonious integration as a hologenome to maintain health. Nutrition plays a crucial role in directly modulating our microbiomes and health phenotypes. Poorly balanced diets can turn the gut microbiome from a partner for health to a "pathogen" in chronic diseases, e. g. accumulating evidence supports the new hypothesis that obesity and related metabolic diseases develop because of low-grade, systemic and chronic inflammation induced by diet-disrupted gut microbiota. Due to the tight integration of gut microbiota into human global metabolism, molecular profiling of urine metabolites can provide a new window for reflecting physiological functions of gut microbiomes. Changes of gut microbiota and urine metabolites can thus be employed as new systems approaches for quantitative assessment and monitoring of health at the whole-body level with the advantage of measuring human health based on the results of interactions between the two genomes and the environment rather than just host genomic information. Large-scale population-based studies in conjunction with these whole-body level systems methods will generate pre-disease biomarkers with predictive power, thus making preventive health management of populations with rapidly changing disease spectrums possible through re-engineering of the imbalanced gut microbiomes with specially designed foods/diets.

L65 Interactive network between nutrition, immunity, the gut microbiota and poultry health

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Keywords: nutrition, microbiota, immunity, gut health, dysbiosis, diet

Summary

Understanding how the diet and nutritional status influence poultry health has been a long standing area of scientific research. The established perception that host defense and nutritional status impact each other must now be expanded to include a third component of this network: the gut microbiota. This biological *ménage a trois* is a multi-faceted relationship that can no longer be considered exclusively dependent on the host, but is also dependent on a second genomic component within the host, the microbiome. There is solid evidence that the microbiome programs host immunity and drives a metabolome that impacts micronutrients and energy balance. In turn, the host immunity shapes the microbiome and host nutritional status influences elements of host defenses and make-up of commensal microbial community.

Introduction

Understanding how the diet and nutritional status influence poultry health has been a long standing area of scientific research. The established perception that host defense and nutritional status impact each other must now be expanded to include a third component of this network: the gut microbiota. This biological *ménage a trois* is a multi-faceted relationship that can no longer be considered exclusively dependent on the host, but is also dependent on a second genomic component within the host, the microbiome. There is solid evidence, mostly from mammalian studies, that the microbiome programs host immunity and drives a metabolome that impacts micronutrients and energy balance. In turn, the host immunity shapes the microbiome and host nutritional status influences elements of host defenses and make-up of commensal microbial community.

Nutrition (diet)-Immunity Interaction

The relationship between nutrition and immunity has been a topic of study in poultry for decades (see reviews[1-3]). Nutrition affects a large number of biological processes vital to the immune response, including gene expression, protein synthesis, metabolism, signal transduction, and cellular proliferation. Cellular metabolism is a fundamental activity that incorporates numerous pathways used to for energy generation and the production of the precursors required for macromolecule production. Thus, immune cell survival and function is inherently associated with nutrition and cellular metabolism of nutrients. Consequently, the dramatic increases in the understanding of the organization of the avian immune system and the factors that regulate immune function have supported the close concordance between host nutritional status and immunity.

Almost all nutrients in the diet play some fundamental role in sustaining an optimal immune response, such that deficient and excessive intakes can have negative consequences on immune status and susceptibility to a variety of pathogens[4]. Innovations over the last 5-10 years have demonstrated that not only nutrition, but diet regulates immune functions and play a critical role in the health of a host[5-7]. Hence, optimized nutrition may be determined by the feed ingredients capable of improving the capacity to resist disease and enhance health. However, based on the current era of poultry production, there is a question of whether the level of nutrients in commercial diets that maximize production is sufficient to maintain competence of immune status and disease resistance. For example, turkeys and broilers have high nutrient density diets devised to sustain rapid growth[8, 9], but possess a reduced systemic pro-inflammatory cytokine response compared to slower growing egg-producing layer-type chickens[10]. Dietary bioactive food components that interact with the immune response have been shown to reduce susceptibili-

ty to infectious diseases. Major classes of macronutrients provide numerous examples, including amino acids such as arginine or lysine[11, 12], lipids such as the omega-3 polyunsaturated fatty acids[13, 14], or novel carbohydrates such as various sources of β-glucans[15-17]. Vitamins such as D and E are commonly used as antioxidants, while zinc and selenium are minerals with a wide spectrum of impacts on the immune system. Lastly, there is accumulating evidence for modulation of the avian immune response in the prevention of infectious diseases by probiotics that will not be discussed in these proceedings (reviewed in 18).

Gut microbiome interaction

Within the host interactive network, the physiological complexity of nutrition and diet is not solely confronted by the host. The complexity of the nutritional interaction within an animal is made substantially greater by the fact that animals play host not only to invading pathogens, but also to entire communities of commensal and symbiotic microbes that receive their nutrition from the host and in turn contribute essential nutrients and play a role in immune defense[19]. This second "genome" component of vertebrates, the gut microbiota, has been shown to have profound and unanticipated effects on immune defense and inflammatory responses. Increasing evidence shows that the nutritional value of food is influenced by the structure and operations of gut microbial community, and that food, in turn, shapes the microbiota and its vast collection of microbial genes (gut microbiome). Therefore, to define the nutritional value of foods and nutritional effects on host immunity, we need to know more about gut microbial communities as well as the avian mucosal immune system, how components of the microbiota affect mucosal immunity, and about how the metabolism of foods consumed by the gut microbial community affects the avian mucosal immunity. Dietary factors are not only essential for animal health, but also for the maintenance of the microbial population. Factors from dietary, microbial or host origin within the gut lumen can greatly influence bacterial metabolism and competition. Bacterial metabolism of dietary substances aids intestinal health by producing fuel for colonic epithelial cells, for example butyrate, and making dietary nutrients available for uptake into the body. The genetic composition of the host and dietary feeding pattern both influence the microbial composition in the gut. This dynamic and complex interplay within the diet-host-microbiota triangle can be disturbed by changes in any of these factors. The complex gut microbiome is not a silent organ or simply a collection of passenger microorganisms; rather, intestinal microbial communities represent active participants in vertebrate immunity and physiology. Under normal conditions, the gut microbiota is not pathogenic, but actually confers health benefits to the host. The microbiota aids in the digestion and absorption of nutrients, and stimulates fat storage. Furthermore, the microbiota contributes to the construction of the intestinal epithelial barrier and also competes with pathogenic microbes to prevent their harmful propagation[20]. Unlike the host genome, which is rarely manipulated by xenobiotic intervention, the microbiome is readily changeable by diet, ingestion of antibiotics, infection by pathogens and other life events. The plasticity of the microbiome has been implicated in numerous disease conditions, and an unfavorable alteration of the commensal structure of gut microbiota is referred to as 'dysbiosis'; this includes a reduction in the number of tolerogenic bacteria and an over-growth of potentially pathogenic bacteria (pathobionts. that can penetrate the intestinal epithelium and induce disease in certain genetic or environmental contexts[21]. As previously mentioned, decades of work has provided an understanding of the impact of nutrition on host defense, but research in defining the interactive network of diet and its effects on the gut microbiome, intestinal immunity, and gut health in poultry is in its infancy. In the last 5-10 years an increasing volume of work has begun to delineate the physiological effects of vertebrate endogenous flora, suggesting that the microbiome contributes to the already complex interaction between nutrition and infectious disease, especially in mammals. The established concept that nutritional status, host defense and infection all impact on each other now has to be expanded into a multiple interaction, with the microbiota interacting with all other elements. There is good evidence that the microbiome programs host defense and drives a metabolome that impacts on energy balance, and some micronutrients. In turn, host defense shapes the microbiome, and nutritional status, particularly micronutrient status, helps determine several elements of host defense. Local immune homeostasis in the intestine is critical for both host health and commensal survival and at the same time is required to provide effective defense against harmful pathogens. The intestinal microbiome provides its host with crucial physiological functions that host organisms have not devel-

oped themselves[22]. Microbial metabolism increases energy yield and storage from the diet, regulates fat storage and generates essential vitamins. This is primarily due to the fermentation of indigestible dietary polysaccharides[22]. Overall, the intestinal microbiota favor the renewal and barrier function of the gastrointestinal epithelium[22.] and have significant effects on host energy, gene expression, cell differentiation and xenobiotic metabolism.

Besides making dietary nutrients available to the host, the commensal microbial community prohibits colonization of intruding pathogens; a process called *colonization resistance*[23]. The molecular basis of this phenomenon is still poorly understood, but probably involves competition with adhesion receptors, stabilization of the gut mucosal barrier, competition for nutrients, and the production of anti-microbial substances[23]. Disruption of colonization resistance by gut inflammation can be triggered by enteropathogenic pathogens like *Campylobacter* and *Salmonella*. A reduction in colonization resistance by either pathogens or an antibiotic intervention increases the risk of gut infections by affecting gut immunity, and thereby causes shifts in the intestinal ecosystem[24].

The avian microbiome

The chicken gastrointestinal (GI. tract is home to a complex microbial community that emphasizes the links between diet and health. The GI tract is rich in microbial biodiversity, playing home to ⩾500 phylotypes or ~1 million bacterial genes, which equates to 40-50 times the number in the chicken genome. In fact, most bacteria (> 90-95%. in the chicken cecum have never been cultured in the laboratory and are accessible only through molecular-biological approaches. These bacteria play important roles in the assimilation of nutrients from food, particularly through the release of energy from dietary fiber. Thus, the gut microbiome acts as an additional complex organ, similar to say the liver. The chicken gut microbiome also acts as source of human infections (e.g. *Salmonella, Campylobacter.* and as a reservoir of antibiotic-resistance determinants. A sub-optimal gut microbiome can impede agricultural productivity, as evidenced by the ability of antibiotics to promote growth in chicks. In addition, the microbiome is essential for development of the gut epithelium and mucosal immunity. Together, the collection of metabolic activities in a non-host compartment makes the flora of the gut a separate metabolic organ or 'metabolome'. For example, one such metabolic effect that the gut flora has on intestinal tissue in the host is the production of the short chain fatty acids (SCFA. liberated by polysaccharide breakdown. These SCFAs have profound effects on intestinal integrity as they represent an important metabolic fuel for the intestinal epithelium[25]. The maintenance of a healthy status is complex and relies on a delicate balance between the immune system and the normal endogenous microbiota. The normal microbiota confers many benefits to the intestinal physiology of the host. However, when this balance is upset (dysbiosis), pathogens that arrive or that have already been present but in numbers too small to cause disease take the opportunity to multiply. The intestinal microflora is a positive health asset to poultry health that influences the normal structural and functional development of the mucosal immune system. Mucosal immune responses to resident intestinal microflora require precise control and an immunosensory capacity for distinguishing commensal from pathogenic bacteria.

Manipulation of the flora to enhance the beneficial components represents a promising therapeutic strategy for the future. The flora has a collective metabolic activity equal to a virtual organ within an organ, and the mechanisms underlying the conditioning influence of the bacteria on mucosal homeostasis and immune responses are only beginning to be resolved in poultry. An improved understanding of this hidden organ will reveal secrets that are relevant to human health and to several infectious, inflammatory and neoplastic disease processes.

Antibiotics have a great effect on the host normal microbiota than on the pathogens, also upsetting the balance and inducing a dysbiotic state. The use of sub-therapeutic doses of antibiotics in a broiler diet has been a common practice for promoting growth or preventing diseases with dietary antibiotics as a factor influencing the digestive microbiota[26-28]. Antibiotic treatment impacts bacterial colonization of the vertebrate gut by a site and antibiotic dependent mechanism[29]. Recovery of total bacterial numbers occurs within 1 week after antibiotic withdrawal, but alterations in specific bacterial groups persist for several weeks. Additionally, susceptibility to intestinal colonization by intestinal pathogens (*Salmonella* and *Campylobacter.* and bacterial-induced mucosal inflammation persists when animals are infected several weeks after withdrawal of antibiotics, correlating with subtle alterations in the intestinal mi-

crobiome involving alterations of specific bacterial groups. The results of these experiments confirm that the microbiota is integral to mucosal host protection and that antibiotics can have prolonged adverse effects on intestinal colonization resistance.

Microbiome-Immunity Interaction

The establishment of a normal microbiota in young poultry constitutes a key component to maintain good health, through colonization resistance mechanisms, and has implications for the development of the gut and for full maturation of the mucosal immune system[19, 30-32]. The contribution of the normal endogenous microbiota to the overall poultry health and immune status has underestimated. Both nutrition and orally ingested drugs pass the gastrointestinal mucosa and may affect the balance between the mucosal immune system and microbial community herein; thereby, affecting composition of the microbial community and the status of local immune system that controls microbial composition and maintains mucosal integrity. The communication between microbiota and immune system is principally mediated by interaction of bacterial components with pattern recognition receptors expressed by intestinal epithelium and various local antigen-presenting cells, resulting in activation or modulation of both innate and adaptive immune responses. Interaction between microbial community and host is known to play a crucial role in the mucosal homeostasis and health status of the host. In addition to providing a home to numerous microbial inhabitants, the intestinal tract is also an active immunological organ, with more resident immune cells than anywhere else in the body. They are organized in lymphoid structures called Peyer's patches and isolated lymphoid follicles such as the cecal tonsils. Macrophages, dendritic cells (DC), various subsets of T cells, B cells and the secretory IgA they produce all contribute to the generation of a proper immune response to invading pathogens, while keeping the resident microbial community in check without generating an overt inflammatory response to it[33, 34]. IgA-producing plasma cells, intraepithelial lymphocytes (IELs. and γδT cell receptor (TCR)-expressing T cells (γδT cells. are lymphocytes that are uniquely present in the mucosa. In addition, of the γδT cells in the intestinal lamina propria, there are significant numbers of IL-17-producing T (Th17. cells[35]. and regulatory T (Treg)[36-38]. cells. The accumulation and function of these mucosal leukocytes are regulated by the presence of intestinal microbiota. By regulating these immune cells, intestinal microbiota enhances the mucosal barrier function and allows the host to mount robust immune responses against invading pathogens, and simultaneously maintains immune homeostasis. Indeed, when properly guided by the microbiota, the mucosal immune system maintains a state of non-responsiveness to dietary antigens and harmless commensal microbes[30]. In addition to the immune cells, the intestinal epithelial cells also contribute to the mucosal immunity[39]. Only one single layer of epithelial cells separates the densely colonized and environmentally exposed intestinal lumen from the largely sterile subepithelial tissue. The epithelium has evolved to maintain homeostasis in the presence of the enteric microbiota. It also contributes to rapid and efficient antimicrobial host defense in the event of infection with pathogenic microbes. Both epithelial antimicrobial host defense and homeostasis rely on signaling pathways induced by innate immune receptors demonstrating the active role of epithelial cells in the host – microbial interplay. Enterocytes have been shown to express pattern recognition receptors (PRR), both extracellular Toll-like receptors (TLR. and intracellular NOD-like receptors (NLR. that sense conserved bacterial products. While in a normal state, TLRs and NLRs remain relatively unresponsive to the myriad bacteria overlying the mucosa. In a state of infection, injury, or another assault they initiate a cascade of events that contributes to the induction of inflammatory host response[40]. This interaction of epithelial cells with professional immune cells illustrates the integrated function within the mucosal tissue. Lastly, there is also a layer of mucus overlying the intestinal epithelium that forms a physical barrier between the mucosa and the resident microbiota[41], minimizing both microbial translocation and excessive immune activation by the resident microbes. In addition, specific bacterial species have been linked to vital roles in mucosal immunity. Polysaccharide antigen of *Bacteroides fragilis*, a prominent member of the gut microbiota, promotes the expansion of splenic T-helper cells and regulates the Th1/Th2 cytokine production, as well as restoring the splenic architecture to that of conventionally raised mice[42]. *Bacteroides thetaiotaomicron*, another ubiquitous microbiota species, affects host gene expression resulting in a number of effects in a number of organ systems[42]. The presence of higher numbers of bacteria belonging to the phylum Bacteroidetes has been shown to be associated with the development of IL-17 producing T-helper cells[43]. Different *Lactobacillus* species, also

important members of the gut microbiota, differentially activate dendritic cells, inducing them to produce different arrays of inflammatory cytokines, thus playing an important role in the modulation of the Th1, Th2 and Th3 balance. Moreover, *Lactobacillus*-stimulated dendritic cells proceed to activate natural killer (NK. cells, thus potentiating gastrointestinal immunity. Segmented filamentous bacteria (SFB. are implicated in the induction of the intestinal IgA[32]. and activation of intraepithelial lymphocytes and induction of MHC class II expression on intestinal epithelial cells[44].

Microbiome-Nutrition Interaction

Bacterial colonization differs among individual animals, but certain bacterial strains are regularly recovered from different individuals. Although the main bacterial composition is maintained, it is likely that the colonic microbiota transiently respond to dietary intake and host physiology[19, 30, 46, 47]. Digested particles, like cellulose, resistant starch, and endogenous products, such as mucins, are essential energy sources that support the intestinal microbiota and may allow biofilms to develop at the intestinal epithelial interface[39]. In addition to mucosal defenses mediated either directly by GI tract-associated cells or by antimicrobial cellular products, there is a layer of mucus overlying the intestinal epithelium that forms a physical barrier between the mucosa and the resident microbiota[41], minimizing both microbial translocation and excessive immune activation by the resident microbes. The gut microbiota degrades dietary components releasing metabolites that can be either beneficial or detrimental to health. Bacterial cross-feeding is a key component of this interaction, defining the true end-products of bacterial metabolism. The gut microbiota has a considerable adaptive capacity, both in terms of composition and function, to respond to both dietary changes and to the presence of other specific bacteria, and to revert to baseline upon the removal of any transient pressure. Thus, the gut microbiome enlarges the host genome and enhances host metabolic potential. Dietary modulation can significantly alter the microbiota community and metabolic activity, and consequently impacts on nutrient bioavailability and host metabolism. The future challenge to establish coherent links between the bioconversion of non-digestible food ingredients, their bioavailability and their downstream effects on the host metabolism may be achieved by metabolomics. Metabolomics studies focusing on microbe-host mutualism have demonstrated that metabolomics is capable of detecting and tracking diverse microbial metabolites from different non-digestible food ingredients, of discriminating between phenotypes with different inherent microbiota and of potentially diagnosing infection and gastrointestinal diseases. Integrative approaches such as the combined analysis of the metabolome in different biofluids together with other -omics technologies will cover exogenous and endogenous effects and hence show promise to generate novel hypotheses for innovative functional feeds impacting animal gut health and immunity.

In summary, nutritional components, gut microbiota, and the mucosal immune response are linked and play a role in host health and disease etiology. The relationship among these interactive determinants is not fully understood in poultry. Microbial interventions can be induced by addition of prebiotics or other nutrients, by the administration of antibiotics, and probiotics. All of these interventions affect different bacterial species. It is critical to unravel the mechanisms of recognition and modulation induced by the different commensal bacterial species. As the understanding of the cross talk between the mucosal immune system and the microbial community is developing, it is hoped that interventions can become more specific. Perhaps by identifying a core microbiota in each type of commercial poultry, interventions that adjust the microbiome will lead to increased growth and productivity due to reduction in infectious and non-infectious diseases.

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L66 Genome editing and its applications in poultry

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Abstract

Poultry has been valued as a source of food protein for human being over 8,000 years. In research area, on the other hands, poultry including chicken have been emphasized as an animal model due to its unique development and reproduction system. Whole genome/transcripts sequencing analysis and newly developed genome editing technologies gave us to opportunities to explore insight of the biological phenomenon. Especially, efficient and precise genome editing tools such as ZFN, TALEN and CRISPR/Cas9 system has been actively utilized for genome editing in several organisms. In poultry, genome editing technology combined with germ cell culture system has been widely utilized to establishment diverse genome-edited lines. Recently reported knockout chicken mediated by TALEN and CRISPR/Cas9 suggest that programmable genome editing in poultry could contribute the development of novel lines for diverse research area including disease control, bioreactor production, modification of egg component and stem cell research. Therefore, in the future, highly efficient genome editing technology will open a new era in poultry biotechnology and industry as well as human society.

Keywords: poultry, genome editing, primordial germ cell

Introduction

Poultry has various advantages as an animal model in multidiscipline research as well as food resource. In the past, poultry species been utilized to study embryology, immunology and toxicology. Because the poultry embryo develops in eggs, the researchers could easily access and effectively manipulate to embryos at early stage without maternal effects. With the completion of chicken genome project in 2004, the value of poultry has been enormously increased (Wallis *et al.* 2004). Furthermore, studies on specific gene functions and epigenetic mechanisms have been possible based on identified gene structures and whole genome/transcripts sequencing analysis. Moreover, rapid development of genome editing technology including Zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN) and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) 9 has facilitated the genome editing in poultry. These technologies have been applied for precise gene targeting and subsequent production of genome-edited poultry. The genome editing technologies are expected to contributing establishment of novel poultry lines for human society, for examples, production of disease resistant poultry, establishment of bioreactors and model animals. Therefore, here, we will introduce recent progress of biotechnology and its future applications in poultry.

Germline−competent cells in poultry

Establishment of germline competent cell is the critical step for genome editing. In this regard, there have been many studies to establish germline competent cells *in vitro* including blastodermal cells, embryonic stem cells (ESCs), spermatogonial stem cells (SSCs) and primordial germ cells (PGCs). The blastodermal cells can easily be accessed and isolated from stage X embryo compare to SSCs or PGCs. Studies revealed that the isolated blastodermal cells have germline competency as well as pluripotency, however, the efficiency of germline transmission was relatively low (Petitte *et al.* 1990, Carsience *et al.* 1993). ESCs derived from stage X embryo cells *in vitro* also have germline-competency and potential to induce somatic chimerism. Therefore it has been considered as germline competent cell for genome editing although ESCs have low germline competency (Pain *et al.* 1999). As an alternative, SSCs have been studied as germline competent cells. SSCs are promising cell types, because the cells are the nature adult stem cells in testis that produce sperm continuously during whole life. Furthermore, SSCs-mediated genome editing system is a time-effective method compare to blastodermal cells, ESCs and PGCs because

time required for sexual maturation of recipient in this system is short (Brinster & Nagano 1998). In poultry, the transplantation study revealed that the testicular cells transplanted into recipient testis could produce donor-derived progenies. However, the cells also showed low germline competency (Lee *et al.* 2006, Trefil *et al.* 2006). Therefore, the development of *in vitro* culture system for SSCs with high germline competency is needed for genome editing of SSCs.

Most promising cells for genome editing in poultry is the PGCs. Because PGCs migrate through embryonic blood vessels and settled in embryonic gonads, it is easily isolated from the developing embryos. Moreover, the PGC-mediated genome editing system shows high germline transmission efficiency compare to ESCs and SSCs because of their long-term cultivable characteristics (van de Lavoir *et al.* 2006, Park & Han 2012). Until now, only chicken PGCs can be maintained *in vitro* for long time without loss of germline competency by modulating FGF, insulin, SMAD and MEF/ERK signaling pathway (Choi *et al.* 2010, Whyte *et al.* 2015). Understanding and further progress in the nature of germ cell may provide the clues for development of *in vitro* culture system of other poultry species. By combined system with the long-term culture of PGCs and precise genome editing technology in poultry, the highly valuable poultry lines are expected to establish easily. Therefore, it is strongly required to develop the powerful and consensus *in vitro* culture system of PGCs in poultry.

Genome editing in poultry

To produce the genome edited poultry, there have been many efforts to manipulate the oviparous avian embryos that have unique morphological and physiological characteristics compare with mammalian embryo. Owing to these distinct characteristics, it is hard to apply to the poultry genome such as pronuclear injection or embryonic stem cell-mediated transgenesis used in mammalian transgenesis system. For this reason, various germline modification system in poultry have been developed by plenty of researchers since first transgenic chicken were produced by viral injection into stage X embryo (Salter *et al.* 1986). Embryo mediated transgenesis system by the direct induction of virus had several limitations, including unintended somatic cell expression, low efficiency of germline transmission and silencing effect of integrated transgene after several generations (Bosselman *et al.* 1989, McGrew *et al.* 2004). Non-virus mediated transgenic chicken production using transposition into germline competent PGCs has been developed avoiding the limitations. Germline transmission efficiency was significantly increased, and the transgenic chicken shows high level of exogenous gene expression without silencing effects (Macdonald *et al.* 2012, Park & Han 2012).

Gene targeting using programmable genome editing tools also applied in poultry. ZFN, TALEN and CRISPR/Cas9 system are genome editing tool which could induce double strand break on targeted loci (Kim *et al.* 1996, Boch *et al.* 2009, Jinek *et al.* 2012). By the tools, genome editing on diverse organisms including domestic animals have been reported (Hauschild *et al.* 2011, Park *et al.* 2014, Whitworth *et al.* 2014). In poultry, gene targeting was firstly reported in chicken by homologous recombination (Schusser *et al.* 2013), and genome edited chickens were reported by TALEN and CRISPR/Cas9 system (Park *et al.* 2014, Oishi *et al.* 2016). The reports indicate that precise and efficient genome editing could be applied in poultry.

Past, present and future applications in poultry

Improvement of economical traits in poultry

Establishment of superior lines of domestic animal is the ultimate goals in agricultural breeding. Traditional breeding system was largely depends on naturally occurred mutations of genetic information which could enhance economical traits. The system needs extensive genotyping and phenotyping requiring time and large costs. In contrast, precision breeding mediated by genome editing technology can easily establish the lines that have desired traits without transmission of undesired alleles (Xiong *et al.* 2015). One of the promising targets is myostatin gene. A mutation on myostatin gene that inhibits muscle growth resulted in double-muscling including domestic animal (Grobet *et al.* 1997, Kambadur *et al.* 1997, McPherron *et al.* 1997, Mosher *et al.* 2007). Therefore, mutations of the genes by genome editing technologies in poultry are expected to development of double-muscling poultry lines. In addition, prevention of avian viral disease that potentially threatening human society and poultry production has been

performed by genome editing technology. Transgenic chickens expressing short-hairpin RNA (shRNA) targeting avian influenza (AI) virus gene related with viral transmission was produced and showed decreased viral transmission ability (Lyall *et al.* 2011). Not only for AI, avian viral disease including avian leukosis virus (ALV) and Marek's disease virus (MDV) could be prevented by disturbing host-virus interactions or viral environmental change (Yao & Nair 2014). Therefore, the genome editing technology is expected to contribute the establishment of high-value poultry lines.

Egg protein modification

Based on rapid advances in biotechnology, the overall market value of biopharmaceutical has being gradually increasing. The chicken is also a great model as a bioreactor (Han 2009). Chicken lays more than 300 eggs per a year so that pharmaceutical proteins could be yielded economically. Also, chicken egg proteins consist of about 10 kinds of major proteins so that separation and purification of target proteins is made easier. Moreover, protein modifications in chicken such as N-glycosylation pattern are similar to those of human and unique, so therapeutic agents from chicken eggs could be more bioactive (Kojima *et al.* 2015). Based upon advantages above, production of therapeutic proteins from transgenic chicken including β-galactosidase and β-lactamase, interferon-α-2b, interferon-β-1a, granulocyte-colony stimulating factor, human FSH, human erythropoietin (EPO) and human EGF have been reported (Mozdziak *et al.* 2003, Rapp *et al.* 2003, Kamihira *et al.* 2005, Lillico *et al.* 2007, Harel-Markowitz *et al.* 2009, Penno *et al.* 2010, Park *et al.* 2015). If genome editing technology overcomes developing industrial system, the pharmaceutical industry will undergo a revolutionary change.

Not only for functional protein expression in egg, but also eggs and meat of poultry itself are the good nutrient resources. Poultry have provided lipids, proteins, carbohydrates, minerals and other nutrients as eggs and meat so that large amounts of poultry products have been consumed in the world (Kovacs-Nolan *et al.* 2005). Although eggs have beneficial nutrients, some of the allergenic egg proteins including ovomucoid, ovalbumin, ovotransferrin and lysozyme can pose quality of life concerns, especially in young children (Caubet & Wang 2011). Also, persons who suffer from atherosclerosis or hyperlipidemia need to limit their intake of high level of cholesterol contained in eggs. So, such allergy-inducing components could be eliminated by genome editing technologies for reducing allergenicity. Recent reports showed that programmed genome edited chickens containing gene mutations of ovalbumin and ovomucoid by TALEN and CRISPR/Cas9 were generated (Park *et al.* 2014, Oishi *et al.* 2016). Furthermore, several key regulators for the form of very low-density lipoproteins in ovary could be modified for reducing lipid content of the eggs (Schneider 2009). The system suggests that genome editing in poultry could contribute to produce the customized eggs avoiding harmful effects.

Poultry as a model animal

Poultry has been utilized for an animal model in various research fields as well as a source of high quality proteins. Because of a distinct developmental character, the identification of B-lymphocyte was conducted and also graft-versus-host reaction was the first time studied in poultry embryo (Murphy 1914, Glick 1956). In researches of developmental endocrinology, chicken embryos have been used to investigate the cellular differentiation and maturation in endocrine glands, and ontogeny changes during embryonic glandular development and their target organs (De Groef *et al.* 2008). Not only poultry embryos but also mature hens have been used for an animal model of reproductive system. In hens, the oviduct epithelial cells secrete large amounts of egg-white proteins almost every day. Therefore, the oviduct system of chicken has been widely examined to study hormonal regulation and gene expression. Also, spontaneously occurring of ovarian cancer in mature hen due to daily ovulation shows similarity with human ovarian cancer in terms of its marker expression and developmental pattern. Thus, the aged hens have been consider to be ideal model for revealing and overcoming of human ovarian cancer (Johnson & Giles 2013). Consequently, combining the advantages of poultry for a model animal and development of biotechnology could facilitate the value of poultry as an animal disease model.

Stem cell and germ cell research

In mammals, various reporter systems have been used for stem cell and germ cell researches (Western *et al.* 2008, Nakaki *et al.* 2013, Irie *et al.* 2015). Reporter genes such as green, blue and red fluorescent

protein regulated by promoters or regulatory sequences of stem cell or germ cell specific marker genes were inserted in cell-based or research model animal. Those models were used for indicators of induction and sorting of various types of stem cells or germ cells. However, avian models for stem cell and germ cell research are less common. Genome editing tools together with *in vitro* culture system of avian PGCs and transgenesis will provide stem cell and germ cell research model for variety of purposes as mammals (Choi *et al.* 2010, Park & Han 2012, Park *et al.* 2014, Whyte *et al.* 2015, Oishi *et al.* 2016). Also, knockout studies using genome editing technologies in specific gene functions would be solved; for example, pre-determined model of germ cells which is not fully revealed in poultry species (Tsunekawa *et al.* 2000, Lee *et al.* 2016).

Conclusions

Poultry is the valuable resources not only for industrial areas but also research fields. Especially, rapid development of biotechnology and *in vitro* culture system of germline competent cells has increased the value of poultry in diverse area not only for research fields but also industrial fields. Highly efficient and precise genome editing tools are actively adapted in poultry species, and the tools are expected to wide applicable possibilities of poultry including disease control, egg protein modification and stem cell researches. In the near future, we expect that genome editing will create the new bio industry in poultry.

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L67 The core technology for the sustainability of animal production, environment and green energy

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Introduction

Anaerobic digestion or biogas technology is recognized for its multiple functions and benefits. It breaks down organic wastes, produces biogas energy, captures and reduces green house gases (GHG) emission, reduces odors, controls pathogens, sanitizes the farm environment, recycles nutrients for organic fertilizer and generates value added co-products. Because of the history of development and recent improvement, the technology is maturing and its market emerging, especially in China and European countries (Shih, 2012,2015). The interest in the U.S. is also on the rise (USDA-EPA-DOE, 2014). On the other hand, two major issues still remain that hamper commercial development. First, the commonly used large-scale digester systems are expensive in construction and complex in operation. Typically, it is not affordable by farmers without government subsidy. Second, the large amount of daily effluent from the digester, or digestate, is hard to manage for utilization or discharge. With forty years of research and experience in the field, this author has recently developed a new system called Holistic Digester™ for animal waste. It is simple to build, easy to operate, adaptable for large and small animal farms to claim all benefits of anaerobic digestion. In the long run, it will enable and enhance the sustainability of our animal and agricultural production, environment and green energy.

Reducing GHG emission

On December 12, 2015, an Agreement was signed by 195 nations in Paris for a global effort for low-carbon economy and energy (New York Times). The effort is to reduce emission of green house gas (GFG) by less use or replacement of fossil fuels. The target is to lower climate temperature by 2 ºC by year 2050. Converting waste biomass, including the huge amounts of farm animal wastes into biogas as renewable energy will reduce the emission of GHG. A joint publication by USDA-EPA-DOE (2014) pointed out that the use of biogas from animal manure is much more favorable in terms of GHG reduction compared using conventional fuels such as gasoline and natural gas. The analysis indicated that when biogas is produced and used as transportation fuel, the net reduction of GHG emissions is significant.

Effluent or digestate

Discharge and utilization of the daily large volume of digestate from a large digester system present great challenges. The nutrients contents in the digestate, on the other hand, are of high value as soil amendments and organic fertilizer to grow plants and crops when properly managed. Many methods, such as centrifugation, sieving, sedimentation, evaporation, membrane filtration have been used to separate or concentrate the digestate into liquid fertilizer. The separated liquid has to be further treated for safe discharge or irrigation on farmland. Solid residue is converted by a conventional process into compost. None of those separation methods are simple and inexpensive. The composting process is always associated with strong odor and loss of nitrogen, which is key nutrient. Concentrated digestate has been demonstrated of higher quality than the regular aerobic compost, because of the high retention rate of nitrogen, phosphorus and potassium (NPK) contents and other benefits for plant growth.

Dairy waste digestion

The dairy industry in the U.S. has used and studied biogas technology for many years. They have determined that it is hard to reach breakeven on the total costs of anaerobic digestion of cow manure if collecting only biogas and carbon credits (as CDM). But, when an integrated approach is taken collecting all the products, including the value of the organic fertilizer from the digestate, the economics of the to-

tal system turned profitable (USDA-EPA-DOE, 2014) as seen in Fig.1. For the US dairy industry, the collective revenues generated from the nutrients-rich fiber and NPK recovery account 48% of the total $2.9 billion market potential of biogas technology.

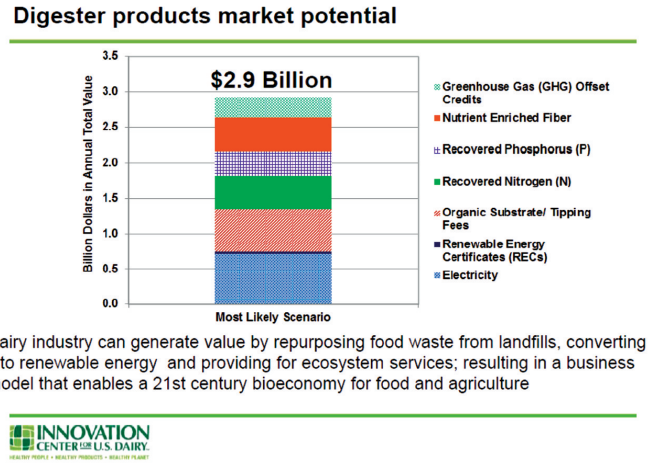

Fig. 1. market potential of multiple benefits of anaerobic digestion from dairy waste. (USDA−EPA−DOE, 2014)

Poultry waste digestion

This author experienced the same financial gain from a UNDP project in China. In 1991, he was invited to help design and operate a thermophilic poultry waste digester in Liu-min-ying village, Beijing. This digester was constructed for 50,000 laying hens that produced 5 tons of chicken manure daily. It was installed in 1992 and has been in operation for more than 20 years. The initial investment was paid back in less than three years because of the high market value and quality of the new fertilizer converted from the digestate. A lesson was learned. In order to make the biogas system work or and financially feasible, all products and benefits, including energy, carbon credits, organic fertilizer and environmental sanitation, have to be fully developed. In both dairy and poultry cases, it is noteworthy that almost 50% of the total value can be created by converting the digestate into organic fertilizer.

Discovery and Development of New Products

Anaerobic digestion is not only a bioprocess for waste treatment and biogas production, but also a rich resource of bio-products. In the late 80's, serendipitously, feather degradation was first observed during the operation of a poultry waste digester on the farm. After two years of painstaking search, a feather-degrading bacterium was isolated that can break down feathers. Subsequently, the keratinase enzyme was purified and the gene encoding this novel enzyme was isolated and sequenced. With fermentation scale up, the enzyme was produced in a quantity sufficient for application research. It was demonstrated that the keratinase can be used for processing feathers to make feather meal more digestible. It can break down prion protein, the putative cause of bovine spongiform encephalopathy, commonly known as mad cow disease. Finally, it was discovered the keratinase as a feed additive can improve the digestibility of feed protein and promote the growth of young chicks. This series of studies have generated a total of 9 U.S. and international patents. In year 2000, a biotechnology company called BioResource International (BRI) was established to commercialize this unique enzyme to help poultry producers worldwide save feed costs and now the enzyme product is on the market worldwide. (see reviews, Shih, 1993, 2012, 2015 and website: www.briworldwide.com).

The discovery of keratinase followed by technology and commercial development is a perfect model of translational research from science to commerce. On the other hand, it demonstrated the potential wealth of anaerobic digestion for enzyme discovery, gene mining and possibilities of other novel bioproducts.

Economics

Biogas technology is a maturing technology. Based on currently available, yet limited, information, the author has proposed a virtual model for cost and return estimation. A hypothetical farm raising one million laying hens and producing 100 tons of fresh manure daily can be outfitted with a conventional CSTR large digester system to process the manure daily. Biogas can be produced at a reasonable rate and converted into electricity while digestate can be processed into organic fertilizer. Preliminary estimations are presented in Table 1. Interestingly, the Return of Investment (ROI) of the large biogas system has a breakeven time only 4 years. Though hypothetical, it is encouraging for follow-up study and improvement.

Table 1. Estimation of ROI for a conventional anaerobic digester

Investment:		
General construction		$0.40 M
Major equipment (digesters, gas storage, etc.)		1.30
Other facilities (de-grit, de-sulfur, post-treat, etc.)		2.00
Power generator system (1 MegaW)		1.00
Design, adjustment, service, etc.		0.40
Operational, 1st yr.		1.50
	Total:	$6.60 M
Income, per year		
Power generation ($20,000 kWh/day)		$0.73 M
CDM (40,000 ton CO_{2e}/yr)		0.40
Bio-organic fertilizer (400 tons/day)		1.50
Saving from waste management fee per yr		0.50
Operational, per year		(1.50)
	Net:	$1.63 M

Return of Investment (ROI) : 6.6/1.63 = 4 years breakeven time

Notes: 1) Assuming a farm with 1,000,000 laying hens.
2) Electricity, $0.10 per kWh.
3) Organic fertilizer, $10.00 per ton.
4) All estimates in US$.

However, there are four limitations or challenges. First, the initial cost of construction and installation is high (US$6.6 million). Second, the scale is too large to meet smaller farms' need. Third, the skill level needed for operation and maintenance of the digester system is high. Fourth, the conversion cost of digestate to fertilizer is not in the calculation. A new design and innovative technology, therefore, is important to lower the production cost of fertilizer.

New technologies

In addition to his first digester design in 1996 (Shih), this author has filed three more patents in the last three years. They are ready for licensing and co-development and briefly described as follows:

1.Hydrolytic Degritter (patent pending, filed in 2013). This is a device and method for pre-treatment of animal waste to remove sandy materials, or grits, before entering the anaerobic digester.

2.Secondary Solid-Phase Anaerobic Digestion for More Biogas Production (U.S. Patent 9,242,881, 2016; filed in 2013). Biomass is used for secondary solid-phase digestion with liquid digestate from the primary digester to produce more biogas.

3.System and Method for Anaerobic Digestion of Animal Wastes (Patent pending, filed in 2015). This is a novel design of a compact and complete digester system including the degritter, the primary anaerobic digester and the secondary digester. While producing both biogas and organic fertilizer, the system is low-cost to build, easy to operate and suitable for both large and small animal farms. The trade name of the new digester system is Holistic Digester™.

Conclusion

In conclusion, anaerobic digestion or biogas technology is a maturing technology and emerging market. However, there is room for improvement for practical applications and commercial development. Based on his forty years of experiences, this author invented a new Holistic Digester™ system, which is compact, low-cost and easy to operate. Most importantly, it produces green energy, reduces carbon emission, and recycles the nutrients to organic fertilizer. It is a holistic or integrated solution to waste problems and, at the same time, promises the sustainability of animal and agricultural production, environment and green energy.

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On behalf of the XXV World's Poultry Congress Organizing Committee, we would like to thank the sponsors for their support which has been essential for the logistic organization of the WPC2016 in Beijing.

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