



LOHMANN INFORMATION

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**LOHMANN
TIERZUCHT**

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Dear colleagues and friends,

Adequate food supply for the growing world population is a major concern of governments and NGOs in industrial and developing countries. Will the development of food production keep pace with the increasing population in developing countries and the growing demand of high quality food, especially food of animal origin, of the industrialized countries? Resources of food production are limited and there is a strong competition for these resources from different directions. The main compartments of the allocation of resources are defined by the so-called 6-F framework: **F**ood for the human population, **F**eed for animals, **F**uel, **F**iber, **F**lower (one amount of plans and natural), and **F**un. In the public discussion the complex issue of nutrition is focused on the competition of feed versus food and the simplified solution is the conversion of the human population from omnivorous to vegetarian nutrition. Abandoning meat consumption and livestock production and would not only solve the problem of food supply for the human population, but also protect farm animals from suffering and reduce environmental pollution. Interestingly the High Level Panel of Experts for Food Security and Nutrition (HLPE) of the committee on world food security (CFS) has recently addressed the particular role of livestock within the global framework of agricultural development and food production. HLPE as an independent international group of experts, in its report to number 10 on "Sustainable agricultural development for food security and nutrition: what roles for livestock?" came to the conclusion that *"The livestock sector is a powerful engine for the development of agriculture and food systems. It drives major economic, social and environmental changes in the food system worldwide, and provides an entry point for understanding the issues around sustainable agriculture development as a whole"*.

While the HLPE report addresses the value of rights of production in the general frame of food production, the first article of the present issue is focused on the importance of food of animal origin on the supply of essential micronutrients. As pointed out by **H. K. Biesalski**, **the importance of meat as a source of essential micronutrients** is generally underestimated. Due to the high bioavailability of vitamins and trace elements, meat and especially liver are highly recommended as essential components of balanced diets, especially for groups with higher needs, such as pregnant women, children and elderly people.

Commercial egg producers have become used to flocks of modern laying hens which lay at rates above 90% for many months, which indicates that most hens are laying an egg a day for extended periods. **B. Kaspers** reviews **the physiology of egg formation**

and calls attention to the hen's needs to lay an egg a day and new techniques which are used to study the composition of egg components in more detail and contribute to the development of new products based on egg components.

Emerging **antibiotic-resistant pathogens** threaten the health of humans and animals. The role of livestock in this scenario is being discussed controversially. There is, however, no doubt that the application of antibiotics has to be reduced. **L. J. Vegad** summarized recent publication and concludes that more careful use of antibiotics and alternatives to the use of antibiotics in livestock production will reduce risks for animals and humans.

H.-W. Windhorst analysed the **development of global egg production** and the underlying driving forces. Intensive production systems in newly industrialized countries contribute the major part of the increase of global egg production. The driving forces for this development are population growth and increasing income. Small-scale poultry production development systems are still prevailing in most Least Developed Countries. There is still considerable increase in production in this sector. The development is often disturbed by unstable political and economic conditions.

Peri-natal temperature treatment shows surprising long lasting effects in broilers, ducks and possibly other poultry species. **B. Tzschentke** and **I. Halle** present results of recent studies, which show that mild increase in temperature during a certain time of incubation has positive effects on hatchability and chick quality, later performance, body composition and general adaptability in broilers. Similar results are obtained in ducks by short time reduction of the incubation temperature. These results will encourage further studies in this area and influence the development of hatching and brooding techniques under commercial conditions.

At a time when the European Poultry Industry is focused on minimizing risks of the current avian influenza wave, we hope you find some of the articles in this issue useful "food for thought" and look forward to your comments and suggestions for future issues.

**Merry Christmas and a Happy, Healthy
and Successful New Year!**



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Meat as a healthy and valuable source of micronutrients

Abstract

During the last decades meat and especially liver have been looked upon as unhealthy food with high fat content and cancerogenic potential. In addition, its content of highly valuable micronutrients has mostly been ignored. As a result, the mean uptake and serum levels of several micronutrients in the population are below the recommended levels. In the meantime, the contamination of liver with heavy metals and other contaminants has fallen far below the allowed thresholds and sometimes even below the detection limit while its content of micronutrients like iron, folate, selenium or zinc are still high. As a further advantage, the bioavailability of many micronutrients often is better from meat and liver than from plant sources. These advantages and the low content of contaminants in meat and liver leads us to conclude that meat and liver should be considered as a regular part of mixed diets with vegetables and fruits as the major components to ensure an optimal supply with micronutrients.

Keywords

Micronutrients, meat, liver, human nutrition, human health

Introduction

Many people consider meat, especially red meat, as unhealthy food, mainly due to its postulated "high" fat content and its very recently posted "reputation" that red meat is a promoter of colon cancer. As a consequence, low or no meat intake is often re-

commended to avoid or minimize the risk of cancer, obesity and metabolic syndrome. Ethical arguments against intensive animal farming and its impact on global climate change contribute to the negative image of meat, but will not be addressed in this review.

These more or less justified arguments against meat consumption ignore the fact that meat is a valuable source for some essential micronutrients such as iron, selenium, vitamins A, B12 and folic acid. Some of these are not present in plants at all or have such a low bioavailability that eating

only plant-derived food may be inadequate to meet the daily requirement.

The fact that the world wide inadequacy of some micronutrients (hidden hunger) is related primarily to iron, zinc, vitamin A and folic acid is a result of rather poor diet diversity with the major component being starchy food and with a very low supply of meat and meat derived products (Biesalski 2013). Meat is an important food as an essential part of a mixed diet that ensures adequate delivery of micronutrients and amino acids even in low amounts. If meat and meat derived products are completely avoided the risk of inadequate supply and subsequent health relevant negative effects (increased infectious diseases, impairment of the immune system, anemia etc.) may occur.

Meat as an important source of micronutrients

Vitamin A and vitamin B12 occur only in meat and can hardly be compensated for by plant-derived provitamins: a provitamin B12 does not exist and provitamin A, β -carotene, would have to be taken up in highest amounts to meet the daily vitamin A recommendation (1mg) due to its poor conversion rate of 1:12 (12 mg provitamin A needed to achieve 1 mg Vitamin A).

Iron has a higher bioavailability when derived from meat as hem iron than plant-derived non-hem iron. Similarly, folic acid from meat (especially liver) and eggs has a nearly ten-fold higher bioavailability than from vegetables. Consequently low or no intake of meat (including liver) poses a risk for inadequate supply and symptoms of deficiency. Meat is also an excellent source for Thiamine (B1) and Riboflavin (B2). These nutrition facts lead to the question whether people with "normal" eating habits or only special groups of people are at risk for deficiencies of meat-derived micronutrients.

One risk group are elderly people who are generally considered at risk to develop vitamin and trace element deficiencies, especially for the vitamins A, D, E, and for folate as well as iron and calcium (Anderson 2001; Bates et al. 2002; Martins et al. 2002; Viteri and Gonzalez 2002). Recently a meta-analysis summarized the data from 41 studies with community-dwelling older adults (ter Borg et al. 2015). 30 % were below the Estimated Average Requirement (EAR) for Vitamin A, 95 % for vitamin D, 40 % for folate, Vitamin B1 and B2 and 15 % for iron. Per definition 50 % below the EAR and 50 % above the EAR are not at risk for deficiency. Adding two standard deviations to the EAR is used to calculate the reference value. Consequently, according to the definition of the EAR a supply below results in an increasing risk for developing a deficiency with clinical consequences. Malnutrition is far more common among institutionalized and chronic hospitalized elderly compared to free-living subjects in the community and the prevalence of malnutrition is associated with the severity of morbidity, functional impairments and mental state. Thiamine and folate status need special attention in this respect, as a deficiency of these nutrients is associated with depression and impaired cognition and dementia. Indeed, supplementation with B-vitamins resulted in a significant positive impact on mood and cognitive vigilance (von Arnim et al. 2013).

Pregnant women are another group at risk for micronutrient deficiencies. Their adequate nutrition plays an important role in the well-being of mother and child and also influences the health of the offspring not only during his intrauterine life but also during childhood and adolescence (intra-uterine or prenatal programming). While the enhanced requirements during pregnancy are met by a balanced diet

and physiological adaptive processes, the micronutrient status of vitamin A, D, folic acid, iron, and zinc may become compromised without supplementation (Draper et al. 1993; Fogelholm 1999; Saletti et al. 2000) especially when meat is avoided, which can be frequently seen in women in the child bearing age.

Selected micronutrients from meat and liver

Meat and liver are excellent sources for a number of micronutrients: low-fat pork contains 1.8 mg iron and 2.6 mg zinc; pig liver contains 360 mg magnesium, 20 mg iron and 60 μ g selenium per 100 g. Thus, meat and liver (100g/day) can cover up to 50 % of the Recommended Daily Allowance (RDA)¹ for iron, zinc, selenium, vitamins B12, B1, B2, B6, and 100 % of vitamin A. Another reason to include liver in the diet is that newest results show porcine as well as bovine liver to be almost free of harmful substances like heavy metals (a former reason to avoid the uptake of liver) which are far below the allowed threshold or even below the technical detection limit (DGE 2004).

Vitamin A

The best source for vitamin A is liver. Liver of pork and beef contains 6.0 μ g and 20 μ g of vitamin A respectively (table 1). However, liver consumption is low and restricted to some areas in Germany where it is a traditional food. The argument that liver should be avoided in a healthy diet has been based primarily on suspected contaminants in the liver (e.g. hormones, xenobiotics, metals etc.) which nowadays are seldom detected (see above). If β -carotene from vegetables were the only source of vitamin A, more than 500 g mixed and β -carotene rich vegetables per day must be consumed to reach the recommended 1 mg retinol. This amount of vegetables

¹ The Recommended Daily Allowance (RDA) is the average daily dietary intake level, which is sufficient to meet the nutrient requirement of 97 – 98 % of healthy people. https://fnic.nal.usda.gov/sites/fnic.nal.usda.gov/files/uploads/recommended_intakes_individuals.pdf

Table 1 Selected mineral and vitamin contents in different animal products

Nutrient	Porc ¹	Liver ¹					
		Beef ¹	Broiler breast ²	Turkey breast ²	Duck ²	Pork	Beef
Ca (mg)	5.1	5.7	15.0	25.0	11.0	7.6	5.8
Mg (mg)	26	23	29.0	24.0	16.0	24	21
Fe (mg)	1.0	2.1	1.0	1.9	2.7	17	6.9
Zn (mg)	2.4	4.3	1.0	2.9	1.9	6.5	4.8
Se (µg)	12	5.4	27.6	37.2	20.0	56	21
Vit C (mg)	ND	ND	0	0	0	23	32
Vit A (µg)	6.0	20	21	0	63	36 000	18 000
Vit D (µg)	na	na	na	na	na	na	1.7
Vit E (µg)	410	480	300	300	700	601	746
Folate (µg)	2.5	3.0	4.0	7.0	6.0	136	592
B1(mg)	0.9	0.057	0.1	0.1	0.2	0.31	0.27
B2(mg)	0.23	0.26	0.1	0.2	0.3	3.2	3.1
B6(mg)	0.575	0.24	0.6	0.4	0.2	0.59	0.96
B12(µg)	2.0	5.0	0.3	0.3	0.3	39	65

¹ Souci-Fachmann Kraut | ² www.nutritiondata.self.com

may actually contain more contaminants than a portion of liver, as their concentration in vegetables and fruits seem to have increased during the late 1990th and early 2000th (DGE 2004). A small portion of liver (100 g) twice a month supplies sufficient vitamin A and is neither toxic nor teratogenic. A poor vitamin A-status which cannot be easily detected via blood analysis due to its homeostatic regulation increases the risk for respiratory tract diseases and impairment of the immune system (Biesalski 2013).

Folate

In European countries the average folate intake in adults was found to be remarkably similar, around 300 µg/day in adult males and 250 µg/day in adult women (De Bree et al. 1997). This is below the former recommended intake level of 400 µg/day (which has recently been reduced

to 300 µg/day) and too low for pregnant women and women planning a pregnancy. For these groups an intake of 600 µg/day is considered protective against Neural Tube Defects (NTD), which appears in around 700 cases/year in Germany. More than 90 % of women of child bearing age have sub-optimal dietary folate intake. Supplementation of folate to the optimal level can reduce the risk to develop NTD by up to 70 %. Data regarding meat derived folate intake and NTD is not available. But it should be considered that meat and liver in particular are sources with better bioavailability than plant derived folate. Due to the fact that females in child bearing age are not adequately supplied with folate, supplements (400 µg/day) are strongly recommended.

Vitamin B12

Vitamin B12 is found only in animal pro-

ducts. In a recent UK study of 250 vegetarian and 250 vegan men, approximately one quarter of vegetarians and more than half of vegans had sub-optimal intakes of vitamin B12. Plasma vitamin B12 levels were low in the vegetarians and extremely low in the vegan group, with more than a quarter below the threshold level where neurological signs may develop (Dror and Allen 2008; Gilsing et al. 2010). Elderly non-vegetarian people are also at risk of vitamin B12 deficiency, due to physiological changes resulting in reduced absorption. In the UK, vitamin B12 status in some people aged 65 and over was inadequate. There was no difference in mean levels between men and women. However, vitamin B12 intake was adequate when compared with UK dietary reference values (Finch et al. 1998). To assure the RDA of vitamin B12 (3 µg/day for adults), again meat (bovine 5 µg/100 g) and especially liver (depending

on the species 30-65 µg/100 g) are the best sources.

Iron

Although iron is one of the most abundant elements in the Earth's crust, iron deficiency is the most common and widespread nutritional disorder in the world. According to the WHO (2008) Iron deficiency adversely affects

- the cognitive performance, behaviour, and physical growth of infants, pre-school and school-aged children;
- the immune status and morbidity from infections of all age groups;
- the use of energy sources by muscles and thus the physical capacity and work performance of adolescents and adults of all age groups.

Table 2 Hazard ratio of red meat, processed meat and poultry consumption (observed and calibrated by 14-hours recall data)² for all-cause mortality

Source	Observed HR	Calibrated HR
Red meat (per 100 g)	1.02 (0.98–1.06)	1.02 (0.98–1.06)
Processed meat (per 50 g)	1.09 (1.06–1.12)	1.18 (1.11–1.25)
Poultry (per 50g)	0.96 (0.92–0.99)	0.95 (0.85–1.04)

→ specifically, iron deficiency anemia during pregnancy increases perinatal risks for mothers and neonates; and increases overall infant mortality. Moreover, iron-deficient animals and humans have impaired gastrointestinal functions and altered patterns of hormone production and metabolism. The latter include those for neurotransmitters and thyroidal hormones which are associated with neurological, muscular, and tempe-

perature-regulatory alterations that limit the capacity to maintain their body temperature of individuals exposed to cold. The iron-concentration in meat is between 1-3 mg/100g. Liver contains between 8 mg/100 g (calf) and 16 mg/100 g (pig). In bread and legumes, the concentration varies between 1 mg up to 8 mg/100 g. Due to biological losses, such as cyclical monthly bleeding of fertile-aged women,

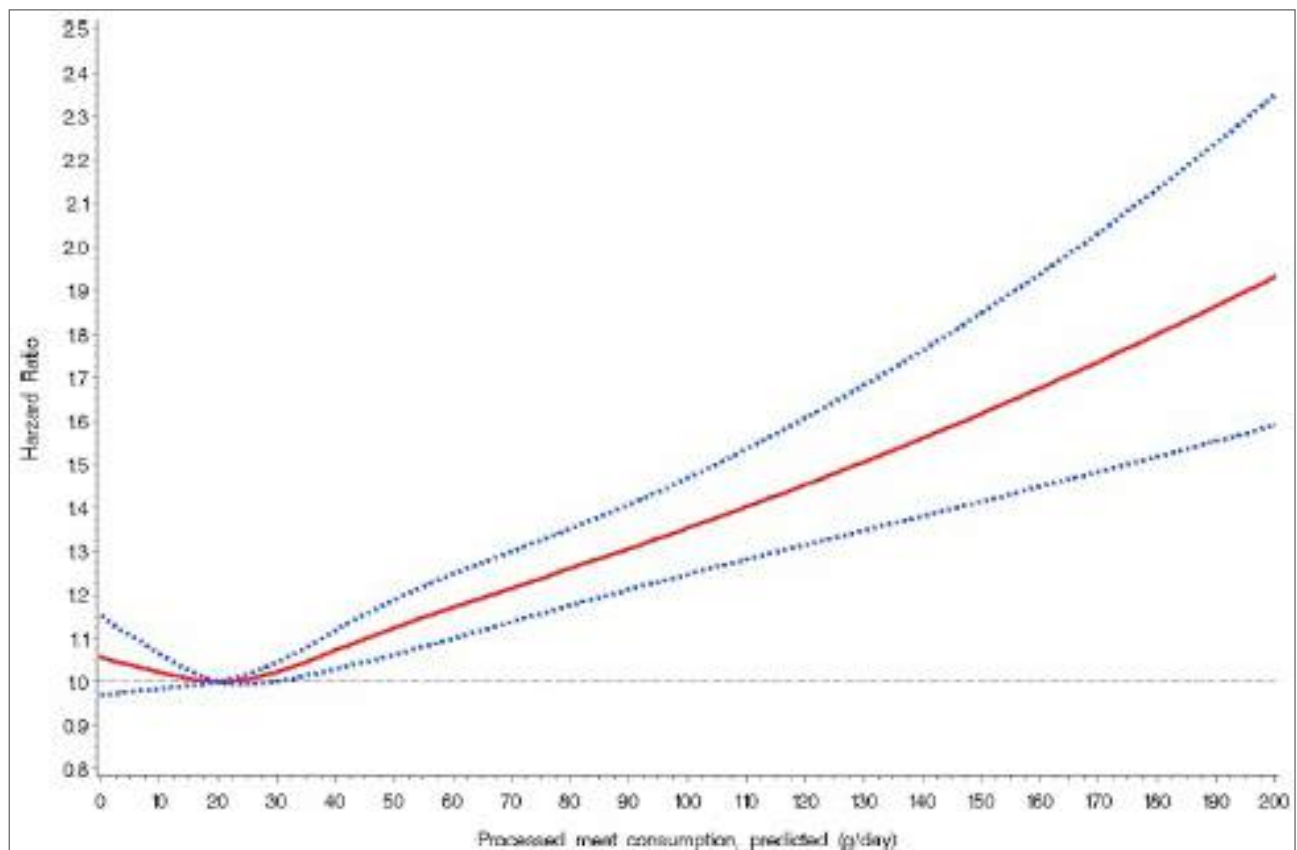


Figure 1: Nonparametric regression curve for the relation of processed meat intake at recruitment with all-cause mortality, European Prospective Investigation into Cancer and Nutrition (EPIC) 1992-2009. Solid line: effect estimate; dotted lines: 95 % confidence interval (Rohrmann et al. 2013). (We acknowledge the permission to reprint the graph)

² A 24-hour dietary recall (24HR) is a structured interview intended to capture detailed information about all foods and beverages, consumed by the respondent in the past 24 hours. This method was used to provide more reliable data on consumption. <https://dietassessmentprimer.cancer.gov/profiles/recall/>

excessive infestation with blood-feeding parasites, or poor bioavailability of iron from plant-based diets, it is estimated that as many as 3-4 billion people, around 20 % in high income and 50-60 % in low income countries may have inadequate iron supply with the diet. At any given time, 1.62 billion people – over 30 % of the world's population – are anemic, mainly due to iron deficiency. In developing countries this is frequently exacerbated by malaria and worm infections (WHO, 2008). The highest prevalence (47.4%) has been found in preschool children and non-pregnant females.

In a recent Irish food consumption survey, almost half of women aged 18-50 year had inadequate iron intakes when compared with national average requirements. Around 25 % of all pregnant women in Europe suffer from iron deficiency. The daily supply is below 60 % of the estimated average requirement (EAR) for pregnancy (Blumfield et al. 2013). Breastmilk is low in iron, in particular when the supply of the mother is inadequate. In a recent study in Germany 21 % of breast fed children were iron deficient and 6% had clear symptoms of anaemia (Dube et al. 2010). During post-weaning age (12 - 23 months) 15 % of male and 18.5 % of female children in Germany had an inadequate supply (below EAR) (Hilger et al. 2015). In the British National Diet and Nutrition Survey, iron intake was found to be low in girls (aged 7-18 years) and decreased with age. Adolescent females (15-18 year) were found to have extremely low intakes of iron when compared to UK dietary reference values.

Depending on the composition of the individual diet the bioavailability of iron can differ 5 to 10-fold. The bioavailability depends on the presence or absence of different ligands (phytates from cere-

al products, tannins from coffee and tea and oxalates from vegetables) which form complexes with iron and zinc and block their absorption. Diets primarily composed of vegetables, rice, beans and corn are associated with poor iron bioavailability. This explains the high incidence of anaemia in developing countries. 100 g pork meat and an even lower amount of liver added to a vegetarian diet increases the iron absorption 3.6 fold.

Zinc

Zinc is essential for more than 100 enzymes and plays an important role within the immune system. Zinc deficient individuals demonstrate slower wound healing and are more prone to infections. Some studies showed that zinc may beneficially affect cold symptoms; however a meta-analysis of randomized controlled trials concluded that there is no evidence for the effectiveness of supplemented zinc in reducing the duration of common cold symptoms (Jackson et al. 2000). Especially in elderly a reduced zinc status is evident (Lukito et al. 2004). In the same group, a higher protein intake (together with slight exercise) stopped sarcopenia, a progressive loss of lean body mass. The RDA for zinc is about 12-15 mg/day. A higher need of zinc is documented (20-25 mg/day) during pregnancy and lactation as well as during chronic inflammatory diseases (Rink and Gabriel, 2000).

Meat contains between 2 and 10 mg of zinc/100g. Plant derived food is also a good source, in particular green leafy vegetables (up to 70 mg/1000 kcal) but due to the more reliable bioavailability meat seems a safer source of zinc.

Considering the nutrition value of vegetarian diets, beside vitamin B12, iron and zinc are the micronutrients of greatest concern.

Bioavailability of iron and zinc is substantially decreased through the interaction with phytate and further constituents of plant food.

Meat and cancer

Meat consumption, especially red meat, is not carcinogenic per se, even if it contains components which are assumed to contribute to cancer formation. On the other hand, persons with a high intake of fruit and vegetables are considered to have a reduced cancer risk because of protecting factors such as carotenoids, flavonoids, further phytochemicals and also folic acid, selenium, zinc and other components. It is not clear why these latter compounds should be less effective if they reach the body via meat. The balance of promoting and protecting factors within the diet is important for the protection against cancer. Furthermore, the insulin-resistance-hypothesis shows that a nutritional behavior leading to a metabolic syndrome (high energy, high glycaemic carbohydrates) might favor colon cancer or even other types of cancer. The "goals for nutrition in the year 2000" (Willett, 1999) give a very good and comprehensive advice: "Current nutritional recommendations for the prevention of cancer include increased consumption of fruits and vegetables; reduced consumption of red meat and animal fat; and avoidance of excessive alcohol".

Very recently the WHO classified red meat as class 2A carcinogenic based on epidemiological data. Class 2A means that there is sufficient evidence of carcinogenicity in humans. However, the data used are based on limited evidence from epidemiological studies. Limited evidence describes a result which shows an association between exposure of red meat and cancer but other reasons for the increased risk cannot be excluded. This limited evidence is

a conclusion in all meta-analysis dealing with red meat intake and cancer risk (Alexander et al 2015; Egeberg et al 2013; Lippi et al 2015; Pham et al 2014). Different epidemiological studies between 2005 and 2013 were used to justify the relation between high meat intake and increased risk for Colorectal Cancer (CRC) as the basis for the cancer risk classification. The European Prospective Investigation into Cancer and Nutrition (EPIC) study, a big human cohort study with more than 500.000 participants showed that the highest daily intake of meat (160 g/day) has a relative risk of 1.35 compared to the lowest intake (Norat et al. 2005). Red meat alone (> 80 g/day) increased the risk slightly (1.17 %), processed meat (>80 g/day) increased the risk up to 1.42 %. A meta-analysis (Chan et al. 2011) with 10 cohort studies documented a relative risk for the highest intake (140 g/day)

of 1.14 % compared to the lowest. Further increase of meat consumption did not change the relative risk. Based on that study the International Agency for Research on Cancer (IARC) distributed the warning and the risk classification (Bouvard et al. 2015). A more detailed examination of the EPIC cohort, however, showed a more differentiated result:

Depending on the model used (uncalibrated or calibrated), only processed meat showed a risk increase of 50 g additional meat/day. The impact of daily processed meat consumption on the risk to develop cancer shows a U-shaped pattern. The lowest consumption of processed meat has the same mortality as a daily intake of approx. 55 g.

In contrast, red meat consumption in the highest quartile vs. lowest did not show

a significant increase in cancer mortality. There was no association between intake of poultry and all-cause mortality.

A very recent meta-analysis with 27 prospective studies and a broader evaluation of the relative risk estimates by specific intake levels documented again a rather weak relation between red meat consumption and CRC risk (Figure 2).

At intake levels > 100 g red meat per day showed a slightly increased risk (1.20 %). The elevation of the relative risk related to servings per day is largely influenced by male participants. Alexander et al. (2015) concluded:

„In the current meta-analysis of red meat intake and CRC (colorectal cancer), we comprehensively examined associations

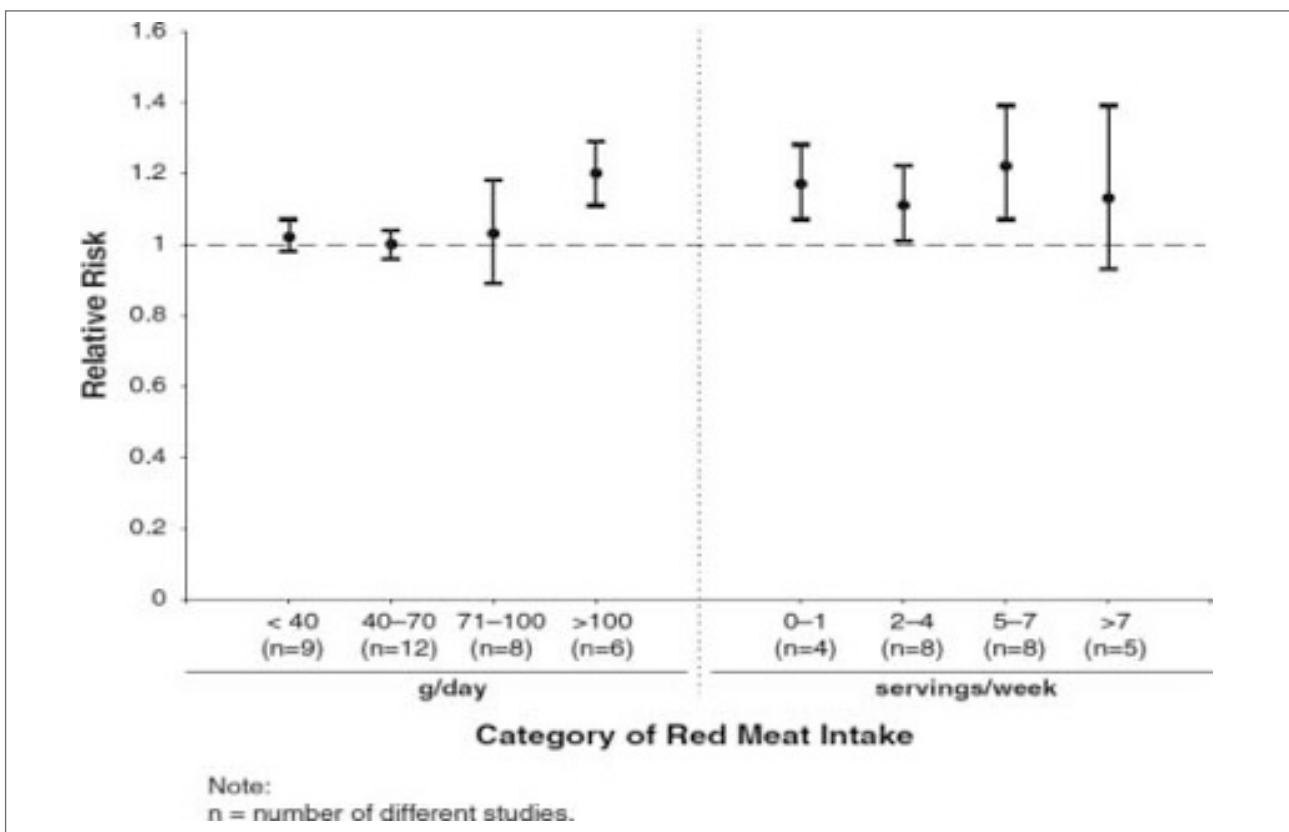


Figure 2: Dose response patterns showing the relative risk to develop Colorectal Cancer (CRC) based on meta-analyses of categorical intake groupings (adapted from Alexander et al. 2015)

by creating numerous subgroup stratifications, conducting extensive sensitivity analyses, and evaluating dose-response using several different methods. Associations between red meat and CRC were weak to nonexistent. Of the highest categories of intake in the individual studies, over one-third of the RRs (relative risks) were 1.0 or lower, and almost half were less than 1.05. Summary associations were weak, with most SRREs (summary relative risk estimates) around the null value or just slightly above. Because of multiple comparisons (i.e., over 70 separate analyses were conducted), some statistically significant correlations could arise by chance alone.

In conclusion, based on the quantitative findings and scientific rationale for interpretation documented in the current meta-analysis, red meat intake does not appear to be an independent predictor of CRC risk“.

The relation between slight but insignificant risk increase and servings per day shows that the best way to be on the safe side with respect to either cancer risk or inadequate micronutrient intake is a mixed and balanced diet. Indeed, a simple and very old suggestion.

Conclusions

Any kind of an unbalanced diet, either avoiding meat or fruit or vegetables may lead to (micro-) nutrient deficiencies and consecutive disorders especially in groups with higher needs (pregnant or lactating women, excessive exercise, growing children, elderly, etc.). At this time one has to assume, that a mixed and balanced diet with higher amounts of fruits and vegetables and moderate amounts of meat (processed and unprocessed) including liver, accompanied with physical exercise is recommended and seems to be the best recipe for prevention of nutritional defi-

ciencies and physiological malfunctions.

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An egg a day – the physiology of egg formation

Abstract

Modern hybrid laying hens can produce more than 300 eggs per year and more than 500 eggs in an extended life cycle. This would not be possible without continuous ovulation, a trait already observed in the ancestors of our modern layers. In the ovary of newly hatched hens more than 12.000 oocytes are present. Of these, only a small proportion will later acquire yolk and develop into mature ovulatory oocytes. With the onset of sexual maturity follicles with different sizes develop by accumulation of lipids resulting in a hierarchy of follicles which ensures the development of only one pre-ovulatory follicle per day. The precise regulation of follicle selection is only partially understood but the pituitary hormones FSH and LH play a dominant role in this process. In contrast, formation of the yellow yolk is well described. With the exception of immunoglobulins all proteins and lipids are synthesized in the liver and transferred from blood stream into the developing follicle. Yolk contains approximately 36% lipids, 17% protein (largely immunoglobulins) and only a minor fraction of free carbohydrates and inorganic components (<1%), the rest is water. Maternal antibodies in the yolk are exclusively of the IgY type. They are made by B-lymphocytes in the spleen and other lymphoid organs, secreted into the circulation and actively transported in the ovary into the yolk. Yolk formation is completed 24 hours before ovulation.

After ovulation the follicle is placed in the infundibulum where the vitelline membranes are formed during the 1 hour passage. They separate the yolk from the album which is secreted by glandular and goblet cells of the magnum during the following 2-3 hours. 90% of the dry matter of the albumen is protein which is exclusively produced by the cells of the magnum. With the passage into the isthmus formation of the eggshell membranes starts which is followed by formation of the eggshell in the uterus. This process is controlled by the ovulation and several neuroendocrine factors which regulate the secretion of osteopontin and the expression of calcium transport proteins and calbindin. Within 11-15 hours 2.0-2.5 g of calcium are deposited in the eggshell. Two third of the daily calcium requirement is derived from intestinal resorption the remaining third is mobilized from unique bone structures called the medullary bone. This bone compartment is restored during the periods between eggshell formation. As in mammals, calcium homeostasis is regulated by 1,25-dihydroxyvitamin D3 and parathyroid hormone.

In recent years proteomics have been used intensively to unravel proteins involved in the formation of the different egg membranes, albumen and the eggshell and identified novel proteins protecting the egg and the embryo from pathogens. In addition, new transcriptomic data help to better understand the regulation of egg formation still only partially understood.

Keywords

chicken egg, production, egg structures, ovary, reproductive tract

Introduction

Modern hybrid laying hens can produce more than 300 eggs per year and more than 500 eggs in an extended life cycle. This would not be possible without continuous ovulation, a trait already inherited in the ancestors of our modern layers (1). In the ovary of newly hatched hens more than 12.000 oocytes are present. Of these, only a small proportion (250-500) will later acquire yolk and develop into mature ovulatory follicles to give rise to egg formation (2). Here I will briefly describe the stepwise development of the main structures of the chicken egg in the ovary and during its passage through the reproductive tract.

Follicle maturation

With the onset of sexual maturity follicles with different sizes develop by accumulation of lipids resulting in a hierarchy which ensures the development of only one pre-ovulatory follicle per day. Sexually mature hens harbor numerous primordial follicles in the cortex of the ovary with up to 1 mm in size. This resting pool of follicles gives rise to a restricted number of primary follicles which further mature into so called pre-hierarchical follicles from which only one follicle (pre-ovulatory) is selected to terminally mature per day (3). The regulation of this selection process is only partially understood. It is assumed that inhibitory signals prevent the expression of the receptor for follicle stimulating hormone (FSH) which is secreted from the pituitary gland. Loss of this inhibitory signal in individual follicles leads to FSH-receptor expression and FSH mediated synthesis of steroid hormones and factors supporting survival of follicle associated cells. Subsequently, the FSH effect diminishes and luteinizing hormone (LH; another pituitary hormone) regulates further follicle maturation and accumulation of yolk. Initially, primordial follicles are recruited to the

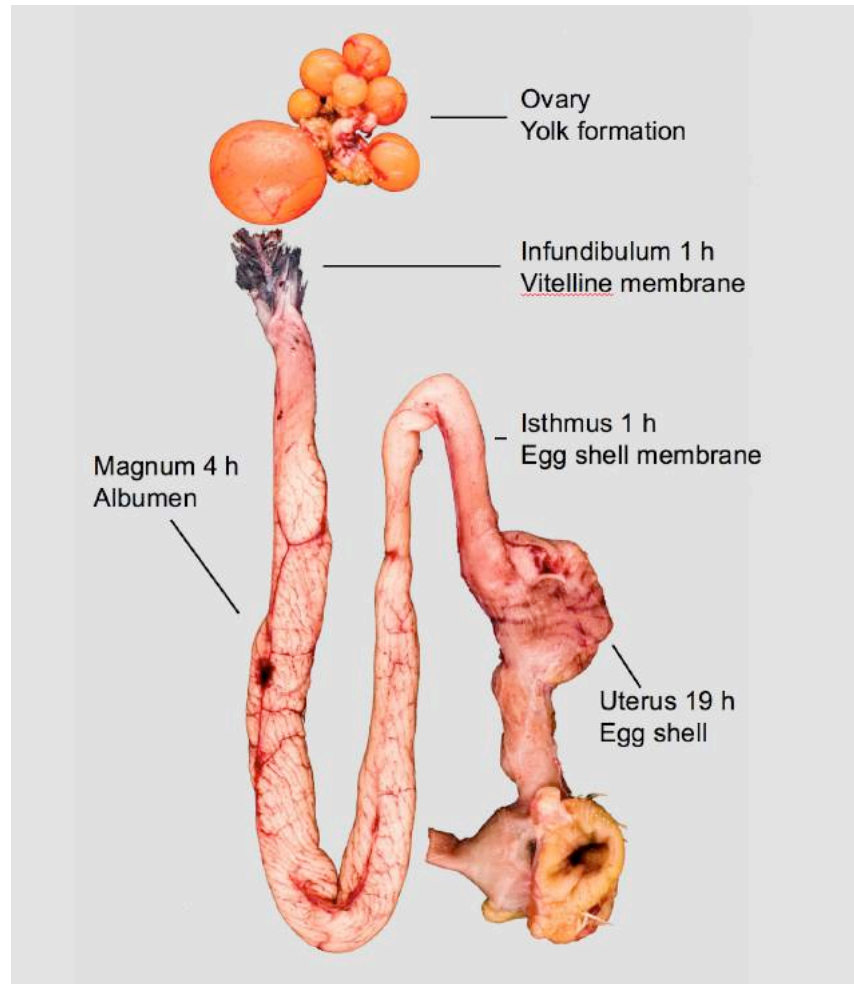


Figure 1: The reproductive tract of the chicken, stepwise egg formation

cortical surface and eventually become connected to the blood supply and nerve system via the follicle stalk. Maturation from primordial follicles via primary follicles into pre-hierarchical follicles is associated with accumulation of white yolk rich in lipoproteins. These follicles are 6-8 mm in diameter and can easily be seen on the ovary surface. The terminal differentiation of such follicles to the pre-ovulatory follicle takes 4-6 days and is characterized by the massive accumulation of yolk (2g per day) a process completed 24 hours prior to ovulation (4).

Yolk composition

Yolk mostly consists of fat (36%), protein

(17%) and water; 65-70% of the fat fraction are triacylglycerols, 25-31% phospholipids and 4-5% cholesterol. The protein fraction is composed of α -livetin (14%; = serum albumen), β -livetin (41%; = glycoproteins) and γ -livetin (45%; = IgY). Carbohydrates, vitamins and inorganic compounds represent less than 1% of the yolk. The characteristic color of the yolk is caused by carotenoids, in particular by xanthophylls (2). Both yolk fats and yolk proteins are synthesized in the liver a process induced by estrogen and testosterone secreted from maturing follicles in the ovary. The fat fraction is transported in the blood as very low density lipoproteins (VLDL). VLDLs bind to a specific receptors called LR8 which

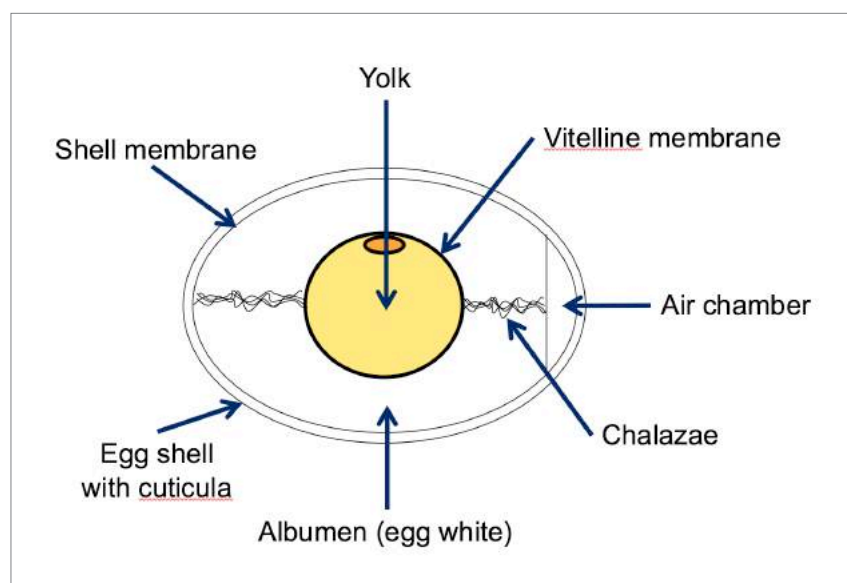


Figure 2: Structures of the chicken egg

is highly expressed on the oocyte membrane and are subsequently endocytosed into the yolk. This receptor has been studied in some detail. In addition to VLDL it transports proteins and protein associated vitamins. Mutations of the LR8 receptor have been observed in hens causing hyperlipidemia and a loss of fat transfer into the oocyte leading to infertility (5).

Immunoglobulin Y (IgY) is the only major yolk protein not being synthesis in the liver. It originates from plasma cells in lymphoid tissue which differentiate from B-lymphocytes during immune responses (6). IgY is actively transported from the blood into the oocyte a process mediated by a specific receptor in the oocyte membrane. The receptor is a member of the mannose receptor family and called FcRY (7). As a consequence of the receptor mediated transfer IgY composition in the yolk precisely reflects blood IgY composition. Up to 100 mg of IgY can be found in the yolk which provides maternal antibodies to the developing embryo and hatchling. In addition, this IgY is a rich source of antibodies for diagnostics and therapy.

Ovulation

15-60 minutes after oviposition the next mature follicle ovulates. Thus, the ovulation cycle takes nearly 24 hours in modern layer lines while it may take up to 27 hours in low producers. Ovulation is preceded by a surge of LH levels in the blood 4-6 hours prior to ovulation which is induced by increasing blood levels of progesterone secreted from the pre-ovulatory follicle. LH regulates the breakdown of connective tissue in the stigma area of the follicle, rupture of the follicle wall and release of the oocyte. The entire process of follicle maturation, hierarchal selection and ovulation has been studied in great detail and more comprehensive reviews including detailed descriptions of regulatory networks can be found in (2,4).

A consequence of the high ovulatory rate in layers is the frequent formation of ovarian tumors. 30-35% of hens older than 2.5 years develop adenocarcinomas (8). Since layers are usually not raised to this age ovarian cancer is rarely seen under commercial conditions.

Formation of the egg in the reproductive tract

Rupture of the follicle wall leads to the release of the ovum which is captured by the infundibulum and thus funneled into the reproductive tract (Figure 1). Fertilization, formation of the vitelline membrane and of the first layer of albumen of the egg takes place in the infundibulum. The majority of albumen is produced during the passage through the magnum and subsequently egg shell membranes are formed in the isthmus. The final step of egg formation, the production of the egg shell, takes place in the uterus or shell gland before the egg is released by oviposition. This entire process takes 24-27 hours with most of the time required for shell formation. The structures of the egg are shown in figure 2. As discussed, 15-60 minutes after oviposition the next ovulation takes place.

Infundibulum

The ovum resides up to one hour in the infundibulum. During this time a membrane similar to the zona pellucida of mammals is formed which separates the ovum from the albumen. Recent studies have shown that at least 137 proteins are involved in the formation of the vitelline membrane most of them with unknown functions. However, it is known that it contains high concentrations of antimicrobial peptides (β -defensin, lysozyme, proteases), thus forming an effective inner barrier against infection (9).

Magnum

Subsequent to the infundibulum the ovum moves through the magnum where albumen formation proceeds during the next 3-4 hours. The main constituent of albumen is water with 88%. 90% of the dry matter are proteins, 6% are minerals and 3.5% is free glucose. In contrast to the yolk proteins, proteins of the albumen are

not produced in the liver but by the mucosa of the magnum. The tubular glands secrete ovalbumin, ovotransferrin, ovomucoid and lysozyme, while goblet cells provide ovomucin and avidin. Synthesis of these proteins is regulated by estrogen and progesterone and continues even in the absence of a forming egg. In contrast, release of the preformed proteins is induced by mechanical stimulation as the forming egg moves down the magnum and is independent of endocrine or neuronal regulation. Albumen is well known for its antimicrobial properties caused by numerous peptides including lysozyme, avidin, cystatin and several defensins (10). At the same time chalazae form a process that starts early in the infundibulum. They are made up of protein fibers which connect the yolk and the shell membrane and thus ensure the central position of the yolk. Due to the rotation of the forming egg during the passage down the magnum the fibers wind to form the characteristic structure.

The albumen is not homogenous but forms areas of different viscosity with a more liquid inner egg white, the thick egg white and chalazae and an outer more liquid layer. This final structure develops as the albumen becomes increasingly hydrated in the distal part of the magnum and the uterus a process mediated by solute secretion (sodium, calcium, magnesium and chloride) and passive water movement.

Isthmus

After transfer into the isthmus formation of the inner and outer shell membranes by a mesh of protein fibers is initiated. These membranes are closely spaced for the most part of the egg but segregate at the blunt end to form the air chamber. While they are impermeable for egg white

they permit the exchange of water, gas (in particular oxygen) and minerals. On their outer surface projections form which are called mammillary cores made up of proteins, carbohydrates and mucopolysaccharides. It is these structures where the formation of the egg shell is initiated.

Uterus (shell gland)

With the arrival of the egg in the uterus hydration of the egg white continues. Water is pumped across the shell membranes which lead to a volume increase and the development of the characteristic ovoid form. Consequently, the forming egg gets in close contact with the uterine wall permitting efficient formation of the egg shell. This process takes up to 19 hours and is characterized by the secretion of an organic matrix of glycoproteins and mucopolysaccharides which becomes calcified to form the egg shell (11). Calcification requires approximately 2 to 2.5 g of calcium per egg which forms calcite crystals, a highly stable form of calcium carbonate (CaCO_3). The crystalline structures grow up from the mammillary cores to form into highly organized palisades giving the unique strength to the egg shell. Layers producing 300 eggs per year require nearly 1.8 kg of calcium matching their own body weight. 60-80% of the calcium is absorbed from the intestine during shell formation if hens are provided with the recommended 3.6-4% of calcium in the feed. The remaining 20-40% of calcium required is mobilized from the bone. Enteral resorption of calcium is regulated by 1,25-dihydroxyvitamin D3 while mobilization of calcium from the bone is under control of 1,25-dihydroxyvitamin D3 and parathyroid hormone (PTH). Calcium mobilized from the bone will be replenished when the uterus is empty and calcium absorbed in the gut is fully available for bone matrix formation. Importantly,

hens develop a unique form of bone matrix called the medullary bone. Two weeks prior to lay a meshwork of interconnecting spicules develops under the control by estrogen and testosterone in the long bones. This structure accounts for up to 12% of the total bone calcium but provides little support for bone stability. However, mobilization of medullary bone is 10-times faster than that of cortical bone and thus can quickly make up for a lack of calcium resorption from the gut (4).

To form the egg shell calcium and bicarbonate (HCO_3^-) must be secreted into the uterine lumen to form the calcite crystals (CaCO_3). Calcium is taken up by epithelial cells in the uterus from the blood by a Ca^{2+} -channel and transferred by a Ca^{2+} -binding protein (calbindin 28K) to the apical membrane where it is actively pumped into the uterus lumen against a concentration gradient. Several Ca^{2+} transporters have been described and recent proteomic studies have added new candidates to the list (12).

To maintain a low Ca^{2+} concentration in the epithelial cytoplasm which is critical for cellular homeostasis Ca^{2+} is not only bound to calbindin but also transported into cellular storage compartments (endoplasmic reticulum) from where it can quickly be released during active shell formation. HCO_3^- is generated from water and carbon dioxide (CO_2) in the cell and transferred into the uterus lumen by a transporter secreting HCO_3^- and taking up Cl^- in exchange. In the lumen Ca^{2+} and HCO_3^- react to calcite crystals to build the mineralized shell matrix.

The final step prior to oviposition is the deposition of a waxy membrane on the egg shell called the cuticula. It is composed of

polysaccharides, lipids and more than 50 proteins many of them with antimicrobial activities. Accordingly, its primary function is to prevent penetration of microbes into the egg and to limit the loss of water from the egg (13).

Oviposition

This process is initiated by the relaxation of the uterine sphincter and the contraction of uterine smooth muscles. It is regulated by neurohypophysial hormones, primarily oxytocin and arginine-vasotocin as well as prostaglandins (e.g. PGF₂α) synthesized locally within the reproductive tract. Within the next 60 minutes after oviposition the next ovulation will take place.

Conclusion

Egg morphology, composition and formation have been studied intensively for more than a century and a large body of literature is available. However, many of the regulatory circuits are only partially understood. New technologies such as next generation sequencing and proteomics (12, 14, 15) may help to gain new insights into the multiple steps of egg formation and the precise composition of yolk, albumen, egg membranes and the egg shell. Reverse genetics are also progressing in the field of avian biology and should finally enable functional in vivo studies of selected genes (13).

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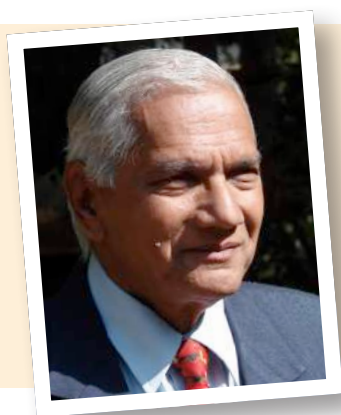
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Antimicrobial Resistance: a Threat to Livestock Production and a Potential Risk for Public Health

Abstract

The widespread and indiscriminate use of antimicrobials has been identified as one of the main reasons for the emergence of antibiotic resistant pathogens. Many of them are multidrug resistant. Antimicrobial resistance continues to expand for a number of different reasons, but the farm animal industry in many countries is forced by public health programs to reduce and eliminate antibiotics in animals as growth promoters. Susceptible bacteria can become resistant in two ways, by genetic mutations, and by acquiring resistance genes from other bacteria. The mechanisms have been discussed. Numerous studies have demonstrated that routine use of antibiotics on the farm promotes drug-resistant superbugs. Threat to public health from the overuse of antibiotics in food animals is real and growing. Humans are at risk both from potential presence of superbugs in meat and poultry and to their migration into the environment, where they can transmit their genetic immunity against antibiotics to other bacteria, including those that make people sick. A simple way to overcome the problem is to stop adding antibiotics to animal feed. Alternatives to antimicrobials to treat or control infections are other important areas that need attention.

Keywords

antibiotic resistance, superbugs, livestock production, public health, growth promoters, food animals

Introduction

Antibiotic-resistant bacteria, difficult to treat, are becoming increasingly common and are causing a global health problem. The phenomenon of antibiotic resistance

is not new. Discoverer of penicillin Nobel laureate Alexander Fleming in his speech in 1928 had warned of resistance against antibiotic penicillin. Much before the antibiotics came into human use, bacteria

had harbored resistance genes (9). This suggests antibiotic resistance arises from an evolutionary process and therefore it cannot be stopped. However, efforts may keep it under control.

The widespread and indiscriminate use of antibiotics throughout the world has resulted in emergence of antibiotic resistant pathogens. Many of them are multidrug resistant (MDR) and pose a serious threat to human and animal health. Today, antibiotic resistance is viewed by the experts as one of the greatest threats to global public health. The international agencies World Health Organization (WHO), World Organization for Animal Health (OIE), and Food and Agriculture Organization (FAO) have called for united global efforts in curtailing indiscriminate and irresponsible use of antimicrobials in livestock, poultry, fishery, agriculture, and human medicine.

Antibiotic Resistance

Antibiotic resistance is the ability of microbes, such as bacteria, to grow in the presence of an antibiotic that would normally kill them, or limit their growth. This occurs when an antibiotic has lost its ability to effectively control or kill bacterial growth. The bacteria then become "resistant" and continue to multiply in the presence of an antibiotic.

Repeated and improper use of antibiotics, both in humans and animals, induces drug resistance and has made some forms of bacteria virtually indestructible to modern medicines (7). More than 70% of the bacteria that cause hospital-acquired infections are resistant to at least one antibiotic, and antimicrobial resistance is a serious threat to public health (6).

Antibiotic resistance continues to expand for a number of reasons, but mainly from the use of antibiotics in animals as growth promoters by the food industry. If animals carry resistant bacteria, then food produced from them may be colonized with these microorganisms. After ingesting these foods people may carry these resistant bacteria and even develop infections. In

poultry, antibiotics are used for three main purposes: therapeutic use to treat sick birds; prophylactic use to prevent infections, and as growth promoters to improve feed utilization and production.

Mechanisms of Antibiotic Resistance

When bacteria are first exposed to an antibiotic, those most susceptible die, leaving surviving bacteria to pass on their resistant features to the succeeding generations (11). Antibiotics cause a selective pressure by killing susceptible bacteria, allowing antibiotic resistant bacteria to survive and multiply (4). Selective pressure is the influence exerted by the antibiotics to promote one group of organisms over another. In this process natural selection follows the rule of survival of the fittest (11). The indiscriminate use of antibiotics in animals and humans has accelerated the pace of resistance against both pathogenic and non-pathogenic bacteria.

Bacteria develop resistance in two ways: (i) by genetic mutations (vertical evolution), and (ii) by acquiring resistance genes from other bacteria (horizontal gene transfer).

(i) Through Genetic Mutations (Vertical Gene Transfer or Vertical Evolution)

Susceptible bacteria can become resistant through mutations in their genes. Mutations are spontaneous changes in the genetic material (DNA). They are thought to occur in about one in one million to one in ten million cells (4). Through mutations bacteria acquire defense mechanisms against antibiotics. Different genetic mutations result in different types of resistance. For example, some bacteria have developed biochemical "pumps" that can remove an antibiotic before it reaches its target, while others have evolved to produce enzymes to inactivate the antibiotic (11).

Bacteria reproduce rapidly, sometimes in as little as 20 minutes. Therefore, it does not take long for the antibiotic resistant bacteria to comprise a large proportion of a bacterial population (11).

Once the resistance genes have developed, they are transferred to all the bacterial progeny during DNA replication. The phenomenon is known as "vertical gene transfer" or "vertical evolution".

(ii) Acquiring Resistance from Other Bacteria (Horizontal Gene Transfer)

Susceptible bacteria can become resistant also by acquiring antibiotic resistance genes from resistant bacteria. Development of resistance occurs by the exchange of small pieces of DNA called plasmids. Plasmids are extra-chromosomal circular molecules of DNA that replicate independently of the bacterial chromosome. Plasmids carry antibiotic resistance genes and have the ability to transfer themselves to other bacteria. They are called "resistance (R) plasmids" and act as vectors to transfer resistance genes.

Bacteria readily exchange plasmids among both related and unrelated species. This way the antibiotic resistance genes from one type of bacteria are incorporated into other bacteria. Non-resistant bacteria acquire resistance genes in three ways: (a) conjugation, (b) transduction, and (c) transformation.

a. Conjugation

Conjugation is the mechanism by which genetic material is transferred through plasmids between two bacterial cells. It requires cell-to-cell contact. A bacterial cell, containing an R plasmid, forms a mating pair with a bacterial cell that does not contain an R-plasmid. They undergo a mating process called "conjugation". By a complex

mechanism, plasmid containing resistant genes is transferred from the plasmid-containing bacterial cell (the donor) to the recipient. This enables the antibiotic susceptible bacteria express resistance as coded by the newly acquired resistance genes.

Most antibiotic resistance in Gram-negative bacteria is acquired through horizontal gene transfer. Genes, encoding antibiotic resistance, are transferred between bacteria via plasmids. Plasmids are often capable of self-movement (conjugation) from one bacterium to another. They are also highly stable once established in a bacterium. Among *E. coli* and *Salmonella enterica* alone, there are more than 30 plasmid types identified and this number continues to grow. Plasmids associated with multidrug resistance are primarily a concern among *E. coli*, *S. enterica*, and *Klebsiella pneumoniae*, although numerous other Gram-negative bacteria have been shown to possess multi-drug resistance-encoding plasmids (5).

b. Transduction

Transduction involves transfer of DNA from one bacterial cell (donor) to another (recipient) by bacteria-specific viruses (bacteriophages). It occurs between two closely related bacteria.

c. Transformation

Transformation occurs from the uptake of short fragments of free DNA from the surrounding medium by non-resistant bacteria. The free DNA is normally present in the surrounding medium from the death and lysis of other bacteria. Bacteria like *Streptococcus* are capable of natural transformation.

Obstacles in the Way

No new effective antibiotic has been developed in the past more than 20 years. Perhaps pharmaceutical companies are not investing required financial resources

for antibiotic drug discovery, because the profit margins have declined. Apparently the antibiotics are becoming ineffective at faster rate due to the emergence of antibiotic resistance, largely from abuse of these drugs. It takes almost 15-20 years to bring a new antibiotic in the market, and the return on investment is uncertain due to the development of new resistance.

Antibiotic Resistance in Livestock

Numerous studies have demonstrated that routine use of antibiotics on the farm promotes drug-resistant superbugs. This is because there are always some bacteria the drug can't kill, and these survive and proliferate (3, 9).

A simple way to overcome the health problems caused by antibiotic resistance is to stop adding antibiotics to animal feed. It was found that when poultry and beef are produced without antibiotics, bacterial resistance quickly declines. Feeding antibiotics to livestock creates an ever-increasing number of antibiotic-resistant bacteria, including many that cause disease in humans.

The US Food and Drug Administration (FDA) say that 80% of all antibiotics sold in the US are fed to food animals. Meat animals are fed antibiotics because doing so increases their weight gain, prevents disease, and makes meat production cheaper.

Many countries have already acted to curb antibiotic feeding to livestock. Most notably, the European Union banned feeding of all medically important antibiotics to livestock in 1998. This was followed with a total ban on all antibiotics in 2006.

Threat to public health from the overuse of antibiotics in food animals is real and growing. Humans are at risk both due to

potential presence of superbugs in meat and poultry and to their migration into the environment, where they can transmit their genetic immunity against antibiotics to other bacteria, including those that make people sick.

Prevention and Control of Antibiotic Resistance

A simple way to overcome the emergence of antibiotic resistance in bacteria is to stop adding antibiotics to animal feed. Ban on the use of antibiotics for growth promotion in some countries has indicated that they could be avoided without any significant impact in livestock production. The experiences from different countries suggest that major reductions can be achieved without significant negative effects on animal health or productivity, and for the long-term benefit of public, environmental, and animal health (10).

However, the veterinarians should still be allowed to use antibiotics to treat animals in case of diseases, but only drugs which are not used for humans; and all treatments should be documented to allow retrospective analysis of field data.

Alternatives to antibiotics to treat or control infections are other important areas that need attention. This may include acidifiers, enzymes, prebiotics and probiotics, boosting host's immune system, and certain other approaches to control infectious agents.

The feed industry, in the meantime, must offer other options to keep animals as healthy as possible.

Improving hygiene and sanitation counter infectious diseases, and is crucial to reducing the rise in drug resistance. The less people get infected, the less they need to use medicines such as antibiotics, and the

less drug resistance arises (9). Improving the general hygiene in all stages of production and thereby reducing the microbial load on food products will also reduce the antimicrobial resistance load (10).

Sensitivity tests should be done to make sure the right antibiotic is prescribed. Also, antibiotics should be administered exactly as prescribed by the veterinarian. Doses should not be skipped and the prescribed course of treatment be completed even when the flocks start feeling better.

Certain essential oils can prevent the transmission of some drug resistance strains of pathogens, especially *Staphylococcus*, *Streptococcus*, and also *Candida*. Essential oils are extracted from different parts of plants such as eucalyptus, clove, tea tree, and lavender.

Herbal medicines have been and should be further explored as alternatives to antibiotics.

Discussion

Antimicrobial resistance in humans is inter-linked with antimicrobial resistance in other populations, especially farm animals, and in the wider environment (12). Resistance can pass between these different populations, and homologous resistance genes have been found in pathogens (12). Farm animals are an important component of this complex system. Despite attempts at reduction, they are exposed to enormous quantities of antibiotics and act as another reservoir of resistance genes. Whole genome sequencing is beginning to quantify the two-way traffic of antimicrobial resistance between the farm and the clinic. Surveillance of bacterial disease, drug usage and resistance in livestock is still relatively poor, though improving, but achieving better antimicrobial control on the farm is challenging (12). There are multiple links between the human, ani-

mal, and environmental compartments that allow not only movement of the bacteria but also of mobile genetic elements (MGEs) and the drugs themselves. The challenge of antimicrobial resistance has even been viewed as somewhat similar to climate change (12).

There is a risk resistant bacteria could pass into the food chain (2). The food borne route is the major transmission pathway for resistant bacteria and resistance genes from food animals to humans (10). Recently a number of antimicrobial resistant pathogens have emerged in the food-production chain, which can transmit to, and cause infections in, humans (10). Farmers need to drastically cut the amount of antibiotics used in agriculture, because of the threat to human health. The use of antibiotic in agriculture is driving up levels of antibiotic resistance, leading to new "superbugs" (2, 3). A ban on the use of growth promoters was implemented throughout the EU in 2006. However, this has not led to any consistent decrease in antibiotic consumption (12). In Europe, the volume of agricultural usage of antibiotics continues to rival that of medical usage and in the USA agricultural usage exceeds medical usage (12). Reducing the levels of antimicrobial consumption in farm animals has not proved straightforward, as the experience of the EU-wide ban on growth promoters has shown (12). Industrial agriculture relies heavily on the widespread use of antimicrobials to improve animal health, welfare and productivity. A complete ban on the use of antimicrobials in farm animals would inevitably have serious repercussions for animal health, welfare and productivity. Growth promotion has become a particularly controversial issue (12).

However, the big question is if we were to ban the use of an antimicrobial drug in

farm animals what would be the impact on levels of resistance in human clinical cases, and to what degree antimicrobial resistance poses a threat to human health. We still are a long way from being able to give clear answers to that kind of question (12). There are surprisingly few published studies which directly address this question (12). The evidence suggests the amount of antimicrobials used in food production internationally is almost the same as that in humans, and in some places is higher. For example, in the US more than 70% of antibiotics that are medically important for humans are used in animals (2, 9). It is estimated that the volumes of antimicrobials used in food animals exceeds the use in humans worldwide, and nearly all the classes of antimicrobials that are used for humans are also being used in food animals, including the newest classes of drugs (10). The majority of scientists see this as a threat to human health (9).

More recently, a long-dreaded superbug has been found in human and animal in US (1, 8). A 49-year-old woman showed the presence of a rare kind of *E. coli* in a urinary tract infection, the first known case of its kind in the United States. It was resistant to many antibiotics, even colistin, which doctors use as a last resort when other antibiotics fail (1). That turned out to be because the organism carried 15 different genes conferring antibiotic resistance. One element included the new, dreaded gene *mcr-1* (8). There was no indication of how the bacteria got into the woman's system. She had not travelled outside the United States within the past five months. The discovery heralds the emergence of truly pan-drug resistant bacteria (8). In his exhaustive review on antimicrobial resistance, O'Neill (2016) describes this discovery of transferable colistin resistance highly disturbing (9).

The US Department of Agriculture believes this to be the first identification of the antibiotic resistance factor MCR in the United States in an animal. It was found in a stored sample of pig intestine (8). The existence of MCR was reported for the first time by British and Chinese researchers who said they had found the gene in people, animals, and meat in several areas of China. Subsequently it has been found in people, animals, or meat in at least 20 countries across the world (8). MCR is so troublesome because it confers protection against colistin. Being a toxic drug, colistin is seldom prescribed in humans (1, 8, 9). As it was used so infrequently bacteria had not adapted to it. But because it is effective, agriculture adopted it instead, using it widely for prevention of diseases in food animals. By the time the medical community discovered that it needed the drug back, resistance to colistin was already moving from agriculture into the human world. However, MCR has not as yet combined with other resistance genes into becoming a completely untreatable organism (8). Some last-resort antibiotics for humans are being used extensively in animals, and there are no replacements currently on the way (2, 9).

Recently, in a literature review, only 5% of the 139 academic papers identified argued there was not a link between antibiotic consumption in animals and resistance in humans, while 72% found evidence of a link (2, 9). The authors concluded these results provide enough justification to reduce the global use of antibiotics in food production to a more appropriate level.

O'Neill (2016), in his report and recommendations on tackling drug-resistant infections globally, states antimicrobial resistance is inevitable. This is because as people keep finding ways to kill the micro-

bes that infect us, those microbes, through evolutionary processes, will mutate to counteract them (9). If not tackled, antimicrobial resistance could have a devastating impact. The seriousness and magnitude of the problem cannot be over-emphasized.

Conclusions

Emergence of antibiotic resistance in bacteria is a serious and challenging problem facing the livestock industry. If not controlled, it may have a devastating impact on livestock health and production. Use of antibiotics on the farm also poses a risk to human health. Antibiotics can promote creation of superbugs which can contaminate meat and chicken that would make it difficult to cure diseases in humans. The alternatives must be explored.

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The role of developing and newly industrialised countries in global egg production

Abstract

The dynamics of egg production between 1993 and 2013 on the basis of four Country Development Groups is analysed in this paper. This term is used to distinguish countries according to their development status. It can be shown that such a classification makes more sense than an analysis on the basis of continents and countries. The classification allows the documentation of the imbalance between their share in global population and their contribution to egg production. The driving force behind the remarkable dynamics in the analysed time period were the newly industrialised or threshold countries. In 2013, they dominated global egg production with a share of 57%. A detailed analysis on the basis of the leading countries in each of the four groups makes it possible to identify countries with a fast growing production, those with a stagnating or even decreasing production volume. It can be shown that the regional concentration of production is very high in all four groups and that in particular in many developing countries absolute growth is still very low because of the development status, lack of capital and Know How and political instability.

Keywords

Laying hens, egg production, developing countries, newly industrialised countries, industrialised countries, regional distribution

Introduction

Compared to the large number of scientific publications dealing with the egg industry in industrialised countries, hardly any papers have been published so far on the role which developing and newly industrialised countries play in global egg production. The following paper will try to close this gap.

Classification of countries according to their development status

In this paper it will be distinguished between the following country development groups (CDG)¹

- Least developed countries (LLDC)
- Less developed countries (LDC)

- Newly industrialised countries (NIC)²
- Old industrialised countries (OIC).

In **table 1**, the contribution of the four CDGs to the global population, the number of laying hens and the volume of egg production is documented. In 2013, 12.5% of the global population lived in the LLDC, but they only contributed 2.0% to global

egg production. On the other hand, OIC only shared 15.2 % of the world population but contributed 24.3 % to the global egg production volume. In the LDC, the share of egg production was lower than that of the global population while in the NIC the contribution to the egg production volume was higher than to the global population.

Development of laying hen flocks

The global number of laying hens increased from 3.7 billion in 1993 to 7.0 billion in 2013 or by 88.6 %. The NIC contributed 69.0 to the growth of the global laying hen population between 1993 and 2013, the LDC 21.0 % and the LLDC 7.5 %. The lowest relative growth rates showed the OIC with only 2.5 %.

A closer look at the spatial dynamics reveals (Figure 1) that the OIC and the LDC lost shares of the global laying hen population in the analysed time period while the LLDC and the NIC gained 1.5 % respectively 10.1 % compared to their contribution in 1993. In 2013, the NIC countries were in an absolutely dominating position, mainly due to the 2.6 billion laying hens in China which contributed almost 38 % to the global laying hen population (see also Figure 2).

Development of egg production

Between 1993 and 2013, global egg production increased from 38.0 mill. t to 68.2 mill. t or by 79.5 %. The relative growth rate was lower than that of the number of laying hens. This is a result of the comparatively low laying rate of the hens in most of the developing countries. The highest relative growth rate showed the LLDC with 176.9 %, followed by the NIC with 123.2 % and the LDC with 87.7 %. Similar to the

Table 1 Contribution of the CDG to the global population, the number of laying hens and to egg production (%) in 2013 (Source: FAO database; own calculations)

CDG	Countries (Number)	Population (%)	Laying hens (%)	Egg production
LLDC	46	12.5	12.4	2.0
LDC	120	20.3	14.4	17.7
NIC	10	52.0	57.6	57.0
OIC	35	15.2	15.6	23.3
Total	211	100.0	100.0	100.0

dynamics in the laying hen population, OIC had the lowest relative growth rate with only 17.1 %, due to an already high production volume in 1993. The highest

absolute growth is found in the NIC with 21.3 mill. t, followed by the LDC with 5.6 mill. t and the OIC with 2.4 mill. t. The NIC contributed 70.7 % to the global growth

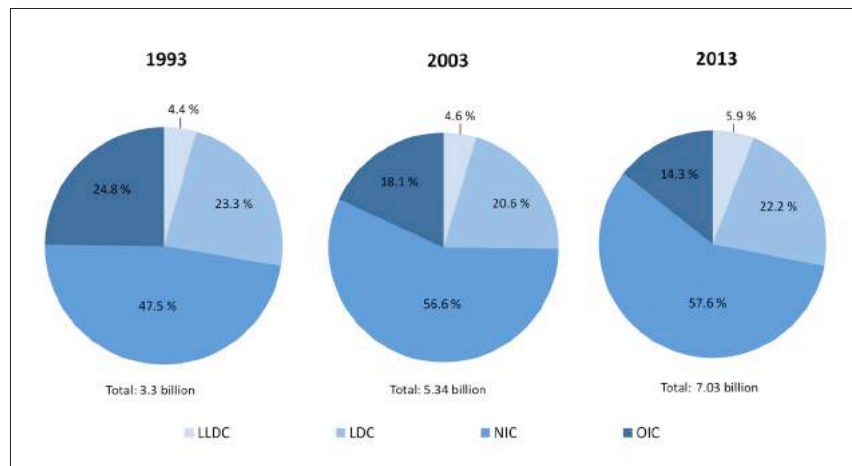


Figure 1 Contribution of the country development groups to the global population of laying hens, showing the significant increase in LLDC and NIC (2013) (Source: own calculations)

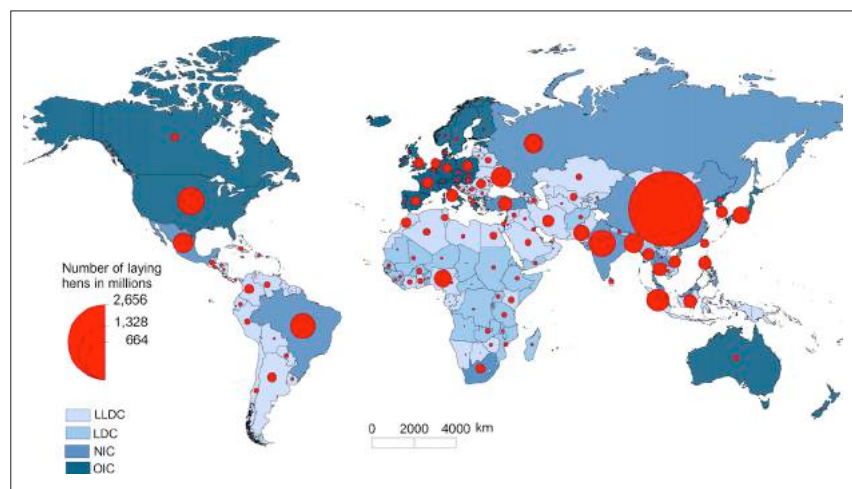


Figure 2 Global distribution of laying hens in 2013 in countries with different levels of development on country development base (Source: FAO database)

Table 2 Development of egg production in the Country Development Groups between 1993 and 2013; data in 1,000 t (Source: FAO database; own calculation)

Year	LLDC	LDC	NIC	OIC	World
1993	494	6,295	17,285	13,963	38,038
2003	773	8,430	29,934	15,203	54,339
2013	1,368	11,864	38,584	16,354	68,170
Increase (%)	176.9	88.5	123.2	17.1	79.5
Increase (1,000 t)	874	5,570	21,299	2,391	30,224
Share (%) of absolute increase	2.9	18.5	70.7	7.9	100.0

of egg production, LDC 18.5 % and OIC 7.9 %. Despite their high relative growth rate, LLDC shared only 2.9 % of the absolute

global growth. Nevertheless the developing countries contributed 21.4 % to the global growth in egg production in the

analysed time period in contrast to only 7.9 % of the old industrialised countries. This clearly documents in combination with the development in the NIC that the growth centres were no longer the OIC but the developing countries and in particular the NIC (Table 2).

The regional shift of the egg production centres from the OIC in Europe and North America to the LDC and NIC in Asia and South America is obvious (Figure 3). The winners were the NIC while the OIC lost about one third of their former share in global egg production. The spatial distribution of egg production in the CDG on country base is documented in Figure 4.

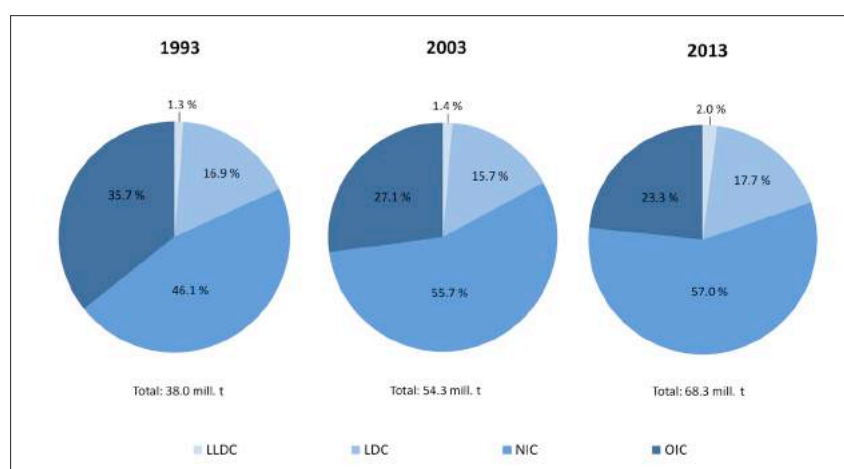


Figure 3 Contribution of the country development groups to global egg production (2013) (Source: own calculations)

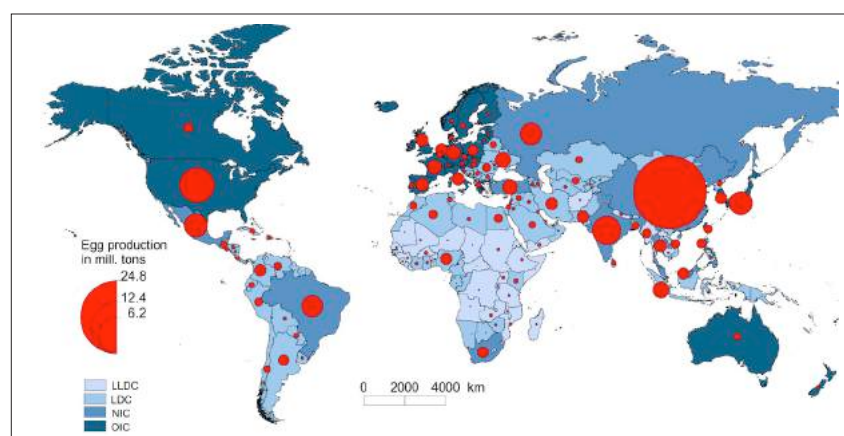


Figure 4 Global distribution of egg production in 2013 at country development base (Source: FAO database)

The main results of the first part of the analysis can be summarized as follows:

- ➔ The global laying hen population grew by 3.3 billion hens or 88.6 % between 1993 and 2013. LLDC and NIC showed the highest relative growth rates.
- ➔ While OIC lost 10.5 % of their former contribution to the global laying hen flocks in the analysed time period, NIC gained 10.1 % and LLDC 1.5 %. The LDC could not maintain their share of 1993 and lost 1.1 %.
- ➔ Global egg production increased by 30.2 mill. t between 1993 and 2013 and reached a volume of 68.3 mill. t in 2013.
- ➔ To the absolute growth NIC contributed 70.8 % and LDC 18.7 %. In contrast, OIC only contributed 7.2 % and LLDC 2.9 %.
- ➔ OIC lost 12.4 % of their share of the global egg production volume in the analysed time period; NIC won 10.9 %, mainly due to the remarkable growth of egg production in China and India. These two countries contributed almost 42 % to the global production volume in 2013.
- ➔ It is obvious that the production centres of egg production shifted from Europe and North America to Asia and Latin America.

Table 3 Contribution of the ten countries with the highest egg production in the CDG of the LLDC and their contribution to the overall egg production in their CDG and to global egg production (2013) (Source: FAO database; own calculations)

Country	Egg production (1,000 t)	Share (%) of CDG	Share (%) of global egg production
Myanmar	382.0	27.9	0.55
Bangladesh	287.0	21.0	0.42
Yemen	67.3	4.9	0.10
Burkina Faso	60.0	4.4	0.09
Zambia	55.0	4.0	0.08
Uganda	47.0	3.4	0.07
Mozambique	45.0	3.3	0.07
Nepal	43.7	3.2	0.06
Enthiopia	41.0	3.0	0.06
Sudan	38.	2.8	0.06
10 countries	1,066.5	77.9	1.56
LLDC total	1,367.5	100.0	2.00

The dynamics at country level

In the following chapter the four CDG will be analysed in more detail on the level of single countries.

Table 3 lists the ten least developed countries with the highest egg production in 2013. It is obvious that Myanmar and Bangladesh are the dominating two countries

Table 4 Contribution of the ten countries with the highest egg production in the CDG of LDC and their contribution to the overall egg production in their CDG and to global egg production (2013) (Source: FAO database; own calculations)

Country	Egg production (1,000 t)	Share (%) of CDG	Share (%) of global egg production
Indonesia	1,223.7	10.8	1.79
Ukraine	1,121.4	9.9	1.64
Colombia	667.6	5.9	0.98
Iran	665.0	5.9	0.97
Nigeria	650.0	5.8	0.95
Pakistan	649.2	5.8	0.95
Argentina	539.2	4.8	0.79
Viet Nam	378.0	3.3	0.55
Peru	349.8	3.1	0.51
Algeria	347.3	3.1	0.51
10 countries	6,591.2	58.0	9.64
LDC total	11,357.3	100.0	16.53

in this group. Together they contributed 48.9 % to the overall egg production volume of the LLDC. But their share of global egg production was less than 1 %.

The dominance of African countries documents the low standard of egg production in this continent. Lack of capital and know how as well as the instable political situation in several of these countries explain the low production volume. As many of the laying hens are kept in backyard flocks, the laying rate is very low. The small farmers mainly use local breeds for egg as well as meat production. In addition, this housing system is a constant risk regarding the introduction and dissemination of Avian Influenza. As in most of these countries no effective veterinary system is available, poultry diseases can cause high mortality rates in the flocks.

The ten countries with the highest egg production of the 120 less developed countries are listed in **Table 4**. Two of these countries, Indonesia and Ukraine, had an egg production volume of 1.2 respectively 1.1 mill. t in 2013. Together they contributed 20.7 % to the total egg production of this CDG. Five countries with more than 500,000 t of egg production followed on ranks 3 to 7. They shared 28.2 % of the production volume of this development group. The regional concentration of egg production in this CDG is very high. The seven leading countries shared 48.9 % of the overall production volume of the CDG, the ten listed countries 58.0 %.

In contrast to the LLDC, in most of the LDC, laying hens are kept in conventional cages, at least on market oriented egg farms. They also use hybrid hens with high laying rates and compound feed. But in almost all countries backyard flocks still play a major role on small farms in rural areas.

Table 5 Contribution of the NIC to egg production in their CDG and to global egg production (2013) (Source: FAO database; own calculations)

Country	Egg production (1,000 t)	Share (%) of CDG	Share (%) of global egg production
China	24,787.7	63.7	36.3
India	3,835.2	9.9	5.6
Mexico	2,516.0	6.5	3.7
Russian Fed.	2,283.6	5.9	3.3
Brazil	2,171.5	5.6	3.2
Turkey	1,031.0	2.6	1.5
Thailand	668.0	1.7	1.0
Malaysia	664.4	1.7	1.0
South Africa	540.0	1.4	0.8
Philippines	427.7	1.1	0.6
NIC total	38,925.3	100.0	57.0

Outbreaks of highly infectious poultry diseases, such as Avian Influenza, are a constant risk not only for the backyard flocks but also for the modern egg farms.

Newly industrialised countries are a group of ten countries in which a shift from a

society in which the primary production sector dominated to a society in which the secondary production sector has the highest share of the gross national product. This shift occurred in the late 20th and the early 21st centuries. The standard of living is higher than in the LDC and LLDC. They

contributed 70.8 % to the growth of global egg production between 1993 and 2013 and shared 57.0 % of the overall global egg production volume in 2013. **Table 5** reveals that within this group China is in a dominating position with a contribution of 63.7 % to the egg production in this CDG and 36.3 % to global production. It is worth mentioning that Turkey's egg industry has grown considerably over the past decade and was already the second most important egg exporting country behind the Netherlands in 2013.

In **Table 6**, the ten leading egg producing countries in the group of OIC are listed. In 2013, they contributed 85.4 % to egg production in this CDG and 24.3 % to global egg production. Because of the already high production volume in 2013, the absolute as well as the relative growth rate was comparatively low. The USA and Japan shared almost half of the total egg production in this development group.

Winners

In the final part of this overview, countries with the highest increase in egg production between 2000 and 2013 are documented.

Table 7 shows that of the ten countries with the highest increase between 2000 and 2013 four were located in Asia, four in the Americas and two in Europe, when one counts the Russian Federation and Ukraine as European countries according to FAO classification. The highest absolute increase had China with 5.9 mill. t, the highest growth rate Ukraine with 125.6 %. The four Asian countries contributed 8.6 mill. t or almost 50 % to the global increase in the analysed time period.

The main results of the second part of the analysis can be summarized as follows:

→ The regional concentration in the four

Table 6 Contribution of the ten leading OIC to egg production in their CDG and to global egg production (2013) (Source: FAO database; own calculations)

Country	Egg production (1,000 t)	Share (%) of CDG	Share (%) of global egg production
USA	5,636	34.2	8.3
Japan	2,522	15.3	3.7
France	944	5.7	1.4
Germany	893	5.4	1.3
Italy	775	4.7	1.1
Spain	743	4.5	1.1
Netherlands	703	4.3	1.0
UK	672	4.2	1.0
Korea, Rep. of	615	3.7	0.9
Poland	558	3.4	0.8
10 countries	14,061	85.4	20.6
OIC total	16,613	100.0	24.3

Table 7 Ten countries with the highest increase in egg production between 2000 and 2013; data in 1,000 t (Source: FAO database; own calculations)

Country	2000	2013	Increase (1,000 t)	Increase (%)
China	18,912	24,788	5,876	31.1
India	2,035	3,835	1,800	88.5
Mexico	1,788	2,562	774	43.3
Brazil	1,509	2,172	663	43.9
USA	4,998	5,636	638	12.8
Ukraine	497	1,121	624	125.6
Indonesia	642	1,244	602	90.7
Russian Fed.	1,895	2,284	389	20.5
Pakistan	344	649	305	88.7
Colombia	386	668	282	73.1
10 countries	33,006	44,959	11,953	34.8
World	51,046	68,262	17,216	33.7

CDG differed considerably.

- In the group of the LLDC, Myanmar and Bangladesh shared 48.9 % of the total egg production. In the group of LDC, no such dominance could be observed. Here, the two leading countries, Indonesia and Ukraine, contributed only 20.7 % to the production volume of the group.
- NIC not only showed the highest absolute growth in egg production between 1993 and 2013, it also had a very high regional concentration with China in a leading position with a share of 63.7 % of the group's egg production volume.
- In the OIC countries, the USA and Japan ranked in the first two positions with a contribution of 49.5 % to the overall production of this CDG.
- Regarding the increase of egg production between 2000 and 2013, Asian countries were in a dominating position.

Perspectives

The preceding analysis could show that

the LLDC and LDC despite their share of 32.5 % of the global population only contributed 19.7 % to global egg production. The relative growth rates were much higher than in the OIC countries. The absolute growth in LDC with 5.6 mill. t between 1993 and 2013 was the second highest behind the NIC. The NIC were the main driving force behind the remarkable growth of the global production volume with an absolute increase of 21.4 mill. t or a contribution of 70.8 % to the absolute global growth. It can be expected that this group will be able to fasten its position in the coming years because of a growing demand in several of the countries, especially the member countries of the BRICS group. The success of egg and poultry meat production in China can perhaps be an example for other threshold and developing countries with a widening middle class and a rising purchasing power. Even though the LLDC had the highest relative growth rate, they will not be able to increase their production volume considerably because of the instable political situation in many

countries, the lack of capital and of Know How as well as the dominance of backyard flocks. The contribution of the OIC to global egg production will further decrease despite the absolute growth in most of the countries. The increase will mainly result from a growing population and not from a fast growth of the per capita consumption, as it is already comparatively high. Trade with shell eggs will also in future be much smaller than that with chicken meat, as they cannot be frozen. This is the reason for the dominance of regional production and trade over comparatively small distances.

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Improvement of robustness and performance in meat-type chickens and ducks by short-term temperature training in the hatcher

Abstract

The thermoregulatory system plays a central role in various body functions of the organism. It not only keeps the body temperature on an optimum level but also interacts with feed intake, growth rate, immune and stress responses. Perinatal temperature training (PTT) with short-term warm loads (+ 1°C, maximum 2 hrs/day) from day 18 until hatching, has shown long lasting effects on different traits. PTT decreased oxygen consumption during final embryonic development, improved hatching performance, changed the secondary sex ratio in favor to male chickens, and improved performance during the post-hatching growing phase by better weight gain and feed conversion. Improvement of hatching results and post-hatching performance was found in small and large scale experiments. Further, long-lasting changes in physiological parameters (HLR, metabolic hormones T3/T4) indicate better stress response through PTT. Similar results have been found in ducks with short-term cold training. It is assumed that PTT during the last days of hatching reduces the metabolic rate. Hence, the birds have more energy available for performance as well as for adaptation, immune and stress responses during environmental challenges. In conclusion, PTT improves robustness and production efficiency. It is a practicable epigenetic tool, in chickens and ducks which enables realization of the genetic determined performance. Through better adaptation to changing environmental conditions PTT also contributes to the bird's welfare.

Keywords

Incubation, broilers, ducks, perinatal temperature, adaptation, performance

Introduction

According to the Agricultural Outlook 2020 poultry meat will be the largest meat sector in the world (OECD/FAO, 2014). Continuous genetic improvement in poultry breeds led to a fast development in

performance of commercial poultry lines. Broilers in particular are characterized by high growth and metabolic rate already during embryonic development (Janke et al.; 2004, Druyan, 2010; Buzala et al., 2015). High performance and increased metabo-

lic heat production result in relative low capacity of heat dissipation and increased susceptibility to stress under changing environmental conditions (Havenstein et al., 2003; Schmidt et al., 2009; Kalmar et al., 2013; Loyau, 2015). The higher risk of high yielding

poultry lines for behavioural, physiological and immunological problems is an important animal welfare issue. Improvement of robustness alongside with high production efficiency has top priority. Recent studies using short-term temperature stimulation during critical developmental periods in the hatching phase (perinatal temperature training, PTT) have shown positive effects on robustness, health and performance in fast growing chickens and ducks.

In the following we review the physiological background of this method and present results of small-scale and large-scale experiments.

Physiological background of PTT

How does temperature training improve robustness and production efficiency?

Temperature regulation plays a central role in the hierarchy of regulatory systems in homeothermic (warm-blooded) animals. The goal of temperature regulation is the maintenance of a constant body core temperature under most conditions. It is the prerequisite for optimum development and performance. The thermoregulatory system integrates different body functions. The close relationship between thermoregulation and other homeostatic physiological functions occur already on cellular level in the regulatory center of the brain, the hypothalamus. Hypothalamic neurons receive and process temperature, metabolic, osmotic and hormonal signals (Boulant and Silva, 1989; Hori, 1991). Hence, adaptation to temperature may also influence feed intake, body weight development, metabolism, immune and stress response, and reproduction (DuRant et al., 2012; Horowitz, 2014; Nassar et al., 2014). Finally, PTT may improve robustness in poultry (Boerjan, 2010; Tzschentke and Tatge, 2012).

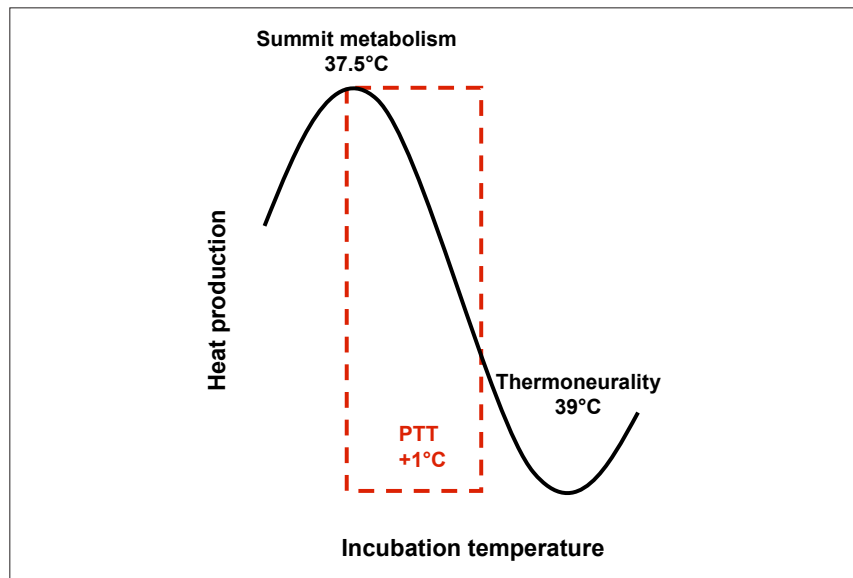


Figure 1: Schematic diagram of the relationship between oxygen consumption and incubation temperature of late term poultry embryos (PTT: perinatal short-term temperature training, temperature range)

Why is the hatching phase optimum for application PTT?

During the last days before hatching the embryo is prepared to respond to environmental stimuli. Physiological mechanisms have evolved from open loop to closed feedback control systems (Tzschentke and Plagemann, 2006; Tzschentke, 2007; Tzschentke and Rumpf, 2011). Closed feedback systems are a prerequisite of efficient PTT. During the perinatal period environmental influences can have long-lasting and obviously transgenerational effects (Tzschentke and Plagemann, 2006; Tzschentke et al., 2014, 2015a). Improvement of temperature adaptation and growth in relation to perinatally experienced chronic temperature changes were already demonstrated in chickens about 30 years ago from Minne and Decuypere (1984) and Decuypere (1984). In our own research in Muscovy ducks we found long-lasting effects of changes in incubation temperature during final embryonic development on post-hatching sensitivity of hypothalamic neuronal mechanisms, which were re-

lated to improved temperature adaptation and stress response (Tzschentke and Basta, 2002; Janke and Tzschentke, 2010).

Why is heat production declined by short-term mild warm PTT?

Mild short-term warm stimulation during final embryonic development decreased oxygen consumption and heat production in layer- and meat-type chicken as well as in Muscovy duck embryos (Nichelmann et al., 1994; Janke et al., 2002; El-Sabry and Tzschentke, 2010). Birds are naturally incubated under conditions of 'summit metabolism' (Nichelmann et al., 1994; Tzschentke and Rumpf, 2011). Heat production decreases with increasing incubation from the natural incubation temperature of about 37.5°C towards thermoneutral conditions of about 39°C (Figure 1). Above thermoneutrality heat production increases again. This shows that the response of heat production to changes in the ambient (incubation) temperature of the embryo is similar to that observed in the post-hatching and adult state (Tzschentke and Nichelmann, 1999; Nichelmann and Tzschentke, 2002).

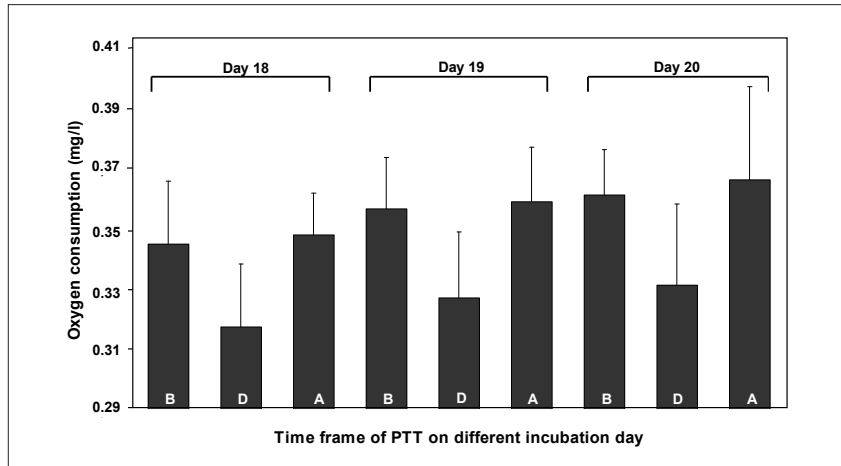


Figure 2: Influence of perinatal short-term temperature training (PTT, + 1°C for 2 hrs daily) on oxygen consumption of Cobb broiler embryos on incubation days 18, 19 and 20 (N=7). Columns represent mean values of oxygen consumption measured for 60 sec. before (B), during (D) and after (A) PTT (El Sabry, M.I., B. Tzschentke, 2010)

During a mild increase in hatching temperature heat loss mechanisms (e.g. respiration, blood circulation) are effective to keep deep body temperature constant (Holland et al., 1998; Nichelmann and Tzschentke, 2002, 2003; Tzschentke 2007). This could be demonstrated in experiments where deep body temperature was measured in the colon of the embryo (Holland et

al., 1998; Tzschentke 2007). We concluded that mild warm PTT decreased oxygen consumption and heat production in embryos of high yielding broilers (Figure 2). The daily PTT training during ‘critical period’ in the development of thermoregulatory system and homeostatic functions. The long-lasting result would be a lower

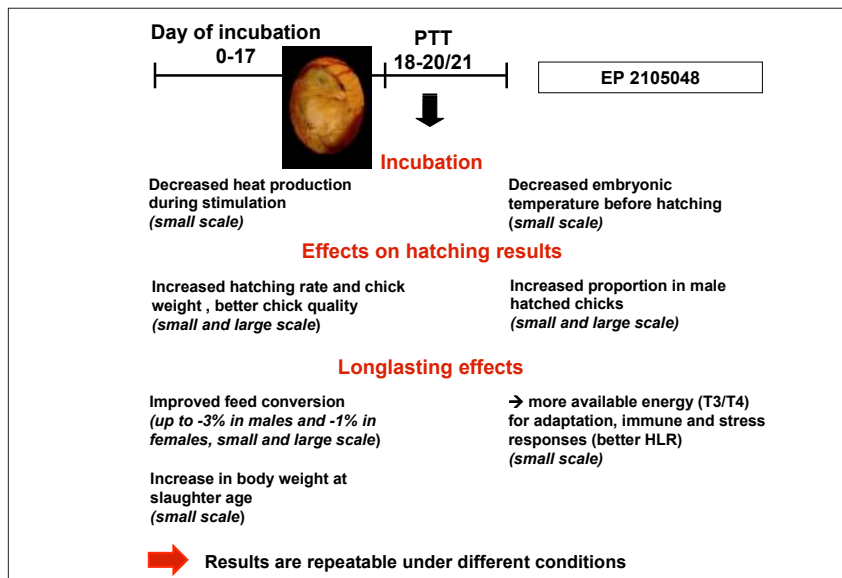


Figure 3: Summary of the results of perinatal short-term temperature training (PTT) on robustness and performance in broiler chickens from small and large scale experiments (results from large scale experiments, Elmehdawi et al., 2015, 2016; image ©Pasreform Hatchery Technologies)

basic metabolism, which enables better weight gain and feed conversion rate. Under these conditions there is energy available to cope with stress and immune challenges. Thus, PTT improves robustness and performance.

Studies on PTT in broilers in small and large scale tests

In small scale experiments (about 1000 eggs per treatment) with Ross 308 broilers (Tzschentke and Halle, 2009) we demonstrated for the first time that PTT (maximum + 1°C, 2 hrs/day) increased hatching rate and body weight of hatched chicks, and changed the secondary sex ratio (percentage of vital male and female chicks hatched in relation to total chicks hatched) (Figure 3). Short-term warm stimulation improved hatchability by more than 1.5% and was associated with a significantly higher proportion of hatched male chickens. In subsequent growth trials a better feed conversion mostly along with better body weight gain up to the slaughter age (35 days) were observed (Tzschentke and Halle, 2009). The positive effects were preferentially found in males (Table 1). Similar effects have been reported by Elmehdawi et al. (2015) in a large scale experiment (incubators for 42,240 eggs) using a stimulation of + 0.5°C for 2 hrs per day from 18th day of incubation until hatching. The proportion of vital hatched male chickens was significantly increased by 2.2 to 3.5% in response to PTT. Examination of the unhatched eggs confirmed the hypothesis that PTT improves the robustness already of the embryos, and especially in the males. The percentage of unhatched male embryos was significantly higher in the control group. Similar effects were found if PTT of +1°C was applied, even the increased percentage in male hatched chickens was not statistically significant higher (Elmehdawi et al., 2016). However, long-term effect of

Table 1: Performance of normally incubated (Control) and perinatal temperature stimulated (4 days, 2 h per day, + 1°C) broiler chickens (n=120/group/sex; Tzschentke and Halle, 2009)

Group Age, days	1 ¹⁾ Control		2 ¹⁾ Short-term warm stimulated	
	Male	Female	Male	Female
Feed intake, g/broiler/day				
1 – 14	35.0 a	33.9 b	35.9 a	33.9 b
15 - 21	89.9 a	81.1 b	92.5 a	81.4 b
22 – 35	155.3 a	128.4 b	156.3 a	127.3 b
1 - 35	93.3 a	80.8 b	95.2 a	80.7 b
Feed to gain ratio, kg/kg				
1 – 14	1.22	1.24	1.22	1.24
15 - 21	1.35	1.39	1.33	1.37
22 – 35	1.65 ab	1.66 a	1.60 c	1.62 bc
1 – 35	1.50 ab	1.51 a	1.47 c	1.49 bc
Body weight, g/broiler				
14	445	428	455	426
21	919 a	845 b	942 a	841 b
35	2270 b	1927 c	2336 a	1938 c
a; b; c; – Means within rows with one common letter do not differ significantly (P> 0.05)				

Table 2: Performance of normal incubated and perinatal temperature stimulated (6 days, 2 h per day; -10C) Pekin ducks (n=72/group/sex; Halle et al., 2012)

Group Age, days	1 ¹⁾ Control		2 ¹⁾ Short-term cold stimulated	
	Male	Female	Male	Female
Feed intake, g/duck/day				
1 – 21	110.9 a	102.5 c	105.bc	108.9 ab
22 – 49	262.0	243.9	241.6	258.7
1 - 49	197.2 a	183.3 b	183.1 b	194.5 ab
Feed to gain ratio, kg/kg				
1 – 21	1.61 a	1.62 a	1.55 b	1.64 a
22 – 49	3.23 a	3.32 a	2.88 b	3.49 a
1 - 49	2.60 b	2.65 ab	2.38 c	2.74 a
Body weight, g/duck				
1	54 b	55 b	58 a	56 b
21	1498 a	1386 b	1476 a	1448 a
49	3773 a	3449 b	3832 a	3526 b
a; b; c; – Means within rows with one common letter do not differ significantly (P> 0.05)				
¹⁾ 1- Control, 2- short-term cold stimulated				

PTT on performance was only found after PTT of + 1°C (Elmehdawi et al., 2016). In this experiment feed conversion was improved by about -2% which was similar to results from the small scale experiment (Tzschentke and Halle, 2009).

First experiments on stress response showed that PTT treated chickens had a significantly lower heterophil to lymphocyte ratio (HLR) in comparison with the normally incubated control birds. During severe stress male broilers showed significantly higher thyroid hormone levels (T3/T4) in the blood, which demonstrate higher ability to mobilize energy for the stress response (Tzschentke et al., 2015b).

Perspectives of PTT application in other poultry species

In other poultry species as Pekin ducks short-term temperature training in the hatching phase was also tested. Whereas short-term mild warm stimulation as in the broiler chickens depressed hatching results in ducks, short-term cold stimulation (1°C below standard incubation temperature for 2 hrs per day) had long-lasting effects on the ducks performance (Halle et al., 2012). Short-term mild cold stimulation during the last days before hatching improved body weight gain and feed conversion during the growing period exclusively in male ducks. Further, at slaughter age male and female ducks from the short-term stimulated group reached statistically higher crude protein content in the breast meat compare to ducks of the control group (**Table 2 and 3**). Obviously, ducks need different temperature stimulation than chickens, because of their better cold tolerance and higher maturity of body functions as termoregulatory heat production already during final incubation (Sallagundala et al., 2006).

Table 3: Content (%) of water, protein and fat in the fresh matter of the breast meat of normally incubated and perinatally temperature stimulated (6 days, 2h per day; -10C) Pekin ducks (n=6/group/sex; Halle et al., 2012)

Group	1 ¹⁾ Control		2 ¹⁾ Short-term cold stimulated	
	Male	Female	Male	Female
Water	76.9 a	76.6 a	76.0 a	75.1 b
Crude fat	1.2 ab	1.0 b	1.3 ab	1.5 a
Crude Protein	21.9 c	22.4 bc	22.7 ab	23.3 a

a; b; c; – Means within rows with one common letter do not differ significantly (P> 0.05)

Conclusions

In conclusion, in broiler chickens robustness alongside with production efficiency can be improved by PTT as a practicable epigenetic tool. Advantages of an epigenetic method would be the relatively simple application of nature like changes in environmental factors during incubation as well as the short-term achievement of practice relevant results (already in the first generation). In chickens, short-term mild warm stimulation during a critical period of body temperature control development may program the basic metabolism on a lower level. This would enable better activation of environmental adaption processes, stress- and immune responses as well as better realization of the genetic determined performance potential. To improve the PTT protocols for other poultry species, more research on embryonic physiology as well as on its long-term effects is necessary.

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