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## Review Article

# Nutritional modulation of health, egg quality and environmental pollution of the layers



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## ABSTRACT

World egg production and consumption have been increasing for the past decades. Traditional strategies in poultry nutrition have made vital contributions to this great growth in quantity. However, current global issues should be considered in modern egg production such as growing populations and food security, food safety and quality, limited resources and environmental problems. The development of knowledge of poultry nutrition and modern biotechnology provides novel nutritional approaches to closely fit the requirement of pullets and laying hens, which will consequently decrease the nutrition excretion and maintain the lower cost of feed. Nutrition has also been widely accepted as a strategy to influence health and diseases of laying hens. The maintenance of good health is an important prerequisite for improving productivity and egg quality. In addition, there are many measures and strategies for minimizing the incidence of egg defects and providing a choice of lifestyle to enhance human health. This paper reviews current research progress on developing innovative technologies and strategies to maximize animal health and performance, improve the quality of egg products and minimize pollution caused by poultry production.

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## 1. Introduction

The poultry industry is one of the fastest growing animal industries globally. The world egg production reached 68.26 Mt in 2013, with an increase of 94.6% from 35.07 Mt in 1990 (FAOSTAT, 2015). Poultry meat and eggs have been suggested as the most important source of food protein for the world population for decades (Magdelaine, 2011). Together with the rapid growth of poultry industry, some global issues impacting on poultry production are becoming increasingly challenging. The world population is expected to reach 9 billion by 2050, which indicates that the demand for food will increase continuously in the upcoming decades. Meanwhile, food producers are experiencing greater

competition for limited land, water and energy sources (Godfray et al., 2010). As for the poultry industry, egg production is now hampered by a heavy shortage of feed ingredients. In addition, pollution problems of animal production show the trend of slowing down the rate of development because of the growing awareness of environmental protection. Thus, to meet the severe challenge, the egg industry should inevitably develop in a more sustainable way, aiming to maximize production at the lowest cost and slightest environmental impacts (Godfray et al., 2010).

Nutrition might hold the key to the problems mentioned above. The development of knowledge of poultry nutrition and modern biotechnology provide novel nutritional approaches to fit closely the requirement of pullets and laying hens, which will consequently decrease the nutrition excretion and maintain the lower cost of feed. However, birds are more susceptible to diseases, possibly due to the increasingly intensive metabolism for egg formation or weakened immune systems (Cherian, 2013), which makes it imperative to shift the focus to the health-maintenance effect of dietary nutrients. Thus, nutritional approaches aim at fully exerting the genetic potential on production with the priority of maintaining birds' health. In addition, consumer awareness should be considered in modern poultry production, and improving

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the quality of eggs is one of the major points of concern in this area. Egg quality is well known to be influenced by certain nutrients and dietary feed formulation. And insufficient or excessive nutrients in feed are responsible for poor-quality eggs. Nutritional approaches aiming to minimize the incidence of egg defects have been developed. Moreover, nutrients could successfully enrich the egg in some minor components of interest for human nutrition, and by this way the nutritional quality of eggs could be increased. With production of eggs with multiple beneficial properties, nutritional strategies have achieved success in providing a choice of lifestyle to enhance health and to increase psychological well-being.

This paper reviews the nutrient requirements of pullets and laying hens, nutritional modulation of egg quality and hens' health, and provides a useful reference for the study of nutrition strategies and sustainable development of laying hen production.

## 2. Nutrient requirements of pullets and laying hens

Laying hen nutrition can be precisely divided in various ways according to the demand of phase feeding and the characteristics of birds at different production periods. It is obvious that the onset of lay is the determining turning point of laying hen nutrition, because the biological characteristics and the production purpose of these 2 phases are totally different: for pullets, the aim of nutrition is to build the solid basis for the future production, and can be detailed as guaranteeing the least mortality, optimum health status, proper sexual mature time, and controllable flock uniformity; whereas for laying hens, it is for optimized performance, most prolonged peak period, and optimum immune functions. Accordingly, the studies and recommendations for these 2 phases are significantly different.

Pullet stage is the most rapid growing period of the hen's whole life. Birds at this period have polytropic developmental features, which demand the requirement parameters to be changing correspondingly. NRC (1994) divided the growing phase of pullets into 4 stages by age, i.e., 0 to 6 wk, 6 to 12 wk, 12 to 18 wk, and 18 wk to the age at the first egg. Nevertheless, different countries and enterprises have their own standards in time division. For example, Chinese Feeding Standard of Chicken (Ministry of Agriculture of the People's Republic of China, 2004) recommends a strategy of 0 to 8 wk, 9 to 18 wk, and 19 wk to onset of lay. In the initial post-hatch phase, newly-hatched chicks can utilize the nutrients from the yolk sac for 72 h (Noy et al., 1996), and afterwards shift to entirely exogenous nutrient ingestion. Thus, in this period, the quality of the feedstuffs is of great importance. Dietary energy level is not the first determining factor to affect the laying performance (Babiker et al., 2011), but higher energy level can increase the body weight at the onset of lay (Hussein et al., 1996). Although there are some studies showing that high level of dietary apparent metabolizable energy can improve laying performance (Bornstein and Lev, 1982), it is agreed that energy should be considered on the basis of reasonable protein supply, or be dealt with using statistical modeling with protein (Kuhi et al., 2012). Physiologically, internal organs, bones and muscles grow rapidly in the pullet phase, thus, pullet diets should firstly provide adequate high quality protein to meet the need of the pullets, thus, protein is the most important factor determining the quality of pullet diet. Leeson et al. (1998) reported that, even though the ratios among the essential amino acids (EAA) were kept unchanged, lowered dietary protein can decrease BW of pullet at the age of the first egg. Thus, dietary protein, for content or for sources, is considered prior to all the other ingredients. Besides, to guarantee the utilization of dietary protein, the balance of amino acids must be considered as a priority. However, with the development of laying hen genetics and breeding and the demand of animal health, these data need to

be updated. Song (2014) suggested that the requirement of methionine of Jing Brown pullets should be 0.49%, 0.42% and 0.29% at the age of 0 to 4 wk, 5 to 8 wk, and 9 to 17 wk, respectively, and similar results were also found on lysine. These values are significantly higher than those given by NRC (1994) and Chinese Feeding Standards of Chicken (Ministry of Agriculture of the People's Republic of China, 2004). In the last 2 decades, requirements of minerals and vitamins for pullets and laying hens were seldom studied. Guo et al. (2008) reported the metabolic alkalosis caused by high dietary calcium content (3.63%) on 5-wk-old pullets. Abdalla et al. (2009) reported that high level of vitamin A (24,000 IU/kg) did not affect the laying performance of young layers at 18 wk of age. Information on mineral and vitamin requirements should also be re-evaluated to satisfy the constantly increasing performance of layers.

As for nutrient requirement of laying hens, it attracts increasing interests to consider the interaction of different nutrients, rather than consider them individually. Undoubtedly, the interaction of dietary protein and energy and the ratio of various AA still hold the central positions in this field. The study of Panda et al. (2012) on Dahlem Red laying hen showed the optimum requirement combination of 2,795 kcal/kg ME, 16% CP, 0.8% lysine, and 0.4% methionine during 28 to 40 wk of age. Hassan et al. (2013) presented a similar optimum combination with 2,750 kcal/kg ME and 16.0% CP. The balance of dietary energy and protein is universally recognized to be even more important than either one of them alone. In recent decades, these kinds of data are still emerging in large numbers. The interaction of AA (Novak et al., 2004; Figueiredo et al., 2012), the relation between AA and trace mineral (Neto et al., 2011), and calcium and phosphorus were also reported. These observations may definitely help establish more refined database that will more fully tap the potential of the birds.

## 3. Nutritional modulation of pullets and laying hens

### 3.1. Nutritional modulation of laying hens' health

In recent years, more and more nutritionists are exploring nutrients' additional benefits such as health-promoting effects rather than their traditional values. Laying hens possess intensive metabolism for the formulation of eggs, thus, they may be more sensitive to adverse circumstances, which, might not be apparent. However, to guarantee the health of birds and consequently egg quality, special attention should be paid to the nutrients in maintaining reasonable immune functions. Antioxidants are a good example, and have long been used to alleviate oxidant stress to maintain health of laying hens in production. The most commonly used ingredients include vitamins E and C (Puthongsiriporn et al., 2001), and trace minerals like Zn, Cu, Mn and Se (Bulbul et al., 2008). Hosseini (2007) reported that antioxidant nutrients in the diet (i.e., vitamin C 200 mg/kg) can strengthen the immune response of laying hens against heat stress and increase egg quality. Bollengier-Lee et al. (1998) and Çiftçi et al. (2005) reported that dietary supplemented with 500 mg/kg vitamin E or 125 mg/kg vitamin E + 200 mg/kg vitamin C, can improve laying performance and egg quality for laying hens exposed to heat stress. All the dosages applied in these studies are far higher than ordinary nutrient recommendations. These observations imply that the recommendations of vitamins and other trace nutrients with complicated bioactivities should be re-reviewed.

Inclusion of some plants or plant extracts into hen's diet, such as resveratrol (Sahin et al., 2010), ginger root (Zhao et al., 2011), aqueous alfalfa (Deng et al., 2012) have also been reported to improve antioxidant status of laying hens. Much attention has been given to the exploration of new alternative antioxidants, evaluation

of their efficiency and safety, and investigation on the physiological and biomedical mechanism of both common and new antioxidants. Of the novel antioxidants, pyrroloquinoline quinone (PQQ) is a potentially effective substance for maintaining redox homeostasis in laying hens, as it exhibits the superior ability than vitamin E in prevention against oxidative stress caused by oxidized sunflower oil (Xu, 2012). Pyrroloquinoline quinone exerts this effect via increasing the activities of antioxidant enzymes by stimulating the transcription coactivator peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1 $\alpha$ ) and nuclear erythroid 2-related factor 2/antioxidant response element (Nrf2/ARE) pathway (Xu, 2012). Conjugated linoleic acid (CLA) is well known for its cancer-preventive and other nutraceutical properties in mammals. Recently, it has been found to enhance the antioxidant capacity of laying hens (Qi et al., 2011). Further investigations have disclosed the mechanism via a series of primary cell culture: t10, c12-CLA but not c9, t11-CLA can increase the activity of antioxidant enzymes, up-regulate the gene expression of antioxidant enzymes by activating the c-Jun N-terminal kinase-nuclear erythroid 2-related factor 2/antioxidant response element (JNK-Nrf2/ARE) signaling pathway, and finally enhance the antioxidant capacity of laying hens (Qi, 2013).

Nutritional modulation for laying hens also covers the prevention or alleviation of certain chronic diseases, especially fatty liver-hemorrhagic syndrome (FLHS). Fatty liver-hemorrhagic syndrome is a typical liver damage caused by oxidative stress on the basis of hepatic steatosis, usually occur in laying hens. It is quite common among cage-reared laying hens and its prevalence in the poultry industry of many countries constitutes a hidden threat to modern intensive poultry production. Dietary L-tryptophan, vitamins (B<sub>12</sub>, E or choline) and lecithin have been reported to alleviate FLHS in laying hens, but there are no effective methods to cure it (Wolford and Polin, 1975). Zou et al. (2007) reported that laying hens tend to exhibit fatty liver due to the damage caused by free radicals and excessive fat in the liver. Recently, PQQ was reported to regulate lipid metabolism and improve mitochondrial function. Studies in our laboratory are underway to quantify whether PQQ can reduce the incidence of FLHS in laying hens. Preliminary results indicate that PQQ can improve the activities of antioxidant enzyme, decrease the lipid content in plasma and liver and prevent mitochondrial injuries, so it has exhibited great potential for the prevention of FLHS in laying hens (Zhao, 2014).

### 3.2. Nutritional modulation of egg quality

Poultry eggs are one of the most attractive targets for nutrition modulation, owing to the extraordinary responsiveness of most of its properties to dietary factors. Diverse and special nutritional approaches are developed targeting the specific properties of eggs. The first concern of egg quality modulation is to reduce the defects of eggs, either on appearance or on flavor/taste. It is well known that macro minerals (calcium and phosphorus) act as eggshell structural component and play essential roles in eggshell function. However, dietary supplementation of trace elements, such as Zn, Cu and Mn, were found to improve eggshell quality, although the independent effect of Mn, Zn, or Cu was not clarified (Mabe et al., 2003). Two recent studies were conducted to investigate the mechanism of dietary Mn and Zn addition on eggshell quality (Xiao et al., 2014; Zhang, 2013). It showed that dietary Mn supplementation could improve eggshell quality by enhancing the glycosaminoglycans and uronic acid synthesis in the eggshell glands, which can affect the ultrastructure of eggshells (Xiao et al., 2014), promote the process of eggshell calcification, and improve layer's physiological status (Xiao et al., 2014). In another study, Zn was found to promote calcium deposition in eggshell gland by

elevation of carbonic anhydrase and osteopontin mRNA expression and increasing carbonic anhydrase activity, which contributed to the improvement of eggshell quality (Zhang, 2013). Consequently, these trace element requirements of layers to achieve optimal eggshell quality need to be reevaluated in the future. Another kind of approach focuses on improvements in egg nutritive values, which have been extensively studied in the last few decades. Attention given to modifying the fatty acid composition of eggs has mainly focused on the elevation of CLA and n-3 polyunsaturated fatty acids (PUFA), including  $\alpha$ -linolenic (ALA, C<sub>18:3</sub> n-3), eicosapentaenoic (EPA, C<sub>20:5</sub> n-3), docosapentaenoic (DPA, C<sub>22:5</sub> n-3) and docosahexaenoic (DHA, C<sub>22:6</sub> n-3) acids. One way to produce n-3 PUFA-enriched eggs is through enrichment of the diet with ALA derived from flaxseed, canola, hempseed, etc., and the other one is supplementation with fish oil or fish meal, which is the major dietary source of DHA (Yannakopoulos, 2007). Enrichment of n-3 PUFA content/concentration in eggs by using different sources is reviewed by Yannakopoulos (2007), Sirri et al. (2011), and Fraeye et al. (2012). Among these approaches, the use of flaxseed and fish oil as sources of n-3 PUFA has been extensively examined. It is possible to increase the n-3 PUFA content by more than 10 fold by feeding hens with 5% to 20% of flaxseed, with the maximum level of n-3 PUFA in eggs reported as 415.4 mg/50 g whole egg (Bean and Leeson, 2003). But at the supplementation level of equal or less than 5%, the n-3 PUFA content did not reach the threshold of 300 mg/egg (Scheideler and Froning, 1996; Nain et al., 2012), which is required for labeling the egg as a source of n-3 PUFA (CFIA, 2003). The transfer efficiency of total n-3 PUFA from flaxseed diet to egg yolk was reported to be 30.5% at the addition level of 3.75%, and 22.2% at the level of 5% (Nain et al., 2012). With the increase in ALA and n-3 PUFA content in eggs, there is a decrease in n-6 PUFA content due to the competition of substrates and biosynthesis enzyme (Jia et al., 2008). Consequently, the ratio of n-6/n-3 PUFA could be decreased to 1.29:1 to 2.5:1 (Cherian and Sim, 1991; Ferrier et al., 1995; Nain et al., 2012). It was reported that enriching egg ALA content is much easier and more economic than enriching DHA in commercial production of n-3 PUFA enriched egg (Surai and Sparks, 2013). While the inefficiency of the conversion of ALA to DHA (0.2%) or long chain n-3 PUFA in healthy individuals Pawlosky et al. (2001) and Burdige and Calder (2005) indicated that bioactive function of n-3 PUFA enriched eggs with DHA may be more promising. Diets containing 3% to 4% fish oil resulted in about 180 to 250 mg DHA/egg, but the transfer efficiency decreased with the increase of addition level (Van Elswyk, 1997). However, as a dietary source of DHA, fish oil has its limitation of susceptibility to oxidation, which will eventually cause undesirable off-flavors in the enriched eggs (Gonzalez-Querra and Leeson, 2000). Thus, there is still much to be investigated to find the optimum way for n-3 PUFA enrichment in consideration of transfer efficiency and cost control.

Conjugated linoleic acid, a mixture of the isomers of linoleic acid, is beneficial to human health for its multiple properties of anti-obesity, antidiabetic, anticancer, antiatherogenic, and immunity improvement. The use of synthetic CLA in laying hen diets to produce eggs enriched with CLA has become a common practice in the table egg industry. Conjugated linoleic acid levels in egg yolks increase in a dose-dependent manner with dietary CLA addition (Shang, 2004). The maximum concentrations of CLA in yolk lipids occurred on 11 d after feeding the CLA-fortified diet (Chamruspollert and Sell, 1999). When dietary CLA was added at more than 5.0%, the content of CLA in the yolk would reach a plateau (Shang, 2004). In addition, the fatty acid composition of egg yolk is significantly altered by dietary CLA supplementation. A recent study in our laboratory showed that feeding CLA-enriched diets resulted in a significant increase in saturated fatty acids (SFA) and CLA,

whereas the proportion of monounsaturated fatty acids (MUFA) and non-CLA PUFA declined significantly. The proportions of linoleic acid, arachidonic acid, and DHA in egg yolk lipids were also reduced as dietary CLA increased (Qi et al., 2011).

Enrichment of eggs with some vitamins and minerals is obtained readily by supplementing the feed with corresponding nutrients. Increase in dietary iodine, selenium, vitamins E, D and A is acknowledged to promote a more than 1.5-fold increase in yolk contents. Nevertheless, previously publications reported vitamin and trace mineral contents in egg varied with the different level in hen feed and administration time. Furthermore, both the efficiency of hens in transferring dietary vitamins to eggs (Naber, 1993) and the response of egg vitamin content to dietary changes varied with vitamins. Vitamin A content of eggs responds more slowly than riboflavin to dietary level, due to the intermediate storage of vitamin A in the liver (King'ori, 2012). For niacin, thiamine, or pyridoxine, the contents of eggs were reported to be insensitive to dietary manipulation (Leeson and Caston, 2003).

Another way to improve nutritional quality of eggs is to reduce the contents of some unwanted components that are considered by customers as health hazards for human. The high cholesterol content of egg, 195 to 250 mg/egg, which induces the rapid decline in per capita consumption of eggs, is a highly controversial topic. It is difficult to decrease the cholesterol content of egg efficiently by alteration of the laying hens' diet with various nutrients, natural products, non-nutritive factors, or pharmacological agents, as the obtained maximal reduction of less than 10% (Rg, 2006). A more recent pioneering finding suggests that daily egg consumption does not show adverse effects on cholesterol in the blood for the majority of people (Fernandez, 2010), but promotes favorable shifts in high density lipoprotein (HDL) lipid composition and function, beyond increasing plasma HDL cholesterol in metabolic syndrome (Andersen et al., 2013).

As discussed above, it is a potential way to improve egg quality by manipulation of hens' diet. However, there are still some questions in the process of improvement that we should consider. Firstly, the accumulation of nutrients in the egg yolk is not unlimited. Secondly, it is possible to produce designer eggs enriched with different nutrients individually or in specific combinations, rather than all nutrients simultaneously. Thirdly, the enrichment of egg nutrients may sometimes negatively affect general egg quality. An undesirable fishy flavor has been occasionally detected from n-3 PUFA enriched eggs or freshly laid eggs, particularly when flaxseed meal, rapeseed meal, or fish meal was used in a laying hen diet. In addition, dietary CLA supplementation was also observed to increase the firmness and height of egg yolk (Qi et al., 2011; Shang, 2004). Thus, more safe and efficient evaluation studies need to be carried out before such dietary supplements can be recommended for the production of functional eggs.

### 3.3. Nutritional approach to reduce poultry pollution

Poultry, together with cattle and pigs, are the major animal species that present serious environmental challenges for agriculture. About 82% and 64% of excess manure nitrogen (N) and phosphorus (P) are generated in association with poultry and egg production (Sims et al., 2005). Nutrient content in feces and urine is influenced by feed composition and feed conversion efficiency. Consequently, various dietary strategies are available or can be developed to minimize environmental pollution. One of the common approaches is the supplementation of certain feed additives aiming at improving the nutrient metabolism and utilization of laying hens. The use of phytase technology for degrading

phytate to release P and other nutrients from plant ingredients has been reported to reduce the excretion of both N and P of laying hens (Augsburger et al., 2007; Sebastian et al., 1998). However, the use of phytase preparations to improve performance and to reduce environmental P pollution has shown less than optimum results, partly due to the potential negative effects of dietary fiber components and to the confounding influence of inadequate knowledge of accurate P requirements and the tendency for the use of excessive safety margins in diet formulation (Slominski, 2011). In addition, diets containing acidifier ingredient (e.g., a mixture of natural zeolite and gypsum [EcoCal]) or inclusion of high-fiber ingredients (e.g., corn distillers dried grains with solubles [DDGS], wheat middlings, or soybean hulls) have been reported to reduce NH<sub>3</sub> emission considerably (Wu-Haan et al., 2007; Roberts et al., 2007). A two-year field study further confirmed that the EcoCal and DDGS diets led to an overall NH<sub>3</sub> emissions reduction of 39.2% and 14.3%, respectively compared to the control diet (Li et al., 2012). Another dietary approach is explored with nutrient reduction to decrease NH<sub>3</sub> formation. Recent advances have been made in decreasing dietary crude protein (CP) content of layer diets employing the ideal protein concept or ideal amino acid profile (Ji et al., 2014). The results indicate that excretion of nitrogen is significantly decreased by the protein-reduced diet (CP level reduced from 18% to 16%) without impairing body weight and production performance of laying hens (21 to 34 wk of age). Latshaw and Zhao (2011) also observed a corresponding decrease of manure N emission when daily protein intake decreased by 2 g per hen, whereas N excretion through eggs is not affected. However, Roberts et al. (2007) found no alleviation of NH<sub>3</sub> emission from layer manure when dietary CP decreased by 1.0%. This contradiction indicated that decreasing pollution by controlling nutrient ingestion requires far more than a simple shrinkage of dietary concentration. Again, the balance between energy and CP may be contributable to this phenomenon. Lowering dietary nutrient density is not only a way for pollution control, but also a way for saving feed resources, which is very promising, and it may be the new standards for future database establishment, given enough studies on proper evaluation methods.

## 4. Conclusion

The poultry industry has made tremendous growth and has brought about a revolution in animal husbandry and food supply over the last 20 years. The great improvement in the efficiency of the egg industry is due to the advance in breeding, management, disease control, and nutrition. Currently, the egg industry is facing new opportunities and challenges, and the efficiency of production is not the only emphasis as in the past. Society and consumer issues, environment pollution, and shortage of feed resources are the major concerns. Although nutritional strategies alone cannot address all challenges, the development of knowledge of poultry nutrition and modern biotechnology provide many measures and strategies for realizing sustainable development of egg industry. Great efforts are presently given into developing innovative technologies and strategies to maximize animal health and performance, and improve the quality of egg products, which are vital to develop feed industry in a sustainable manner. There will be a growing trend in egg industry in the near future.

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