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Effect of dietary phosphorus levels on meat quality and lipid metabolism in broiler chickens

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ABSTRACT

To analyze the influence of dietary phosphorus (P) levels on meat quality and lipid metabolism, a 42-day feeding experiment (P deficient group; normal group; high P level groups of H1 and H2, respectively) using 100 one-day-old broilers was conducted. Results demonstrated that the quality of broiler chicken meat in deficient or high P groups decreased relative to the normal group. High P diets resulted in increased lightness, redness values, shear forces and decreased fatty acid contents and intramuscular fat content in breast meat ($p < 0.01$). Compared with normal group, lower malic enzyme activity, higher fatty acid synthase and AMP-activated protein kinase activities were observed in the treatment groups ($p < 0.05$). Chickens fed with normal diets had the lowest serum total cholesterol and triglyceride levels which differed from that of other treatments ($p < 0.05$). High-P diets significantly decreased the lipid accumulation in the liver ($p < 0.01$), whereas phosphorus levels in breast meat increased significantly ($p < 0.01$). It can be concluded that deficient or higher P levels could affect meat quality and expression of indicators on lipid metabolism of broiler chickens.

1. Introduction

Chicken meat is considered a better choice for health diets because it contains less fat, as well as a higher proportion of polyunsaturated fatty acids (PUFA), when compared with meat from other species (Riovanto, Marchi, Cassandro, & Penasa, 2012). Due to growing concerns on the relationship between diet and health, consumers are increasingly demanding high-quality meat products. Consequently, factors that affect meat quality in chickens are receiving the attention of both researchers and consumers.

Meat quality in chickens is affected by various potential intrinsic and extrinsic factors. Among factors, phosphorus (P) is a critical and expensive mineral in poultry nutrition. This mineral is used in energy pathways and during the synthesis of cell membranes (Hill, Knowlton, Kebreab, France, & Hanigan, 2008). P cannot be synthesized. It must be obtained from dietary sources (Weinera et al., 2001). Studies on effects of dietary P levels on animal growth and bone development showed that high P intake negatively impacts calcium metabolism and bone properties, whereas low P diets will limit the growth of the animals (Roman-Garcia et al., 2010). Currently, P recommendations appear to have exaggerated the availability of P in animals, and many countries are considering revisions to the P recommendations for broiler chicken feed (Yan et al., 2011). In addition, farmers tend to use feed with more P than the recommended level (Lopez, Kanitz, Moreira, Wiltbank, & Satter, 2004). By far, there are barely any reports on the relationship between dietary P levels and meat quality.

Moreover, P is a critical component during glycolysis, energy and lipid metabolism. All enzymes in the body need P (Takeda, Hironori, Hisami, & Taketani, 2012), but few reports describing how P affects lipid metabolism in broiler chickens are available. Lipid metabolism in chickens is affected by the activities of lipase and lipogenesis enzymes, including AMP-activated protein kinase (AMPK), lipase, and main lipogenic enzymes (fatty acid synthase (FAS), and malic enzyme (ME), etc. Among those enzymes, FAS can alter the rates of the biosynthesis and hydrolysis of fatty acids (Smith, Witkowski, & Joshi, 2003), and AMPK is primarily a critical regulator for energy metabolism (Kim, Solis, Arias, & Cartee, 2004), regulating numerous intracellular pathways such as fatty acid oxidation (Jonathan, John, & Bruce, 2012), development of fat cells and the deposition of intramuscular fat (IMF) (Hardie, 2007), and low

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AMPK activity is expected to enhance intramuscular fat (IMF) contents (Underwood et al., 2008). Therefore, we hypothesized that dietary P level may change the enzyme activities in broiler chickens, thus affecting meat quality through lipid metabolism, since fatty acid composition and biosynthesis play a role during the regulation for IMF contents (Camerona et al., 2000).

To investigate the effects of dietary P levels on the meat quality and lipid metabolism of broiler chickens, a 42-day feeding experiment was performed using broilers. The chemical composition, meat color, cooking loss, tenderness, pH values, fatty acid profiles and IMF content were determined to evaluate the differences in meat quality affected by dietary P levels. Besides, to help understand how dietary P levels affect lipid metabolism in broiler chickens, the activities of enzymes including FAS, ME and AMPK in breast meat and liver were determined, trying to provide a foundation for understanding the relation of dietary P levels and meat quality.

2. Materials and methods

2.1. Animals and diet composition

One hundred one-day old female broilers (Arbor Acres) were randomly allocated to 4 groups with 25 birds for each treatment. All birds were housed in metallic pens of identical size (1.4 m × 0.7 m × 0.4 m) with 5 chickens per pen and provided with 12 h light and dark cycles. The feeding experiments were conducted in animal testing grounds of the Institute of Agro-products Processing Science and Technology, Chinese Academy of Agricultural Sciences. The birds were allowed to consume food and water *ad libitum*.

The experiment was divided into four groups according to available phosphorus (AP) concentration of the feed: D (P deficient group), N (normal diet group), H1 (high P group with available P 0.86% and 0.82%), and H2 (high P group with available P 1.16% and 1.10%). The nutrient levels of N group were based on the NRC (1994) recommendations, and chickens were fed by basal diet. And on this basis we set deficient group (D) and adequate groups (H1 and H2) with different P content in the diet. The dietary P levels were mainly obtained by adding potassium dihydrogen phosphate (feed grade) to the feed. The birds were given a diet based on corn and soybean meal, and the diet included both starter and grower phases. The formulation and nutrient levels of the feed are presented in Table 1. Contents of Ca and TP were measured according to the AOAC (1935, 1996), while the others were calculated values.

All chickens were weighed on a weekly basis, in the morning between 7:00 a.m and 8:00 a.m before the feed is offered. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were calculated at the end of growth phase.

At 42 days of age, blood samples were collected from veins under the wings in all live chickens. The serum was separated via centrifugation at 2500g and 4 °C for 15 min. The chicken weights, feed intake and mortality are shown in Table 2. And all the live chickens were slaughtered and eviscerated. The liver and pancreas were removed, rinsed in 0.9% NaCl and immediately stored in liquid nitrogen for biochemical analysis. The chickens were divided after evisceration. The whole breasts of each chicken were cut off, and all samples were stored at −80 °C until further analysis.

2.2. Basic chemical composition and phosphorus contents

Before analysis, frozen samples were thawed at 4 °C and homogenized with a Waring blender (7012G, Texas, USA). Each of the following chemical analyses involved a single sample from each chicken and experimental replication was across all chickens in a particular treatment. The basic chemical composition of the meat was determined according to the methods of AOAC (2000). The moisture contents were determined by oven-drying method at 110 °C for 24 h (AOAC, 950.46B). The total protein content was determined using the Kjeldahl method (AOAC, 928.08), and the total ash content was determined by weight after heating the samples in muffle furnace at 550 °C for 4 h (AOAC, 920.153).

About 0.5–1.5 g of each homogeneous meat sample from each chicken was weighed into crucible before being dried for 1–2 h at 110 °C. The rest of procedure follows that used to determine the total ash content. Afterwards, 5.0 mL of HCl was added to the cooled crucible, and all rinses were transferred though a filter into a volumetric flask. The same procedure was used for the blank. The TP of the breast meat was determined from the ash solution by measuring the yellow color developed through the vanadate-molybdate method at 430 nm (T6 UV–vis spectrophotometer, Purkinje, Beijing, China) (AOAC, 1995). The phosphorus contents were expressed as P.

2.3. Shear forces, cooking losses, meat color and pH values

The frozen breast meat samples were thawed at 4 °C for 24 h and cut into 1 cm × 1 cm × 4 cm along the direction of the muscle fibers. The shear force values were measured on raw meat samples using a Warner Bratzler shear force device (TA-XT2i Plus, probe HDP/BSW, Stable Micro System Ltd., UK) according to the method of Wattanachant, Benjakul, and Ledward (2004).

Approximately 5 g of meat was weighed, wrapped in airtight polythene bag and cooked for 30 min at 80 °C. Afterwards, the samples were cooled at room temperature and analyzed for cooking loss.

Meat color attributes including lightness (L^*), redness (a^*) and yellowness (b^*) were determined using a hand-held Minolta colorimeter (chroma meter CR-400, Konica Minolta Sensing Inc., Japan) standardized against a white calibration plate.

The pH values were determined 24 h post-mortem using a Testo 205 pH meter (Testo Instrument Co. Ltd., Germany) equipped with an insertion electrode.

2.4. Total cholesterol and triglyceride in serum and liver

The serum total cholesterol (TC) and triglyceride (TG) levels were measured using commercial kits (GPO-POD; Applygen Technologies Inc., Beijing, China) with a HITACHI 7020 biochemistry analyzer (HITACHI Ltd., Tokyo, Japan).

The liver lipid extractions were conducted according to the procedures developed by Lin, Chang, Yang, Tzang, and Chen (2013). Briefly, the liver lipids were extracted using chloroform and methanol (2:1, v/v). The TG in the liver was measured using commercial kits with a HITACHI 7020 biochemistry analyzer.

2.5. Intramuscular fat content and fatty acid profiles

The IMF content was measured using the methods described by Folch, Lees, and Sloane-Stanley (1957). The total lipids were extracted in triplicates from each homogenized breast meat sample and used for IMF determination.

The fatty acid composition in meat was determined by gas chromatography. Fatty acid methyl esters (FAME) were prepared by esterification with acetyl chloride in methanol. Approximately 0.5 g of each meat sample was dissolved in 6 mL of acetyl chloride in methanol in a tube that was tightly capped after filling with nitrogen. The tube was then shaken vigorously and treated in a water bath for 2 h at 80 °C. The tube was washed using 3.0 mL