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Methionine supplementation augments tissue n-3 fatty acid and tocopherol content in broiler birds fed flaxseed

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ABSTRACT

Methionine (Met) is the first limiting amino acid in meat-type broiler chicken diets and serves as a lipotropic agent with antioxidant properties. The objectives of the current study is based on the hypothesis that Met supplementation will enhance n-3 fatty acid (FA) status, antioxidant content, lipid stability, and production indices in broilers fed flax. The effect of Met supplementation (50 and 100% above Cobb 500 requirement level) on tissue FA composition, tocopherol (Toc) content, lipid oxidation products, and growth performance of broilers fed flax is investigated. One hundred and twenty (n = 120) day-old Cobb chicks were fed corn-soybean meal-based diet containing 0% flax, (Control), 15% flax (Diet 1), Diet 1 + 50% more Met (Diet 2), and Diet 1 + 100% more Met (Diet 3) for 42 days. Total lipids in liver and adipose tissue was lowest in Diet 3 ($P < 0.05$). Feeding flax led to a reduction total lipids in breast muscle ($P < 0.05$) and was not affected by Met level ($P > 0.05$). α-Linolenic acid (18:3n-3) was highest in thigh muscle and liver of Diet 3 and adipose tissue of chickens fed Diet 2 and Diet 3 ($P < 0.05$). Total long chain (> 20 C) n-3 FA was highest in the breast muscle of chickens fed Diet 2 and Diet 3 ($P < 0.05$). Total saturated FA were lowest in the breast and thigh muscle of Diet 3 fed birds ($P < 0.05$). Addition of Met led to an increase in α-Toc in breast muscle in birds fed Diet 2 and Diet 3 ($P < 0.05$). Lipid oxidation products were lower in the thigh muscle and adipose tissue of birds fed Diet 2 and Diet 3 than Diet 1 and Control ($P < 0.05$). Body weight gain was lowest in birds fed flax ($P < 0.05$). Met supplementation had no effect on weight gain or feed conversion in birds fed flax ($P > 0.05$). No effect of diet on feed consumption was observed ($P > 0.05$). Overall, results from the current study demonstrate that Met supplementation is a novel way to enrich tissues with n-3 FA and Toc in chickens fed flax.

1. Introduction

The cardio-protective and other health-promoting effects of dietary n-3 fatty acids (FA) notably long chain (> 20C) n-3 FA (eicosapentaenoic acid, (EPA, 20:5 n-3), docosahexaenoic acid, (DHA, 22:6 n-3)) have been widely documented in both human and animal studies (Arab-Tehrany et al., 2012; Calder, 2013). Health agencies recommend increased consumption of n-3 FA or marine foods (Lands, 2014). However, due to culture, cost, allergic issues, concerns over sustainability, environmental contamination, and

Abbreviations: FA, fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ALA, α-linolenic acid; DPA, docosapentaenoic acid; ANF, antinutritional factors; Met, Methionine; Toc, tocopherol; TBARS, thiobarbituric acid reactive substances; FCR, feed conversion ratio; SFA, saturated fatty acid MUFA monounsaturated fatty acid; LC, long chain; SEM, standard error of mean

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seasonal availability, Western diets contain limited amounts of marine foods. Broiler chicken meat is the most widely consumed animal protein globally. Incorporating n-3 FA in chicken meat may be an alternative strategy to increase human n-3 FA consumption without changing existing dietary habits. The success of this strategy would depend on developing ways to increase the n-3 FA content of meat above current levels without affecting chicken production performance and meat quality aspects.

α-Linolenic acid (ALA, 18:3 n-3) is the precursor of long chain n-3 FA such as EPA, docosapentaenoic acid (DPA), and DHA. Feeding ALA-rich diets to poultry enhances ALA and other long chain n-3 FA in meat. Among the different dietary sources, flax (*Linum usitatissimum* L.) is one of the richest source of ALA (> 50%). In addition, due to its other nutritional properties such as metabolizable energy (3750 Kcal/kg) and crude protein (22%), flax is commonly added to meat-type broiler chicken diets targeted for n-3 FA enrichment purposes. However, low energy utilization, poor growth performance, and carcass yield were observed in broiler chickens fed flax (Ajuyah et al., 1991; Lee et al., 1991; Ortiz et al., 2001; Alzueta et al., 2003). Such adverse effects were attributed to the several antinutritional factors (ANF) present in flax (e.g. cyanogenic glycosides, trypsin inhibitor, phytic acid, and mucilages) affecting nutrient utilization and growth. Strategies to mitigate ANF in flax will enhance n-3 FA content in meat without affecting chicken productivity and carcass yield.

Cyanogenic glycosides are amino acid-derived plant metabolites present in flax, and upon consumption, are degraded by glucosidase enzyme leading to the release of toxic hydrogen cyanide. The detoxification of hydrogen cyanide is through conversion to thiocyanate in the liver which requires sulfur donors and are provided by sulfur amino acids (Mosharov et al., 2000; Rocha-e-Silva et al., 2010). Methionine (Met) is an essential sulfur-containing amino acid and is the first limiting amino acid in broilers fed corn-soybean based diets. Met acts as a lipotropic agent and plays many roles in the animal body, including participation in protein synthesis, production of other sulfur-containing amino acids, hormones, enzymes, antibodies, and are precursor of glutathione, protecting cells against oxidative stress (Németh et al., 2004; Li et al., 2008; Hasek et al., 2013). In broilers, dietary Met levels affect growth indicators, blood lipid and antioxidant status as well as hormone parameters and carcass quality (Pillai et al., 2006; Zhai et al., 2016). Modern lines of broiler chickens require high-energy, well balanced diets to achieve their full genetic potential. We hypothesized that dietary Met supplementation in broilers fed flax-containing diets will enhance tissue n-3 FA and antioxidant status, lipid stability, and production indices. The objectives of the experiment were to determine the effect of Met supplementation (50 and 100% above Cobb 500 requirement level) on tissue FA composition, lipid and tocopherol (Toc) content, lipid oxidation products measured as thiobarbituric acid reactive substances (TBARS) and growth performance of meat-type broiler chickens fed flax.

2. Materials and methods

An institutional animal care and use committee approved all experimental protocols to ensure adherence to Animal Care Guidelines.

2.1. Birds and dietary treatments

One hundred and twenty (n = 120) one day-old Cobb broiler chicks were obtained from a commercial hatchery and randomly placed in 20 floor pens bedded with wood shavings. Chicks were weighed and assigned to one of the four corn-soybean meal basal diet containing 0% flax (Control), 15% flax (Diet 1), Diet 1 + 50% more Cobb 500 Met requirement (Diet 2), and Diet 1 + 100% more Cobb 500 Met requirement (Diet 3) (Table 1). Each treatment was replicated in five pens with six birds per each pen. The chicks were fed experimental diets (starter, day 1–10), (grower, day 11–22), and finisher, (day 23–42). All diets were isocaloric and isonitrogenous. Diets within each phase were formulated with similar dietary fat, Ca, P and lysine levels to ensure that any observed performance differences were due to dietary flax and Met. The calculated Met concentrations were 0.45, 0.45, 0.68, 0.90% (starter), 0.42, 0.42, 0.63, and 0.87% (grower), 0.39, 0.39, 0.59, and 0.78% at finisher phase for Control, Diet 1, Diet 2 and Diet 3, respectively. On days 10, 22 and 42, chickens and feed were weighed, and body weight gain and feed consumption was recorded for each pen. Average chicken weight and feed conversion ratio (feed:gain) (FCR) were calculated. During the experiment, birds were provided free access to water and feed. The chicks were not vaccinated and were housed in an environmentally controlled facility with a lighting program of 23L:1D.

2.2. Tissue collection

On day 42, two chickens from each pen (10 per treatment) were randomly selected, weighed and euthanized with CO_2 gas. Tissue samples from each chicken including heart, liver, spleen, breast and thigh muscle (pectoralis major and biceps femoris, without skin), and abdominal fat pad (including fat surrounding the gizzard, bursa of Fabricius, and cloaca), were collected, weighed. Tissue samples (liver, fat, breast and thigh muscle) were cleaned with saline, and stored at −20 C until analysis. All analyses were done within one month of sample collection.

2.3. Total lipid and fatty acid analysis

Total lipids were extracted from approximately 2 g of tissues or feed using a 2:1 solution of chloroform and methanol (Folch et al., 1957). Visible fat was removed from the muscle tissue samples before lipid extraction. Total lipid content was determined gravimetrically. FA methyl esters were prepared with boron trifluoride methanol as reported earlier (Cherian et al., 2002). FA analysis was performed with an HP 6890 gas chromatograph (Hewlett-Packard Co., Wilmington, DE) equipped with an autosampler,