Animal Nutrition xxx (2017) 1–8

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Changing dietary n-6:n-3 ratio using different oil sources affects performance, behavior, cytokines mRNA expression and meat fatty acid profile of broiler chickens

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ARTICLE INFO

Article history:
Received 1 May 2017
Received in revised form
25 July 2017
Accepted 5 August 2017
Available online xxx

Keywords:
Broiler
n-6:n-3 PUFA ratio
Performance
Antioxidant status
Meat
Immunity

ABSTRACT

Typical formulated broiler diets are deficient in n-3 poly-unsaturated fatty acids (PUFA) due to widening n-6:n-3 PUFA ratio which could greatly affect performance, immune system of birds and, more importantly, meat quality. This study was conducted to evaluate the effect of modifying dietary n-6:n-3 PUFA ratio from plant and animal oil sources on performance, behavior, cytokine mRNA expression, antioxidative status and meat fatty acid profile of broiler chickens. Birds ($n = 420$) were fed 7 diets enriched with different dietary oil sources and ratios as follows: sunflower oil in control diet (C); fish oil (FO); 1:1 ratio of sunflower oil to FO (C1FO1); 3:1 ratio of sunflower oil to fish oil (C3FO1); linseed oil (LO); 1:1 ratio of sunflower oil to linseed oil (C1LO1); 3:1 ratio of sunflower oil to linseed oil (C3LO1), resulting in dietary n-6:n-3 ratios of approximately 40:1, 1.5:1, 4:1, 8:1, 1:1, 2.5:1 and 5:1, respectively. The best final body weight, feed conversion ratio as well as protein efficiency ratio of broilers were recorded in the C1FO1 and C1LO1 groups. Compared with the control group, the dressing percentage and breast and thigh yield were highest in the C1FO1 and C1LO1 groups. Narrowing the dietary n-6:n-3 ratio increased ($P < 0.05$) n-3 PUFA content of breast meat. Moreover, the breast meat contents of eicosapentaenoic acid and docosahexaenoic acid increased ($P < 0.05$) with increasing dietary FO whereas α-linolenic acid content was higher with LO supplementation. Also, enriching the diets with n-3 PUFA from FO and LO clearly decreased ($P < 0.05$) serum total cholesterol, triglycerides and very low-density lipoproteins and enhanced antioxidative status. The feeding frequency was decreased ($P < 0.05$) in the C1FO1 and C1LO1 groups. Likewise, n-3 PUFA-enriched diets enhanced the frequency of preening, wing flapping and flightiness. Animal oil source addition, compared to plant oil, to broiler diets enhanced the relative mRNA expression of interferon gamma, interleukin-1 beta, interleukin-2 and interleukin-6 genes, especially at low n-6:n-3 ratios. This study has clearly shown that narrowing n-6:n-3 ratio through the addition of FO or LO improved performance and immune response of broilers and resulted in healthy chicken meat, enriched with long chain n-3 PUFA.

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

1. Introduction

The success of the modern poultry industry depends on enhancing growth performance, reducing fat deposition of growing chicks and improving the products offered to consumers. Nutrition plays a strong role in growing chickens, and early ingestion behavior generates feed experience that affects the bird's overall performance (Hale and Green, 1988). Recently,

http://dx.doi.org/10.1016/j.aninu.2017.08.003
2405-6545/

Please cite this article in press as: Ibrahim D, et al., Changing dietary n-6:n-3 ratio using different oil sources affects performance, behavior, cytokines mRNA expression and meat fatty acid profile of broiler chickens, Animal Nutrition (2017), http://dx.doi.org/10.1016/j.aninu.2017.08.003

significant effort has been made to produce poultry products enriched with n-3 poly-unsaturated fatty acid (n-3 PUFA) (Pietras and Orczewska-Dudek, 2013), and modify the potential of the bird's immune response (Swiatkiewicz et al., 2015). The concentration of n-3 PUFA in animal tissues depends mainly on the fatty acid composition of the diet (Bou et al., 2005). The omega-3 fatty acids can decrease the concentrations of C-reactive protein, proinflammatory eicosanoids, cytokines, chemokines and other inflammatory biomarkers (Schwab and Serhan, 2006). It is known that fish oil is an excellent source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (members of the n-3 family), which are precursors of the lipid mediators of inflammation and have anti-inflammatory and immunomodulatory functions (Calder, 2010). On the other hand, vegetable oils (e.g., linseed oil) are rich in α-linolenic acid (ALA), which is the metabolic precursor of EPA and DHA (Kouba and Mourot, 2011). Less than 20% of the world's population consume about 250 mg/day of n-3 PUFA from marine sources (Micha et al., 2014). So, there is a need to make n-3 PUFA available for a greater part of the remaining 80% of the world population. Recent studies have shown that dietary imbalance of n-6:n-3 PUFA ratio can affect human health, especially with high n-6:n-3 PUFA ratio in our modern diets, as it can lead to increased production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) and thus excessively augment inflammation (Simopoulos, 2002). It was recommended that n-6:n-3 PUFA ratio should be nearly 3:1 to 1:1 (Kim et al., 2007). In addition, human conversion of ALA to EPA is low, and to DHA is even lower (Burdge and Calder, 2005). Thus, there is a potential to enrich the human diet with n-3 PUFA by modifying poultry feeding practices to satisfy the human requirements, as both type and ratio of dietary oils affect the deposition of fatty acids in broiler meat. Therefore, the aim of the current study was to improve broiler performance and health, through consumption of specific fatty acids, particularly at the right n-6:n-3 PUFA ratio. This will produce meat that is beneficial to human consumers.

2. Materials and methods

The protocol for animal experiments was approved by the animal care and use committee at Faculty of Veterinary Medicine, Zagazig University.

2.1. Experimental birds and management

A total of 420 day-old Ross 308 broiler chickens were obtained from a commercial hatchery. On arrival, they were weighed and randomly assigned to 7 groups, each consisting of 5 replicates of 12 birds each. Birds were reared in a naturally ventilated open house with sawdust as litter, at a density 10 birds/m^2. Pens were equipped with semi-automatic tube feeders and bell drinkers.

2.2. Experimental diets and design

The birds were fed a basal diet formulated according to Ross 308 broiler nutrition specification. The nutrient composition of the basal diet is shown in Table 1. Seven dietary treatments were prepared using different oil sources (plant and animal) as follows: sunflower oil (C); fish oil (FO); sunflower oil and fish oil at a ratio of 1:1 (C1FO1); sunflower oil and fish oil at a ratio of 3:1 (C3FO1); linseed oil (LO); sunflower oil and linseed oil at a ratio of 1:1 (C1LO1); sunflower oil and linseed oil at a ratio of 3:1 (C3LO1), resulting in dietary n-6:n-3 ratios of approximately 40:1, 1.5:1, 4:1, 8:1, 1:1, 2.5:1 and 5:1, respectively. The different types of oils used in the experiments and their inclusion rates (%) are listed in Table 2. The fatty acid composition of experimental diets is shown in Table 3. The diets were prepared weekly and kept at 4 °C to prevent oxidative rancidity.

Table 1
Ingredients and nutrient composition of the basal diet (% of dry-matter basis).

Item	Starter diet (1–21 d)	Grower diet (22–42 d)
Ingredients, %		
Corn, ground	54.4	60
Soybean meal (48%)	37.2	31.8
Oil	4	4.5
Calcium carbonate	1.3	1.2
Calcium dibasic phosphate	1.5	1.25
NaCl (common salt)	0.5	0.3
L-lysine (78%)	0.24	0.21
DL-methionine (98%)	0.26	0.24
Vitamin and mineral premix[1]	0.6	0.5
Calculated composition, %		
ME, kcal/kg	3,113	3,212
Protein	22.62	20.50
Ether extract	6.30	7.00
Calcium	1.16	1.00
Avail. P	0.54	0.46
Lysine	1.41	1.24
Methionine	0.58	0.53

[1] Provided per kilogram of diet: 12 MIU vitamin A; 4 MIU vitamin D_3; 28 mg vitamin E (DL-α-tocopherol acetate); 3 mg Vitamin K; 2.0 mg menadione; 2 mg thiamine; 4.0 mg riboflavin; 50 mg niacin; 6 mg pyridoxine; 0.015 mg cobalamin; 15.0 mg pantothenic; acid, 6.0 mg folic acid, 0.16 mg biotin, 0.625 mg ethoxyquin, 500 mg $CaCO_3$; 80 mg Fe; 80 mg Zn; 110 mg Mn; 10 mg Cu; 0.7 mg I; 0.3 mg Se (as Na_2SeO_3); antioxidant 0.5 g.

2.3. Growth performance and carcass traits

The body weight, body weight gain, and feed intake of all broiler chickens were recorded weekly and feed conversion ratio (FCR), protein efficiency ratio (PER) and overall performance were calculated. Five birds from each group were selected at the end of the experiment, fasted overnight, weighed and then sacrificed to obtain weight of the dressed carcass, breast, thigh, and abdominal fat yields, expressed as a percentage of body weight. Samples were stored at −20 °C until analysis. Five samples from the breast and thigh muscles, from each experimental group were used for analysis of intramuscular fat and determined by extraction with petroleum ether in a Soxhlet apparatus (Horwitz, 2002).

2.4. Tissue fatty acid analysis and cholesterol

The experimental diets and homogenized freeze-dried breast meat were analyzed for fatty acid composition. For this purpose, total lipids were extracted from homogenized muscle tissue, using a solvent mixture of chloroform and methanol (2:1, vol/vol), which is suitable for quantitative extraction of lipids according to the method of Folch et al. (1957). The fatty acid methyl esters were prepared as described by Ichihara and Fukubayashi (2010) for gas chromatography (GC). The total cholesterol in breast and thigh was determined enzymatically and measured by GC using the method of Allain et al. (1974).

2.5. Determination of lipid parameters and oxidative status

At the end of the experimental period, blood samples were collected from 5 birds per group into tubes without anticoagulant. The separated serum was used for determination of total cholesterol, triglyceride, high-density lipoprotein-cholesterol (HDL-C),

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