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Effect of Feeding High-alpha Linolenic Acid Flaxseed Oil to Broilers and Layers on Liver Biosynthesis of Total Very Long Chain Omega-3 Fatty Acids

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ABSTRACT

Omega-3 fatty acids (n-3 FA) are known to possess beneficial effects on human health especially the long chain members (>20C; VLC n-3 FA). High-α linolenic acid flaxseed oil (FLAX oil) is known to be the richest plant source with n-3 FA (58% ALA vs 50% in regular flaxseed oil). Broilers and layers were fed FLAX oil in order to assess their ability to enrich the breast tissues with n-3 FA. In the broilers experiment, FLAX oil was added at a level of 2.25% of the diet while in laying hens, was added at 4% of the diet. The growth performance was not affected by the dietary treatments in both broilers and layers. The fatty acid profile of the liver was affected by the dietary treatments where the total n-3 FA was higher in laying hens, but the total VLCn-3 FA was greater in broilers.

Keywords: Broilers, layers, omega-3, fatty acid

INTRODUCTION

Omega-3 fatty acids, (n-3 FA) cannot be synthesized in the body so they are essential for humans and animals. In plants, α-linolenic acid, (ALA; 18:3n-3) is widely distributed but after consumption, conversion to the very long-chain n-3 fatty acids, (VLCn-3 FA, >20C) requires a series of elongation and desaturation which is limited. The eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3) are main VLCn-3 FA (Goyens *et al.,* 2006). Increasing dietary n-3 FA intake results in health improvement including reducing the incidence of cardiovascular diseases, inhibiting inflammation, improving and maintain mental and neural development, and diminishing the occurrence of cancer and autoimmune diseases (Shahidi & Ambigaipalan, 2018; Troesch *et al.*, 2020). Naturally, VLCn-3 FA are obtained mainly from fatty fish, such as salmon, mackerel, herring, and tuna, as well as fish oils, but this source is limited for reasons related to ocean sustainability, fish stocks, presence of contaminants and toxins, and the high cost of extraction and purification (Betti *et al.,* 2009; Lenihan-Geels *et al.,* 2013; Strobel *et al.,* 2012). Both meat and egg type chicken are favored for enriching meat and table eggs with n-3 FA to maximize their intake based on the wide consumption of such products among populations (Siró *et al.*, 2008). In addition, the ability of chicken to elongate the FA chain is greater than mammals because chicken have both elongase enzymes (ELOV2 and ELOV5) which elongate stearidonic acid (SDA; 18:4 n-3) to eicosatetraenoic acid (ETA; C20:4 n-3) and eicosapentaenoic acid (EPA; C20:5 n-3) to docosapentaenoic acid (DPA; C22:5 n-3), where ELOV2 only exists in mammals (Balić *et al.*, 2020; Gregory *et al.*, 2013). In chicken, liver is the primary site for FA *de novo* synthesis, with a

limited ability in adipose tissue. Estrogen in laying hens stimulates the liver to synthesize a special very low density lipoprotein (VLDL) targeted to the yolk (VLDLy), which constituted about 93% of the yolk lipids (Walzem *et al.*, 1999).

Synthesis and transfer of VLCn3 FA from vegetable oils is dependent on availability of n-3 supply, capacity of the elongation and desaturation processes, and competition with n-6 FA for the enzymes (Kartikasari *et al.,* 2012). The ALA from flaxseed oil is first desaturated by delta-6-desaturase enzyme (Δ-6D) to SDA then elongated by ELOV2 and ELOV5 to ETA, which is then desaturated by delta-5-desaturase enzyme (Δ-5D) forming EPA. In addition, EPA can be further elongated by ELOV2 and ELOV5 to DPA, which is elongated by ELOV2 forming tetracosapentaenoic acid (TPA; C24:5 n-3) that is desaturated by Δ-6D to tetracosahexaenoic acid (THA; C24:6 n-3), which is converted to DHA by a β-oxidation step (Balić *et al.,* 2020)

The physiological differences between broilers and layers affect the lipid metabolism; where a large number of adipocytes and higher activity of lipase enzymes are reported in broilers which enhances the uptake of fatty acids into adipocytes (Hermier *et al.,* 1989). Furthermore, the higher activity of Δ-9 desaturase activity in broilers, which facilitate the incorporation of fatty acids into VLDL with subsequent transportation to extra hepatic tissues (Legrand *et al.,* 1987). Estrogen, in layers, increases the liver capacity for fatty acid *de novo* synthesis to meet the increased lipid demand for yolk formation (Dashiti *et al.,* 1983).

This study aimed to investigate the liver fatty acid composition of broilers and layers in response to feeding diets contained high ALA flaxseed oil at 2.25 and 4% of broiler and layer diets respectively. Also, the growth performance was monitored in response to treatments

MATERIALS AND METHODS

Birds and Housing

Fifteen one-day-old broiler chicks (Cobb 500) were purchased from a local hatchery and housed in Petersime wired-floor brooder cages [76 cm (w) x 102 cm (d) x 25 cm (h)]. Cages are provided with a feed trough and drip-nipple water line. Light was provided 23 h/d during the first three days, reduced to 16 h/d till the end of the first week, and then increased gradually to 20 h/d by the end of experiment (d 35). The room temperature was set to 35ºC on the first three days of age and decreased gradually to 21ºC at the end of the experiment. The chicks were weighed, wing-tagged on d 11. At of age 21 d, chicks were moved to Petersime finisher batteries [74 cm (w) x 69 cm (d) x 36 cm (h)]. All animal procedures were approved by the Penn State Institutional Animal Care and Use Committee (IACUC #PROTO201800609).

In layers, twenty 52-week-old Hy-Line W-36 White Leghorn hens were housed in individual cages [30 cm (w) × 43 cm (d) × 36 cm (h)] with sloping wire floors in an environmentally controlled-room and provided with 16 hours of light daily. When the hens were 53 weeks old (day 0 of the experiment), the 10 best-performing hens were selected to start the experiment based on 5-day pre-experimental egg weights and egg production (n=10). All animal protocols were approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University (IACUC # 46992).

Diets and Treatments

A commercial pelleted starter diet containing 24% CP and 3100 ME kcal/kg (Purina Animal Nutrition, Arden Hills, MN) was fed from d 0 to 11, then were switched to a mash grower diet starting on d 12. The diet was formulated to meet or exceed the nutrient requirements of the broiler chicken according to (NRC, 1994). The diet was supplemented with 22.5 g/kg FLAX oil (66.5% ALA), (HiOmega 70, Polar Foods, Fisher Branch, MB, Canada) (Table 1). In layers experiment, hens were fed corn-soybean meal-based layer mash diets supplemented with 40 g/kg FLAX oil for 28 days. The diet was formulated to meet or exceed the hens' nutrient requirements as listed in the Hy-Line W-36 Commercial Management Guide, containing 16.9% CP and 3161 ME kcal/kg (Table 1). Feed and water were provided to all animals for *ad libitum* consumption.

Diet's Sampling and Analysis

Representative feed samples were collected and kept at -20ºC until analyzed. Diets were ground with a coffee grinder and thoroughly mixed to

obtain a uniform sample. Feed samples were analyzed by the following AOAC International Official Methods (AOAC, 2000) for crude protein (CP) by 990.03, ether extract (EE) by 2003.05, dry matter (DM) by 930.15, ash by 942.05, crude fiber (CF) by 978.10, calcium (Ca) and available phosphorus (AP) by 985.01, and starch determined enzymatic method (Cumberland Valley Analytical Lab, Waynesboro, PA; Hall, 2009). Fatty acid profile and concentration of oil and diets were determined by gas-liquid chromatography as described below.

Performance and Sampling

Growth performance was assessed by measuring live body weight (LBW), body weight gain (BWG), and feed intake (FI). Broilers birds were weighed individually at 11 and 35 d of age while layers at d 0 and 28 of experiment. Feed intake was calculated, once at the end of experiment, as the difference between the total amount of feed prepared and the amount of feed remaining at the end of the experiment in broilers. On d 35, all birds were euthanized by electric stunning followed by exsanguination, and liver was dissected, weighed, and sampled (1 to 2 g). Liver samples were placed in aluminum foil packets, snap-frozen into liquid nitrogen, and stored at -80ºC until further analysis.

In layers, the production performance was assessed by observing hen-day egg production (HDEP), egg mass (EM), and feed efficiency ratio (FER). Hens were individually weighed on d 28 (LBW) and d 0 of experiment, where the difference between is the BWC. Eggs were collected and weighed daily and stored at 4ºC until processed. Daily feed intake was recorded for each hen and was calculated as the difference between the amount of feed offered on a day and the amount remaining in the feeder on the day after.

Fatty Acid Analysis

A dual methylation procedure was followed to analyze the FA profile of feeds, oil, and liver tissue using 0.5 M sodium methoxide in methanol followed by 5% methanolic HCL (Jenkins, 2010). The individual fatty acids (FA) were separated and quantified by gas-liquid chromatography (GC) using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a fused-silica capillary column (SP2560; 100 m x 25 mm i.d. with 0.2-µm film thickness; Supelco, Inc., Bellefonte, PA) and a flame ionization detector as described by (Elkin *et al.*, 2015). Concentration and methylation efficiencies of FA were determined using dual external standards (13:0 free FA and 17:1 methyl ester; Nu-Chek Prep, Inc., Elysian, MN). Peaks were identified based on purified standards (GLC780, GLC461, GLC 566, and pure 22:1 n-9 [Nu-Chek Prep]; and pure 20:4 n-3 [Caymen Chemical Company, Ann Arbor, MI]), with the exception of stearidonic acid (SDA), which was identified based on liver fatty acids from chickens fed high-SDA soybean oil (Elkin *et al.*, 2015, 2016). An equal weight standard (GLC461; Nu-Chek Prep) was used for calculation of recovery factors.

Statistical analysis

The data obtained was analysed with one-way ANOVA using JMP pro 15 (SAS institute, Cary, NC). The effect of treatment was included in the model and means were separated using a Protected LSD test with significance declared at $P \leq 0.05$. Data points outside of ± 3 Studentized residuals were considered outliers and removed from the analysis.

RESULTS

Growth and production performance

The high ALA flaxseed oil applied either at 2.25 or 4% of the broiler and layer diets had no effects on both growth performance and production performance of broilers and layers (Table 2).

Fatty acid profile

The fatty acid profile of experimental diets reflected the treatments where broiler diets contained 13.4 g/kg while the layers' diet contained 24.9 g/kg as ALA (Table 3). The dietary treatments increased the total liver n-3 FA content with a significantly greater (P=0.01) increase in layers (721 mg/100g in layers vs 518 mg/100g in broilers: Table 4). However, the total VLCn-3 FA was higher in livers of broilers than that of layers (over 100 mg/100g; P<0.01). The individual VLCn-3 FA including EPA and DPA were significantly higher (P<0.001) in the livers of broilers fed diets contained 2.25%

FLAX oil than those of layers fed diets contained 4% FLAX. In contrast, DHA was 27 mg per 100g of fresh tissue numerically higher in livers of laying hens than that in broilers. Similarly, ALA highly reflected the diet in laying hens where the liver content was 335 mg/100g higher than that in broilers (102 mg/100g; $P<0.01$).

Table 1. Ingredients and nutrient composition of experimental diets (g/kg, as-is basis).

	Diets*	
Ingredients	**Broiler diet (d 12 to 35)**	**Layer diet (d 0 to 28)**
FLAX oil	22.5	40
SOY oil	5.4	--
Corn starch	-	90
Ground yellow corn	642.8	138.0
Soybean meal (48% CP)	288.1	281.5
Degermed corn meal	-	276.7
Ground wheat	-	35.5
Alfa alfa meal	-	12.0
Calcium chips	-	63.0
Limestone	10.6	42.0
Dicalcium Phosphate	16.9	-
Monocalcium phosphate	-	12.9
Sodium chloride	-	4.2
Salt	4.3	-
DL-Methionine	3.6	-
Vitamin and mineral Premix	2.5	4.2
L-Lysine-HCL	1.7	-
L-Threonine	1.6	-
Calculated Nutrient		
ME (kcal/kg)	3031	3161
CP	190	169
Calcium	9.0	43.7
Av. Phosphorus	4.1	5.07
Dig. Lysine	10.5	8.48
Histidine, digestible	-	4.12
Dig. Methionine	6.2	6.73
Dig. Threonine	7.5	5.76
Dig. Methionine & Cysteine	8.7	4.39
Analyzed nutrient		
DM	894	893
Moisture	106	107
ME, (kcal/kg)	3031	3161
CP	213	166
CF	43.0	21.0
Ca	9.0	42.9
Av. P	4.4	4.95

* Treatment oils included SOY = conventional soybean oil; FLAX oil= high-ALA flaxseed oil.

Table 2. Growth performance of broilers and layers fed diets contained high ALA flaxseed oil

Growth performance, g	Broilers	Layers
Start LBW (d- 12)	395	1739
Final LBW (d- 35)	2364	1773
BWC[1]	1967	33.6
Total FI	3240	2794
Daily FI	-	99.8
FCR	1.67	-
HDEP[2]	-	92.9
Egg weight (g)	-	60.4

[1] body weight change= final LBW- start LBW
[2] HDEP= hen-day-egg production = number of eggs laid/ (number of hens × days)

Table 3. omega-3 fatty acid profile of diets (g/kg, as-is basis) and oils (as % of fatty acids).

	Diets		Oil
FA[2]	Broiler diet	Layer diet	FLAX
Total FA	45.9	50	100
PUFA			
n-3 FA			
18:3n-3 (ALA)	13.4	24.9	66.5
∑n-3	13.4	24.9	66.5
n-6 FA			
18:2n-6 (LNA)	17	10.8	11.3
18:3n-6 (GLA)	ND	0.09	0.12
∑n-6	17.0	10.9	11.5
∑n-6: ∑n-3	1.26	0.438	0.173
∑PUFA	30.4	35.8	78

Table 4. Omega-3 fatty acid profile of livers (mg/100g of fresh tissue) of broilers and layers fed diets supplemented with high ALA flaxseed oil.

FA	Broilers	layers	SEM	P-value
18:3n-3 (ALA)	102[b]	437[a]	78	0.001
18:4n-3 (SDA)	6.07	3.59	0.91	0.142
20:3n-3	7.88	9.37	0.63	0.088
20:4n3 (ETA)	5.85[a]	2.71[b]	0.46	<0.0001
20:5n-3 (EPA)	114[a]	33.5[b]	3.51	<0.0001
22:5n-3 (DPA)	79.8[a]	27.0[b]	4.25	<0.0001
22:6n-3 (DHA)	156	183	10.9	0.105
∑VLCn-3	364[a]	255[b]	16	0.0002
∑n-3	518[b]	721[a]	36	0.01

DISCUSSION

The FLAX oil had no negative effects on the growth performance and production parameters of broilers and laying hens. These findings agreed with those of (Elkin *et al.,* 2015, 2016; Ferrini *et al.,* 2010; Mridula *et al.,* 2014) who observed no adverse effects on broilers resulting from feeding them flaxseed oil. Also, (Ehr *et al.,* 2017; Huang *et al.,* 2018; Kartikasari *et al.,* 2012; Nain *et al.,* 2012) reported that feeding laying hens on diets contained extruded flaxseed, flaxseed meal or oil had no effects on laying parameters. In contrast, inclusion of 10% flaxseed in layers diets for 16 weeks resulted in higher feed intake and egg production (Cherian & Quezada, 2016).

The ability of chicken to elongate and desaturate the 18-C n-3 FA to the VLC n-3 FA is limited because of either direct deposition without undergoing further desaturation and elongation or competition for enzymes (Mirshekar *et al.,* 2015; Wood *et al.,* 2008). The biotransformation of ALA to the long chain members depends on the availability of enzymes (elongases and desaturases), concentration of n-3 FA substrate, and the amount of n-6 FA in the diet (Gregory *et al.,* 2013).

The higher liver content of total n-3 FA of layers resulted from the increased deposition of dietary ALA where the liver ALA constituted about 70% of dietary ALA. The lower concentration of ALA in liver of broilers may be due to lower dietary ALA concentration, oxidation, or conversion to VLCn-3 FA. Also, the greater liver ALA in layers maybe caused by direct deposition of dietary ALA without undergoing metabolism. Similarly, the lower concentration of VLCn-3 FA in layers liver is supporting the explanation that increasing dietary concentration of polyunsaturated n-3 FA

inhibits the biotransformation to EPA and DHA caused by saturation of enzymes involved in elongation and desaturation process (Kartikasari *et al.,* 2012). In a study conducted by (Elkin *et al.,* 2015), laying hens were fed diets contained 50 g/kg flaxseed oil and resulted in liver total n-3 and total VLCn-3 FA if 698 mg/100g, respectively.

CONCLUSION

Supplementation of diet with high-ALA-flaxseed oil increased the liver total n-3 FA in broilers and layers with a higher content in layers. Increasing the oil level above 2.25% decreased the total liver VLC n-3 FA in laying hens indicating that the optimal supplementation level of the oil should be less than 4% of the layers diet.

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