Enriching ISA brown and Shaver white breeder diets with sources of n−3 polyunsaturated fatty acids increased embryonic utilization of docosahexaenoic acid[1]

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ABSTRACT There is limited information on feeding egg-type chick breeders n−3 polyunsaturated fatty acids (**PUFA**) and its impact on hatching egg quality and embryonic fatty acid (**FA**) utilization. We investigated the effects of feeding brown and white egg-type chick breeders diets containing sources of n−3 PUFA on egg composition, apparent embryonic FA utilization, and intestinal FA transporter in hatchlings. Twenty-six-week-old ISA brown and Shaver white breeders were fed either 1) control (**CON**); 2) CON + 1% of microalgae (DMA, *Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid (**DHA**); or 3) CON + 2.60% of coextruded full-fat flaxseed and pulse mixture (FFF, 1:1 wt/wt) as a source of α-linolenic acid (**ALA**). Test diets had similar total n−3 and n−6:n−3 ratio. Eggs were hatched, and residual yolk (**RY**) samples taken for FA analyses. Apparent embryonic FA utilization was calculated by subtracting concentration of FA in RY from concentration of FA in yolk before incubation. There was an interaction between strains and diets ($P < 0.05$) on DHA in phospholipid and triglyceride fractions of yolk. Both n−3 PUFA sources increased DHA to a greater extent in Shaver white than in ISA brown. The interactive effect of strains and diets ($P = 0.019$) on embryonic utilization of ALA was such that DMA and FFF reduced ALA utilization, and this pattern was more prevalent in Shaver white birds than in ISA brown birds. There was no interaction between strains and diets on DHA utilization ($P > 0.05$). Embryos from hens fed n−3 PUFA sources used less total FA in phospholipid fraction ($P < 0.001$), and they preferentially used more DHA than CON embryos. Shaver white embryos used more ($P < 0.05$) ALA and DHA than ISA brown embryos. Although data suggested Shaver white had higher propensity of depositing DHA than ISA brown, irrespective of strain, feeding n−3 PUFA modified embryonic pattern of FA utilization toward utilization of DHA.

Key words: breeder feeding, n−3 polyunsaturated fatty acid, embryonic fatty acids utilization, egg-type chick breeder

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INTRODUCTION

Unlike mammals, growth and development of the avian embryo are dependent on nutrients deposited in fertile eggs. The egg nutrients are transferred to the developing embryo through specialized tissue, the yolk sac membrane (**YS**), formed from the gut in the first week of incubation (Bauer et al., 2013; Yadgary et al., 2014). The epithelial cells of the YS function as the mediators for nutrient transport from the contents of the yolk to the blood circulation of developing embryo (Moran, 2007). Nutrient and oxygen availability, as well as embryo temperature, are the primary drivers for embryonic metabolism and development in chickens (Everaert et al., 2008; Nangsuay et al., 2015). Embryonic utilization of egg nutrients has a direct effect on embryo growth, hatchability, and post-hatch performance (Uni and Ferket, 2004; Uni et al., 2005). However, there is dearth of knowledge on the rate and mechanisms of nutrient utilization during embryogenesis (Uni et al., 2005; Yadgary et al., 2014).

It is well recognized that there are differences between meat and egg-type chickens in terms of nutrients

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utilization for growth and development during embryogenesis (Everaert et al., 2008; Ho et al., 2011; Nangsuay et al., 2015). Different strains of egg-type chickens show remarkable post-hatch differences in feed intake, body growth and development, sexual maturity, egg production efficiency, and skeletal health among many others (Singh et al., 2009; Bain et al., 2016; Khanal et al., 2019). To our knowledge, there is no information on the impact of breeder diet on fertile egg composition and subsequent effects on embryonic nutrients utilization in egg-type chickens of different genetic backgrounds. Yolk lipids are the main source of energy in the last week of incubation and in newly hatched chicks via residual yolk (**RY**) (Cherian, 2015). Furthermore, long-chain polyunsaturated fatty acids (**PUFAs**) such as docosahexaenoic acid (22:6 n−3; **DHA**) and eicosapentaenoic acid (20:5 n−3; **EPA**) are critical for optimal cell, tissue, and organ development (Koppenol et al., 2014a,b; Yadgary et al., 2014). These fatty acids are needed for prenatal and postnatal development because of their vital roles in the synthesis of structural lipids (Maldjian et al., 1995; Mennitti et al., 2015). Previous research showed that the chicken embryo preferentially used PUFA, particularly DHA, between day 15 and 19 of incubation as compared to all other examined fatty acids (Yadgary et al., 2014). Further investigations revealed substantial upregulation of membrane fatty acid transporters and cytosolic fatty acid–binding proteins (**FABP**) linked to uptake of PUFA (Yadgary et al., 2014). This suggested that chick embryo may have specific requirements for n−3 PUFA.

In a typical breeder diet, linoleic acid (**LA**) constitutes over 50% of the total FA compared with ∼3–3.5% of α-linolenic acid (**ALA**) because of high inclusion of corn and other sources of dietary lipids with a high concentration of n−6 fatty acids (Cherian, 2008). Lack of desaturases in avian species results in the inability of hen to insert double bonds beyond δ-9 carbon (Brenner, 1971). Consequently, ALA and LA cannot be synthesized *de novo* and must be supplied through the diet. Feeding hens n−3 FA–rich feedstuffs (such as flaxseed, marine sources) has become one of the most popular and sustainable means for supplying affordable n−3 FA–fortified eggs for human consumption (Elkin et al., 2015). Several studies have shown that feeding broiler breeder with diets containing n−3 FA sources enriched yolk with n−3 FA (Cherian, 2015; Koppenol et al., 2015a,b; Delezie et al., 2016). However, there is little information on the effects of feeding egg-type chick breeder diets with n−3 FA sources on the composition of fertile eggs and embryonic uptake of FA. We hypothesized feeding egg-type chick breeders with diets supplemented with n−3 PUFA sources may change the egg composition and patterns of FA utilization during embryogenesis. Thus, the aim of this study was to evaluate the impact of feeding white and brown egg-type chick breeders with diets rich in n−3 PUFA and subsequent effects on egg composition, embryonic FA utilization, intestinal FA transports and receptor, as well as hepatic malondialdehyde (**MDA**) level in hatchlings as an indicator of lipid-oxidation.

MATERIALS AND METHODS

Birds and Management

The experimental protocol (#3675) was approved by the University of Guelph Animal Care Committee, and birds were cared for in accordance with the Canadian Council on Animal Care guidelines (CCAC, 2009). Day-old breeders of ISA brown and Shaver white (240 ♀ and 30 ♂ per strain) were procured from Hendrix Genetic Canada (Kitchener, ON, Canada) and kept at the Arkell Poultry Research Station, University of Guelph. The birds were housed in separate floor pens by strain and gender in groups of 30. At 16 weeks of age (**WOA**), females of each strain were equally distributed based on the body weight in floor pen of 600 × 188 cm equipped with 21 nipple drinkers and 3 round pan feeders. Males of each strain introduced to each flock to a total of 31 birds (27 ♀ and 4 ♂).

The room temperature was initially set at 34°C on the first day and reduced by 2°C per week until constant temperature of 21°C was reached. The lighting program started on the first day at 40 lux, 02:00 to 18:00 h, and was reduced on the 28th D to 10 lux, 08:00 to 20:00 h up to 16 WOA. During the laying period, birds received 14 h of incandescent light (20 lux, 03:00 to 1,700 h) and 10 h of dark period. The vaccination program included infectious bronchitis (spray), Marek's disease (injection), and Immucox for coccidiosis control (gel droplet) on the first day, followed by Newcastle-infectious bronchitis vaccine (spray) at 3 WOA, Infectious Laryngotracheitis Vectormune FP-LT-AE (wing web) at 6 WOA, and Newcastle-infectious bronchitis at 10 (spray) and 16 (intramuscular) WOA. Birds were fed with commercial fine crumble starter (0–6 WOA, 2,900 kcal/kg AME, 21.0% CP, 1.06% Ca, and 0.77% P) and coarse crumbles (7–16 WOA, 2,900 kcal/kg AME, 18.0% CP, 1.00% Ca, and 0.78% P). During the laying period, birds were fed commercial feed with 2,875 kcal/kg AME, 18.0% CP, 4.24% Ca, and 0.68% P until 25 WOA. All feeds were supplied by Floradale Feed Mill (Floradale, ON, Canada). All eggs produced at week 25 were counted and weighed to establish baseline egg production for each strain.

At 26 WOA, birds were distributed within each strain into 3 dietary treatments (Table 1): 1) control (**CON**), a corn, soybean meal, wheat, and corn gluten diet; 2) CON plus 1% of dried microalgae (*Aurantiochytrium limacinum*) supplement (DMA) as a rich source of DHA (0.04% ALA; 0.2% EPA; 17.9% DHA as-is basis with 3.1% moisture [Alltech Canada, Guelph, Ontario, Canada.]); 3) CON plus 2.6% of coextruded full-fat flaxseed and pulse mixture (FFF, 1:1 wt/wt) as a source of ALA (10.5% ALA; 0.0% EPA; 0.0% DHA as-is basis with 5.2% moisture, LinPRO; O & T Farms Ltd., Regina, SK, Canada). The inclusion of 1% of DMA was

Table 1. Composition of the experimental diets for the ISA brown and Shaver white breeders from 26 to 31 wk of age, as-fed basis.

Item	CON	DMA	FFF
Ingredient, g/kg			
Corn grain	522.35	516.65	509.06
Soybean meal	233.41	234.67	224.70
Wheat	50.00	50.00	50.00
Corn gluten (60.4% crude protein)	36.85	34.42	35.75
DMA[1]	-	10.00	-
FFF[2]	-	-	26.00
Soybean oil	20.40	17.48	17.49
Limestone coarse	37.93	37.92	37.92
Limestone fine	63.22	63.20	63.21
Monocalcium phosphate	15.03	14.94	14.95
Vitamin and trace mineral premix[3]	12.00	12.00	12.00
DL-Methionine, 99%	1.59	1.62	1.62
L-Lysine HCl, 78%	0.74	0.70	0.77
L-Threonine, 98%	0.09	0.09	0.14
Salt	2.65	2.65	2.69
Sodium bicarbonate	2.07	1.98	2.03
Choline Chloride, 60%	1.52	1.52	1.52
Ethoxyquin[4]	0.15	0.15	0.15
Calculated composition			
Metabolizable energy, kcal/kg	2,800	2,800	2,800
Crude protein, %	18.20	18.20	18.20
Calcium, %	4.00	4.00	4.00
Available phosphorus, %	0.38	0.38	0.38
SID[5] Lysine, %	0.80	0.80	0.80
SID Methionine, %	0.42	0.42	0.42
SID Methionine + cystine, %	0.65	0.65	0.65
SID Threonine, %	0.56	0.56	0.56
SID Tryptophan, %	0.18	0.18	0.18
Sodium, %	0.17	0.17	0.17
Chloride, %	0.25	0.25	0.25
$\sum$n−3, %	0.20	0.42	0.43
$\sum$n−6, %	2.47	2.31	2.37
$\sum$n−6:$\sum$n−3	12.35	5.50	5.51

[1]Microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid (DHA), Alltech Canada, Guelph, Ontario, Canada.

[2]Coextruded full-fat flaxseed and pulse mixture (1:1 wt/wt), as a source of α-linolenic acid (ALA), O & T Farms Ltd., Saskatoon, Saskatchewan, Canada.

[3]Provided in kg of diet: vitamin A (retinol), 10,000 IU; vitamin D_3 (cholecalciferol), 3,000 IU; vitamin E, 100 mg; vitamin K_3 (menadione), 5.0 mg; vitamin B_1 (thiamin), 4.0 mg; vitamin B_2 (riboflavin), 10.0 mg; vitamin B_3 (niacin), 50.0 mg; vitamin B_5 (pantothenic acid), 20.0 mg; vitamin B_6 (pyridoxine), 4.0 mg; vitamin B_9 (folic acid), 2.0 mg; vitamin B_{12} (cyanocobalamin), 30.0 mg; biotin, 200 mcg; choline, 400.0 mg; Mg, 110 mg; Zn, 80 mg; Fe, 40.0 mg; Cu, 10.0 mg; I, 1 mg, Se, 0.31 mg.

[4]SANTOQUIN, Novus International Inc., Saint Charles, MO.

[5]Standardized ileal digestible.

previously shown to double enrichment of n−3 PUFA (DHA) in laying hens (Ao et al., 2015). The inclusion level of FFF was chosen to ensure diets 2 and 3 were formulated to have a similar concentration of total n−3 PUFA (Table 2). All diets were formulated to meet or exceed the breeder's genetic specification (Parent Stock Management Guide: ISA Brown, 2018; Parent Stock Management Guide: Shaver White, 2018)

Experimental Procedures

Birds were fed respective diets for 30 D, enough timeline for the peak of n−3 PUFA deposition in the egg (Neijat et al., 2016). Samples of eggs were collected to confirm n−3 PUFA enrichment. A total of 3,109 eggs were collected (∼500 eggs per diet), individually marked and subsequently stored at 4°C until incubation (within 8 D of the collection). Egg production was recorded daily, and feed intake was recorded to the end of egg collection. All eggs produced in the last 3 consecutive days of egg collection were weighed. Twenty eggs per diet were collected at the beginning and at the end of the egg collection period for quality analyses. Eggs were incubated and hatched in a commercial-grade incubator and hatcher (Nature Form, Jacksonville, FL) at the Arkell Poultry Research Station (Guelph, ON). Briefly, eggs were incubated at 37.5°C with 55% humidity to day 19 and then transferred to the hatcher set at 36.9°C with 66% humidity. Incubator conditions were the same for all strains by positioning one egg tray (90 eggs) of CON, DMA, and FFF of both strains at one level of the six used levels (central level and 2 levels above and 3 levels below the central level). Eggs were candled on day 19 (E19). Eight eggs per diet were randomly collected and weighed and embryos were dissected. The small intestine samples were kept at –80°C for further analyses. The separation of different parts of the small intestine obtained at E19 was not possible because of small size. On the day of hatch (**DOH**), chicks were counted and sexed. All males were euthanized by using CO_2. Ten birds from each diet were euthanized for tissue samples. Jejunum segments, starting from the end part of the duodenum and ending at the yolk sack residue (Meckel's diverticulum), were stored at −80°C. The entire liver and RY were taken and stored at −20°C for further analyses.

Sample Processing and Analyses

Whole Egg Separation Egg components, including yolk and albumen, were separated by using a plastic hand-held egg separator (Pierce Chicken IgY Purification Kit # 44918; Thermo Fisher Scientific, Waltham, MA). Paper napkins were used to eliminate the adhering albumen tissues, and then the yolks were weighed. The eggshells were washed with water, dried overnight at 105°C, and weighed (Akbari Moghaddam Kakhki et al., 2016). The albumen weight was calculated by subtracting yolk and shell weights from the whole egg weight.

Concentration of Fatty Acids in Feed and Yolk Feed samples were ground and submitted for FA analyses in a commercial lab (Activation Laboratories, Ancaster, ON) according to O'fallon et al. (2007). Briefly, digestion of samples was performed with a combination of water, methanol, and NaOH. Subsequently, hexane extraction was performed to concentrate the newly produced fatty acid methyl ester in the organic layer. The extract was analyzed using Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with an SP-2560 capillary column (24056; Sigma Aldrich, St. Louis, MO), where the now-separated individual fatty acid methyl ester were identified and quantified.

Lipid extraction and separation in egg yolk was performed based on the method described by Reza-López et al. (2009). Briefly, samples of egg yolk and RY were weighed and mixed with KCL, chloroform, and

Table 2. Analyzed fatty acid profile of experimental diets, as-fed basis.

	CON		DMA[1]		FFF[2]	
Item	% Of total fatty acids	% Of dry matter	% Of total fatty acids	% Of dry matter	% Of total fatty acids	% Of dry matter
14:0	0.00	0.00	0.57	0.03	0.10	0.00
14:1	0.00	0.00	0.00	0.00	0.00	0.00
15:0	0.00	0.00	0.16	0.01	0.04	0.00
15:1	0.00	0.00	0.00	0.00	0.00	0.00
16:0	10.35	0.47	16.37	0.74	10.19	0.44
16:1	0.00	0.00	0.11	0.01	0.15	0.01
17:0	0.00	0.00	0.15	0.01	0.11	0.00
17:1	0.00	0.00	0.00	0.00	0.04	0.00
18:0	1.47	0.07	1.85	0.08	1.93	0.08
18:1n−9	31.62	1.45	23.75	1.08	30.11	1.31
18:2n−6	47.63	2.18	45.68	2.07	41.36	1.80
20:0	0.00	0.00	0.79	0.04	0.34	0.01
18:3n−6	0.00	0.00	0.09	0.00	0.15	0.01
20:1	0.00	0.00	0.28	0.01	0.60	0.03
18:3n−3	6.30	0.29	5.52	0.25	9.26	0.40
21:0	0.00	0.00	0.00	0.00	0.03	0.00
20:2	0.00	0.00	0.00	0.00	0.04	0.00
22:0	0.00	0.00	0.12	0.01	0.17	0.01
20:3n−6	0.00	0.00	0.00	0.00	0.00	0.00
22:1n−9	0.00	0.00	0.00	0.00	0.02	0.00
20:3n−3	0.00	0.00	0.07	0.00	0.02	0.00
20:4n−6	0.00	0.00	0.05	0.00	0.06	0.00
23:0	0.00	0.00	0.00	0.00	0.03	0.00
22:2	0.00	0.00	0.00	0.00	0.00	0.00
24:0	0.00	0.00	0.07	0.00	0.11	0.00
20:5n−3	0.00	0.00	0.08	0.00	0.05	0.00
24:1	0.00	0.00	0.00	0.00	0.06	0.00
22:5	0.00	0.00	0.00	0.00	0.00	0.00
22:6n−3	0.00	0.00	3.80	0.17	0.25	0.01
∑n3	6.30	0.29	9.48	0.43	9.58	0.46
∑n6	47.63	2.18	45.89	2.08	45.60	1.81
∑n6:∑n3		7.56		4.84		4.76
Total	100.00	4.57	100.00	4.56	100.00	4.49

[1]Microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid (DHA).
[2]Coextruded full-fat flaxseed and pulse mixture (FFF, 1:1 wt/wt), as a source of α-linolenic acid (ALA).

methanol. Homogenized samples were spun at 836 g for 10 min, followed by transferring the chloroform layer. Samples were spotted on thin-layer chromatography silica gel plate (P01011; Sigma Aldrich, St. Louis, MO) and sit in a glass Thin-Layer Chromatography (**TLC**) tank containing a solvent mixture of petroleum ether, ethyl ether, and acetic acid. Bands corresponding to phospholipids and triglycerides were visualized under UV light after spraying a fine mist of 8-anilino-1-naphtalene-sulfonic acid. After addition of 2 mL of hexane, and 2 mL of BF3-MeOH to separated lipid classes, the samples were methylated at 100°C for 1.5 h and spun at 10 g for 10 min. Extracted hexane was transferred into gas chromatography vials and analyzed using Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a flame ionization detector and separated on a fused-silica capillary column (127-32H2; Technologies, Santa Clara, CA). Samples were injected in 200:1 split mode. The injector and detector ports were set at 250°C. Fatty acid methyl esters were eluted using a temperature program set initially at 150°C and held for 0.25 min, increased at 35°C/min and held at 170°C for 3 min, increased at 9°C/min to 225°C, and finally increased to 80°C/min to 245°C and held for 2.2 min. The carrier gas was hydrogen, set to a 30-mL/min constant flow rate.

Tissue Protein Extraction and Analyses For protein extraction, the whole small intestine was taken at E19 (0.11 ± 0.013 g), and jejunum samples were taken at DOH (0.12 ± 0.010 g), were weighed, and then placed into a free-standing microcentrifuge tube (02-682-558; Thermo Fisher, Waltham, MA), followed by addition of T-PER Tissue Protein Extraction Reagent (sample weight × 15; 78510; Thermo Fisher, Waltham, MA) supplemented with Halt Protease Inhibitor cocktail (78430; Thermo Fisher, Waltham, MA). For liver samples, 0.1 ± 0.01-g samples were weighed, and phosphate-buffered saline (PBS, 28372; Thermo Fisher, Waltham, MA) was added to the tubes (samples weight × 15). Then, 0.1 ± 0.01 acid-washed glass beads (≤106 μm; G4649-100G; Sigma Aldrich, St. Louis, MO) were added, followed by homogenization with a bead mill for one cycle of 150 s at 3 m/s (15-340-163; Fisher Brand bead mill-24; Thermo Fisher, Waltham, MA). Homogenized samples were then centrifuged at 10,000 × *g* for 15 min at 4°C. Supernatants were analyzed for protein concentration based on the method described in the study by Smith et al. (1985) by using a Pierce BCA protein assay kit (23225; Thermo Fisher, Waltham, MA) and kept at −80°C for further analyses. The concentration of G-protein–coupled receptor 120 (**GPR 120**), small intestine FABP, and hepatic MDA was measured in duplicate using

ELISA kits that followed the recommended assay procedures (GPR 120: ECKG0026 and I-FABP: ECKF0037; ABclonal, Woburn, MA, and MDA: RDR-MDA-Ge; Reddot Biotech Inc., Kelowna, BC).

Calculations and Statistical Analyses

Apparent embryonic FA utilization was calculated by subtracting concentration of FA in RY from the concentration of FA in yolk before incubation. The ratio of daughter FA to parent FA was used for calculating enzymes activity (Nain et al., 2012). The activity of enzymes involved in the biosynthesis of long-chain n−6 PUFA (Δ6-desaturase + elongase + Δ5-desaturase activity) and the biosynthesis of long-chain n−3 PUFA (Δ6-desaturase + elongase + Δ5-desaturase activity) were calculated based on the following formula as described by Nain et al. (2012).

$$\text{biosynthesis of long}-\text{chain } n{-}6 \text{ PUFA} = \frac{20:4n{-}6 \text{ (mg)}}{18:2n{-}6 \text{ (mg)}}$$

$$\text{biosynthesis of long}-\text{chain } n{-}3 \text{ PUFA} = \frac{20:5n{-}3 \text{ (mg)}}{18:3n{-}3 \text{ (mg)}}$$

Data were tested for normality with univariate plot normal procedure of SAS 9.4 and subsequently subjected to a two-way ANOVA in a 2 (ISA brown and Shaver white) × 3 (CON, DMA, and FFF) factorial arrangement using Tukey test by the GLIMMIX procedure of SAS 9.4. Significance was declared at $P < 0.05$. A tendency was declared at $P \leq 0.10$.

RESULTS

Fatty Acids Concentration in Experimental Diets

Supplementation with DMA and FFF modified the FA profile of the diets as shown in Table 2. The dominant FA were LA, 18:1n−9 and 16:0 across all the diets. Diet containing DMA had a higher relative percent composition of DHA (3.8 vs. 0.0% of FA) and lower ALA (5.1 vs. 6.3% of FA) than CON. The FFF had higher relative percent composition of ALA (10.3 vs. 6.3% of FA) than CON.

Production Performance

Feed intake, egg production, mass, and weight were neither affected by the interaction between strain and diets nor the main effect of diets ($P > 0.05$; Supplementary Table 1). Feed intake was higher for ISA brown hens than for Shaver white breeders (113 vs. 108 g/b/D; $P = 0.039$). Egg production, mass, and weight were not influenced by strain ($P > 0.05$). Average egg production, mass, and weight were 93.2%, 54.5 g/b/D, and 50.8 g, respectively, for ISA brown. Corresponding values for Shaver white were 94.8%, 52.89 g/b/D, and 50.12 g.

Egg Components

The interactive effect of strain and diets did not affect egg weight and its components (Table 3; $P > 0.05$). ISA brown hens had heaver eggs ($P = 0.027$) and eggshell weight ($P = 0.023$) than Shaver white. However, strain did not influence albumen, yolk, and eggshell measurements ($P > 0.05$).

Fatty Acids Profile of Yolk Phospholipid Fraction

There was an interaction ($P < 0.05$) between strain and diets on the relative percent composition of 20:3n−6, arachidonic acid (AA; 20:4n−6), 22:4n−6, 22:5n−6, DHA, ∑n−6, and ∑n−6:∑n−3 in the phospholipid fraction of yolk (Table 4). However, this interactive effect was not systematic and did not follow the same patterns for all observations. Supplemental DMA increased the DHA by 39.0% compared with CON in ISA brown, and the corresponding value was 45.3% for Shaver white. Feeding FFF increased DHA by 19.0% and 40.8% in ISA brown and Shaver white hens, respectively. Supplementation with DMA decreased the ratio of ∑n−6:∑n−3 by 82.9% and 28.7% compared with CON in ISA brown and Shaver white, respectively. Feeding FFF reduced the corresponding values by 45.7% in ISA brown and 28.2% in Shaver white.

The FA profile in the yolk phospholipid was influenced by the strain ($P < 0.05$). Shaver white had a higher relative value of 20:2n−6, 20:3n−6, AA, EPA, 22:5n−3, and DHA in the FA content of their yolk phospholipid than ISA brown, which resulted in a higher relative percent composition of ∑n−3 and a lower ratio of ∑n−6:∑n−3 ($P < 0.05$). Yolk phospholipid fraction of ISA brown contained a higher relative percent composition of 16:00, 18:1n−9, LA, 22:4n−6, and total FA in phospholipid fraction (mg/g dry matter of yolk) than Shaver white.

Dietary addition of DMA reduced the relative percent composition of ALA compared with CON ($P = 0.006$). The relative percent composition of EPA was increased in response to the inclusion of FFF ($P = 0.001$). Supplementation with DMA and FFF increased the relative amount of ∑n−3 ($P < 0.001$). Supplemental DMA and FFF decreased the total FA in phospholipid fraction (mg/g dry matter of yolk) by 18.0% and 16.0%, respectively ($P < 0.001$).

Fatty Acids Profile of Yolk Triglyceride Fraction

There was an interaction between strain and diets in percent composition of DHA ($P = 0.003$) and ∑n−6 ($P = 0.032$) to total FA (Table 5). Supplementation with DMA increased the relative percent composition

Table 3. Effects of dietary treatments on the egg components of Shaver white and ISA brown breeders.[1]

Items		Albumen		Yolk		Eggshell	
		%	g	%	g	%	g
Strain	Diet						
ISA brown	CON[2]	63.45	36.88	26.44	15.39	10.11	5.88
ISA brown	DMA[3]	64.05	39.21	25.77	15.77	10.18	6.23
ISA brown	FFF[4]	64.04	37.08	25.65	14.84	10.30	5.97
Shaver white	CON	63.41	35.98	26.73	15.10	9.87	5.58
Shaver white	DMA	62.82	36.15	27.02	15.52	10.26	5.89
Shaver white	FFF	64.48	36.32	25.36	14.27	10.15	5.72
SEM		0.673	1.022	0.645	0.431	0.187	0.154
Main effect							
Strain							
ISA brown		63.85	37.72	25.95	15.33	10.20	6.03[a]
Shaver white		63.57	36.15	26.37	14.96	10.10	5.73[b]
SEM		0.476	0.565	0.381	0.356	0.108	0.089
Diet							
CON		63.43	36.43	26.58	15.24	9.99	5.73
DMA		63.43	37.68	26.39	15.65	10.22	6.06
FFF		64.26	36.70	25.51	14.56	10.23	5.84
SEM		0.476	0.700	0.472	0.316	0.132	0.109
Probabilities (P value)							
Strain		0.616	0.053	0.441	0.297	0.509	0.023
Diet		0.373	0.411	0.220	0.052	0.352	0.099
Strain × diet		0.462	0.428	0.503	0.924	0.680	0.963

Values with uncommon superscripts within each column are significantly different ($P < 0.05$).
Abbreviation: SEM, standard error of the mean.
[1]Data are means of 16 egg samples per each treatment.
[2]Control.
[3]Microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid (DHA).
[4]Coextruded full-fat flaxseed and pulse mixture (FFF, 1:1 wt/wt), as a source of α-linolenic acid (ALA).

of DHA by 123% and 275% in ISA brown and Shaver white, respectively, compared with CON. The relative percent composition of DHA was increased by 30.8% and 242% in response to the inclusion of FFF in ISA brown and Shaver white, respectively. Supplemental DMA reduced total FA in triglyceride fraction (mg/g dry matter of yolk) by 19.3% compared with CON in ISA brown, while supplementation with FFF did not affect the total FA in triglyceride fraction. Supplementation with DMA and FFF decreased total FA in triglyceride fraction in Shaver white by 39.8% and 40.3% compared with CON, respectively.

There were higher relative percent composition of 18:4n−3, 20:2n−6, 20:3n−6, 20:3n−3, 22:2n−6, 22:5n−3, and DHA in the yolk triglyceride of Shaver white than that of ISA brown, which resulted in a lower ratio of ∑n−6:∑n−3 than that of the ISA brown ($P < 0.05$).

Supplemental DMA decreased the relative percent composition of ALA, 22:5n−3, and increased the relative percent composition of 20:2n−6 and 20:3n−6 compared with CON ($P < 0.05$). Supplementation with FFF increased the relative percent composition of 18:4n−3 and 20:3n−3 and increased the relative percentage composition of 22:5n−3 compared with CON ($P < 0.05$).

Calculated n−3 and n−6 PUFA Desaturation and Elongation

There was an interaction between strain and diets in the activity of n−6 PUFA desaturation and elongation ($P = 0.009$; Table 6). Supplementation with DMA reduced n−6 PUFA desaturation and elongation activity in the phospholipids in ISA brown, while their activity was increased in response to supplementation with DMA and FFF in Shaver white. Shaver white had a higher activity of desaturation and elongation of n−3 and n−6 in phospholipid fraction and n−3 in triglyceride fraction than ISA brown ($P < 0.05$). Supplementation with DMA enhanced the activity of n−3 desaturation and elongation enzymes in phospholipid ($P < 0.001$) and triglyceride ($P = 0.029$) fractions.

Apparent Embryonic Utilization of Fatty Acids of Phospholipid Fraction

There was an interaction ($P < 0.05$) between strain and diets in the apparent utilization of 18:3n−6, ALA, 20:3n−6, AA, and total phospholipid FA (Table 7). The interactive effect did not follow a pattern in either strain. The ISA brown embryos from DMA and FFF diets used less 18:3n−6, 20:3n−6, AA, and total phospholipid. Shaver white embryos used less ALA and more 20:3n−6, while they maintained equal total phospholipid utilization as CON embryos. Shaver white embryos used more 18:3n−6, ALA, 20:3n−6, AA, EPA, 22:5n−3, and DHA and subsequently used 11.1% more phospholipids than ISA brown embryos ($P < 0.05$). Diets influenced utilization of FA; among n−6 FA, 22:4n−6 utilization was decreased by feeding FFF and DMA relative to CON ($P < 0.05$). Among the n−3 group, the utilization of DHA was increased in DMA and FFF

Table 4. The effects of dietary treatments on the fatty acid profile of the egg yolk phospholipid fraction in ISA brown and Shaver white breeders (% composition).

	ISA brown			Shaver white				Strain			Diet				*P* value		
Treatment	CON[1]	DMA[2]	FFF[3]	CON	DMA	FFF	SEM	ISA	Shaver	SEM	CON	DMA	FFF	SEM	Strain	Diet	Strain × diet
16:0	27.28	28.4	28.42	27.02	26.97	27.36	0.488	28.00^{a}	27.12^{b}	0.281	27.13	27.69	27.86	0.345	0.021	0.343	0.930
18:0	16.74	14.91	16.45	12.82	18.2	17.07	1.379	16.03	16.03	0.839	14.78	16.56	16.76	0.975	0.821	0.143	0.120
18:1n−9	24.50	25.69	23.84	22.64	21.19	22.49	1.164	24.64^{a}	22.11^{b}	0.672	23.57	23.39	23.17	0.823	0.012	0.584	0.605
18:2n−6	15.47	15.09	15.19	14.56	14.62	14.19	0.284	15.25^{a}	14.46^{b}	0.172	15.02	14.86	14.69	0.217	0.002	0.398	0.891
18:3n−6	0.08	0.03	0.05	0.12	0.14	0.12	0.012	0.05^{b}	0.13^{a}	0.007	0.10^{a}	0.09^{b}	0.09^{b}	0.009	<0.001	0.042	0.492
18:3n−3	0.28	0.38	0.36	0.49	0.22	0.27	0.052	0.34	0.33	0.032	0.39^{a}	0.30^{b}	0.32^{a,b}	0.040	0.687	0.006	0.076
18:4n−3	0.04	0.05	0.04	0.03	0.03	0.02	0.007	0.04	0.03	0.004	0.04	0.04	0.03	0.005	0.064	0.897	0.205
20:2n−6	0.26	0.23	0.24	0.27	0.29	0.28	0.012	0.24^{b}	0.28^{a}	0.006	0.27	0.26	0.26	0.008	<0.001	0.072	0.519
20:3n−6	0.15^{b}	0.10^{b}	0.10^{b}	0.18^{b}	0.36^{a}	0.28^{a,b}	0.018	0.12^{b}	0.27^{a}	0.011	0.17^{b}	0.23^{a}	0.19^{b}	0.013	<0.001	<0.001	0.001
20:4n−6	5.98^{a}	4.00^{b}	5.05^{a,b}	5.87^{a,b}	6.12^{a}	5.54^{a,b}	0.287	5.01^{b}	5.84^{a}	0.167	5.93^{a}	5.06^{b}	5.30^{a,b}	0.190	<0.001	0.001	0.017
20:3n−3	0.05	0.05	0.07	0.04	0.04	0.05	0.009	0.06	0.04	0.005	0.05	0.05	0.06	0.006	0.188	0.654	0.281
20:5n−3	0.04	0.08	0.09	0.11	0.10	0.10	0.009	0.07^{b}	0.10^{a}	0.005	0.08^{b}	0.09^{a,b}	0.10^{a}	0.007	<0.001	0.001	0.082
22:2n−6	0.06	0.04	0.04	0.05	0.05	0.03	0.008	0.05	0.04	0.005	0.06	0.05	0.04	0.006	0.748	0.075	0.593
22:4n−6	0.50^{a}	0.37^{b}	0.47^{a,b}	0.44^{a,b}	0.32^{b}	0.38^{a,b}	0.029	0.45^{a}	0.38^{b}	0.018	0.47^{a}	0.35^{b}	0.43^{a,b}	0.021	0.009	0.023	0.007
22:5n−6	0.77^{a}	0.52^{b}	0.41^{c}	0.65^{a,b}	0.42^{c}	0.64^{a,b}	0.053	0.57	0.57	0.027	0.71^{a}	0.47^{b}	0.53^{a,b}	0.035	0.890	<0.001	0.023
22:5n−3	0.06	0.07	0.03	0.09	0.18	0.12	0.019	0.05^{b}	0.13^{a}	0.012	0.08^{b}	0.13^{a}	0.08^{b}	0.014	<0.001	0.021	0.207
22:6n−3	4.80^{c}	6.67^{a,b}	5.71^{b}	5.47^{b,c}	7.95^{a}	7.70^{a}	0.323	5.73^{b}	7.04^{a}	0.203	5.14^{c}	7.31^{a}	6.71^{b}	0.230	<0.001	<0.001	<0.001
∑n−3	4.45	7.29	6.30	6.23	8.52	8.26	0.301	6.04^{b}	7.67^{a}	0.183	5.39^{b}	7.91^{a}	7.28^{a}	0.230	<0.001	<0.001	0.499
∑n−6	23.27^{a}	19.90^{c}	21.57^{b}	21.21^{b,c}	22.53^{a,b}	21.77^{a,b}	0.386	21.58	21.84	0.233	22.24^{a}	21.22^{b}	21.67^{a,b}	0.383	0.429	0.036	<0.001
∑n−6:∑n−3	5.14^{a}	2.81^{c,d}	3.53^{b}	3.41^{b,c}	2.65^{d}	2.66^{d}	0.150	3.83^{a}	2.91^{b}	0.087	4.27^{a}	2.73^{b}	3.10^{b}	0.106	<0.001	<0.001	<0.001
Total[4]	30.27	22.79	24.61	29.50	27.89	26.92	1.166	28.10a	25.89^{b}	0.710	29.89^{a}	25.34^{b}	25.77^{b}	0.890	0.029	<0.001	0.053

Values with uncommon superscripts within each column are significantly different ($P < 0.05$).
Abbreviation: SEM, standard error of the mean.
[1]Control.
[2]Microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid (DHA).
[3]Co-extruded full fat flaxseed and pulse mixture (FFF, 1:1 wt/wt), as a source of α-linolenic acid (ALA).
[4]Expressed as mg of phospholipid per gram of yolk dry matter.

Table 5. The effects of dietary treatments on the fatty acid profile of the egg yolk triglyceride fraction in ISA brown and Shaver white breeders (% composition).

	ISA brown			Shaver white				Strain			Diet				*P* value		
Treatment	CON[1]	DMA[2]	FFF[3]	CON	DMA	FFF	SEM	ISA	Shaver	SEM	CON	DMA	FFF	SEM	Strain	Diet	Strain × diet
16:0	25.21	25.11	24.09	25.25	22.34	21.58	1.668	24.80	23.06	0.986	25.23	23.73	22.84	1.221	0.217	0.737	0.346
16:1n−9	2.84	2.80	2.80	1.68	1.84	1.96	0.310	2.81	1.83	0.183	2.26	2.32	2.38	0.226	<0.001	0.912	0.910
18:0	5.60	7.20	5.18	7.02	7.59	6.98	0.640	5.99	7.20	0.354	6.31	7.40	6.08	0.439	0.021	0.288	0.147
18:1n−9	45.81	42.15	46.30	45.78	43.78	41.54	1.531	44.75	43.70	0.846	45.80^{a}	42.97^{b}	$43.92^{a,b}$	1.04	0.383	0.021	0.845
18:2n−6	16.46	17.07	17.38	15.16	17.8	16.02	0.676	16.97	16.33	0.399	15.81	17.44	16.70	0.495	0.261	0.443	0.070
18:3n−6	0.05	0.03	0.03	0.07	0.16	0.1	0.015	0.04^{b}	0.11^{a}	0.008	0.06^{b}	0.10	0.07	0.010	<0.001	<0.001	0.102
18:3n−3	1.22	1.08	1.84	1.74	1.19	1.09	0.131	1.38	1.34	0.078	1.48^{a}	1.14^{b}	1.47^{a}	0.096	0.709	<0.001	0.930
18:4n−3	0.08	0.07	0.06	0.09	0.11	0.07	0.009	0.07^{b}	0.09^{a}	0.005	0.09^{a}	0.09^{a}	0.07^{b}	0.006	0.023	0.004	0.188
20:2n−6	0.13	0.15	0.12	0.16	0.26	0.22	0.022	0.13^{b}	0.21^{a}	0.012	0.15^{b}	0.21^{a}	$0.17^{a,b}$	0.014	<0.001	0.020	0.055
20:3n−6	0.03	0.03	0.01	0.04	0.17	0.09	0.027	0.02^{b}	0.10^{a}	0.015	0.04^{b}	0.10^{a}	$0.05^{a,b}$	0.018	<0.001	0.016	0.080
20:4n−6	0.21	0.79	0.27	0.25	0.35	0.28	0.246	0.42	0.29	0.136	0.23	0.57	0.28	0.168	0.497	0.449	0.363
20:3n−3	0.09	0.04	0.04	0.08	0.10	0.08	0.018	0.06^{b}	0.09^{a}	0.009	0.09^{a}	$0.07^{a,b}$	0.06^{b}	0.010	0.047	0.022	0.624
20:5n−3	0.03	0.04	0.03	0.05	0.10	0.03	0.027	0.03	0.06	0.016	0.04	0.07	0.03	0.018	0.100	0.061	0.191
22:2n−6	0.06	0.04	0.03	0.06	0.15	0.20	0.044	0.04^{b}	0.14^{a}	0.024	0.06	0.10	0.12	0.030	0.009	0.233	0.301
22:4n−6	0.18	0.21	0.09	0.12	0.13	0.24	0.035	0.16	0.16	0.021	0.15	0.17	0.17	0.025	0.864	0.005	0.408
22:5n−6	0.12	0.14	0.06	0.09	0.19	0.42	0.119	0.11	0.23	0.066	0.11	0.17	0.24	0.081	0.174	0.211	0.530
22:5n−3	0.02	0.03	<0.01	0.02	0.14	0.18	0.028	0.03^{b}	0.11^{a}	0.016	0.02^{c}	0.09^{b}	0.18^{a}	0.020	<0.001	0.007	0.056
22:6n−3	0.13^{b}	0.29^{b}	0.17^{b}	0.24^{b}	0.66^{a}	0.58^{a}	0.066	0.20^{b}	0.49^{a}	0.037	0.19^{b}	0.48^{a}	$0.38^{a,b}$	0.062	<0.001	0.002	0.003
∑n−3	1.56	2.44	2.15	2.03	2.41	2.32	0.391	2.05	2.25	0.221	1.80	2.42	2.23	0.267	0.522	0.249	0.803
∑n−6	$17.24^{a,b}$	$18.45^{a,b}$	$17.98^{a,b}$	$17.79^{a,b}$	15.95^{b}	19.37^{a}	0.737	17.89	17.70	0.435	17.52	17.20	18.68	0.539	0.761	0.140	0.032
∑n−6:∑n−3	11.07	9.97	8.86	9.05	6.65	8.71	0.741	9.97^{a}	8.13^{b}	0.419	10.06	8.31	8.78	0.507	0.003	0.053	0.099
Total[4]	29.57^{a}	24.78^{b}	$29.04^{a,b}$	32.22^{a}	23.05^{b}	22.96^{b}	1.18	27.80	26.08	0.649	30.89^{a}	23.92^{b}	$26.00^{a,b}$	0.831	0.069	<0.001	0.002

Values with uncommon superscripts within each column are significantly different ($P < 0.05$).
Abbreviation: SEM, standard error of the mean.
[1]Control.
[2]Microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid (DHA).
[3]Co-extruded full fat flaxseed and pulse mixture (FFF, 1:1 wt/wt), as a source of α-linolenic acid (ALA).
[4]Expressed as mg of triglyceride per gram of yolk dry matter.

Table 6. Effects of dietary treatments on calculated biosynthesis of fatty acids in Shaver white and ISA brown breeders.[1]

Items		n−6 PUFA biosynthesis[2]		n−3 PUFA biosynthesis[3]	
		PH	TG	PH	TG
Strain	Diet				
ISA brown	CON	0.387[a]	0.014	0.126	0.020
ISA brown	DMA[4]	0.242[b]	0.014	0.286	0.033
ISA brown	FFF[5]	0.334[a,b]	0.015	0.219	0.015
Shaver white	CON	0.361[a,b]	0.016	0.237	0.023
Shaver white	DMA	0.419[a]	0.020	0.461	0.039
Shaver white	FFF	0.412[a]	0.017	0.394	0.026
SEM		0.031	0.001	0.031	0.006
Main effect					
Strain					
ISA brown		0.321[b]	0.015[b]	0.211[b]	0.023
Shaver white		0.397[a]	0.018[a]	0.364[a]	0.029
SEM		0.018	0.0007	0.019	0.003
Diet					
CON		0.374	0.015	0.181[b]	0.022[b]
DMA		0.331	0.017	0.373[a]	0.036[a]
FFF		0.373	0.016	0.307[a,b]	0.021[b]
SEM		0.023	0.0009	0.025	0.004
Probabilities (P value)					
Strain		0.006	0.004	<0.001	0.187
Diet		0.300	0.419	<0.001	0.029
Strain × diet		0.009	0.135	0.514	0.850

Values with uncommon superscripts within each column are significantly different ($P < 0.05$).

Abbreviations: CON, control; PH, phospholipid; TG, triglyceride; SEM, standard error of the mean.

[1]Data are means of 16 egg samples per each treatment.

[2]n−6 PUFA biosynthesis pathway enzyme activity (Δ6-desaturase, elongase, and Δ5-desaturase) was calculated as ratio of 20:4 n−6 to 18:2 n−6.

[3]n−3 polyunsaturated fatty acid biosynthesis pathway enzyme activity (Δ6-desaturase, elongase, and Δ5-desaturase) was calculated as ratio of 20:5 n−3 to 18:3 n−3.

[4]Microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid (DHA).

[5]Coextruded full-fat flaxseed and pulse mixture (FFF, 1:1 wt/wt), as a source of α-linolenic acid (ALA).

($P = 0.001$). Utilization of total phospholipid was lower for embryos from breeders fed with DMA and FFF than for those from breeders fed with CON ($P < 0.001$).

Apparent Embryonic Utilization of Fatty Acids of Triglyceride Fraction

There was an interaction between strains and diets ($P < 0.05$) in utilization of 18:3n−6, ALA, 20:3n−6, and 22:5n−3 (Table 8). However, similar to phospholipid utilization, the interactive effect was not systematic. Shaver white embryos from DMA and FFF used less ALA ($P < 0.001$) and more 22:5n−3 ($P = 0.015$) and tended ($P = 0.105$) to use more DHA. Shaver white embryos used less triglyceride than ISA brown ($P = 0.020$). The main effect of diet was significant for the utilization of AA, where embryos from DMA used more AA than CON and FFF ($P < 0.001$).

Fatty Acids Transporters and Hepatic Malondialdehyde

There was no interaction between strain and diets ($P > 0.05$) on intestinal and jejunum GPR 120 and FABP, as well as on hepatic MDA (Table 9). The concentration of GPR 120 was higher in the jejunum of Shaver white embryos than that in ISA brown ($P = 0.046$). The effect of strain was also observed in hepatic MDA, where Shaver white had higher MDA (8.07%; $P = 0.005$) than ISA brown embryos. The concentration of GPR 120 on E19 and DOH was increased by dietary inclusion of DMA ($P > 0.05$). The concentration of FABP was neither affected by the main effect of strain nor the dietary treatments ($P > 0.05$). Dietary treatment did not affect hepatic MDA ($P > 0.05$).

DISCUSSION

Dietary FA profile confirmed that DMA was a rich source of DHA, whereas FFF was a rich source of ALA. Supplementation with either source enriched diets with the other FA to a lesser extent than DHA and ALA. ISA brown breeders had a tendency to lay heavier eggs than Shaver white, resulting in a higher absolute eggshell weight. The differences between white and brown hens were previously reported by Singh et al. (2009), where brown strains had heavier egg and eggshell weight than white strains. Similarly, Nain et al. (2012) observed no changes in egg and yolk weight in response to supplementation of up to 15% LinPRO (same as FFF in the present study) in the diet of 65 WOA Lohmann White Leghorn hens. It has been reported that production performance (egg production percentage, weight, and FCR), eggshell, and yolk percentage were not affected by supplementation of up to 3% microalgae (same as DMA in the present study) in diets of 45 WOA Hy-Line W-36 laying for 36 WOA (Ao et al., 2015).

Despite the dietary ALA being 2 times more in the FFF diet than the DMA diet, the total ALA content was identical in eggs. Nain et al. (2012) reported 426% and 769% increase in ALA content (mg/egg) by supplementing 7.5% and 15% LinPRO, respectively. However, based on our confirmatory test for FA deposition in FFF eggs, the corresponding value (based on the percentage of FA to total fat) was 70% and 60% in ISA brown and Shaver white, respectively. In the present study, feeding DMA increased DHA content by 30.8% and 83.3% in phospholipid and triglycerides, respectively. The corresponding values for FFF fed birds were 21.7% and 50.0% for phospholipid and triglycerides, respectively. This difference in DHA levels in the yolk might be because the % composition of DHA was 17-fold higher in the DMA than in the FFF in the present study. Similar to our observations, Ao et al. (2015) observed a linear increase in DHA % composition without any changes in egg fat content when the supplemental levels of microalgae were increased by up to 3% in diets of 45 WOA Hy-line W-36 hens for 36 WOA. Conversely, supplementation with 7.5% LinPRO for 18 D increased DHA (mg/egg) by 36.6% in 65 WOA Lohmann White Leghorn hens (Nain et al., 2012).

Based on findings of the present study, Shaver white breeders deposited more n−3 and less n−6 FA in their eggs than ISA brown breeders. Similar to the present

Table 7. The effects of dietary treatments on the apparent utilization of phospholipid throughout the embryonic period in Shaver white and ISA brown breeders.

Treatment		18:3n−6	18:3n−3	20:3n−6	20:4n−6	20:5n−3	22:4n−6	22:5n−3	22:6n−3	Total[1]
Strain	Diet									
ISA brown	CON	0.17^{a,b}	0.62^{a}	0.34^{b}	13.54^{a}	0.08	1.05	0.11	9.49	226.38^{a}
ISA brown	DMA[2]	0.03^{c}	0.63^{a}	0.15^{c}	4.81^{c}	0.13	0.45	0.08	11.51	151.13^{b}
ISA brown	FFF[3]	0.06^{b,c}	0.50^{b}	0.17^{c}	7.67^{b,c}	0.13	0.67	0.02	9.87	151.57^{b}
Shaver white	CON	0.25^{a}	0.99^{a}	0.36^{b}	10.67^{b}	0.24	0.88	0.17	11.37	206.55^{a}
Shaver white	DMA	0.27^{a}	0.39^{b}	0.68^{a}	11.74^{a,b}	0.20	0.42	0.28	15.86	193.79^{a}
Shaver white	FFF	0.20^{a}	0.47^{b}	0.53^{a}	11.21^{a,b}	0.21	0.61	0.23	15.22	187.52^{a}
SEM		0.018	0.105	0.041	0.999	0.020	0.066	0.039	0.809	9.178
Main effect										
Strain										
ISA brown		0.09^{b}	0.58^{b}	0.22^{b}	8.67^{b}	0.11^{b}	0.72	0.07^{b}	10.29^{b}	176.36^{b}
Shaver white		0.24^{a}	0.62^{a}	0.52^{a}	11.18^{a}	0.21^{a}	0.63	0.23^{a}	14.14^{a}	195.96^{a}
SEM		0.004	0.064	0.024	0.608	0.012	0.040	0.024	0.493	5.585
Diet										
CON		0.21^{a}	0.81a	0.35^{b}	12.10^{a}	0.16	0.97^{a}	0.14	10.43^{b}	216.46^{a}
DMA		0.15^{b}	0.51^{b}	0.41^{a}	8.28^{b}	0.16	0.44^{c}	0.18	13.69^{a}	172.46^{b}
FFF		0.13^{b}	0.49^{b}	0.35^{b}	9.44^{b}	0.17	0.63^{b}	0.13	12.55^{a}	169.55^{b}
SEM		0.005	0.078	0.031	0.764	0.015	0.050	0.030	0.573	7.010
Probabilities (P value)										
Strain		<0.001	0.021	<0.001	0.005	<0.001	0.102	<0.001	<0.001	0.020
Diet		0.033	<0.001	0.002	0.002	0.950	<0.001	0.356	0.001	<0.001
Strain × diet		0.032	<0.001	<0.001	<0.001	0.065	0.533	0.130	0.117	0.003

Values with uncommon superscripts within each column are significantly different ($P < 0.05$).
Abbreviations: CON, control; SEM, standard error of the mean.
[1]Including all phospholipid of all the measured fatty acids.
[2]Microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid (DHA).
[3]Coextruded full-fat flaxseed and pulse mixture (FFF, 1:1 wt/wt), as a source of α-linolenic acid (ALA).

study with regard to the n−6 family, Ao et al. (2015) observed a linear decrease in LA and AA by increasing the inclusion level of microalgae up to 3% in the diet of 45 WOA W-36 Hy-line for 36-week. In the present study, there was no detectable 20:2n−6, AA, and 22:5n−6 in CON diet. However, the higher % composition of AA and 22:5n−6 were incorporated into the phospholipid fraction in CON compared with DMA and FFF, indicating the *de novo* desaturation and elongation of AA and 22:5n−6 from the LA. Enzymes such as Δ-6

Table 8. The effect of dietary treatments on the apparent utilization of triglyceride throughout the embryonic period in Shaver white and ISA brown breeders.

Treatment		18:3n−6	18:3n−3	20:3n−6	20:4n−6	20:5n−3	22:4n−6	22:5n−3	22:6n−3	Total[1]
Strain	Diet									
ISA brown	CON[2]	0.11^{b}	2.69^{a,b}	0.07^{b}	0.38	0.06	0.34	0.02^{b}	0.25	217.67
ISA brown	DMA[3]	0.03^{b}	1.90^{b}	0.04^{b}	1.16	0.07	0.30	0.04^{b}	1.78	164.22
ISA brown	FFF[4]	0.02^{b}	3.52^{a}	0.00^{b}	0.22	0.06	0.12	0.00^{b}	0.23	185.70
Shaver white	CON	0.14^{b}	3.71^{a}	0.07^{b}	0.45	0.10	0.24	0.05^{b}	0.50	215.73
Shaver white	DMA	0.25^{a}	1.83^{b}	0.26^{a}	0.43	0.17	0.26	0.21^{a}	0.97	149.84
Shaver white	FFF	0.13^{b}	1.59^{b}	0.12^{a,b}	0.31	0.05	0.68	0.29^{a}	0.87	147.93
SEM		0.027	0.289	0.039	0.086	0.042	0.184	0.044	0.661	8.033
Main effect										
Strain										
ISA brown		0.06^{b}	2.70	0.03^{b}	0.59	0.06	0.25	0.02^{b}	0.75	189.20^{a}
Shaver white		0.18^{a}	2.38	0.15^{a}	0.40	0.11	0.40	0.18^{a}	0.78	171.17^{b}
SEM		0.017	0.160	0.023	0.022	0.025	0.102	0.026	0.374	3.099
Diet										
CON		0.13	3.20^{a}	0.07	0.41^{b}	0.08	0.29	0.03	0.37	216.70^{a}
DMA		0.14	1.86^{b}	0.15	0.80^{a}	0.12	0.28	0.13	1.37	157.03^{b}
FFF		0.08	2.56^{a}	0.05	0.27^{b}	0.05	0.41	0.14	0.55	166.81^{b}
SEM		0.022	0.191	0.029	0.030	0.032	0.130	0.033	0.486	4.298
Probabilities (P value)										
Strain		<0.001	0.139	0.001	0.558	0.167	0.301	<0.001	0.960	0.020
Diet		0.129	<0.001	0.060	0.003	0.233	0.720	0.053	0.264	<0.001
Strain × diet		0.007	<0.001	0.028	0.065	0.506	0.123	0.015	0.505	0.289

Values with uncommon superscripts within each column are significantly different ($P < 0.05$).
Abbreviation: SEM, standard error of the mean.
[1]Including all phospholipid of all the measured fatty acids.
[2]Control.
[3]Microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid (DHA).
[4]Co-extruded full fat flaxseed and pulse mixture (FFF, 1:1 wt/wt), as a source of α-linolenic acid (ALA).

Table 9. Effects of the dietary treatments on ileum G-protein-coupled receptor 120 (GPR 120), intestinal fatty acid-binding protein (FABP), and hepatic malondialdehyde (MDA) in 19 D of embryonic period (E19) and day of hatch (DOH) of Shaver white and ISA brown breeders.[1]

		E19				DOH					
		Intestinal GPR 120[2]		Intestinal FABP[3]		Jejunum GPR 120		Jejunum FABP		Hepatic MDA	
Items		ng/mg	ng/mg protein	ng/mg	ng/mg protein	ng/mg	ng/mg protein	ng/mg	ng/mg protein	ng/mg	ng/mg protein
Strain	Diet										
ISA brown	CON	24.09	3.52	0.28	0.04	20.56	1.82	1.28	0.14	222	4.45
ISA brown	DMA[4]	69.42	6.49	0.30	0.03	34.42	3.06	1.28	0.14	229	4.24
ISA brown	FFF[5]	50.81	4.90	0.28	0.03	19.49	1.73	1.28	0.14	228	4.70
Shaver white	CON	31.24	3.15	0.32	0.03	23.82	2.12	1.29	0.15	224	4.71
Shaver white	DMA	86.37	8.70	0.34	0.04	34.36	3.05	1.26	0.12	233	4.94
Shaver white	FFF	70.83	7.31	0.34	0.04	33.28	2.96	1.27	0.13	228	4.90
SEM		13.394	1.540	0.043	0.005	3.413	0.303	0.012	0.001	4.900	0.162
Main effect											
Strain											
ISA brown		48.10	4.97	0.28	0.03	24.82^{b}	2.20^{b}	1.28	0.14	226	4.46^{b}
Shaver white		62.82	6.38	0.33	0.03	30.48^{a}	2.71^{a}	1.27	0.13	228	4.85^{a}
SEM		7.732	0.889	0.025	0.003	1.970	0.175	0.007	0.0006	2.829	0.094
Diet											
CON		27.66^{b}	3.33^{b}	0.30	0.03	22.19^{b}	1.97^{b}	1.29	0.14	223	4.58
DMA		77.90^{a}	7.59^{a}	0.32	0.03	34.39^{a}	3.05^{a}	1.27	0.13	231	4.59
FFF		60.82^{a}	6.10^{a,b}	0.31	0.03	26.39^{a,b}	2.34^{a,b}	1.28	0.13	228	4.80
SEM		9.471	1.089	0.030	0.004	2.413	0.214	0.009	0.0008	3.465	0.115
Probabilities (P value)											
Strain		0.186	0.267	0.160	0.288	0.047	0.046	0.360	0.417	0.665	0.005
Diet		0.002	0.027	0.913	0.859	0.003	0.002	0.485	0.486	0.121	0.331
Strain × diet		0.882	0.604	0.943	0.574	0.116	0.110	0.471	0.452	0.951	0.251

Values with uncommon superscripts within each column are significantly different ($P < 0.05$).
Abbreviations: CON, control; SEM, standard error of the mean.
[1]Data are means of 10 samples per each treatment.
[2]The sensitivity of the assay was 0.1 ng/mL.
[3]The sensitivity of the assay was 0.1 ng/mL.
[4]Microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid (DHA).
[5]Coextruded full-fat flaxseed and pulse mixture (FFF, 1:1 wt/wt), as a source of α-linolenic acid (ALA).

desaturase, elongase 5, and Δ-5 desaturase have been shown to be involved in the process of elongation and desaturation of LA and ALA (Cherian et al., 2007).

The inhibitory effect of n−3 FA on δ-6 desaturase activity might be one reason for the reduction of AA and 22:5n−6 in DMA and FFF treatments, along with a lower dietary composition of LA as the precursor in DMA and FFF than in CON. The 18-carbon precursors of n−6 PUFA and n−3 PUFA substrates compete for the same enzymatic machinery of desaturation and elongation to get converted into long-chain FA (Watkins, 1991). In case of equal quantity, n−3 FA are metabolized as the preferred substrate to that of n−6 series by these desaturases and elongase enzymes (Cherian, 2008). The observation of the better ability of Shaver white in retaining n−3 PUFA was consistent with higher activity of n−3 and n−6 desaturation and elongation enzymes in either fraction in Shaver white than in ISA brown breeders. The lower calculated activity of n−6 desaturation and elongation in phospholipid of ISA brown in response to supplementation with DMA and higher activity of n−3 desaturation and elongation in the phospholipid and triglycerides fractions in either strain may partially explain the reduction of n−6 members. Similar to the findings of the present study, despite different dietary levels, a dose-dependent reduction in activity of desaturation and elongation in n−6 FA was reported by the inclusion of 7.5% and 15% LinPRO (Nain et al., 2012) and up to 3% microalgae in hens (calculated based on the provided data; Ao et al., 2015). Regarding the higher potential of triglycerides in retaining long-chain n−3 FA than phospholipid, Neijat et al. (2017) observed that the triglyceride fraction had a better ability to deposit n−3 PUFA than the phospholipid faction even though these 2 major lipid classes have been shown to possess the ability to retain long-chain PUFA. The discrepancies in results of various studies on the effect of dietary FA concentration on egg FA profile may be attributed to different ages (Koppenol et al., 2014b) and supplemental level (Nain et al., 2012; Ao et al., 2015), and based on the findings of the present study, the bird strain can also be an important factor.

The egg fat acts as a vital source of energy and essential FA, such as LA and ALA, during embryogenesis and early posthatch period (Noble and Cocchi, 1990). There is a rapid uptake of different lipid components by the embryo, which has been shown to start from the second week of incubation (Maldjian et al., 1995; Yadgary et al., 2014; Cherian, 2015). Throughout the embryonic period, half of the lipid content in the yolk becomes incorporated into embryonic tissue (Speake et al., 1998). The required energy for embryo development has been shown to be supplied by β-oxidation of the remaining FA, mainly saturated FA (Yalçin et al., 2012). Preference of the embryo to consume FA that are AA and DHA rather than other C-16 and C-18 FA led to higher accumulation of PUFA in several tissues (Speake et al., 1998). The major PUFA in the avian

central nervous system is DHA (Cherian, 2008), which is incorporated into membrane phospholipids, and has been shown to make up around 17% of the total phospholipids contents (Maldjian et al., 1996). In the present study, embryos from DMA and FFF diets used more DHA from the phospholipid fraction amounting to 8% and 7% phospholipid, respectively. Less utilization of ALA might be attributed to the lower yolk content and the use of ALA as the precursor for the synthesis of n−3 PUFA (Cherian and Sim, 1993). Similarly, Cherian and Sim (1993) and Lin et al. (1991) observed that there was a preferred utilization of DHA in Leghorn white embryos. In the present study, the ability of embryo to consume n−6 PUFA was influenced by diets. The lower utilization of the n−6 family (mainly LA, AA, and 22:4n−6) by embryos from DMA and FFF might be attributed to lesser yolk content (Koppenol et al., 2014a) and preference of the embryo in utilization of DHA.

Shaver white embryos used 11.1% more FA from phospholipid and 10.5% less FA from triglycerides than ISA brown. Genetic selection for various traits in different strains of poultry influence not only the posthatch performance variables but also prehatch characteristics (Everaert et al., 2008). The difference in embryonic metabolism and physiological events such as thyroid hormones, corticosterone, air cell pCO_2 (Everaert et al., 2008), and body composition (protein and fat content; Pal et al., 2002) may play a role. In addition, the phenotypic differences among strains arise from the first few days of the embryonic period (Ho et al., 2011). The embryo relies on the egg nutrients for growth, which can provide the potential for maternal impact to influence the development of breed-specific phenotypes (Ho et al., 2011).

Utilization of total phospholipids and triglyceride was lower in embryos from DMA and FFF than in CON. The retained dry matter by the embryos has been associated with the yolk FA profile (Peebles et al., 1999). This connection highlights the vital role of yolk FA content in modifying nutrient utilization by the embryo (Yalçin et al., 2008). In avian species, the yolk and YS act as the main reservoirs for long-chain PUFA. The YS membrane eventually develops elaborate folds and a microvillus structure (Yadgary et al., 2011; Bauer et al., 2013). The YS membrane plays a vital role in the delivery of required nutrients for embryo development (Romanoff, 1960; Moran, 2007; Bauer et al., 2013; Yadgary et al., 2014; Cherian, 2015). The functional ability of the YS is dependent on numerous factors: 1) morphological and structural changes (Romanoff, 1960) and 2) nutrient digestion and transportation changes (Yadgary et al., 2013). Nutrient transportation in the YS can be conducted via transporters for amino acids or receptor-mediated endocytosis of lipoproteins (Yadgary et al., 2013). Maternal feeding of conjugated LA reduced the ability of the embryo to take in lipid out of the YS, which resulted in higher embryo mortality due to lack of energy (Yadgary et al., 2013). There is scarce information about the effect of nutrients, especially FA on YS membrane characteristics (Leone et al., 2010).

Jejunum and ileum are the main sites for FA digestion (Tancharoenrat et al., 2014) and absorption (Hurwitz et al., 1973) in poultry. FABP family are involved in transporting FA by expediting FA through extracellular and intracellular membranes (Chmurzyńska, 2006). It has been reported that the level of dietary fat is correlated with the expression of FABP in poultry (Katongole and March, 1980). In the present study, neither strain nor dietary treatment affected the concentration of FABP. Intestinal GPR 120 has been reported to be regulated by dietary long-chain FA (Mo et al., 2013), which is expressed in the intestine, adipose tissue (Koren et al., 2014), and macrophages (Mo et al., 2013). In the present study, the concentration of intestinal GPR 120 was dependent on strain and dietary treatments. The GPR 120 is a membrane-bound n−3 PUFA sensor (Talukdar et al., 2010). Activated GPR 120 has been shown to initiate a signaling pathway, which interferes with activation of nuclear factor-kappa B as a transcription factor involved in upregulation of proteins associated with inflammatory cytokines. To our knowledge, there is no previous report on GPR 120 in poultry. In the present study, Shaver white embryos fed with either DMA or FFF had a higher concentration of intestinal GPR 120 than CON. The expression of GPR 120 has been correlated with the dietary n−3 PUFA content (Talukdar et al., 2010), and in the present study, DMA and FFF had higher dietary n−3 PUFA than CON. When DMA and FFF were added in the diets, the MDA was not affected. MDA is a by-product of lipid peroxidation and acts as an indicator of oxidative stress. When a difference in strains was assessed, the Shaver white breeders had higher hepatic MDA than ISA brown breeders, illustrating Shaver white embryos tolerated higher level of oxidative stress during embryonic period than ISA brown embryos.

The results from the present study suggest that dietary supplementation with either source of n−3 PUFA could modify yolk FA profile toward more DHA either directly with DMA or indirectly with FFF as the DHA must be transformed from ALA. The difference between strains in deposition and synthesis of n−3 long-chain PUFA is another factor that needs to be considered in the feeding and management strategies for producing n−3 FA-enriched eggs. Preferential utilization of DHA by embryos of either strain suggests a high requirement of DHA during the embryonic period. Owing to the important role of n−3 PUFA, particularly DHA in metabolic and physiological mechanisms, more research is needed to characterize subsequent posthatch growth and development in egg-type chicks from breeders fed with n−3 PUFA-rich diets.

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SUPPLEMENTARY DATA

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