Effects of feeding ISA brown and Shaver white layer breeders with sources of n-3 fatty acids on hatching egg profiles, apparent embryonic uptake of egg components, and body composition of day-old chicks

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ARTICLE

Effects of feeding ISA brown and Shaver white layer breeders with sources of *n*-3 fatty acids on hatching egg profiles, apparent embryonic uptake of egg components, and body composition of day-old chicks

Reza Akbari Moghaddam Kakhki and Elijah G. Kiarie

Abstract: Effects of feeding ISA brown and Shaver white breeders sources of *n*-3 fatty acids (FA) on egg components, apparent embryonic uptake (AEU) of egg components, and hatching body composition were examined. A total of 240 females and 30 males per each strain were fed either: (1) control (CON); (2) CON + 1% of dried microalgae (DMA), as a source of docosahexaenoic acid; or (3) CON + 2.60% of dry extruded product consisting of full-fat flaxseed (FFF), as a source of α-linolenic acid for 30 d. Eggs were incubated and the residual yolks (RY) sampled at hatch for AEU of dry matter (DM), minerals, and organic matter (OM). Feeding *n*-3 FA sources reduced the AEU of OM and minerals resulting in a higher ratio of RY to body weight ($P = 0.002$). Feeding FFF increased body fat and decreased lean in Shaver white hatchlings compared with CON ($P < 0.05$). The body mineral was reduced by feeding DMA compared with other treatments ($P < 0.05$). The change in body composition in response to feeding of *n*-3 sources was associated with the change in AEU of DM, OM, and minerals, not the concentration of these components in the yolk.

Key words: breeder feeding, *n*-3 polyunsaturated fatty acids, embryonic nutrients utilization, body composition.

Résumé : Les effets d'alimentation des pondeuses ISA brunes et Shaver blanches des sources d'acides gras (FA — « fatty acids ») *n*-3 sur les composantes des œufs, l'absorption embryonnaire apparente (AEU — « apparent embryonic uptake ») des composantes des œufs, et de la composition corporelle à l'éclosion ont été étudiés. Un total de 240 femelles et 30 mâles pour chaque souche ont reçu soit : (1) témoin (CON — « control ») ; (2) CON + 1 % de microalgues séchées (DMA — « dried microalgae »), comme source d'acide docosahexaénoique oo ; (3) CON + 2,60 % de produit sec extrudé consistant de graine de lin entier (FFF — « full-fat flaxseed »), comme source d'acide α-linoléique pendant 30 j. Les œufs ont été incubés, et les matières résiduelles du jaune (RY — « residual yolks ») ont été échantillonnées à l'éclosion pour des échantillons d'AEU des matières sèches (DM — « dry matter »), des minéraux, et des matières organiques (OM — « organic matter »). L'alimentation avec des sources de FA n-3 a réduit l'AEU des OM et des minéraux ayant comme effet un ratio plus élevé de RY au poids corporel ($P = 0{,}002$). L'alimentation avec le FFF a augmenté le gras corporel et a diminué la viande maigre chez les petits nouvellement éclos de type Shaver blanc par rapport aux CON ($P < 0{,}05$). Les minéraux corporels ont été réduits en alimentant au DMA par rapport aux autres traitements ($P < 0{,}05$). Le changement de composition corporelle en réponse à l'alimentation des sources d'acides gras *n*-3 était associé aux changements d'AEU des DM, OM, et minéraux, et non à la concentration de ces composantes dans le jaune. [Traduit par la Rédaction]

Mots-clés : alimentation des pondeuses, acides gras polyinsaturés *n*-3, utilisation embryonnaire des éléments nutritifs, composition corporelle.

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Introduction

Embryo growth, hatchability, and post-hatch performance are dependent on nutrients deposited in fertile eggs (Uni and Ferket 2004; Uni et al. 2005). Fatty acids (FA) supply energy for embryogenesis (Cherian 2015) and are involved in other diverse physiological roles in developing embryos. Long-chain polyunsaturated FA (PUFA), 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. 2014). These FA are required for prenatal and postnatal development due to their vital roles in the synthesis of structural lipids (Mennitti et al. 2015). We previously showed that feeding layer breeders diets supplemented with sources of α-linolenic acid (ALA) (flaxseed) or DHA (microalgae) increased DHA concentration in hatching egg yolk and increased embryonic utilization of DHA (Akbari Moghaddam Kakhki et al. 2020*a*). Moreover, the data showed Shaver white embryos utilizing more DHA than ISA brown (Akbari Moghaddam Kakhki et al. 2020*a*).

There is a little knowledge of the rate and mechanism of nutrient utilization during embryogenesis in different strains (Uni et al. 2005). The available literature is mainly limited to the effect of feeding breeders diets supplemented with *n*-3 FA sources on FA profile in eggs, tissue, and embryonic FA uptake (Cherian 2015). We hypothesized that feeding layer breeders diets supplemented with sources of *n*-3 PUFA may change the egg yolk composition, nutrient utilization and, subsequently, body composition. This experiment aimed to evaluate the impact of feeding ISA brown and Shaver white layer breeders diets rich in *n*-3 PUFA and the subsequent effects on egg yolk composition, embryonic nutrient utilization, and body composition at hatch.

Materials and Methods

Birds and management

The experimental protocol (No. 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 throughout the experiment (CCAC 2009). A total of 270 ISA brown and Shaver white (240 females and 30 males per strain) were weighed individually and categorized into six body weight (BW) groups. Based on the number of birds in each category, a defined number of birds from each category were assigned to each pen, ensuring the coefficient of variation among pens stayed below 2%. At 26 wk of age (WOA), every three pens were assigned to one of the three dietary treatments as previously described in Akbari Moghaddam Kakhki et al. (2020*a*): (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 contained 17.9% DHA (Alltech, Nicholasville, KY, USA); and (3) CON plus 2.6% of a 1:1 (*w*/*w*) co-extruded full-fat flaxseed (FFF) and pulse mixture containing 10.5 % ALA (LinPRO®, O & T Farms Ltd, Regina, SK, Canada). The addition of 1% of DMA in the diet of layer hens was reported to double the enrichment of *n*-3 PUFA in eggs (Ao et al. 2015). The inclusion level of FFF was chosen to give a similar concentration of the total *n*-3 and *n*-6 in diets.

All diets were formulated to meet the breeder nutritional specifications (Parent Stock Management Guide: ISA Brown 2018; Parent Stock Management Guide: Shaver White 2018). Birds were fed experimental diets for a month, followed by the collection of egg samples to confirm *n*-3 PUFA enrichment (Akbari Moghaddam Kakhki et al. 2020*a*). Feeding with *n*-3 PUFA sources for four weeks has been reported as enough time to reach the plateau of the deposition of *n*-3 PUFA in eggs (Yin et al. 2008; Neijat et al. 2017). Twenty eggs per diet were collected at the beginning and at the end of the egg collection period for analyses of hatching egg composition. A total of 3109 eggs were collected over 8 d, marked, and stored at 4 °C until incubated. Breeder housing and hatchery conditions were previously described (Akbari Moghaddam Kakhki et al. 2020*a*).

All the samples were procured from the same birds and sampling procedure, as described in our previous report (Akbari Moghaddam Kakhki et al. 2020*a*). Briefly, on day 14 of incubation, all eggs were candled and categorized as live, infertile, or early mortality. On days 14 and 19 of the incubation, eight eggs per treatment were randomly collected, weighed, then embryo dissection followed. The residual yolk (RY) was carefully removed, weighed, and stored at −20 °C for further analyses. On day of hatch (DOH), chicks were counted and sexed. All un-hatched eggs were opened and late mortality was recorded. Ten females from each diet were euthanized for RY and liver samples, which were kept at −20 °C for future analyses. Ten birds were sacrificed with carbon dioxide and intact bodies were kept at −20 °C for measuring body composition.

Sample processing and analyses

The egg yolk was separated from albumen based on the method described by Akbari Moghaddam Kakhki et al. (2020*a*). Both RY and yolk samples were freeze-dried and weighed to measure dry matter (DM). A portion (1 ± 0.05 g) of dried egg yolk and RY was weighed, ashed at 600 °C for 12 h (Akbari Moghaddam Kakhki et al. 2018*b*), then reweighed for measuring the concentration of ash, calcium, and phosphorous. The whole body of day-old pullets was weighed and whole-body fat as well as lean percentage were measured using Prodigy dual-energy X-ray absorptiometry (GE Healthcare, Madison, WI, USA) equipped with enCORE software (version 14.0, GE Healthcare, Madison, WI, USA) as described by Akbari Moghaddam Kakhki et al. (2018*a*). The bodies were then freeze-dried, weighed, ground, and ashed for

measuring total mineral, calcium, and phosphorous. The concentration of calcium and phosphorous in diet, yolk, and body of pullet was measured according to the described method by Khanal et al. (2019). Briefly, samples were ashed as previously described, and a portion of ash (0.05 ± 0.01 g) wet digested with a mixture of 5 mL concentrated hydrochloric and 100 μL nitric acids for 24 h at 120 °C. The digested samples were transferred into 100 mL volumetric flask, ringed, and topped to volume with double deionized water. An aliquot of 15 mL was submitted for reading the concentration of calcium and phosphorous using inductively coupled plasma atomic emission absorption spectroscopy (Varian Inc., Palo Alto, CA, USA).

Calculations and statistical analyses

The RY to egg weight (EW) ratio (g:g) was calculated by dividing the recorded weight of RY by the weight of the egg multiplied by 100. The yolk and RY organic matter (OM) was calculated by subtracting ash from DM content. The apparent embryonic uptake (AEU) of DM, minerals, and OM was calculated by subtracting the respective concentration in RY from concentration in yolk samples before incubation. The RY weight was subtracted from the DOH chick BW to derive RY-free BW. Data were tested for normality with UNIVARIATE plot normal procedure of SAS version 9.4 and subsequently subjected to a two-way analysis of variance in a 2 (ISA brown and Shaver white) × 3 (CON, DMA, and FFF) factorial arrangement using the GLIMMIX procedures of SAS version 9.4. Appropriate means were separated using the TUKEY method and significance was declared at $P < 0.05$.

Results

Yolk weight and concentration of DM, OM, total mineral, calcium, and phosphorous were neither affected by the interaction between strain and diets nor the main effect of strain and diet ($P > 0.05$, Table 1). The main effect of strains on yolk weight and DM content was such that ISA brown breeders had heavier yolks with higher DM content compared with Shaver white brown breeders ($P < 0.05$). Yolks from birds fed DMA tended to have higher ash concentration ($P = 0.07$) than yolks of breeders fed CON and ALA.

The was no ($P > 0.05$) interaction between strain and diets on RY weight and concentration of DM as well as the ratio of RY:EW during the embryonic period and in the day-old chicks (Table 2). ISA brown showed greater RY weight on day 14 of incubation ($P = 0.024$) and DM concentration ($P < 0.001$) in DOH compared with Shaver white. At day 19 of incubation, DM concentration was greater in RY of FFF compared with CON ($P = 0.023$), whereas DMA had similar ($P > 0.05$) value to both CON and FFF. Compared with CON, yolks from breeders fed either source of *n*-3 FA had greater RY weight ($P < 0.001$) and RY:BW ratio ($P = 0.002$) at hatch (Table 2).

The AEU of DM, OM, and ash are shown in Table 3. There was an interaction ($P = 0.026$) between strain and diets on uptake of DM with the effects of diets being seen in ISA brown embryos only. In this context, embryos from hens fed *n*-3 FA sources had lower DM uptake than embryos from hens fed CON. There was no interaction ($P > 0.05$) between strain and diets on AEU of OM and ash. The main effect of diets was that CON embryos had higher AEU of OM ($P = 0.002$) than *n*-3 FA embryos and uptake of ash ($P = 0.015$) than DMA embryos. The ISA brown embryos consumed less ($P = 0.004$) yolk ash compared with Shaver white embryos (Table 3).

The interaction ($P < 0.001$) between strain and diets on RY-free BW of hatchlings relative to respective CON, *n*-3 FA sources increased RY-free BW in ISA brown, whereas FFF reduced RY-free BW in Shaver white (Table 4). The DOH liver weight was dependent ($P < 0.001$) of strain and diets with diet effects observed for ISA brown DOH only. In this context, *n*-3 FA DOH had lighter liver than CON DOH. There was an interaction between strain and diets on body lean ($P = 0.001$) and fat ($P = 0.005$) percentage in DOH with diet effects being seen in Shaver white DOH only. Relative to the DOH from CON breeders, Shaver white DOH from FFF breeders showed lower body lean and higher body fat. There was no interaction ($P > 0.05$) between stain and diet on body ash and phosphorous. Although body ash was higher in Shaver white hatchlings compared with ISA brown hatchlings ($P = 0.045$), this was not reflected in body calcium and phosphorus ($P > 0.05$). Hatchlings from breeders fed DMA had lower body ash, calcium, and phosphorous compared with CON ($P < 0.05$).

Discussion

Developing embryos receive nourishment from the shell, albumen, and yolk reserves. Therefore, egg composition is vital for embryonic growth. In addition, optimum embryo development is reflected in the quality, health, production, and welfare of chicks throughout the post-hatch life (Onbaşılar et al. 2017). Egg yolk is the primary source of minerals such as phosphorus, iron, and zinc, whereas the eggshell is the main component of calcium and magnesium for the embryo growth (Yair and Uni 2011). Other egg components (allantoic fluid, albumen, etc.) contain low amounts of minerals (Yair and Uni 2011). Feeding breeders *n*-3 FA sources did not influence yolk composition, including OM, ash, calcium, and phosphorous, demonstrating dietary *n*-3 FA cannot modify the concentration of minerals in the yolk. This builds on our previous observations indicating that feeding DMA and FFF to ISA brown and Shaver white pullets did not influence yolk, albumen, and shell weight, but increased the proportion of yolk DHA (Akbari Moghaddam Kakhki et al. 2020*a*). To our knowledge, the available reports on the effects of feeding *n*-3 FA on egg composition are limited to the FA profile in eggs. Nain et al. (2012) observed an increase in *n*-3 FA

Table 1. Effects of feeding ISA brown and Shaver white layer breeders sources of docosahexaenoic acid and α-linolenic acid on egg yolk composition of hatching eggs ($n = 10$).

Items		Dried yolk (g)	Dry matter (%)	Organic matter (%)[a]	Minerals (%)		
					Ash	Ca	P
Strain	Diet						
ISA brown	CON	7.91	51.34	96.76	3.32	0.21	0.63
ISA brown	DMA[b]	7.92	50.16	96.72	3.39	0.21	0.64
ISA brown	FFF[c]	7.26	48.91	97.04	3.28	0.18	0.61
Shaver white	CON	7.23	47.95	96.89	3.34	0.20	0.61
Shaver white	DMA	7.43	47.70	96.58	3.38	0.20	0.63
Shaver white	FFF	7.07	49.58	96.76	3.33	0.20	0.61
SEM		0.220	0.547	0.116	0.033	0.006	0.010
Main effect							
Strain							
ISA brown		7.69a	50.14a	96.84	3.33	0.20	0.63
Shaver white		7.24b	48.44b	96.74	3.35	0.20	0.62
SEM		0.127	0.200	0.064	0.019	0.004	0.006
Diet							
CON		7.57	49.65	96.83	3.33	0.20	0.62
DMA		7.67	48.93	96.65	3.39	0.21	0.63
FFF		7.17	49.24	96.90	3.31	0.19	0.61
SEM		0.156	0.372	0.076	0.024	0.005	0.007
Probabilities (*P* value)							
Strain		0.016	<0.001	0.292	0.503	0.389	0.121
Diet		0.064	0.192	0.085	0.066	0.157	0.130
Strain × Diet		0.533	0.061	0.202	0.661	0.067	0.660

Note: Values with lowercase letters within each column are different ($P < 0.05$). Ca, calcium; P, phosphorus; CON, control; DMA, dried microalgae; FFF, full-fat flaxseed; SEM, standard error of mean.

[a]Organic matter was calculated by subtracting yolk ash content from the dry matter of yolk.

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

[c]Co-extruded FFF and pulse mixture (1:1 *w*/*w*), as a source of α-linolenic acid.

concentration in egg in response to feeding 15% FFF for 18 d in 65 WOA Lohmann White Leghorn hens, whereas there was no effect on egg and yolk weight. Ao et al. (2015) reported an increase in *n*-3 FA in eggs without any changes in egg fat content and yolk percentage when the supplemental levels of DMA were increased up to 3% in diets of 45 WOA Hy-line W-36 hens.

The RY meets the demand for nutrients in the newly hatched chicks during the first few days of life (Şahan et al. 2014). About 60% of egg nutrients are absorbed throughout the embryonic period, and the remaining 30% play an essential role in supplying energy for the newly hatched birds over the first few post-hatch days (Nangsuay et al. 2011). However, RY can also host bacteria leading to yolk sac infection (Khan et al. 2004). In the current study, the hatchlings from breeders fed diets supplemented with *n*-3 FA had heavier leftover RY at hatch, illustrating the possible change in the embryonic uptake of nutrients in response to breeder-feeding *n*-3 FA. We previously reported reduced embryonic utilization total FA in phospholipid and triglyceride fractions in embryos from breeders fed DMA and FFF (Akbari Moghaddam Kakhki et al. 2020*a*). This observation elucidated the reduction in liver weight of ISA brown DOH from DMA compared with those from CON. It has been reported that hepatic weight was associated with the early mobilization of yolk lipids because the liver plays a vital role in the remodeling of RY lipids into lipoprotein particles that are exported into circulation (Wolanski et al. 2007).

The reason for the increase in body fat and a decrease in body lean in DOH of Shaver white breeders fed *n*-3 FA is difficult to interpret. Dietary FA have been shown to modify abdominal fat deposition in broilers, such that male broilers fed a diet supplemented with linseed as a source of ALA, had lower abdominal fat compared with those fed diets supplemented with tallow or olive oil (Crespo and Esteve-Garcia 2001). In contrast, dietary supplementation of *n*-3 and *n*-6 PUFA has been reported to increase FA β-oxidation and reduce hepatic fat content compared with saturated fat by inhibiting the activity of hepatic FA synthase and enhancing the activities of Carnitine palmitoyltransferases (Fouad and El-Senousey 2014).

Table 2. Effects of feeding ISA brown and Shaver white layer breeders sources of docosahexaenoic acid and α-linolenic acid on RY dried weight during the embryonic period.[a]

		Day 14 of embryonic period			Day 19 of embryonic period			Day of hatch		
Items		DM (g)	DM (%)	RY:EW[b]	DM (g)	DM (%)	RY:EW[c]	DM (g)	DM (%)	RY:BW[d]
Strain	Diet									
ISA brown	CON	2.76	47.76	11.41	5.03	48.92	21.14	1.05	53.03	6.67
ISA brown	DMA[e]	2.91	47.23	11.09	4.30	49.49	16.80	1.93	54.32	8.89
ISA brown	FFF[f]	3.21	47.06	12.67	5.21	49.77	20.07	1.99	53.56	9.02
Shaver white	CON	2.25	44.56	11.54	4.66	46.54	18.86	1.27	51.82	7.63
Shaver white	DMA	2.50	46.80	10.13	4.62	48.20	19.45	1.63	51.91	8.87
Shaver white	FFF	2.49	46.25	10.79	5.06	50.74	19.92	1.71	51.06	8.73
SEM		0.290	1.258	1.110	0.461	1.02	1.454	0.127	0.600	0.530
Main effect										
Strain										
ISA brown		2.96a	47.35	11.72	4.85	49.40	19.33	1.65	53.64a	8.19
Shaver white		2.41b	45.87	10.82	4.78	48.49	19.41	1.54	51.59b	8.41
SEM		0.164	0.711	0.628	0.243	0.536	0.840	0.073	0.346	0.306
Diet										
CON		2.50	46.16	11.47	4.84	47.73b	20.00	1.16b	52.42	7.15b
DMA		2.70	47.02	10.61	4.46	48.84ab	18.13	1.78a	53.11	8.88a
FFF		2.85	46.66	11.73	5.14	50.26a	19.99	1.85a	52.31	8.88a
SEM		0.199	0.861	0.760	0.305	0.673	1.028	0.090	0.424	0.374
Probabilities (*P* value)										
Strain		0.024	0.148	0.317	0.847	0.229	0.949	0.273	<0.001	0.620
Diet		0.489	0.787	0.556	0.279	0.023	0.341	<0.001	0.353	0.002
Strain × Diet		0.853	0.487	0.658	0.711	0.167	0.247	0.082	0.493	0.471

Note: Values with lowercase letters within each column are different ($P < 0.05$). RY, residual yolk; DM, dry matter; EW, egg weight; BW, body weight; CON, control; DMA, dried microalgae; FFF, full-fat flaxseed; SEM, standard error of mean.

[a]Data are means of eight samples per each treatment for days 14 and 19 of embryonic period and 10 samples on the day of hatch.

[b]Percentage of RY to EW at day 14 of embryonic period (g:g).

[c]Percentage of RY to EW at day 19 of embryonic period (g:g).

[d]Percentage of RY to body weight at hatch (g:g).

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

[f]Co-extruded FFF and pulse mixture (1:1 *w*/*w*), as a source of α-linolenic acid.

Supplementation of amino acids in mice diets before mating decrease body mass and lean mass, whereas it increased the fat mass in their offspring (Buckley et al. 2005). The authors attributed these observations to *n*-3 and *n*-6 FA modification of glucose tolerance, insulin sensitivity, signaling, and lipid metabolism (Buckley et al. 2005).

Feeding breeder *n*-3 FA reduced the concentration of ash, calcium, and phosphorous in hatchlings. It is worth noting that eggshell weight was not affected by feeding breeders *n*-3 FA (Akbari Moghaddam Kakhki et al. 2020*a*). In addition, the eggshell concentration of calcium was 4.0%, 4.2%, and 4.1%, and the concentration of phosphorus was 0.7%, 0.8%, and 0.7% for CON, DMA, and FFF, respectively. The inadequate embryonic uptake of minerals has been associated with the low bone mineral content at hatch, leading to the impairment in the development of the skeletal system and other critical organs (Yair and Uni 2011). Various factors have been reported to alter AEU of nutrients, including breeder flock age, egg composition, egg size, egg storage duration and condition, and incubation conditions. Nangsuay et al. (2013) observed heavier RY and DM in 53 WOA breeders compared with 29 WOA. Egg size is correlated to the age and whether there are any impacts of the difference in egg size and egg composition, regardless of breeder age, on RY weight and composition at hatch. It has been reported that day-old chicks from larger eggs had a heavier RY than those from smaller eggs, but without differences in DM concentration, showing RY weight appears to be determined by egg size rather than by breeder flock age, and that RY composition is more determined by breeder age than by egg size (Nangsuay et al. 2011, 2015). Egg storage longer than 7 d has been associated with a longer incubation duration and lighter RY at hatch (van der Wagt et al. 2020). Higher incubation temperature than 37.8 °C can reduce AEU and increase RY weight (Molenaar et al. 2010). However, our results showed that the profile of FA in yolk might affect the

Table 3. Effects of feeding ISA brown and Shaver white layer breeders sources of docosahexaenoic acid and α-linolenic acid on apparent embryonic uptake of dry matter, organic material, and ash ($n = 10$).

Items		Dry matter (%)[a]	Organic matter (%)[b]	Ash (%)[c]
Strain	Diet			
ISA brown	CON	86.55a	87.19	73.25
ISA brown	DMA[d]	73.59bc	74.02	65.76
ISA brown	FFF[e]	71.20c	72.20	67.83
Shaver white	CON	81.27ab	77.53	80.80
Shaver white	DMA	76.49bc	78.74	70.95
Shaver white	FFF	78.23abc	80.69	76.50
SEM		2.221	2.383	2.837
Main effect				
Strain				
ISA brown		77.11	77.85	68.95b
Shaver white		78.66	78.89	76.08a
SEM		1.282	1.317	1.638
Diet				
CON		83.91a	82.50a	77.02a
DMA		75.04b	76.24b	68.35b
FFF		74.72b	76.46b	72.17ab
SEM		1.570	1.632	2.006
Probabilities (*P* value)				
Strain		0.398	0.258	0.004
Diet		<0.001	0.002	0.015
Strain × Diet		0.026	0.105	0.824

Note: Values with lowercase letters within each column are different ($P < 0.05$). CON, control; DMA, dried microalgae; FFF, full-fat flaxseed; SEM, standard error of mean.

[a]Percentage of consumed dry matter during embryonic period to the total dry matter of yolk.

[b]Percentage of consumed organic matter during embryonic period to the total organic matter of yolk.

[c]Percentage of consumed ash during embryonic period to the total ash of yolk.

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

[e]Co-extruded FFF and pulse mixture (1:1 *w*/*w*), as a source of α-linolenic acid.

uptake of various nutrients, especially minerals. Overall, it is not clear whether the differences in body composition were due to the inability of the embryo to utilize nutrients in RY or a change in programming the putative mechanisms underlying determinants of body development.

Hatchability, chick quality, and production are influenced by the breeder nutrition (Chang et al. 2016), age (Latour et al. 1998), egg storage, incubation conditions (Tona et al. 2003; Yalcin et al. 2008), and strain (Şahan et al. 2014). Şahan et al. (2014) and Wolanski et al. (2007) reported that RY characteristics and uptake of nutrients is dependent on the strains. The growth and development of embryos and hatchlings are dependent on nutrients deposited in the egg. Therefore, the physiological status of the hatchlings is drastically affected by the nutrition of the breeder hens, which will influence chick metabolism and production performance (Chang et al. 2016). Increasing egg production by breeding for lay persistency and stability in egg quality are the main goals and priorities (Bain et al. 2016). Making hens lay for longer appears to be the most logical approach for the efficient utilization of resources, both financial and environmental (Bain et al. 2016). Breeding companies are now targeting extended laying cycles to deliver continued commercial and economic benefits. However, the persistency in lay requires consideration of how to maintain birds' health and welfare in longer laying cycles. Therefore, genetic companies should not only focus on extending the laying cycle but

Table 4. Effects of feeding ISA brown and Shaver white layer breeders sources of docosahexaenoic acid and α-linolenic acid on RY-free BW, liver, and body composition of day-old pullets.[a]

Items		RY-free BW (g)	Liver (%)	Body composition (%)				
				Lean[b]	Fat[b]	Mineral	Ca	P
Strain	Diet							
ISA brown	CON	27.44d	0.17a	77.53ab	21.39ab	7.86	0.93ab	0.74
ISA brown	DMA[c]	35.55a	0.12c	75.31b	23.71a	7.73	0.89b	0.70
ISA brown	FFF[d]	37.18a	0.12c	77.41ab	21.81a	8.19	0.96ab	0.75
Shaver white	CON	34.70ab	0.13bc	79.76a	18.97b	8.48	1.01a	0.77
Shaver white	DMA	32.21bc	0.14abc	77.55ab	21.42ab	7.86	0.90b	0.70
Shaver white	FFF	29.70cd	0.16ab	75.20b	23.86a	8.14	0.91b	0.71
SEM		0.657	0.008	0.613	0.595	0.139	0.021	0.015
Main effect								
Strain								
ISA brown		33.39a	0.14	76.75	22.30	7.93b	0.93	0.73
Shaver white		32.20b	0.14	77.50	21.42	8.16a	0.94	0.73
SEM		0.379	0.004	0.368	0.357	0.080	0.012	0.009
Diet								
CON		31.07b	0.15a	80.43a	20.18b	8.17a	0.97a	0.75a
DMA		33.88a	0.13b	78.17b	22.56ab	7.80b	0.90b	0.70b
FFF		33.44a	0.14ab	76.87b	22.83a	8.16a	0.94ab	0.73ab
SEM		0.465	0.005	0.433	0.421	0.098	0.015	0.011
Probabilities (*P* value)								
Strain		0.031	0.428	0.146	0.080	0.045	0.408	0.953
Diet		<0.001	0.022	<0.001	<0.001	0.013	<0.005	0.003
Strain × diet		<0.001	<0.001	0.001	0.005	0.055	0.011	0.108

Note: Values with lowercase letters within each column are significantly different ($P < 0.05$). RY-free BW, residual yolk-free body weight; Ca, calcium; P, phosporus; CON, control; DMA, dried microalgae; FFF, full-fat flaxseed; SEM, standard error of mean.

[a]Data are means of 10 samples per each treatment.

[b]The minimum detectable amount was 0.1 g.

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

[d]Co-extruded FFF and pulse mixture (1:1 *w*/*w*), as a source of α-linolenic acid.

also on improving bird resilience and coping with longer productive life (Fernyhough et al. 2020). The potential of breeder nutrition on hatchling quality can play an important role in the bird's health and further production. Different strategies such as diluted breeder diets, modifying the concentration of vitamins, major and trace minerals have been reported to alter the quality of hatchlings and their subsequent performance (Chang et al. 2016). For instance, the follow-up of the current study on the hatchlings showed that skeletal properties at 18 and 42 WOA responded differently to breeder feeding with *n*-3 PUFA (Akbari Moghaddam Kakhki et al. 2020*b*, 2020*c*). Furthermore, commercial strains can be categorized based on their efficiency in utilizing nutrients from RY (Wolanski et al. 2007; Şahan et al. 2014). For instance, Şahan et al. (2014) calculated the range of RY weight in 10 different commercial strains (anonymous strains) from 3.70 to 5.50 g, accounting for approximately 10%–14% of chick BW at DOH. Thus, the effect of strain should be considered as an important criterion in maternal feeding strategies because different strains may have a different rate of nutrients utilization from RY.

The effect of supplementing breeder diets with sources of *n*-3 FA on BW, liver weight, and body composition demonstrated the potential of breeder-feeding strategy on hatchling quality. The quality of the newly hatched chick is a significant factor in determining its livability, growth, performance, and health. Supplementation of *n*-3 FA sources into breeder diets did not change the yolk concentration of OM and minerals, indicating the subsequent change in hatchling quality was not associated with the effect of *n*-3 FA on the yolk. However, embryonic uptake of DM, OM, and mineral was reduced in response to feeding breeders sources of *n*-3 FA, leading to a change in body composition and increase in leftover RY, which might increase the chance of yolk infection. Thus, the impact of feeding breeder sources of *n*-3 FA should be considered based on the quality of day-old pullets and the subsequent effect of productivity and health. Moreover, due to the difference among strains in

responding to breeder feeding of *n*-3 FA, it is necessary to consider the breeder line, among other factors.

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