# n-3 fatty acids fed to ISA brown and Shaver white breeders and their female progeny during rearing: Impact on egg production, eggshell, and select bone attributes from 18 to 42 weeks of age

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**ABSTRACT** The impact of feeding sources of n-3 fatty acids (**FA**) to ISA brown and Shaver white breeders and their female offspring during rearing on egg production, eggshell, tibia, and keel bone attributes was examined. Breeders were fed Control (**CON**) or CON + 1% dried microalgae (**DMA**: *Aurantiochytrium limacinum*) as the source of docosahexaenoic acid or CON + 2.6% of a coextruded mixture of full-fat flaxseed (**FFF**) and pulses as a source of α-linolenic acid. Day-old offspring were fed 1) breeder CON-pullet CON (**CON-CON**), 2) breeder CON-pullet DMA (**CON-DMA**), 3) breeder CON-pullet FFF (**CON-FFF**), 4) breeder DMA-pullet CON (**DMA-CON**), 5) breeder DMA-pullet DMA (**DMA-DMA**), 6) breeder FFF-pullet CON (**FFF-CON**), and 7) breeder FFF-pullet FFF (**FFF-FFF**). At 18 wk of age (**WOA**), pullets were fed a common layer diet to 42 WOA for egg production and bone quality assessments. There was no ($P > 0.05$) interaction between strains and diets and the main effect of diets on egg production, egg mass, and eggshell quality. There was an interaction ($P = 0.008$) between strain and diet on egg weight (**EW**); however, the strain effect on EW ($P < 0.001$) was such that ISA brown had heavier eggs than Shaver white. Shaver white had higher ($P < 0.001$) eggshell %, eggshell, and tibia breaking strength (**BS**), as well as tibia ash concentration compared with ISA brown hens. In contrast, ISA brown hens exhibited heavier ($P < 0.05$) tibia and keel bones. Feeding breeders DMA and pullets both sources of n-3 FA increased tibia medullary ash concentration compared with other diets ($P < 0.001$). Shaver white hens showed greater decline in tibia BS (83.7 vs. 96.3%) and ash content (84.1 vs. 94.3%) than ISA brown hens from 18 to 42 WOA ($P < 0.05$). Strain and diets exhibited independent effects on eggshell, tibia, and keel attributes. Provision of α-linolenic acid and docosahexaenoic acid to breeders and offspring improved tibia medullary ash concentration at 42 WOA.

**Key words:** breeder feeding, pullet feeding, egg production and quality, bone quality, n-3 fatty acids

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## INTRODUCTION

The high demand for eggs has put increased pressure on the individual laying hen, which has resulted in the modern hen experiencing bone deterioration throughout the laying cycle, leading to a high occurrence of osteoporosis (Whitehead, 2004). Keel bone fractures are another skeletal issue that compromises the welfare and health of layers (Casey-Trott et al., 2015). To prevent the development of osteoporosis in laying hens, the peak bone mineral density of structural bones should ideally be achieved and sustained before the onset of lay (Kuroda et al., 2017). In addition to counteracting the development of osteoporosis, a healthy skeletal system has a direct impact on eggshell quality. It has been reported that around 60% of calcium in an eggshell is derived from diet, whereas the rest 40% of calcium is supplied by the skeletal system (Elaroussi et al., 1994). Eggshell strength is one of several key elements in ensuring the safety and integrity of the egg contents. About 10% of downgraded eggs at grading stations are rejected because of inferior eggshell quality (Kemps et al., 2006).

Nutrition and environmental stimuli of breeding hens have profound effects on progeny growth and metabolism (Uni et al., 2005). The lipid component of a fertilized egg is the one that constitutes more than 30% of the yolk (Brenner, 1971). Egg fat is a major source of energy



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and essential fatty acids (**FA**) during embryogenesis and early posthatch as it contains linoleic acid (**LA**) and α-linolenic acid (**ALA**) (Noble and Cocchi, 1990). Linoleic acid and ALA are involved in the production of a variety of FA, and yet in all avian species, LA and ALA cannot be synthesized *de novo* and must be supplied by the diet (Brenner, 1971). The efficacy of the production of long-chain n-3 and n-6 polyunsaturated FA (**PUFA**) from LA and ALA is dependent on the concentration of both groups of FA because of the involvement of the same desaturase and elongase enzymes (Brenner, 1971). An example of an n-3 long-chain PUFA synthesized from ALA is docosahexaenoic acid (**DHA**), which is crucial for many biological processes. Currently, little consideration is given to the composition of the breeder hen dietary FA composition and what effect it may have on reproduction and/or offspring (Cherian, 2011). The addition of FA to a growing hen's diet, especially n-3 PUFA, is believed to possess various health benefits, most notably increasing bone mineral content and attenuating age-related bone loss (Boeyens et al., 2014).

Previous reports demonstrated that feeding ISA brown and Shaver white pullet breeders ALA and DHA enhanced embryonic utilization of DHA (Akbari Moghaddam Kakhki et al., 2020a) and delayed prehatch tibia mineralization (Akbari Moghaddam Kakhki et al., 2020b). Further investigations showed that feeding ALA and DHA to breeders and pullets supported cortical development in Shaver white pullets at 18 wk of age (**WOA**) but not in ISA brown pullets (Akbari Moghaddam Kakhki et al., 2020b). There is minimal research on the effect of feeding n-3 FA during breeder and rearing phases and the subsequent impact on eggshell and bone quality in hens. Bone remodeling has been shown to make hens susceptible to osteoporosis. Therefore, the hypotheses of this study were that feeding n-3 FA to the breeder, pullets, or both would improve eggshell and bone quality at 18 WOA through 42 WOA. Furthermore, it was hypothesized that these effects may be strain specific. The present study aimed to follow the pullets from previous studies (Akbari Moghaddam Kakhki et al., 2020a,b) to the laying house phase. Therefore, the objective was to investigate the effects of feeding n-3 FA to breeders and their female progeny during rearing or feeding n-3 FA to breeders only on egg production, eggshell, and bone quality in ISA brown and Shaver white hens.

## MATERIALS AND METHODS

Birds were cared for under the Canadian Council on Animal Care guidelines (CCAC, 2009). The Animal Utilization Protocol (#3675) was approved by the University of Guelph Animal Care Committee.

### Birds and Management

ISA brown and Shaver white breeder birds (243 females and 36 males per strain) were distributed at 26 WOA into 3 dietary treatments as previously described in the study by Akbari Moghaddam Kakhki et al. (2020a): 1) corn-soybean meal–based diet identified as control (**CON**), 2) CON plus 1% of dried microalgae (**DMA**, *Aurantiochytrium limacinum*) supplement as a rich source of DHA (Alltech, Nicholasville, KY), and 3) CON plus 2.6% of a 1:1 (wt/wt) coextruded full-fat flaxseed (**FFF**) and pulse mixture as a source of ALA (LinPRO, O & T Farms Ltd., Regina, SK, Canada). The 1% DMA inclusion level was chosen as previous reports demonstrated an increase in the enrichment of n-3 PUFA by twofold in layer hens (Ao et al., 2015). The inclusion level of FFF was selected to give an equal concentration of the total n-3 and n-6 FA to the DMA diet. The housing system, lighting regiment, and feeding regiment offered before the experimental diets were fed are described in the study by Akbari Moghaddam Kakhki et al. (2020a). After 30 D on the experimental diet, 10 eggs were collected and submitted for FA analyses to confirm n-3 PUFA deposition as previously described (Akbari Moghaddam Kakhki et al., 2020a). n-3 FA deposition in eggs was tested, confirming breeder-feeding of DMA and FFF increased the concentration of DHA and ALA, respectively, compared with CON (Akbari Moghaddam Kakhki et al., 2020b). After n-3 confirmation in eggs, 1,549 eggs of ISA brown and 1,560 eggs of Shaver white hens were collected, marked, and incubated at 37.5°C with 55% humidity for 19 D and hatched set at 36.9°C with 66% humidity in a commercial-grade incubator and hatcher (Nature Form, Jacksonville, FL) at the Arkell Poultry Research Station (Guelph, ON, Canada).

On the day of hatch (**DOH**), female hatchlings were weighed individually, transported to the brooding room, and distributed into posthatch treatments, ensuring that there was no significant difference in body weight among each group as previously described in Akbari Moghaddam Kakhki et al. (2020b). At the DOH and with respect to breeder diet, female progeny were assigned to the following diets: 1) breeder CON-pullet CON (**CON-CON**), 2) breeder CON-pullet DMA (**CON-DMA**), 3) breeder CON-pullet FFF (**CON-FFF**), 4) breeder DMA-pullet CON (**DMA-CON**), 5) breeder DMA-pullet DMA (**DMA-DMA**), 6) breeder FFF-pullet CON (**FFF-CON**), and 7) breeder FFF-pullet FFF (**FFF-FFF**). Posthatch diets (Table 1) were balanced to meet or exceed nutrient requirements (Table 2) from hatch to prelay (Commercial Product Guide-ISA Brown, 2018; Commercial Product Guide-Shaver White, 2018. Diets containing DMA and FFF in breeder and progeny phases had the same total amount of total n-3 FA and n-6:n-3 FA ratio (Akbari Moghaddam Kakhki et al., 2020a,b).

Twelve birds of the same breeder diets were placed in 20″ × 30″ cages (Ford Dickson Inc., Mitchell, ON, Canada) with 5 replications for each posthatch diet and kept until 42 WOA. Information about the housing system, temperature, and lighting regiment is given in the study by Akbari Moghaddam Kakhki et al. (2020b). In accordance with the Canadian National Farm Animal Care Council, a minimum space allowance of 67.0 square

**Table 1.** Composition of the experimental diets for the ISA brown and Shaver white pullets, as feed basis.

| | Starter | | | Grower | | | Developer | | | Prelay | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item[1] | CON | DMA | FFF | CON | DMA | FFF | CON | DMA | FFF | CON | DMA | FFF |
| Ingredient, g/kg | | | | | | | | | | | | |
| Corn grain | 611.13 | 598.39 | 590.44 | 478.89 | 473.00 | 464.40 | 419.42 | 435.13 | 417.64 | 474.52 | 490.23 | 472.74 |
| Soybean meal | 311.16 | 327.00 | 317.09 | 253.76 | 251.80 | 243.80 | 175.92 | 176.16 | 167.82 | 209.80 | 209.90 | 201.57 |
| Wheat | - | - | - | 70.00 | 70.00 | 70.00 | 60.00 | 50.00 | 60.00 | 60.00 | 50.00 | 60.00 |
| Corn gluten, 60.4% CP | 18.83 | 6.45 | 7.59 | - | - | - | - | - | - | - | - | - |
| DMA[2] | - | 10.00 | - | - | 10.00 | - | - | 10.00 | - | - | 10.00 | - |
| FFF[3] | - | - | 26.50 | - | - | 26.00 | - | - | 24.50 | - | - | 24.50 |
| Wheat middlings | - | - | - | 130.00 | 130.00 | 130.00 | 275.20 | 265.57 | 265.69 | 147.40 | 137.77 | 137.89 |
| Soybean oil | 1.50 | 1.50 | 1.50 | 10.00 | 7.00 | 7.40 | 9.00 | 3.00 | 4.00 | 9.00 | 3.00 | 4.00 |
| Limestone fine | 17.98 | 17.90 | 17.90 | 19.50 | 20.00 | 20.00 | 20.15 | 20.06 | 20.09 | 58.41 | 58.33 | 58.36 |
| Monocalcium phosphate | 19.25 | 19.09 | 19.10 | 15.16 | 15.50 | 15.50 | 16.42 | 16.41 | 16.40 | 17.58 | 17.57 | 17.56 |
| Poultry premix[4] | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| DL-Methionine, 99% | 2.38 | 2.48 | 2.48 | 2.83 | 2.80 | 2.90 | 3.18 | 3.18 | 3.20 | 3.23 | 3.24 | 3.25 |
| L-Lysine HCl, 78% | 0.89 | 0.51 | 0.57 | 2.05 | 2.10 | 2.10 | 2.82 | 2.83 | 2.87 | 2.35 | 2.35 | 2.39 |
| L-Threonine, 98% | 0.24 | 0.19 | 0.24 | 0.94 | 0.90 | 1.00 | 1.20 | 1.19 | 1.24 | 1.03 | 1.43 | 1.46 |
| Salt | 1.86 | 1.95 | 1.99 | 0.69 | 0.70 | 0.70 | 1.45 | 1.45 | 1.48 | 1.43 | 1.02 | 1.06 |
| Sodium bicarbonate | 3.75 | 3.50 | 3.55 | 5.16 | 5.10 | 5.10 | 4.21 | 4.13 | 4.18 | 4.21 | 4.13 | 4.18 |
| Choline chloride, 60% | 0.89 | 0.89 | 0.89 | 0.89 | 0.90 | 0.90 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 | 0.89 |
| Ethoxyquin[5] | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |

Abbreviations: CON, control; DMA, dried microalgae; FFF, full-fat flaxseed.

[1]The experimental diets of layer breeders are presented in the study by Akbari Moghaddam Kakhki et al. (2020a).

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

[3]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.

[4]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.

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

inches for white birds and 75.0 square inches for brown birds after age of maturity must be provided (National Farm Animal Care Council, 2017). After 18 WOA, the population of each cage was reduced to 8 birds for ISA brown and 9 birds for Shaver white, giving 75 and 67 square inches for ISA brown and Shaver white hens. After the onset of lay, all birds from each dietary treatment were fed a commercial diet without supplemental source of n-3 FA containing 39.16% LA, 2.79% ALA (of total fat), and 4.55% total fat throughout the laying phase (Floradale Feed Mill, Floradale, ON, Canada).

## Sampling

ISA brown and Shaver white pullets were kept separately in 2 cage aisles in one house. Based on the original schedule, eggshell quality analysis was planned to start from 22 WOA. However, a mechanical malfunction of

**Table 2.** Calculated nutritional composition of the experimental diets for the ISA brown and Shaver white pullets, as fed basis.

| | Starter | | | Grower | | | Developer | | | Prelay | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item[1] | CON | DMA | FFF | CON | DMA | FFF | CON | DMA | FFF | CON | DMA | FFF |
| Metabolizable energy, kcal/kg | 2,900 | 2,900 | 2,900 | 2,800 | 2,800 | 2,800 | 2,700 | 2,700 | 2,700 | 2,700 | 2,700 | 2,700 |
| Crude protein, % | 21.00 | 21.00 | 21.00 | 19.10 | 19.10 | 19.10 | 17.20 | 17.20 | 17.20 | 17.20 | 17.20 | 17.20 |
| Calcium, % | 1.10 | 1.10 | 1.10 | 1.10 | 1.10 | 1.10 | 1.10 | 1.10 | 1.10 | 2.50 | 2.50 | 2.50 |
| Analyzed calcium, % | 1.15 | 1.15 | 1.17 | 1.20 | 1.18 | 1.77 | 1.20 | 1.20 | 1.21 | 2.52 | 2.51 | 2.49 |
| Available phosphorus, % | 0.48 | 0.48 | 0.48 | 0.40 | 0.40 | 0.40 | 0.44 | 0.44 | 0.44 | 0.45 | 0.45 | 0.45 |
| Analyzed phosphorus, % | 0.79 | 0.79 | 0.79 | 0.71 | 0.71 | 0.71 | 0.80 | 0.80 | 0.79 | 0.83 | 0.83 | 0.84 |
| SID[2] Lysine, % | 1.00 | 1.00 | 1.00 | 0.98 | 0.98 | 0.98 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 |
| SID Methionine, % | 0.53 | 0.53 | 0.53 | 0.53 | 0.53 | 0.53 | 0.54 | 0.54 | 0.54 | 0.54 | 0.54 | 0.54 |
| SID Methionine + cystine, % | 0.78 | 0.78 | 0.78 | 0.76 | 0.76 | 0.76 | 0.76 | 0.76 | 0.76 | 0.76 | 0.76 | 0.76 |
| SID Threonine, % | 0.67 | 0.67 | 0.67 | 0.66 | 0.66 | 0.66 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 |
| SID Tryptophan, % | 0.23 | 0.23 | 0.23 | 0.21 | 0.21 | 0.21 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 |
| Sodium, % | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 |
| Chloride, % | 0.23 | 0.23 | 0.23 | 0.16 | 0.16 | 0.16 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| $\sum$n-3, % | 0.07 | 0.31 | 0.33 | 0.13 | 0.35 | 0.36 | 0.11 | 0.30 | 0.31 | 0.11 | 0.31 | 0.31 |
| $\sum$n-6, % | 1.63 | 1.64 | 1.69 | 1.82 | 1.66 | 1.73 | 1.54 | 1.26 | 1.35 | 1.69 | 1.41 | 1.50 |
| $\sum$n-6: $\sum$n-3 | 23.29 | 5.29 | 5.12 | 14.00 | 4.74 | 4.81 | 14.00 | 4.20 | 4.35 | 15.36 | 4.55 | 4.84 |

Abbreviations: CON, control; DMA, dried microalgae; FFF, full-fat flaxseed.

[1]The analyzed fatty acids profile is presented in Akbari Moghaddam Kakhki et al. (2020b).

[2]Standardized ileal digestible.

**Table 3.** Effects of feeding sources of docosahexaenoic and α-linolenic acids to ISA brown and Shaver white breeders and their pullets on laying hen egg production from 25 to 42 wk of age.[1,2]

| Items | | Hen-day egg production, % | Egg weight, g | Egg mass, g/b/d |
|---|---|---|---|---|
| Strain | Diet[3] | | | |
| ISA brown | CON-CON[4] | 91.59 | 65.44[a] | 59.77 |
| ISA brown | CON-DMA | 93.96 | 64.02[a,b] | 60.22 |
| ISA brown | CON-FFF | 94.31 | 63.64[a,b] | 60.04 |
| ISA brown | DMA-CON | 95.89 | 63.81[a,b] | 61.21 |
| ISA brown | DMA-DMA | 92.23 | 66.54[a] | 61.32 |
| ISA brown | FFF-CON | 90.77 | 65.11[a] | 59.18 |
| ISA brown | FFF-FFF | 95.48 | 64.66[a,b] | 61.77 |
| Shaver white | CON-CON | 93.40 | 59.00[c] | 55.20 |
| Shaver white | CON-DMA | 94.51 | 59.55[c] | 56.36 |
| Shaver white | CON-FFF | 94.29 | 61.39[b,c] | 58.03 |
| Shaver white | DMA-CON | 90.31 | 59.25[c] | 53.50 |
| Shaver white | DMA-DMA | 94.24 | 59.17[c] | 55.81 |
| Shaver white | FFF-CON | 94.90 | 58.41[c] | 55.51 |
| Shaver white | FFF-FFF | 95.71 | 59.58[c] | 57.07 |
| SEM | | 1.809 | 0.681 | 1.248 |
| Main effect | | | | |
| Strain | | | | |
| ISA brown | | 94.12 | 64.81[a] | 60.58[a] |
| Shaver white | | 94.35 | 59.40[b] | 55.82[b] |
| SEM | | 0.393 | 0.262 | 0.480 |
| Breeder diet | | | | |
| CON | | 94.17 | 62.17 | 58.27 |
| DMA | | 93.88 | 62.19 | 57.96 |
| FFF | | 94.82 | 61.94 | 58.38 |
| SEM | | 0.514 | 0.341 | 0.624 |
| Offspring diet | | | | |
| CON-CON | | 93.51 | 62.22 | 57.48 |
| CON-DMA | | 94.24 | 61.78 | 58.29 |
| CON-FFF | | 94.30 | 62.52 | 59.04 |
| DMA-CON | | 94.52 | 61.53 | 57.35 |
| DMA-DMA | | 93.23 | 62.86 | 58.56 |
| FFF-CON | | 94.04 | 61.76 | 57.34 |
| FFF-FFF | | 95.59 | 62.12 | 59.42 |
| SEM | | 0.705 | 0.482 | 0.882 |
| Probabilities (*P*-value) | | | | |
| Strain | | 0.686 | <0.001 | <0.001 |
| Breeder diets | | 0.396 | 0.839 | 0.882 |
| Offspring diet | | 0.381 | 0.276 | 0.276 |
| Strain × breeder diet | | 0.662 | 0.121 | 0.152 |
| Strain × offspring diet | | 0.216 | 0.008 | 0.723 |

Values with uncommon superscripts within each column are significantly different ($P < 0.05$).
[1]Data are means of 5 replications per each treatment.
[2]Laying hens during 25 to 42 wk of age were not fed the experimental diets. These diets were only fed in the breeder and progeny pullet stage.
[3]CON: control; DMA: dried microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid; FFF: coextruded full-fat flaxseed and pulse mixture (1:1 wt/wt), as a source of α-linolenic acid.
[4]The day-old female pullets from breeders fed CON, DMA, and FFF were divided into 3 (CON, DMA, and FFF), 2 (CON and DMA) and 2 (CON, FFF) posthatch treatments, respectively. The order shows the breeder diet and pullet diet (e.g., breeder diet-pullet diet, CON-CON).

the watering system and subsequent water shortage occurred on 21 WOA in cage aisle of Shaver white birds, resulting in mortality and drop in egg production. Before the incident, the egg production was 61.4%, which dropped to 26.8% because of the water shortage. The same cage density (7 Shaver white and 6 ISA brown) was maintained for both strains starting at 22 WOA. Body weight was measured at 22, 23, and 24 WOA as an indicator for the recovery. Birds were given 2 wk of recovery time to reach the same egg production rate as before the incident. Starting at 25 WOA egg numbers were recorded daily, and half of the produced eggs were randomly collected, labeled, weighed, and kept at 4°C for eggshell quality analysis in every 4 wk. The last eggshell quality measurement was performed on the last day of the 41st wk. The eggshell quality testing was performed within the 48 h after egg collection. At 42 WOA, birds were weighed and then palpated for tibia and keel bone sampling. Hens with a hard eggshell in the shell gland were chosen as described by Akbari Moghaddam Kakhki et al. (2018a). Keel bone and left and right tibias were dissected, defleshed, and stored at −20°C for further analysis.

## Sample Analyses

Eggshell thickness (**EST**) and eggshell breaking strength (**ESBS**) were measured in accordance with the method followed by Mwaniki et al. (2018). Eggshell thickness (mm) was measured using a high-resolution

**Table 4.** Effects of feeding sources of docosahexaenoic and α-linolenic acids to ISA brown and Shaver white breeders and their pullets on laying hen eggshell quality from 25 to 42 wk of age.[1,2]

| Items | Eggshell thickness, mm | Eggshell breaking strength, kgf | Eggshell Weight, g | Eggshell % |
|---|---|---|---|---|
| Main effect | | | | |
| Strain | | | | |
| ISA brown | 0.429 | 4.24[b] | 6.40 | 9.9[b] |
| Shaver white | 0.430 | 5.01[a] | 6.50 | 11.1[a] |
| SEM | 0.002 | 0.051 | 0.039 | 0.069 |
| Breeder diet[3] | | | | |
| CON | 0.428 | 4.63 | 6.45 | 10.5 |
| DMA | 0.433 | 4.63 | 6.42 | 10.5 |
| FFF | 0.427 | 4.62 | 6.48 | 10.5 |
| SEM | 0.002 | 0.068 | 0.053 | 0.091 |
| Offspring diet[4] | | | | |
| CON-CON | 0.427 | 4.67 | 6.46 | 10.4 |
| CON-DMA | 0.430 | 4.64 | 6.42 | 10.4 |
| CON-FFF | 0.426 | 4.57 | 6.47 | 10.6 |
| DMA-CON | 0.432 | 4.63 | 6.38 | 10.4 |
| DMA-DMA | 0.435 | 4.63 | 6.46 | 10.6 |
| FFF-CON | 0.428 | 4.66 | 6.43 | 10.5 |
| FFF-FFF | 0.426 | 4.57 | 6.51 | 10.5 |
| SEM | 0.003 | 0.097 | 0.075 | 0.129 |
| Probabilities ($P$-value) | | | | |
| Strain | 0.901 | <0.001 | 0.094 | <0.001 |
| Breeder diets | 0.088 | 0.989 | 0.772 | 0.971 |
| Offspring diet | 0.886 | 0.921 | 0.854 | 0.395 |
| Strain × breeder diet | 0.516 | 0.240 | 0.606 | 0.928 |
| Strain × offspring diet | 0.863 | 0.620 | 0.281 | 0.432 |

Values with uncommon superscripts within each column are significantly different ($P < 0.05$).
[1]Data are means of 5 replications per each treatment.
[2]Laying hens during 25 to 42 wk of age were not fed the experimental diets. These diets were only fed in the breeder and progeny pullet stage.
[3]CON: control; DMA: dried microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid; FFF: coextruded full-fat flaxseed and pulse mixture (1:1 wt/wt), as a source of α-linolenic acid.
[4]The day-old female pullets from breeders fed CON, DMA, and FFF were divided into 3 (CON, DMA and FFF), 2 (CON and DMA) and 2 (CON, FFF) posthatch treatments, respectively. The order shows the breeder diet and pullet diet (e.g., breeder diet-pullet diet, CON-CON).

nondestructive device with precision ultrasound (ORKA Food Technology Ltd., Ramat HaSharon, Israel), and ESBS was measured by Force Reader (ORKA Food Technology Ltd., Ramat HaSharon, Israel). Eggs then were cracked open, and the eggshells were washed by water, dried for 24 h at 105°C, and weighed as described by Akbari Moghaddam Kakhki et al. (2020a).

The left tibia sample dry weight, ash content, and ash concentration in epiphysis, medullary, and cortical subparts were measured in accordance with the followed by Akbari Moghaddam Kakhki et al. (2018a). Right tibia samples were used for measuring breaking strength (**BS**) using an Instron material tester (Instron crop, Canton, MA) with the crosshead speed of 2 mm/s, in accordance with the method followed by Khanal et al. (2019).

## Calculations and Statistical Analyses

The normality of data was tested using UNIVARIATE plot normal procedure (SAS, version 9.4, Cary, NC). To demonstrate how dietary treatments modified bone attributes through the lay cycle compared with 18 WOA, the measured bone attributes (total bone and subparts) at 42 WOA were divided by previously reported values of pullet phase (Akbari Moghaddam Kakhki et al., 2020b), multiplied by 100 and reported as a change index (**CI**, Akbari Moghaddam Kakhki et al., 2018b). Data were subjected to nested factorial arrangement for including a two-way ANOVA in 2 strains (ISA brown and Shaver white) and 3 breeder diets factorial arrangement as well as a two-way ANOVA in 2 strains and 7 posthatch diets factorial arrangement using GLIMMIX procedure (SAS, version 9.4). Dry weight, ash content of tibia, and keel bone were normalized based on BW (Akbari Moghaddam Kakhki et al., 2018a). Egg weight was considered as a covariate for analyzing data of EST and ESBS. Significance was declared at $P < 0.05$.

# RESULTS

## Egg Production and Eggshell Quality

Hen-day egg production was not affected by strain, breeder diet, offspring diet, or interactions ($P > 0.05$; Table 3). Egg weight was affected by the interaction between diet and strain ($P = 0.008$). This interactive effect was not systematic and mainly demonstrated strain differences. The ISA brown hens had 5.41 g heavier eggs than Shaver white ($P < 0.001$). Egg mass was not affected by the interaction between strain and breeder

**Table 5.** Effects of feeding sources of docosahexaenoic and α-linolenic acids to ISA brown and Shaver white breeders and their pullets on attributes of the whole tibia from 42-wk-old laying hens.[1,2]

| Items | | BW, g | Tibia: Breaking strength, N[3] | Weight, g[4] | Ash weight, g | Ash concentration, % |
|---|---|---|---|---|---|---|
| Strain | Breeder diet | | | | | |
| ISA brown | CON | 2,272.5 | 117.7[a,b,c] | 3.71[a] | 1.63[a] | 44.02 |
| ISA brown | DMA | 2,309.2 | 109.8[b,c] | 3.38[b,c] | 1.55[a,b] | 44.97 |
| ISA brown | FFF | 2,267.7 | 101.2[c] | 3.57[a,b] | 1.55[a,b] | 44.40 |
| Shaver white | CON | 1,894.1 | 122.9[a,b] | 3.02[d] | 1.35[c] | 44.70 |
| Shaver white | DMA | 1,894.0 | 132.2[a,b] | 3.04[c,d] | 1.40[b,c] | 46.08 |
| Shaver white | FFF | 1,854.4 | 134.6[a] | 3.21[c,d] | 1.49[a,b,c] | 46.38 |
| SEM | | 43.756 | 5.618 | 0.066 | 0.042 | 0.527 |
| Main effect | | | | | | |
| Strain | | | | | | |
| ISA brown | | 2,283.1[a] | 109.6[b] | 3.55[a] | 1.58[a] | 44.46[b] |
| Shaver white | | 1,880.8[b] | 129.9[a] | 3.09[b] | 1.41[b] | 45.72[a] |
| SEM | | 28.64 | 3.058 | 0.047 | 0.027 | 0.351 |
| Breeder diet[5] | | | | | | |
| CON | | 2,083.3 | 120.3 | 3.37 | 1.49 | 44.36 |
| DMA | | 2,101.6 | 121.0 | 3.21 | 1.48 | 45.52 |
| FFF | | 2,061.1 | 117.9 | 3.39 | 1.52 | 45.39 |
| SEM | | 37.89 | 3.973 | 0.058 | 0.031 | 0.457 |
| Offspring diet[6] | | | | | | |
| CON-CON | | 2,120.2 | 114.6 | 3.29 | 1.47 | 44.46 |
| CON-DMA | | 2,069.9 | 121.1 | 3.42 | 1.51 | 44.19 |
| CON-FFF | | 2,059.9 | 125.1 | 3.39 | 1.50 | 44.43 |
| DMA-CON | | 2,060.5 | 121.2 | 3.25 | 1.48 | 45.52 |
| DMA-DMA | | 2,142.6 | 120.8 | 3.24 | 1.47 | 45.53 |
| FFF-CON | | 2,036.6 | 115.9 | 3.41 | 1.53 | 45.04 |
| FFF-FFF | | 2,085.6 | 119.9 | 3.30 | 1.51 | 45.74 |
| SEM | | 53.59 | 5.618 | 0.088 | 0.053 | 0.646 |
| Probabilities (*P*-value) | | | | | | |
| Strain | | <0.001 | <0.001 | <0.001 | <0.001 | 0.017 |
| Breeder diets | | 0.752 | 0.841 | 0.071 | 0.614 | 0.096 |
| Offspring diet | | 0.678 | 0.731 | 0.732 | 0.943 | 0.952 |
| Strain × breeder diet | | 0.909 | 0.025 | 0.035 | 0.022 | 0.543 |
| Strain × offspring diet | | 0.188 | 0.383 | 0.642 | 0.338 | 0.103 |

Values with uncommon superscripts within each column are significantly different ($P < 0.05$).
[1]n = 5.
[2]Laying hens during 25 to 42 wk of age were not fed the experimental diets. These diets were only fed in the breeder and progeny pullet stage.
[3]Breaking strength N/kg BW.
[4]g/kg BW.
[5]CON: control; DMA: dried microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid; FFF: coextruded full-fat flaxseed and pulse mixture (1:1 wt/wt), as a source of α-linolenic acid.
[6]The day-old female pullets from breeders fed CON, DMA, and FFF were divided into 3 (CON, DMA, and FFF), 2 (CON and DMA) and 2 (CON, FFF) posthatch treatments, respectively. The order shows the breeder diet and pullet diet (e.g., breeder diet-pullet diet, CON-CON).

diet, strain and offspring diet, or the main effect of breeder and offspring diets ($P > 0.05$). ISA brown hens had 4.76 g heavier egg mass than Shaver white ($P < 0.001$). The EST and absolute eggshell weight were not affected by the interaction between strain and breeder diet, the interaction between strain and offspring diet, or the main effect of strain, breeder diet, and offspring diet (Table 4; $P > 0.05$). Shaver white hens had greater ESBS and percentage of eggshell to egg weight than ISA brown ($P < 0.001$). However, the interaction between strain and breeder diet, the interaction between strain and offspring diet, or the main effect of breeder diet and offspring diet did not affect ESBS and the percentage of the eggshell ($P > 0.05$).

## Tibia and Keel Bones Attributes

Body weight and the majority of the tibia attributes were not influenced by the interaction between strain and offspring diet or the main effect of breeder and offspring diets ($P > 0.05$; Tables 5 and 6). ISA brown hens were 402.3 g heavier than Shaver white hens ($P < 0.001$; Table 5). Shaver white hens had 18.5 and 1.3% higher whole tibia BS and ash concentration, respectively, than ISA brown hens ($P < 0.05$). In addition, Shaver white hens had 14.9 and 12.1% lower whole tibia and ash weight ($P < 0.05$). Tibia BS, weight, and ash weight were affected by the interaction between strain and offspring diets, which was not systematic and mainly demonstrated strain differences ($P < 0.05$).

ISA brown hens had heavier tibia epiphysis than Shaver white hens ($P < 0.001$; Table 6). ISA brown hens showed heavier tibia medullary (0.46 vs. 0.39 g/kg BW, $P < 0.01$) than Shaver whites, whereas Shaver white hens had greater tibia medullary ash concentration (21.4 vs. 17.4%, $P < 0.01$) than ISA brown hens. ISA brown had 14.2 and 11.4% greater tibia cortical weight and ash content than Shaver white, whereas Shaver white hens had 2.7% higher tibia cortical ash concentration ($P < 0.001$) than ISA brown hens.

**Table 6.** Effects of feeding sources of docosahexaenoic and α-linolenic acids to ISA brown and Shaver white breeders and their pullets on attributes of tibia subparts from 42-wk-old laying hens.[1,2]

| | Epiphysis | | | Medullary | | | Cortical | | |
|---|---|---|---|---|---|---|---|---|---|
| Items | Wt, g[3] | Ash wt, g[4] | Ash c, %[5] | Wt, g | Ash wt, g | Ash c, % | Wt, g | Ash wt, g | Ash c, % |
| Main effect | | | | | | | | | |
| Strain | | | | | | | | | |
| ISA brown | 1.90[a] | 0.72[a] | 38.06 | 0.46[a] | 0.08 | 17.37[b] | 1.21[a] | 0.78[a] | 64.62[b] |
| Shaver white | 1.64[b] | 0.62[b] | 37.92 | 0.39[b] | 0.08 | 21.36[a] | 1.06[b] | 0.70[b] | 66.34[a] |
| SEM | 0.031 | 0.013 | 0.509 | 0.007 | 0.002 | 0.399 | 0.022 | 0.014 | 0.293 |
| Breeder diet[3] | | | | | | | | | |
| CON | 1.80 | 0.67 | 37.10 | 0.42 | 0.08[b] | 18.48[b] | 1.15 | 0.75 | 65.13 |
| DMA | 1.73 | 0.67 | 38.59 | 0.42 | 0.09[a] | 21.42[a] | 1.09 | 0.72 | 65.93 |
| FFF | 1.77 | 0.68 | 38.74 | 0.43 | 0.08[b] | 18.63[b] | 1.16 | 0.76 | 65.56 |
| SEM | 0.041 | 0.017 | 0.662 | 0.009 | 0.003 | 0.528 | 0.029 | 0.019 | 0.381 |
| Diet[6] | | | | | | | | | |
| CON-CON[7] | 1.77 | 0.68 | 38.27 | 0.41 | 0.06[c] | 13.68[c] | 1.11 | 0.73 | 65.63 |
| CON-DMA | 1.80 | 0.66 | 36.27 | 0.43 | 0.09[a,b] | 20.71[a,b] | 1.19 | 0.77 | 64.72 |
| CON-FFF | 1.82 | 0.66 | 36.76 | 0.41 | 0.09[a,b] | 21.04[a,b] | 1.16 | 0.75 | 65.04 |
| DMA-CON | 1.73 | 0.67 | 38.53 | 0.43 | 0.10[a] | 22.58[a] | 1.08 | 0.71 | 66.02 |
| DMA-DMA | 1.73 | 0.67 | 38.64 | 0.41 | 0.08[a,b] | 20.26[a,b] | 1.10 | 0.72 | 65.85 |
| FFF-CON | 1.81 | 0.69 | 38.55 | 0.44 | 0.08[a,b] | 18.07[b] | 1.17 | 0.76 | 65.26 |
| FFF-FFF | 1.73 | 0.67 | 38.93 | 0.42 | 0.08[a,b] | 19.20[b] | 1.15 | 0.75 | 65.85 |
| SEM | 0.059 | 0.024 | 0.936 | 0.013 | 0.004 | 0.747 | 0.040 | 0.026 | 0.539 |
| Probabilities (*P*-value) | | | | | | | | | |
| Strain | <0.001 | <0.001 | 0.878 | <0.001 | 0.250 | <0.001 | <0.001 | <0.001 | <0.001 |
| Breeder diets | 0.462 | 0.771 | 0.102 | 0.602 | 0.004 | <0.001 | 0.191 | 0.278 | 0.277 |
| Offspring diet | 0.877 | 0.891 | 0.633 | 0.543 | <0.001 | <0.001 | 0.751 | 0.884 | 0.712 |
| Strain × breeder diet | 0.104 | 0.110 | 0.948 | 0.834 | 0.930 | 0.748 | 0.065 | 0.088 | 0.762 |
| Strain × offspring diet | 0.713 | 0.085 | 0.075 | 0.640 | 0.286 | 0.062 | 0.627 | 0.811 | 0.432 |

[1]Values with uncommon superscripts within each column are significantly different ($P < 0.05$). n = 5.
[2]Laying hens during 25 to 42 wk of age were not fed the experimental diets. These diets were only fed in the breeder and progeny pullet stage.
[3]Weight presented as g/kg of body weight.
[4]Ash weight.
[5]Ash concentration percentage.
[6]CON: control; DMA: dried microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid; FFF: coextruded full-fat flaxseed and pulse mixture (1:1 wt/wt), as a source of α-linolenic acid.
[7]The day-old female pullets from breeders fed CON, DMA, and FFF were divided into 3 (CON, DMA, and FFF), 2 (CON and DMA) and 2 (CON, FFF) posthatch treatments, respectively. The order shows breeder diet and pullet diet (e.g., breeder diet-pullet diet, CON-CON).

The main effect of breeder diets on tibia subparts was such that ash weight and ash percentage in offspring were increased by breeder-feeding of DMA relative to the CON and FFF ($P < 0.005$). The main effect of the offspring diet on medullary ash content and ash concentration was such that hens from CON-CON had the lowest values compared with other dietary treatments ($P < 0.001$). Hens fed DMA-CON had higher ($P < 0.001$) tibia medullary ash concentration than hens fed FFF-CON and FFF-FFF. There was no interaction between strains and breeder diets, the interaction between strain and offspring diets, or the main effect of breeder and offspring diets on keel bone attributes ($P > 0.05$; Table 7). The strain effect was such that ISA brown hens had 23.5, 29.1, and 1.1% more keel weight, ash content, and ash concentration, respectively, than Shaver white hens ($P < 0.05$).

## *Evolution of Tibia Attributes From 18 to 42 WOA*

There was no strain, breeder diet, offspring diet, and interaction effects on CI of body and whole tibia weight between 18 and 42 WOA ($P > 0.05$; Table 8). There was neither interaction between strain and breeder diet and between strain and offspring diet nor the main effect of breeder and offspring diets on the CI of whole tibia BS, ash content, and ash concentration ($P > 0.05$). However, the strain effect was such that Shaver white hens showed greater decline in whole tibia BS (83.7 vs. 96.3%) and ash content (84.1 vs. 94.3%) at 42 WOA relative to 18 WOA than ISA brown hens. Moreover, the CI of whole tibia ash concentration was lower in Shaver white hens (117 vs. 128%) than in ISA brown hens. There was no interaction effect between strain, breeder, and offspring diets ($P > 0.05$) on the CI of tibia epiphysis and medullary weight, ash content, and ash concentration (Table 9). Only strain effect was observed on the CI of tibia cortical attributes, in this context. Shaver whites exhibited the lower 42 WOA decline in cortical weight (70.1 vs. 78.7%), ash content (75.1 vs. 89.1%), and ash percentage (107.1 vs. 113.9%) than ISA brown hens ($P < 0.05$).

## DISCUSSION

The quality of eggshell has a considerable impact on the profitability of the egg industry (Lichovnikova, 2007). In the present study, supplementing either breeder progeny or both diets with n-3 FA did not affect egg production and eggshell quality, which was consistent with the previous report regarding the impact of

**Table 7.** Effects of feeding sources of docosahexaenoic and α-linolenic acids to ISA brown and Shaver white breeders and their pullets on keel bone attributes from 42-wk-old laying hens.[1,2]

| Items | Weight, g[3] | Ash weight, g[4] | Ash concentration, % |
|---|---|---|---|
| Main effect | | | |
| Strain | | | |
| ISA brown | 2.68[a] | 1.11[a] | 41.93[a] |
| Shaver white | 2.17[b] | 0.86[b] | 39.79[b] |
| SEM | 0.066 | 0.022 | 0.752 |
| Breeder diet[3] | | | |
| CON | 2.47 | 1.00 | 40.48 |
| DMA | 2.32 | 0.97 | 41.91 |
| FFF | 2.48 | 0.98 | 40.40 |
| SEM | 0.086 | 0.029 | 0.995 |
| Diet[4] | | | |
| CON-CON[5] | 2.33 | 0.97 | 41.50 |
| CON-DMA | 2.44 | 1.04 | 42.69 |
| CON-FFF | 2.63 | 0.98 | 37.25 |
| DMA-CON | 2.42 | 1.05 | 43.25 |
| DMA-DMA | 2.22 | 0.90 | 40.56 |
| FFF-CON | 2.55 | 0.97 | 39.50 |
| FFF-FFF | 2.41 | 0.99 | 41.30 |
| SEM | 0.123 | 0.041 | 1.408 |
| Probabilities (*P*-value) | | | |
| Strain | <0.001 | <0.001 | 0.049 |
| Breeder diets | 0.345 | 0.804 | 0.468 |
| Offspring diet | 0.291 | 0.095 | 0.071 |
| Strain × breeder diet | 0.137 | 0.080 | 0.453 |
| Strain × offspring diet | 0.418 | 0.963 | 0.340 |

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

[1]n = 5.

[2]Laying hens during 25 to 42 wk of age were not fed the experimental diets. These diets were only fed in the breeder and progeny pullet stage.

[3]Weight presented as g/kg of body weight.

[4]CON: control; DMA: dried microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid; FFF: coextruded full-fat flaxseed and pulse mixture (1:1 wt/wt), as a source of α-linolenic acid.

[5]The day-old female pullets from breeders fed CON, DMA, and FFF were divided into 3 (CON, DMA, and FFF), 2 (CON and DMA) and 2 (CON and FFF) posthatch treatments, respectively. The order shows breeder diet and pullet diet (e.g., breeder diet-pullet diet, CON-CON).

feeding n-3 FA on egg production and eggshell quality (Amini and Ruiz-Feria, 2007; Nain et al., 2012; Wu et al., 2018). Progressive deterioration of structural bone throughout the laying cycle increases the susceptibility of fractures and osteoporotic mortality (Whitehead, 2004). Dietary interventions after the onset of lay have been reported not to be effective in alleviating the adverse effect of aging on structural bones (Akbari Moghaddam Kakhki et al., 2018b). It has been suggested that nutritional strategies aiming to minimize osteoporosis should not be focused on the late phase of lay cycle or when there is a high risk of osteoporosis and that they should preferably be implemented in the early stages of skeletal development (Akbari Moghaddam Kakhki et al., 2018b; Whitehead and Fleming, 2000).

Among nutritional interventions, n-3 FA is believed to possess various health benefits such as increasing bone mineral content and attenuating age-related bone loss (Boeyens et al., 2014). The n-3 PUFA are thought to modify bone resorption and formation (Moon et al., 2012) through increasing intestinal Ca absorption (Moon et al., 2012), synthesizing bone collagen, circulating insulin-like growth factor 1, decreasing locally produced prostaglandins (**PG**; Mazzuco et al., 2005), and inflammatory cytokines (Moon et al., 2012). A previous report showed that breeders fed the same sources of n-3 FA reduced the bone mineral content in hatchlings and increased collagen type II (Akbari Moghaddam Kakhki et al., 2020b). Addition of DMA and FFF to both breeders and their progeny increased cortical formation in Shaver White pullets. However, the addition of DMA and FFF to breeder diet or their progeny did not affect bone development in ISA brown and Shaver white pullets (Akbari Moghaddam Kakhki et al., 2020b). In the present study, all birds of the previous phases were fed commercial diets at the onset of lay, to examine the impact of prehatch and pullet-phase feeding sources of n-3 FA on the tibia epiphysis and cortical attributes post-peak of egg production. Hens from breeders fed DMA or pullets fed n-3 FA sources had greater ash content and ash percentage in their tibia medullary. However, the same pattern of response was not observed in any other parameters including performance, eggshell quality, and tibia BS. As a result, the effects on medullary attributes on other

**Table 8.** Effects of feeding sources of docosahexaenoic and α-linolenic acids to ISA brown and Shaver white breeders and their pullets on the change of the whole tibia attributes in the 42-wk-old laying hen relative to their 18-wk-old pullet attributes %.[1,2]

| Items | Bodyweight | Tibia | | | |
|---|---|---|---|---|---|
| | | Breaking strength[3] | Weight | Ash weight | Ash concentration |
| Main effect | | | | | |
| Strain | | | | | |
| ISA brown | 129.0 | 96.3[a] | 74.1 | 94.3[a] | 127.9[a] |
| Shaver white | 132.4 | 83.7[b] | 72.3 | 84.1[b] | 116.6[b] |
| SEM | 2.650 | 4.304 | 1.477 | 2.219 | 2.132 |
| Breeder diet[3] | | | | | |
| CON | 131.4 | 86.4 | 73.6 | 87.3 | 118.7 |
| DMA | 132.9 | 96.7 | 72.8 | 90.2 | 124.3 |
| FFF | 127.5 | 88.7 | 73.0 | 91.3 | 125.6 |
| SEM | 3.503 | 5.694 | 1.921 | 2.885 | 2.771 |
| Diet[4] | | | | | |
| CON-CON[5] | 133.7 | 90.9 | 71.7 | 87.1 | 121.3 |
| CON-DMA | 130.9 | 89.0 | 73.8 | 87.4 | 118.7 |
| CON-FFF | 129.4 | 79.4 | 75.3 | 87.2 | 116.0 |
| DMA-CON | 131.8 | 94.1 | 69.7 | 89.2 | 128.5 |
| DMA-DMA | 134.1 | 99.2 | 75.9 | 91.1 | 120.2 |
| FFF-CON | 124.8 | 91.0 | 77.0 | 93.5 | 120.7 |
| FFF-FFF | 130.3 | 86.5 | 68.9 | 89.0 | 130.4 |
| SEM | 4.958 | 8.051 | 2.717 | 4.080 | 3.919 |
| Probabilities ($P$-value) | | | | | |
| Strain | 0.382 | 0.043 | 0.508 | 0.003 | <0.001 |
| Breeder diets | 0.531 | 0.374 | 0.937 | 0.527 | 0.118 |
| Offspring diet | 0.897 | 0.821 | 0.119 | 0.951 | 0.210 |
| Strain × breeder diet | 0.988 | 0.305 | 0.195 | 0.441 | 0.398 |
| Strain × offspring diet | 0.112 | 0.754 | 0.706 | 0.819 | 0.742 |

Values with uncommon superscripts within each column are significantly different ($P < 0.05$).
[1]n = 5.
[2]Laying hens during 25 to 42 wk of age were not fed the experimental diets. These diets were only fed in the breeder and progeny pullet stage.
[3]Calculated by dividing week 42 values by wk 18 values and multiplied by 100. The data of 18 WOA values were obtained from Akbari Moghaddam Kakhki. et al. (2020b).
[4]CON: control; DMA: dried microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid; FFF: coextruded full-fat flaxseed and pulse mixture (1:1 wt/wt), as a source of α-linolenic acid.
[5]The day-old female pullets from breeders fed CON, DMA and FFF were divided into 3 (CON, DMA, and FFF), 2 (CON and DMA) and 2 (CON and FFF) posthatch treatments, respectively. The order shows the breeder diet and pullet diet (e.g., breeder diet-pullet diet, CON-CON).

physiologic and production aspects remain to be answered. There was no change in structural parts in response to breeder and pullet-phase feeding of n-3 FA, showing that the positive impact of n-3 FA on structural bone did not last over 24 wk.

There are contradictory results in mammalian studies regarding the effect of prenatal and early life feeding of n-3 on bone development in later life. Korotkova et al. (2004) tested the effect of feeding rats in their late pregnancy diets containing either 70 g of linseed oil (as a source of n-3 FA), soybean oil (as a source of n-6 and n-3 FA), or sunflower seed oil (as a source of n-6 FA) on bone development in their offspring at 30 D. Their results showed that maternal feeding of a diet containing soybean oil increased BW, femur length, cortical density, thickness, and area (Korotkova et al., 2004). Although the PUFA level in diets was not presented, the authors concluded the lower total amount of PUFA in the diet of linseed oil was responsible for the lower bone parameters compared with the soybean oil group (Korotkova et al., 2004). Korotkova et al. (2005) observed that feeding essential FA–deficient diets to rats during late gestation and throughout lactation increased BW, cortical content, area, and thickness, while it decreased the trabecular bone density of femur in 44-day-old pups. However, the bone attributes were not normalized based on the BW (Korotkova et al., 2005). These differences in the results might be related to various factors including the type of FA (Kruger and Schollum, 2005), FA dosage (Kruger and Schollum, 2005), duration of intervention (Cohen and Ward, 2005), and the health status of target population (Anez-Bustillos et al., 2018).

Occurrence of fractures in keel bone is highly dependent on the housing system, strain, and management styles (Casey-Trott et al., 2015). These differences could be a reason why there was a lack of effect of n-3 FA on the keel bone in the present study. It has been reported that n-3 PUFA–supplemented diets can reduce Keel bone fracture likely without a detrimental effect on production depending on the quantities of n-3 FA as a high level of n-3 FA may lead to health and production detriments (Toscano et al., 2015). However, whether the protective effects of n-3 PUFA can translate to bone metabolism remains unknown and must be further explored (Toscano et al., 2015).

Feed intake, body growth and development, sexual maturity, egg production efficiency, skeletal health (Khanal et al., 2019), and eggshell quality (Ketelaere

**Table 9.** Effects of feeding sources of docosahexaenoic and α-linolenic acids to ISA brown and Shaver white breeders and their pullets on the change of tibia subpart attributes in the 42-wk-old laying hen relative to their 18-wk-old pullet attributes %.[1,2]

| Items | Epiphysis | | | Medullary | | | Cortical | | |
|---|---|---|---|---|---|---|---|---|---|
| | Wt[3] | Ash wt[4] | Ash c[5] | Wt | Ash wt | Ash c | Wt | Ash wt | Ash c |
| Main effect | | | | | | | | | |
| Strain | | | | | | | | | |
| ISA brown | 72.4 | 91.4 | 126.6 | 88.9 | 3,287.8 | 4,099.8 | 78.28[a] | 89.0[a] | 114.2[a] |
| Shaver white | 70.7 | 85.7 | 121.3 | 104.7 | 3,051.3 | 3,204.5 | 70.47[b] | 75.7[b] | 107.3[b] |
| SEM | 1.716 | 2.774 | 2.632 | 8.633 | 647.90 | 585.0 | 2.103 | 2.022 | 1.998 |
| Breeder diet[3] | | | | | | | | | |
| CON | 72.1 | 86.3 | 120.1 | 101.1 | 2,782.3 | 2,855.2 | 74.46 | 80.7 | 109.1 |
| DMA | 70.3 | 88.4 | 125.9 | 101.0 | 3,849.2 | 4,300.9 | 72.86 | 82.0 | 112.9 |
| FFF | 72.0 | 92.0 | 127.6 | 86.89 | 2,877.0 | 3,800.2 | 75.81 | 84.2 | 110.2 |
| SEM | 1.82 | 3.783 | 2.842 | 10.421 | 841.65 | 773.90 | 2.781 | 2.627 | 2.632 |
| Diet[6] | | | | | | | | | |
| CON-CON[7] | 70.0 | 86.8 | 124.3 | 97.7 | 3,659.8 | 2,993.2 | 72.2 | 81.9 | 114.3 |
| CON-DMA | 71.0 | 82.9 | 116.8 | 94.3 | 2,095.1 | 2,699.1 | 77.4 | 82.4 | 106.7 |
| CON-FFF | 75.2 | 89.3 | 119.1 | 108.2 | 2,591.9 | 2,873.4 | 73.7 | 78.0 | 106.5 |
| DMA-CON | 68.5 | 88.9 | 130.4 | 95.9 | 5,853.3 | 6,676.2 | 68.2 | 78.9 | 115.5 |
| DMA-DMA | 72.2 | 88.0 | 121.5 | 106.2 | 1,845.0 | 1,925.7 | 77.5 | 85.1 | 110.3 |
| FFF-CON | 76.9 | 94.3 | 120.6 | 89.6 | 2,544.7 | 3,182.3 | 79.2 | 87.8 | 111.3 |
| FFF-FFF | 67.1 | 89.7 | 134.7 | 84.2 | 3,209.5 | 4,418.1 | 72.4 | 80.6 | 109.2 |
| SEM | 3.154 | 5.190 | 4.923 | 16.151 | 1,190.3 | 1,094.5 | 3.934 | 3.713 | 3.830 |
| Probabilities (*P*-value) | | | | | | | | | |
| Strain | 0.560 | 0.192 | 0.170 | 0.168 | 0.725 | 0.261 | 0.011 | <0.001 | 0.019 |
| Breeder diets | 0.822 | 0.496 | 0.201 | 0.605 | 0.587 | 0.331 | 0.762 | 0.598 | 0.534 |
| Offspring diet | 0.150 | 0.877 | 0.160 | 0.956 | 0.167 | 0.051 | 0.297 | 0.409 | 0.402 |
| Strain × breeder diet | 0.252 | 0.624 | 0.782 | 0.783 | 0.199 | 0.492 | 0.337 | 0.264 | 0.911 |
| Strain × offspring diet | 0.725 | 0.424 | 0.184 | 0.989 | 0.538 | 0.824 | 0.637 | 0.494 | 0.944 |

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

[1]n = 5.

[2]Laying hens during 25 to 42 wk of age were not fed the experimental diets. These diets were only fed in the breeder and progeny pullet stage.

[3]Dry weight. Calculated by dividing week 42 values by wk 18 values and multiplied by 100. The data of 18 WOA values were obtained from Akbari Moghaddam Kakhki. et al. (2020b).

[4]Ash weight.

[5]Ash concentration.

[6]CON: control; DMA: dried microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid; FFF: coextruded full-fat flaxseed and pulse mixture (FFF, 1:1 wt/wt), as a source of α-linolenic acid.

[7]The day-old female pullets from breeders fed CON, DMA, and FFF were divided into 3 (CON, DMA, and FFF), 2 (CON and DMA) and 2 (CON, FFF) posthatch treatments, respectively. The order shows the breeder diet and pullet diet (e.g., breeder diet-pullet diet, CON-CON).

et al., 2002) differ between strains of the layer hen. In the present study, the strains possessed different traits and characteristics in egg production, eggshell quality, tibia, and keel bone. ISA brown hens showed higher egg weight and egg mass but lower eggshell quality. Tibia in Shaver white hens was stronger than in ISA brown hens, while ISA brown hens maintained higher ash content and ash percentage in their keel one. Previously, our findings showed that Shaver white pullets at 18 WOA had higher tibia and femur ash concentration than ISA brown pullets (Akbari Moghaddam Kakhki et al., 2020b). In addition, the difference in bone metabolism among strains was demonstrated not only by the lack of effect of feeding of n-3 FA on tibia characteristics in ISA brown pullets vs. an effect in the Shaver white pullets but also by the higher activity of osteoclast and lower activity of osteoblast in ISA brown than in Shaver white hens (Akbari Moghaddam Kakhki et al., 2020b). However, the rate of mineral mass loss among treatments and strains was measured by calculating CI value based on the difference between sampling at 42 WOA and 18 WOA. Lower CI values for tibia BS, ash content, ash percentage, and cortical bones in Shaver white than in ISA brown hens demonstrated the faster erosion rate and depletion of mineral in the tibia of Shaver white hens compared with ISA brown. This difference emphasizes the importance of considering the role of the genetic effect on studying nutritional strategies to improve bone quality in poultry.

In our study, the incidence of water system malfunction occurs only in Shaver white hens, which could be associated with the difference among strains by effecting BW. Body weight measurement before the incidence was 1436.8 g (Akbari Moghaddam Kakhki et al., 2020b). The BW after the incidence was 1655.5 g, or 15.22% higher than the BW at 18 WOA. The average BW in those found dead was only 4.9% higher than the BW at 18 WOA. After the recovery period (23 WOA), the BW was 1719.8 g, or 19.7% higher than the BW at 18 WOA values. At 24 WOA, the BW increase relative to

18 WOA was calculated as 20.1%. Although the feed intake was not measured, feed intake went back to normal based on a visual assessment daily. In addition, the production performance was reached to recommended values presented in their breed guideline Commercial Product Guide-Shaver White (2018). Therefore, the effect of the water system malfunction was negligible on measured parameters.

Feeding breeder and their female progeny during pullet-phase sources of n-3 FA did not affect egg production and eggshell quality in ISA brown and Shaver white hens post-peak of egg production. Keel bone, tibia, its epiphysis, and cortical attributes in ISA brown and Shaver white hens post-peak of egg production were not influenced by either breeder-feeding or offspring feeding of n-3 FA source. However, the effect of the n-3 FA source on tibia medullary characteristics was dependent on the phase of feeding and type of n-3 FA source. Only breeder-feeding of DMA increased the tibia medullary ash content and percentage while feeding both sources of n-3 during pullet-phase increased ash content and percentage in tibia medullary of 42 WOA ISA brown and Shaver white hens. These results showed that the positive effect of feeding n-3 FA on the development of tibia cortical and epiphysis did not last for 24 wk after removal of the n-3 sources. The strains showed differences in their production performance, eggshell quality, tibia, and keel bone characteristics, highlighting the importance of considering the strain effect in designing and interpreting the results of nutritional and management studies.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Co-authors K. R. P. and J. M. are employees of Alltech Canada, and O & T Farms, respectively.

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