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Significance of successive feeding of sources of n-3 fatty acids to broiler breeders and their progeny on growth performance, intestinal lesion scores, lymphoid organs weight and plasma immunoglobulin A in broiler chickens challenged with *Eimeria*

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ABSTRACT The study examined the effects of successive feeding of sources of n-3 PUFA to broiler breeders (**BB**) and their progeny in broiler chickens challenged with *Eimeria*. The BB were fed: 1) control (CON), corn-soybean meal diet, 2) CON + 1 % microalgae (**DMA**), as a source of DHA and 3) CON + 2.50% co-extruded full fat flaxseed (**FFF**), as a source of ALA. Eggs were hatched at 34, 44, and 54 wk of age. Posthatch treatments (BB-progeny) were: CON-CON, DMA-CON, FFF-CON, DMA-DMA and FFF-FFF with diets formulated for starter (d 1 −10) and grower/finisher (d 11−42) phases. All chicks were orally challenged with *Eimeria* (E. *acervulina* and E. *maxima)* on d 10. Relative to CON, DMA and FFF increased concentration of n-3 PUFA by ≥ 2-fold in hatching eggs and progeny diets. There were no ($P > 0.05$) interactions between treatment and BB age on d 0 to 10 growth. In general, BB age affected ($P < 0.05$) growth performance throughout the study. In the starter phase, successive exposure to DHA and ALA improved FCR over CON-CON ($P < 0.01$). The interaction between treatment and BB age in grower/finisher was such that DHA exposure to younger BB resulted in poor growth performance ($P < 0.05$) relative to exposure to older BB. In contrast, exposure to ALA had similar ($P > 0.05$) growth performance irrespective of BB age. Moreover, successive exposure to ALA resulted in higher BWG, breast weight and lower FCR compared to successive exposure to DHA ($P < 0.05$). There were no ($P > 0.05$) interactions between treatment and BB age on the intestinal lesion scores, lymphoid organ weights and concentration of plasma immunoglobulin A (**IgA**). Successive exposure to DHA resulted in higher ($P = 0.006$) jejunal lesion scores than CON-CON birds. The results showed that successive exposure of DHA and ALA improved FCR relative to non-exposed birds in the starter phase. However, responses in the grower/finisher phase depended on n-3 PUFA type, with birds on successive ALA exposure supporting better growth and breast yield than birds on successive DHA exposure.

Key words: omega-3 fatty acid, broiler breeder and chick, coccidiosis, growth performance, immunocompetence

2024 Poultry Science 103:103796
https://doi.org/10.1016/j.psj.2024.103796

INTRODUCTION

Advances in genetics, nutrition, health, and management have collectively contributed to remarkable biological and economic efficiency seen in modern broiler chickens (Havenstein et al., 2003; Gous, 2010; Krupa et al., 2017; Merks, 2018; Torrey et al., 2021). However, broiler chickens are often afflicted with physiological and metabolic disorders such as lameness, poor immunocompetence, and myopathies that exert economic losses (Julian, 2005; Santos et al., 2021; Santos et al., 2022a, b). These disorders are exacerbated by evolving pressure for cessation of production practices such as indiscriminate use of preventative antimicrobial growth promoters (**AGP**) (Bean-Hodgins and Kiarie, 2021; Mak et al., 2022). From a nutrition perspective, tremendous efforts have been dedicated towards the development of specialized starter feeding programs based on a range of digestible and functional ingredients such as specialty soy products and advanced feed processing (Barekatain and Swick, 2016; Kiarie and Mills, 2019; Kiarie et al., 2021). Efforts have also been dedicated to specialty feed additives as alternatives to AGP (Kiarie et al., 2013; Kiarie et al., 2016; Kiarie et al., 2019). However, one area that has not received much attention is the role of breeder nutrition and its subsequent impact on progeny

Received January 24, 2024.
Accepted April 22, 2024.

[1]Present address: Jones Feed Mills Ltd., 1024 Alfred Street, Linwood, Ontario N0B 2A0, Canada.

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performance. This area is critical as genetic progress towards a shorter life to slaughter weight has elevated the importance of the embryonic and immediate post-hatch period in broiler chickens production (Ferket, 2012; Wijtten et al., 2012).

The hen diet can manipulate the nutrients deposited in the hatching egg that are critical for embryonic development (Uni and Ferket, 2004; Uni et al., 2005; Ferket, 2012). Specifically, the egg fat component is a vital source of energy and essential fatty acids such as linoleic (**LA**) and α-linolenic (**ALA**) fatty acids (**FA**) during embryogenesis and early post-hatch (Noble and Cocchi, 1990; Speake et al., 1998). In this context, lipids constitute over 30% of the yolk and are one of the significant components of fertile eggs. There is a rapid uptake of different lipid components by the embryo starting from the 2nd week of incubation and continuing until the residual yolk is completely absorbed (Cherian, 2015; Akbari Moghaddam Kakhki et al., 2020a). Studies have shown that n-3 polyunsaturated FA (**n3-PUFA**) such as eicosapentaenoic acid (**EPA**), docosapentaenoic acid (**DPA**) and docosahexaenoic acid (**DHA**) are preferentially incorporated in the phospholipid membranes during late stages of embryogenesis (Koppenol et al., 2014; Yadgary et al., 2014). Moreover, n-3 PUFA play critical role in regulating biological processes, including the development of vital organs such as the skeleton, brain, and gastrointestinal and engendering transgenerational effects on growth and behavior (Cherian, 2015). As such, the potential of n3-PUFA in stimulating bone, brain, and immune cell development at embryonic through to early phases of chicks is a tremendous opprtunity to improve poultry productivity and welfare (Thanabalan and Kiarie, 2021).

In avian species, ALA and LA cannot be synthesized *de novo* and have to be supplied in the diet (Brenner, 1971; NRC, 1994). This essentiality is due to the inability of the hen to insert double bonds (due to the lack of desaturases) beyond δ-9 carbon (Brenner, 1971). Once a double bond is inserted at the 3rd and 6th carbon (from CH_3 end locations), the hen can add more double bonds and form a longer chain **n-3 PUFA** (Brenner, 1971). However, a standard broiler breeder (**BB**) diet has more LA (over 50% of total FA) than approx. 3 to 3.5% of n3-PUFA (Cherian, 2008). This is due to the predominance of corn and other sources of dietary fat that are high in n-6 FA and is reflected by the absence of long-chain n-3 PUFA in commercial hatching eggs (Cherian, 2008). Similar desaturation and elongation enzymes are involved in the synthesis of n-6 and n-3 PUFA, and as such, competitive inhibition of the enzymes will occur, depending on which substrate is present at the highest concentration (Brenner, 1971). This ratio or balance between n-6 to n-3 FA is necessary for the optimum synthesis of long-chain PUFA (Brenner, 1971). Enriching diets with n-3 PUFA decreases the competition between ALA and LA for elongation to their long chain and active metabolites and increases n-3 PUFA metabolites.

Currently, little consideration is given to the FA composition of the BB diets and their effect on reproductive performance (Cherian, 2011, 2015; Thanabalan and Kiarie, 2021). Furthermore, the current industry practice of feeding BB diets high in n-6 FA limits the supply of essential and healthier n-3 PUFA to the hatchlings. Several studies have shown that BB fed sources of n-3 PUFA significantly enriched yolk (Cherian, 2015; Koppenol et al., 2015a,b; Delezie et al., 2016; Thanabalan et al., 2020). Other studies have reported feeding BB sources of n-3 PUFA increased transfer of passive maternal antibodies (Wang et al., 2002; Wang et al., 2004), proliferation and maturation of B cells (Koppenol et al., 2015a; Koppenol et al., 2015b), and reduced proinflammatory metabolites (Hall et al., 2007; Cherian et al., 2009) and embryonic mortality (Saber and Kutlu, 2020). We recently showed that chicks hatched from BB fed a flaxseed rich diet had a higher brain-to-body weight ratio (larger brain size) (Whittle et al, 2024a). In further research, we demonstrated that laying hens breeders feed sources of n-3 PUFA hatched chicks with higher tibial ash than chicks of breeders fed a standard control diet (Akbari Moghaddam Kakhki et al., 2020a). It appears that feeding poultry breeders diets enriched with n-3 PUFA may have tremendous impact on immunocompetence and skeletal development. However, little is known about the impact of enriching BB diets with n-3 PUFA on progeny performance and metabolism through slaughter weight when subjected to an enteric pathogen. Therefore, the objective of the study was to investigate the effect of successive feeding sources n-3 PUFA on broiler breeders and their progeny on growth performance, intestinal lesion scores, lymphoid organs weight and plasma immunoglobulin A in broiler chickens challenged with *Eimeria*.

MATERIALS AND METHODS

The University of Guelph Animal Care Committee (**AUP** # 3675) reviewed and approved the animal use protocol, and birds were cared for according to the Canadian Council on Animal Care guidelines (**CCAC**, 2009).

Birds and Housing

The broiler chickens for the current study were derived from a long-term study involving the placement of d old BB for the rearing and laying phases at Arkell Poultry Research Station, University of Guelph (Guelph, ON). The details for the birds, housing and management during the rearing and laying phases have been reported (Thanabalan and Kiarie, 2022; Thanabalan, 2023). Briefly, 588 pullets (Ross 708) and 60 cockerels were procured from Aviagen (Aviagen Inc., Huntsville, AL). The pullets and cockerels were reared in separate floor pens until 22 wk of age (woa). At the beginning of 22 woa, the birds were transitioned to floor pens in a laying house and were grouped based on laying diets (60♀and 10♂/pen) (Figure 1). The broiler chicks were hatched from eggs collected from BB at 34-, 44- and 54- woa and were

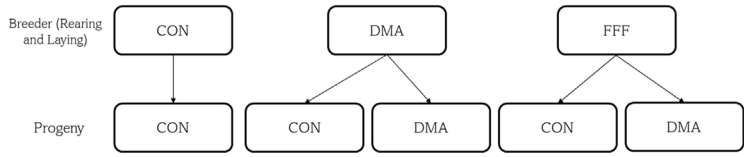

Figure 1. Dietary treatment layout for the broiler breeder and progeny phases. Day-old female broiler breeder pullets were divided into dietary treatments: 1) control (**CON**); 2) Microalgae (*Aurantiochytrium limacinum*) fermentation product, docosahexaenoic acid source (**DMA**); and 3) co-extruded full-fat flaxseed and pulses mixture (50/50, wt/wt), α-linolenic acid source (**FFF**). Offspring from the CON treatment remained on CON, and offspring from the DMA and FFF treatments were divided into 2 posthatch treatments, CON and DMA or CON and FFF. The concentration of total n-3 PUFA and ratio of n-6: n-3 were similar among DMA and FFF diets in both phases.

designated Hatch 1, 2, and 3, respectively. Six eggs per pen treatment group were collected and submitted for fatty acids profile analyses at each hatching egg collection time point. The eggs were incubated and hatched in a commercial-grade incubator and hatcher (Nature Form, Jacksonville, FL) at 37.5°C with 55% humidity to d 19 and then transferred to the hatcher set at 36.9°C with 66 % humidity. Fertile eggs were identified on d 19 of incubation through candling and transferred to the hatcher. Upon hatching, all chicks were wing-feather sexed, weighed, and vaccinated against bronchitis (spray), Marek's disease (subcutaneous) and coccidiosis (spray) in the hatchery. Chicks were placed in 90 metabolic cages (76 cm width, 51 cm depth, and 56 cm height) (Ford Dickson Inc., Mitchell, ON, Canada) based on body weight within the breeder treatment group (8 chicks per cage) for Hatch 1 and 2 and 5 chicks per cage for Hatch 3 due to the poor hatchability. In each hatch placement, half of the cages were for pullets and the other half for cockerels. The room temperature was initially set at 32°C on d 1 and gradually decreased to 29°C by d 14. There were 2 tiers of cages each with independent lighting. The lighting program was 23 h of light, 1 h of dark (20+ lux) for d 1 to 3, and 20 h of light, 4 h of dark (10-15 lux) for d 4 to 42. Each cage had 2 nipple drinkers connected to a common water line supplying the whole room and an independent trough feeder (70 cm length, 8.5 cm width and 9 cm depth).

Diets

An illustration of the dietary allocation during laying phase and linkage with the current experiment (progeny phase) is shown in Figure 1. Briefly, in each phase, 3 diets were formulated: 1) control, a corn-soy diet (**CON**), 2) CON + 1 % microalgae (**DMA**, *Aurantichytrium limacinum*) as a source of DHA (Ao et al., 2015), [Alltech Canada, Guelph Ontario, Canada], and 3) CON + 2.88-3.07% co-extruded full-fat flaxseed and pulse mixture (**FFF**, 1:1 wt/wt) as a source of ALA (linPRO, O & T Farms Ltd., Regina, SK, Canada). The DMA and FFF diets were formulated such that the ratio of total n- 3 to n-6 FA were similar. All diets met or exceeded the nutrient specifications for Ross 708 (Aviagen, 2019) in 2 phases: starter (d 1−10) and grower/finisher (d 11−42) (Table 1). The detailed formulation of laying phases were previously reported (Thanabalan, 2023).

Experimental Procedures and Sampling

The allocation of treatment in the progeny phase considered diets fed to BB during laying, as illustrated in Figure 1. The chicks hatched from BB fed CON continued with the CON diet for the progeny phase. Chicks hatched from BB fed DMA or FFF were respectively split into CON and DMA, CON and FFF for the progeny phase. As such the 5 posthatch treatments identities (BB-progeny) were: CON-CON, DMA-CON, FFF-CON, DMA-DMA, and FFF-FFF. For each hatch, the treatments were allocated within sex in a completely randomized design to give 18 replicate cages per treatment (9 replicates/sex/treatment). The feed and water were offered on an *ad libitum* basis. Body weight (**BW**) and feed intake (**FI**) were recorded on d 10, 15, and 42. The mortality count and BW of dead birds were recorded as they occurred for calculation of mortality corrected feed conversion ratio (**FCR**). On d 10, two birds closest to the cage average BW received a higher oral dose of 1 mL of *Eimeria culture* (100,000 oocysts of *E. acervulina* and 25,000 oocysts of *E. maxima*). The remaining birds received a low oral dose of 1 mL of *Eimeria* culture (25,000 oocysts *E. acervulina* and 5,000 oocysts *E. maxima*). *Eimeria* culture was from the laboratory of Dr. John Barta, Department of Pathobiology, University of Guelph. The methodology for the *Eimeria* culture propagation, preparation and titration was described by Shirley (1995). The high dose allowed for quantification of the acute immune response, while the low dose provided a low-grade impact on intestinal cells, which can impair nutrient absorption and growth akin to subclinical challenge and has been validated in our previous research in the same cages (Kim et al., 2017; Akbari Moghaddam Kakhki et al., 2019; Lu et al., 2019; Kim et al., 2022).

On d 15, the 2 birds that received high *Eimeria* dose were bled from the wing vein into heparin-coated blood tubes. The tubes with blood were placed on ice immediately and transported to the lab for plasma separation. The birds were subsequently euthanized via cervical dislocation and necropsied. The duodenum and jejunum were dissected and scored for intestinal lesions as described by Price and Guerin, 2014, using a scale of 0 (none) to 4 (high) Johnson and Reid, 1970. The liver, bursa, and spleen were dissected, blotted dry with a paper towel and weighed. On d 42, two birds per cage were randomly selected and euthanized, and the breast muscle was dissected, blotted dry and weighed. The left tibia was dissected, fresh weight recorded, and subsequently stored at -20°C until further analyses.

Table 1. Composition and analyzed provisions of experimental diets, as fed basis.

Item	Starter			Grower and finisher		
Ingredient, %	Control	DMA	FFF	Control	DMA	FFF
Corn	45.3	44.8	44.0	42.6	42.5	41.4
Soybean meal	34.4	34.4	33.2	32.2	31.9	30.9
Wheat	10.0	10.0	10.0	10.0	10.0	10.0
Pork meal	3.00	3.00	3.00	3.00	3.00	3.00
Corn oil	2.95	2.42	2.50	6.94	6.40	6.45
Monocalcium phosphate	1.42	1.41	1.41	1.72	1.71	1.65
DMA[1]	-	1.00	-	-	1.00	-
FFF[2]	-	-	2.88	-	-	3.07
Vitamin and trace mineral premix[3]	1.00	1.00	1.00	1.00	1.00	1.00
Limestone fine	0.70	0.70	0.70	1.24	1.24	1.27
DL-Methionine	0.36	0.37	0.37	0.36	0.36	0.36
L-Lysine HCL	0.32	0.33	0.33	0.32	0.33	0.31
Sodium bicarbonate	0.27	0.27	0.27	0.19	0.19	0.18
L-Threonine	0.17	0.17	0.17	0.15	0.15	0.15
Salt	0.12	0.12	0.12	0.22	0.22	0.22
Multi-carbohydrase enzyme[4]	0.05	0.05	0.05	0.05	0.05	0.05
Ethoxyquin[5]	0.02	0.02	0.02	0.02	0.02	0.02
Calculated Provisions						
Apparent metabolizable energy, kcal/kg	3,000	3,000	3,000	3,100	3,100	3,100
Crude protein, %	23.0	23.0	23.0	20.0	20.0	20.0
SID Lys, %	1.28	1.28	1.28	1.15	1.15	1.15
SID Met + Cys, %	0.95	0.95	0.95	0.89	0.89	0.89
SID Trp, %	0.25	0.25	0.25	0.23	0.23	0.23
SID Thr, %	0.86	0.86	0.86	0.77	0.77	0.77
Calcium, %	0.96	0.96	0.96	0.87	0.87	0.87
Available phosphorus, %	0.48	0.48	0.48	0.43	0.43	0.43
Sodium, %	0.16	0.16	0.16	0.16	0.16	0.16
Analyzed Provisions						
Dry matter, %	87.1	86.9	87.3	88.4	88.7	87.2
Crude protein, %	22.7	22.3	22.8	20.2	20.0	20.5
Crude fat, %	8.74	8.78	8.95	9.24	9.40	9.53
Fatty Acids, mg/g fat						
Linoleic acid (LA, C18:2n-6)	518	493	501	533	495	508
alpha-Linolenic acid (ALA, C18:3n-3)	22.8	18.8	47.5	21.8	18.7	46.8
Docosahexaenoic acid (DHA, C22:6n-3)	0.10	18.3	5.10	0.10	20.1	2.40
Σn-3	23.8	39.0	53.7	22.6	40.8	50.0
Σn-6	519	494	503	534	496	509
Σn-6:Σn-3	21.8	12.7	9.35	23.6	12.2	10.2

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

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

[3]Provided in kg of diet: vitamin A (retinol), 10,000 IU; vitamin D3 (cholecalciferol), 3,000 IU; vitamin E, 100 mg; vitamin K3 (menadione), 5.0 mg; vitamin B1 (thiamin), 4.0 mg; vitamin B2 (riboflavin), 10.0 mg; vitamin B3 (niacin), 50.0 mg; vitamin B5 (pantothenic acid), 20.0 mg; vitamin B6 (pyridoxine), 4.0 mg; vitamin B9 (folic acid), 2.0 mg; vitamin B12 (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]Provided 2,800 U of cellulase, 400 U of mannanase, 50 U of galactanase, 1,000 U of xylanase, 600 U of glucanase, 2,500 U of amylase, and 200 U of protease per kilogram of diet. (Superzyme OM; Canadian Bio-Systems Inc., Calgary, Alberta, Canada)

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

Sample Processing and Analyses

Experimental diets were finely ground in a coffee grinder and analyzed using Association of Official Agricultural Chemists methods (AOAC, 2005) for dry matter (930.15), crude protein (935.11), and crude fat (920.39) in a commercial laboratory (SGS Canada Inc, Guelph, ON, Canada). The fresh hatching eggs were cracked open, and the yolk and albumen were manually separated. The yolk was subsequently homogenized (Fisherbrand 850 Homogenizer, Fisher Scientific, Canada). Samples of yolk and diets were submitted to a commercial lab (Actlabs, Hamilton ON, Canada) for fatty acids analyses as described by O'Fallon et al. (2007). The concentration of fatty acids was expressed as mg/g of fat in the yolk. Blood samples were centrifuged at 2,500 × *g* for 15 min at 4°C, and plasma was stored at -80°C until all hatches were completed. The plasma samples were used for immunoglobulin A (**IgA**) quantification using a commercial Chicken IgA ELISA kit, following the manufacturer's instructions (E33-104, Bethyl Laboratories, TX). The tibia samples were thawed, defleshed, and weighed before being oven-dried at 105°C for 24 h and weighed to determine dry matter content. The dried samples were ashed at 600°C for 12 h to determine ash content without defatting.

Calculations and Statistical Analyses

The organ weights (liver, spleen, bursa, and breast) were expressed relative to body weight. The data were analyzed using PROC GLIMMIX (SAS 9.4) with treatment, broiler breeder age and associated interactions as

fixed factors. Chick sex was used as a covariate and significance was declared at $P < 0.05$.

RESULTS

Chemical Composition of the Experimental Diets

The analyzed crude protein was comparable among the diets within the phase (Table 1). The concentration of ALA was higher in FFF diets than non-FFF diets and similarly the concentration of DHA was higher in DMA diets than non-DMA diets. Both DMA and FFF diets had more than 2-fold concentration of total n-3 PUFA relative to CON diet in both phases. As a result, the total n-6: n-3 FA ratio in the starter phase was 21.8, 12.7, and 9.35 for CON, DMA and FFF diets, respectively. The corresponding total n-6: n-3 FA ratio in the finisher was 23.6, 12.2, and 10.2, respectively.

Fatty Acids Concentration in the Hatching Eggs

The concentrations of FA in the yolk are presented in supplementary Table 1. There were interactions between diet and BB age on the concentration of ALA ($P < 0.001$), DHA ($P < 0.001$), and total n-3 PUFA ($P < 0.001$). The concentrations of ALA and DHA were higher in eggs sampled from BB fed FFF and DMA, respectively. However, among the BB fed FFF, the concentration of ALA was lower at 54 woa relative to at 34 and 44 woa. On the other hand, the concentration of DHA was lower in BB fed DMA at 44 woa relative to at 34 and 54 woa. Overall, independent of BB age, the concentration total n-3 PUFA was higher ($P < 0.001$) in the eggs of birds fed DMA and FFF relative to BB fed CON (Supplementary Table 1). Relative to CON BB, the concentrations of total n-3 PUFA were 3.1- and 2.8-fold higher in DMA and FFF eggs, respectively. Consequently, albeit observed interactions ($P < 0.001$) between diet and BB age, the hatching eggs from BB fed sources of n-3 PUFA had lower n-6 to n-3 PUFA ratio than eggs of BB fed CON diet.

Growth Performance

There was no ($P > 0.05$) interaction between treatment and BB age or the main effect of treatment on hatchling weight (Table 2). The BB age effect ($P < 0.001$) was such that chicks hatched from older BB were heavier. There were no ($P > 0.05$) interactions between treatment and BB age on BW, BWG, FI, and FCR in the starter phase. The treatment effect was such that birds in DMA-DMA group had higher ($P \leq 0.006$) BWG and d 10 BW than CON-CON birds whereas birds in the other treatment groups were intermediate. Birds in DMA-CON group consumed more ($P = 0.004$) feed than birds in DMA-DMA and FFF-FFF groups in the starter phase. Birds in DMA-DMA, FFF-FFF, and FFF-CON had lower ($P < 0.01$) FCR than CON-CON birds in the starter period. There was no interaction ($P = 0.114$) between treatment and BB age on the final BW (d 42). Birds in FFF-CON group were heavier ($P = 0.009$) than birds in DMA-CON group but neither differed from the birds in the other groups. The final BW (d 42) was 2,630, 2,570, 2,807, 2,607, 2,614 and 2,793 g/bird for CON-CON, DMA-CON, FFF-CON, respectively. There was an interaction between treatment and BB age on BWG ($P = 0.031$) and FCR ($P = 0.012$) but not in feed intake ($P = 0.496$) in the grower/finisher period (d 11 −42). The interaction on BWG was such that the DMA-CON and DMA-DMA birds hatched from BB at 34 woa had lower BWG and higher FCR than counterparts hatched from BB at 44 and 54 woa. In addition, among the birds hatched from BB at 34 woa, the DMA-CON birds had lower BWG and higher FCR than either FFF-CON or FFF-FFF birds, but none ($P > 0.05$) differed with CON-CON birds. Birds exposed to FFF had similar ($P > 0.05$) BWG and FCR irrespective of BB age. Independent of BB age, FFF-FFF birds had higher BWG and lower FCR than DMA-DMA birds in grower/finisher period. There were no ($P > 0.05$) effects of treatment on feed intake in the grower/finisher phase. In general, BB age affected growth performance throughout the study. Chicks hatched from BB 44 at woa were heavier at d 10 and 15 than chicks from BB at either 34 or 54 woa. However, chicks hatched from BB at 54 woa had the highest d 42 BW linked to higher BWG and feed intake between d 11 and 42.

Intestinal Lesion Scores, Organs Weight and Plasma Immunoglobulin A,

There were no ($P > 0.05$) interactions between treatment and BB age on the intestinal lesion scores, organ (liver, bursa, and spleen) weights and concentration of plasma immunoglobulin A (**IgA**) in 15-d old broiler chickens (Table 3). There was no ($P = 0.818$) treatment effects on duodenal lesion scores, however, DMA-DMA birds showed higher ($P = 0.006$) than CON-CON birds. There was a breeder age effect ($P < 0.001$) on duodenal lesion scores such that birds from BB at 54 woa showed higher scores than birds from younger BB. There was no ($P > 0.05$) treatment effect on liver, bursa, and spleen weight in 15 d old broiler chickens. Livers of broilers hatched from BB at 34 and 54 woa were both heavier (33.4 and 32.9 mg/g, respectively), than livers of broilers hatched from BB at 44 woa, 29.8 mg/g ($P < 0.001$). The birds hatched from BB at 44 and 54 woa had lower ($P < 0.001$) concentration of plasma IgA than birds from younger (34 woa) BB.

Breast Weight and Tibia Ash Content

There was no ($P > 0.05$) interaction between treatment and BB age on breast weight and tibia ash content in 42-d old broiler chickens (Table 4). The treatment effect ($P < 0.001$) was such that FFF-FFF birds had

Table 2. Effect of feeding sources of n-3 PUFA to broiler breeders and/or their offspring on growth performance of broiler chicks hatched from 34-, 44- and 54-wk old boiler breeders and challenged with *Eimeria* on d 10 of age.

Effects			Body Weight, g/bird			BWG, g/bird		FI, g/bird		FCR	
Treatment		Day:	0	10	42	0-10[1]	11-42[2]	0-10[1]	11-42[2]	0-10[1]	11-42[2]
Breeder	Offspring										
CON	CON		43.7	266.6^{b}	2630^{ab}	222.9^{b}	2366	314.2^{ab}	3400	1.428^{a}	1.450
DMA[3]	CON		44.2	281.2^{ab}	2570^{b}	236.9^{ab}	2256	320.0^{a}	3371	1.381^{ab}	1.541
FFF[4]	CON		44.0	276.4^{ab}	2807^{a}	232.5^{ab}	2531	281.1^{ab}	3500	1.211^{bc}	1.399
DMA	DMA		44.0	287.7^{a}	2614^{ab}	243.7^{a}	2326	278.6^{b}	3531	1.160^{c}	1.536
FFF	FFF		43.8	279.0^{ab}	2793^{ab}	235.2^{ab}	2514	277.1^{b}	3356	1.186^{c}	1.347
	SEM		0.306	3.74	5.78	3.823	55.47	10.243	85.32	0.047	0.040
Breeder age, week											
		34	40.5^{a}	259.7^{c}	2422^{c}	219.2^{c}	2143	285.4^{b}	3357^{b}	1.319^{a}	1.606
		44	43.4^{b}	292.5^{a}	2697^{b}	249.1^{a}	2404	262.9^{b}	3312^{b}	1.068^{b}	1.380
		54	47.9^{c}	282.3^{b}	2929^{a}	234.3^{b}	2648	334.2^{a}	3625^{a}	1.432^{a}	1.377
		SEM	0.237	2.90	44.7	2.961	42.97	7.935	66.08	0.036	0.031
Interactions											
Breeder	Offspring	Age									
CON	CON	34	40.1	245.7	2382	205.6	2136^{bcd}	294.5	3362	1.437	1.596^{abc}
DMA	CON	34	41.2	269.0	2175	227.5	1810^{d}	301.5	3293	1.408	1.850^{a}
FFF	CON	34	39.9	254.3	2661	214.4	2407^{abc}	266.3	3493	1.234	1.476^{bc}
DMA	DMA	34	40.9	269.5	2229	228.6	1960^{cd}	273.5	3340	1.198	1.711^{ab}
FFF	FFF	34	40.2	260.3	2662	220.1	2402^{abc}	291.3	3299	1.318	1.395^{bc}
CON	CON	44	43.3	278.8	2622	235.5	2343^{abc}	286.6	3114	1.234	1.330^{c}
DMA	CON	44	43.3	285.9	2720	242.6	2434^{ab}	291.8	3132	1.211	1.289^{c}
FFF	CON	44	43.9	292.2	2677	248.3	2385^{abc}	269.9	3399	1.088	1.431^{bc}
DMA	DMA	44	43.2	309.0	2763	265.8	2454^{ab}	224.7	3624	0.854	1.477^{bc}
FFF	FFF	44	43.3	296.7	2703	253.4	2406^{abc}	241.4	3292	0.954	1.373^{c}
CON	CON	54	47.9	275.4	2885	227.5	2618^{ab}	361.5	3723	1.614	1.422^{bc}
DMA	CON	54	48.0	288.8	2814	240.7	2525^{ab}	366.6	3690	1.524	1.483^{bc}
FFF	CON	54	48.1	282.8	3084	234.7	2801^{a}	307.0	3609	1.310	1.290^{c}
DMA	DMA	54	47.7	284.5	2849	236.8	2565^{ab}	337.6	3628	1.427	1.419^{bc}
FFF	FFF	54	48.0	280.0	3013	232.0	2733^{a}	298.5	3478	1.285	1.272^{c}
SEM			0.530	6.47	100	6.62	96.03	17.7	147.7	0.081	0.068
Probabilities											
Treatment			0.868	0.004	0.009	0.006	0.002	0.004	0.489	<0.01	<0.01
Age			<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.003	<0.01	<0.01
Treatment x Age			0.754	0.482	0.114	0.563	0.031	0.371	0.496	0.478	0.012

[1]Prechallenge phase, d 0 to 10
[2]Postchallenge phase, d 11 to 42
[3]Microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid (DHA).
[4]Co - extruded full-fat flaxseed and pulse mixture (1:1 wt/wt), as a source of α-linolenic acid (ALA).Within a column by factor of analyses (treatment, broiler breeder age of interactions), LSmeans assigned different letter superscripts differs, $P < 0.05$.

Table 3. Effect of feeding sources of n-3 PUFA to broiler breeders and/or their offspring on concentration of plasma immunoglobulin A (**IgA**), intestinal lesion scores and immune organ weights in 15-day-old broiler chicks hatched from 34-, 44- and 54-wk old boiler breeders and challenged with *Eimeria* on d 10 of age.

Effects			Intestinal lesion scores		Organ Weights, mg/g BW			
Treatment breeder	Offspring		Duodenum	Jejunum	Liver	Bursa	Spleen	Plasma IgA, μg/mL
CON	CON		3.44	2.19^{b}	32.2	1.82	1.08	120.2
DMA[1]	CON		3.47	2.56^{ab}	31.2	1.91	1.00	121.5
FFF[2]	CON		3.50	2.58^{ab}	32.4	2.05	0.96	118.4
DMA	DMA		3.50	2.83^{a}	32.1	1.74	1.00	122.1
FFF	FFF		3.58	2.42^{ab}	32.4	1.98	1.04	121.1
SEM			0.088	0.126	6.04	0.096	0.046	2.09
Breeder age, week		34	3.28^{b}	2.45	33.4^{a}	1.98	1.06	129.8^{a}
		44	3.28^{b}	2.45	29.8^{b}	1.86	0.94	115.3^{b}
		54	3.93^{a}	2.65	32.9^{a}	1.86	1.04	116.9^{b}
		SEM	0.059	0.097	4.68	0.075	0.33	1.62
Probabilities								
Treatment			0.818	0.006	0.707	0.102	0.339	0.738
Age			<0.001	0.296	0.001	0.388	0.056	<0.001
Treatment x Age			0.979	0.353	0.320	0.674	0.527	0.983

[1]Microalgae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid (**DHA**)
[2]Co - extruded full-fat flaxseed and pulse mixture (1:1 wt/wt), as a source of α-linolenic acid (**ALA**)Within a column by factor of analyses (treatment, broiler breeder age of interactions), LSmeans assigned different letter superscripts differs, $P < 0.05$.

Table 4. Effect of feeding sources of n-3 PUFA to broiler breeders and/or their offspring on breast weight and tibia ash content in 42-day-old broilers hatched from 34-, 44- and 54-wk old boiler breeders and challenged with *Eimeria* on d 10 of age.

Effects				
Treatment breeder	Offspring		Breast weight, g/kg BW	Tibia ash, % DM
CON	CON		226.2^{ab}	39.97
DMA[1]	CON		226.7^{ab}	38.83
FFF[2]	CON		209.0^{b}	38.85
DMA	DMA		207.6^{b}	38.91
FFF	FFF		238.5^{a}	39.01
SEM			4.57	0.32
Breeder age, week		34	200.7^{c}	38.16
		44	217.6^{b}	39.09
		54	246.5^{a}	40.08
SEM			3.53	0.269
Interactions				
Breeder	Offspring	Age		
CON	CON	34	203.4	38.86^{bc}
DMA	CON	34	207.5	37.40^{c}
FFF	CON	34	178.7	38.42^{bc}
DMA	DMA	34	195.7	37.06^{c}
FFF	FFF	34	218.1	39.09^{bc}
CON	CON	44	213.0	38.10^{bc}
DMA	CON	44	218.4	39.28^{bc}
FFF	CON	44	205.8	38.76^{bc}
DMA	DMA	44	204.4	40.30^{ab}
FFF	FFF	44	246.4	39.02^{bc}
CON	CON	54	262.2	42.94^{a}
DMA	CON	54	254.0	39.82^{abc}
FFF	CON	54	242.5	39.38^{abc}
DMA	DMA	54	222.7	39.37^{abc}
FFF	FFF	54	251.0	38.91^{bc}
SEM			7.89	0.604
Probabilities				
Treatment			<0.001	0.127
Age			<0.001	<0.001
Treatment x Age			0.374	<0.001

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

[2]Co - extruded full-fat flaxseed and pulse mixture (1:1 wt/wt), as a source of α-linolenic acid (**ALA**).Within a column by factor of analyses (diet, broiler breeder age of interactions), LSmeans assigned different letter superscripts differs, $P < 0.05$.

heavier breast than FFF-CON and DMA-DMA birds but similar to CON-CON or DMA-CON birds. The breast weight increased with BB age with breast weight birds from BB at 34, 44 and 54 woa being, 200.7, 217.6, and 246.5 g/kg BW respectively. There was an ($P <$ 0.001) interaction between treatment and BB age on tibia ash content. In this context, tibia ash content of CON-CON birds was greater than for FFF-FFF birds for BB at 54 woa but not in younger BB.

DISCUSSION

The goal of BB production is to produce as many quality eggs as possible to maximise hatchability and ensure that the chicks can tolerate field placement challenges and achieve performance targets (Zuidhof et al., 2017). The impact of BB nutrition on egg production, egg size and shell quality, mortality, fertility, and hatchability has been well documented (Leeson and Summers, 2005; Ipek and Sozcu, 2015; Chang et al., 2016). Many of these studies focused on manipulation of breeder macro nutrients (energy, protein/amino acids, fat, minerals) (Spratt and Leeson, 1987; Enting et al., 2007; Ciacciariello and Tyler, 2013; Moraes et al., 2014; van Emous et al., 2015; Moraes et al., 2019; Bakhshalinejad et al., 2024). However, awareness on the importance of maternal diet on offspring lifetime performance, welfare and health is increasing (Thanabalan and Kiarie, 2021; Whittle et al., 2024b,c). In the current study, microalgae (**DMA**) was used as a DHA source and co-extruded full-fat flaxseed (**FFF**) as an ALA source and extended previous studies that demonstrated that these feedstuffs enriched egg yolks with n-3 PUFA (Neijat et al., 2016; Akbari Moghaddam Kakhki et al., 2020a; Thanabalan et al., 2020; Maina et al., 2023). As expected, BB fed FFF increased deposition of ALA with lesser extent of DHA conversion, whereas feeding DMA resulted in greater deposition of DHA (Neijat et al., 2016; Akbari Moghaddam Kakhki et al., 2020a). However, an interaction between diet and BB age on the deposition of n-3 PUFA was observed. In general enrichment of eggs with n-3 PUFA is a gradual process and typically plateaus by 4 wk of postfeeding (Neijat et al., 2016; Moran et al., 2019). The BB in the current study started receiving the diets at 23 woa, as such the enrichment was expected to have plateaued by the time of first sampling (34 woa). Therefore, it is rather difficult to pinpoint the basis of the observed patterns of age-dependent- deposition of n-3 PUFA.

The hatching egg or chick quality is affected by many factors, however, BB physiology has a huge influence, and comparative data from chicks of younger and older BB shows differences in hatch weight, growth, intestinal development, resistance to enteric diseases and immunocompetence (Hudson et al., 2004; Gous, 2010; Mahmoud and Edens, 2012; Ipek and Sozcu, 2015). In agreement with the current study, old BB flocks produce a greater number of heavier chicks linked to heavier eggs (Suarez et al., 1997; Sklan et al., 2003; Ulmer-Franco et al., 2010; Maina et al., 2022). As such the possibility of bolstering post-hatch growth performance of broiler chickens from younger BB through breeder or post-hatch nutrition is an important frontier to the poultry industry (Maina et al., 2022). Successive provision of DHA and ALA improved FCR relative to control in the starter. Previous research indicated that provision of n-3 PUFA to BB improved offspring performance in the early post-hatch periods providing evidence that the developing embryo benefited from the availability of n-3 PUFA during early perinatal period (Koppenol et al., 2015a,b). The same studies showed that independent of provision on n3-PUFA to BB, post-hatch feeding of n-3 PUFA had much more strong improvement on growth performance. Collectively suggesting that successive n-3 PUFA supplementation to BB and their progeny was more efficient and beneficial.

The interaction between treatment and BB age on BWG revealed differential responses linked to sources of n-3 PUFA in the grower/finisher phase. Specifically, exposing DHA to younger (34 wk old BB) or their

progeny resulted in poor growth performance in broiler chickens relative to counterparts from older (≥ at 44 wk old BB). In contrast, the birds exposed to ALA had similar growth performance irrespective of BB age. Moreover, successive exposure to n3-PUFA showed ALA had superior growth performance relative to DHA in the grower/finisher phase. Although we cannot rule out possible implications of the *Eimeria* challenge in the grower/finisher phase, these observations were somewhat intriguing and difficult to explain. However, there were peculiar differences between BB only and successive exposure to n-3 PUFA. In starter phase, DMA-CON birds ate more feed than DMA-DMA and FFF-FFF birds whereas FFF-CON birds had better FCR than CON-CON birds but commensurate to birds subjected to successive exposure of DHA or ALA. In grower/finisher phase, FFF-CON birds had higher final BW than DMA-CON birds and had higher BWG than DMA-DMA birds. Whereas DMA-CON birds had poor FCR relative to FFF-FFF birds. Although we did not measure blood concentration or tissue retention of n-3 PUFA in chicks, such phenomenon may relate to differences between half-life of ALA and DHA. For example, rodent model studies demonstrated that plasma DHA turnover was faster and the half-life shorter relative to ALA (Metherel et al., 2016; Metherel et al., 2018).

Coccidiosis pathogenesis entails *Eimeria* invasion of the intestinal cells as part of the life cycle. The resulting intestinal damage impair nutrients digestion and absorption, gut barrier function and ultimately leads to bacterial infections particularly necrotic enteritis caused by C*lostridium perfringens* (Chapman, 2014; Kiarie et al., 2019). Collectively both coccidiosis and necrotic enteritis results in annual losses of more than $10 billion to the global poultry industry (Blake et al., 2020). Discovery of the protective effects of n-3 PUFA against protozoa infections were originally demonstrated in rodent models (Godfrey, 1957, 1958). Later research in poultry demonstrated significant reduction in intestinal lesion scores and mucosa parasitic activity in broiler chickens fed n-3 PUFA rich feedstuffs such as fish oil and flaxseed (Allen et al., 1997; Korver et al., 1997; Adhikari et al., 2020). The antiparasitic activity of n-3 PUFA is associated with lethal oxidative stress emanating from autooxidation of the highly unsaturated FA once incorporated into the parasite cell membranes (Adhikari et al., 2020). As such a typical response to dietary n-3 PUFA in broiler chickens exposed to *Eimeria* is the reduction in the severity of intestinal lesions linked to significant inhibition of parasite development in the mucosa (Allen et al., 1997; Korver et al., 1997; Adhikari et al., 2020). Although there were no treatment differences in duodenal lesion scores; jejunal lesion scores were somewhat higher in DMA-DMA broilers. The interaction between dietary n-3 PUFA and coccidiosis infection model often gives conflicting results in response to different *Eimeria* species. Feeding broiler chickens diets containing fish oil (rich in DHA) ameliorated growth-depressing effects of an *E. tenella* infection but had no influence on coccidiosis indications of inflammation at the intestinal mucosa level (Korver et al., 1997). Feeding broiler chickens diets containing 0.175% DHA rich microalgae had no impact on growth performance and intestinal lesion scores in responses to coccidiosis vaccine (with 10X Coccivac-B52 vaccine containing mixture of E. *acervulina, E. Maxima, E. mivati* and *E. tenella*) (Fries-Craft et al., 2021). Although dietary n-3 PUFA (ALA, EPA, and DHA) consistently reduced *E. tenella* replication and associated cecal lesions, it showed inconsistent impact on small intestinal lesions induced by *E. maxima* (Allen et al., 1997; Adhikari et al., 2020). Other studies indicated n-3 PUFA exacerbated intestinal lesions at high *E. maxima* doses (for example, Allen et al., 1997). Factors that have been associated to the differences in intestinal lesions in response to interaction between n-3 PUFA and *Eimeria* species included parasite invasion site (ceca vs. the middle portion of the small intestine), immune reactions of host and parasite metabolism (Allen et al., 1997; Korver et al., 1997).

We anticipated that the responses to provision of n-3 PUFA will be extended to systemic responses in the peripheral blood and lymphoid organs. Previous reports indicated chicks hatched from BB fed n-3 PUFA enriched diet incorporated higher levels of n-3 PUFA in the immune tissue and influenced humoral and cell-mediated immune responses (Wang et al., 2000; Wang et al., 2002). The thymus, spleen, and bursa of fabricus are the major lymphoid organs in poultry. Increase in immune tissue mass during an infection may be linked to immune status (Chen et al., 2018). As n-3 PUFA plays a role on immune modulation and membrane biogenesis (Wang et al., 2000); we anticipated impact of maternal and/or progeny feeding on n-3 PUFA on lymphoid organ mass (Smith and Hunt, 2004). In general, the impact of n3-PUFA on lymphoid organs in poultry is somewhat conflicting. For example, pullets fed 5% linseed oil or fish oil had heavier bursa, thymus, and spleen at 4 wk of age compared to pullets fed 5% animal fat (Wang et al., 2000). Supplementation of DMA and FFF did not have effects on thymus, spleen and bursa weight during the rearing phase of pullets (Thanabalan and Kiarie, 2022). However, pullets fed animal fat exhibited heavier bursa than those fed n-3 FA at 8 wk of age (Wang et al., 2000). Supplementation of broiler diets with 3 to 5% fish oil had no effect on spleen weight, increased thymus weight and decreased bursa weight (Al-Khalifa et al., 2012). Feeding 1 to 5% FFF to pullets from hatch through to 16 woa reduced thymus and bursa weight when birds were younger (<8 woa) but improved bursa weight after 12 woa (Lee et al., 2023). It appears that the response of lymphoid organs to dietary n-3 PUFA depended on dose, type (ALA, EPA, DHA) and bird age. Further investigations are warranted to elucidate impact of n-3 PUFA on the lymphoid organ functionality. Increased immunoglobulins in hatching eggs could benefit young chicks by reinforcing immune defense (Ulmer-Franco et al., 2012; Lu et al., 2019). Contrary to the current study, previous research showed that feeding BB sources of n-3 PUFA increased transfer of passive maternal antibodies (Wang et al., 2002; Wang

et al., 2004), B cell proliferation and maturation (Koppenol et al., 2015a,b), reduced proinflammatory metabolites (Hall et al., 2007; Cherian et al., 2009) and embryonic mortality (Saber and Kutlu, 2020). However, it is noteworthy that most of these studies fed purified/extracted n-3 PUFA oils whereas the current study used feedstuffs rich in n-3 PUFA that also contained other components that could have elicited confounding or blunting immunomodulatory effects. For example, microalgae are a rich source of n3-PUFA, carotenoids, B vitamins, and functional non-starch polysaccharides (Świątkiewicz et al., 2015). Individually, these compounds have been documented to exert immunomodulation effects (Eggersdorfer and Wyss, 2018; Fries-Craft et al., 2021). On the other hand, flaxseed is rich in ALA but the fiber fraction is highly soluble and fermentable and has been documented to alter microbial activity releasing metabolic fuels, biosynthetic precursors and metabolic signaling molecules (Kiarie et al., 2007; Ndou et al., 2017; Leung et al., 2018; Ndou et al., 2018).

Breast is a high value meat product, therefore, the possibility of improving yield and enriching with n-3 PUFA through breeder and offspring nutrition is an attractive proposition. Previous research reported no difference or decrease in breast yield in broiler chickens fed n-3 PUFA. For example, feeding 10 or 17% flaxseed to broiler chickens resulted in significant decrease in breast yield compared to control-fed birds (Zuidhof et al., 2009). Whereas Bharath et al. (2016) reported no effects of feeding broiler chickens 3.28% linseed or fish oils on breast yield. It was also noteworthy that successive ALA exposure improved breast yields relative to successive exposure to DHA or feeding ALA to BB only. These discrepancies on feeding ALA and DHA on breast yield in broiler chickens are difficulty to explain given DHA (the dominant n3-PUFA in DMA) is more potent biologically. However, there are many proposed mechanisms through which n-3 PUFA may influence protein synthesis and muscle accretion. For example, enhanced protein synthesis and myogenesis through enhanced fluidity of the cell membranes and attendant increase in amino acids uptake (Rossato et al., 2020). Prenatal and early life exposure to fish oil in swine increased 5′ adenosine monophosphate-activated protein kinase activity and glucose transporters in the intestinal tissue, improving growth performance (Gabler et al., 2007; Gabler et al., 2009). Bone mineralization is a concern in broilers due to rapid and disproportionate muscle accretion relative to the maturation of skeletal system (Fleming, 2008). Enriching hatching eggs with n -3 PUFA and subsequently increasing availability to the developing embryo could influence skeletal development post-hatch (Mennitti et al., 2015; Akbari Moghaddam Kakhki et al., 2020b; Thanabalan and Kiarie, 2021; Thanabalan et al., 2022). Mechanisms associated with beneficial effects of n3-PUFA on bone quality included stimulation of intestinal calcium absorption, modulation of bone marrow cells and attenuation of osteoclasts activity (Thanabalan et al., 2022). Successive feeding of sources of n-3 PUFA in the current study did not result in marked differences in tibia ash content in 42-day-old broiler chickens.

Research on feeding broiler breeders functional feedstuffs to impact offspring might be the next frontier for enhancing resilience and immunocompetence in broiler chickens. Just like any study, our experiment had some logistical limitations related to feed spillage, particularly in the starter phase. Moreover, because of spacing limitations in the research facility and adequacy of chicks as broiler breeders aged, we did not include unchallenged group, as such it is not possible to delineate the responses of *Eimeria* and treatments. However, the study provided valuable insights for integrating broiler breeder nutrition to lifetime performance of broiler chickens. Successive exposure of DHA and ALA to breeders and their progeny improved FCR in starter phase relative to birds not exposed to these fatty acids anytime in their lifetime. Moreover, successive exposure to DHA tended to improve starter phase growth. Suggesting that successive feeding of DHA to BB and progeny was more beneficial compared to feeding BB only. However, in grower/finisher phase were dependent on ALA supported better growth and breast yield. However, these effects were not linked to modulation of immunocompetence as indicated by intestinal lesion scores, lymphoid organs weight and plasma IgA concentration.

ACKNOWLEDGMENTS

The research was funded by Ontario Agri-Food Innovation Alliance, Natural Sciences and Engineering Research Council of Canada-CRD Program, Egg Farmers of Canada, Egg Farmers of Ontario, Alltech Canada, and O & T Farms Ltd.

DISCLOSURES

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. R. Dreger is an employee of O & T Farms Ltd.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2024.103796.

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