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ORIGINAL ARTICLE

In ovo exposure to omega-3 fatty acids does not enhance omega-3 long-chain polyunsaturated fatty acid metabolism in broiler chickens

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The content of omega-3 long-chain polyunsaturated fatty acids ($n-3$ LCPUFA) in chicken meat can be boosted by feeding broilers a diet containing α-linolenic acid (ALA, from flaxseed oil), some of which is converted by hepatic enzymes to $n-3$ LCPUFA. However, most of the accumulated $n-3$ polyunsaturated fatty acid (PUFA) in meat tissues is still in the form of ALA. Despite this, the levels of chicken diets are being enhanced by the inclusion of vegetable and marine sources of omega-3 fats. This study investigated whether the capacity of chicken for $n-3$ LCPUFA accumulation could be enhanced or inhibited by exposure to an increased supply of ALA or $n-3$ LCPUFA *in ovo*. Breeder hens were fed either flaxseed oil (High-ALA), fish oil (high $n-3$ LCPUFA) or tallow- (low $n-3$ PUFA, Control) based diets. The newly hatched chicks in each group were fed either the High-ALA or the Control diets until harvest at 42 days' post-hatch. The $n-3$ PUFA content of egg yolk and day-old chick meat closely matched the $n-3$ PUFA composition of the maternal diet. In contrast, the $n-3$ PUFA composition of breast and leg meat tissues of the 42-day-old offspring closely matched the diet fed post-hatch, with no significant effect of maternal diet. Indeed, there was an inhibition of $n-3$ LCPUFA accumulation in meat of the broilers from the maternal Fish-Oil diet group when fed the post-hatch High-ALA diet. Therefore, this approach is not valid to elevate *n*-3 LCPUFA in chicken meat.

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Key words: chicks, fish oil, flaxseed oil, maternal diet, meat, n – 3

Introduction

The omega-3 long-chain polyunsaturated fatty acids ($n-3$ LCPUFA) have a number of important health benefits in humans, in particular in relation to inflammatory conditions such as rheumatoid arthritis and protection against cardiovascular diseases.[1,2] These effects rely on the incorporation of $n-3$ LCPUFA into the phospholipid fraction of the cell membrane, and subsequent release in the free fatty acid pool to give rise to bio-active mediators.[3] This has led to recommendations from a number of health agencies for humans to increase their consumption of fish and seafood, the richest dietary sources of these fatty acids. Despite this, consumption of these sources remains low in western countries,[1,4] and fish and seafood are also not environmentally sustainable sources of these fats.[5] In contrast to seafood, the global consumption of poultry, especially chicken, is steadily increasing and this is now the most popular type of meat in many societies.[6] This has led to suggestion that one strategy to increase dietary $n-3$ LCPUFA intake in western countries, and one which would avoid placing additional pressure on global marine resources, is to increase the $n-3$ LCPUFA content of chicken meat.[7,8]

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Chicken meat is naturally low in fat (~2.0%), and a poor source of $n-3$ polyunsaturated fatty acids (PUFA) at only ~2.5% of total fatty acids including $n-3$ LCPUFA at ~1.3%.[9] However, we and other researchers have demonstrated that increasing the amount of $n-3$ PUFA in the diet, by feeding chickens diets supplemented with flaxseed (*Linum usitatissimum*) oil [high in the short-chain $n-3$ PUFA, α-linolenic acid (ALA)], results in substantial increases in the ALA content of the meat, without increasing the overall fat content.[10] Importantly, chickens also possess the hepatic enzymes required to synthesize the $n-3$ LCPUFA; eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), from ALA.[7,11] Consequently, replacing standard fat sources in formulated chicken diets such as corn oil, canola oil, soybean oil (all rich in $n-6$ PUFA), animal fat or their blends, with flaxseed oil reduces the dietary $n-6$: $n-3$ PUFA ratio and increases the $n-3$ LCPUFA content of chicken tissues[9,10,12–14], as $n-6$ and $n-3$ PUFA precursors compete for the same hepatic enzymes in their elongation and desaturation pathways.[10,15] Despite the success of High-ALA diets for increasing the level of $n-3$ LCPUFA in chicken meat, the actual levels of $n-3$ LCPUFA which are achieved (76.5–108 mg/100 g meat)[9] are still substantially lower than those in oily fish (e.g. herring, salmon and mackerel), which contains >1500 mg in the similar amount of meat.[16]

Improving the efficiency through which High-ALA diets improve chicken meat $n-3$ LCPUFA content relies on altering

the processes which regulate ALA conversion to $n-3$ LCPUFA and/or deposition of fatty acids into different lipid fractions.[17,18] The egg yolk is the main reserve of energy and the sole source of essential fatty acids during embryogenesis,[19] and previous studies have demonstrated that feeding layer hens a diet enriched in ALA increases the $n-3$ LCPUFA content of the eggs[20–23] and of the chicks at hatch.[22] In addition, feeding hens a diet enriched with fish oil results in higher $n-3$ PUFA levels in cardiac tissues in their chicks.[24] However, no previous studies have determined the effect of *in ovo* exposure to an increased supply of $n-3$ PUFA on the chicken's subsequent capacity for ALA-derived $n-3$ LCPUFA accumulation in meat tissues. Therefore, the aim of the present study was to determine the effect of maternal dietary treatments that would expose developing chicks *in ovo* to an elevated level of $n-3$ LCPUFA, or its precursor (ALA) on the capacity of chickens to accumulate $n-3$ LCPUFA when fed a High-ALA diet post-hatch.

Methods

Maternal dietary treatments

A total of 324 of broiler breeder hens of the Cobb 500 strain were housed in the HiChick Breeding Company facility (Bethyl, SA, Australia). Before the study all the hens received the same commercial breeder diet. Hens were allocated to one of three dietary groups ($n = 108$/group): Control (basal diet mixed with 4% w/w beef tallow), High-ALA (basal diet mixed with 4% w/w flaxseed oil) or Fish-Oil (basal diet mixed with 4% w/w fish-oil) (Fig. 1.). The breeder basal diet used for all feeds was purchased from Lauke Mills, Australia. The three diets contained the same proportions of fat, carbohydrate and protein, and differed only in their fatty acid composition (Table 1). All three experimental diets contained the same levels of vitamins and minerals and these either met or exceeded the recommended levels.[25] Each group of hens was housed separately with eight roosters from the same strain, and fed the diets for the duration of the experiment (Fig. 1).

Egg sampling

All eggs that were laid in the 5th week of the maternal diet regimen (Control, $n = 132$ eggs; High-ALA, $n = 148$ eggs; Fish-Oil, $n = 80$ eggs) were collected and stored at holding temperature to temporarily prevent initiation of embryonic development. The yolk from five eggs selected at random from each maternal diet group were collected and stored at −18°C for subsequent fatty acid analysis. The remaining eggs were transferred to the South Australian Research and Development Institute (Roseworthy, SA, Australia) and immediately placed in incubators under standardized conditions (38°C and 55% humidity, increasing to 60% in the last 4 days of incubation). After 1 week of incubation, the fertility of each egg was assessed, and non-fertile eggs and eggs with dead embryos were discarded.

Chick hatch and sampling

Chicks were hatched at ~21 days after the start of the incubation, and at 1-day old were feather-sexed into pullets and cockerels[26]

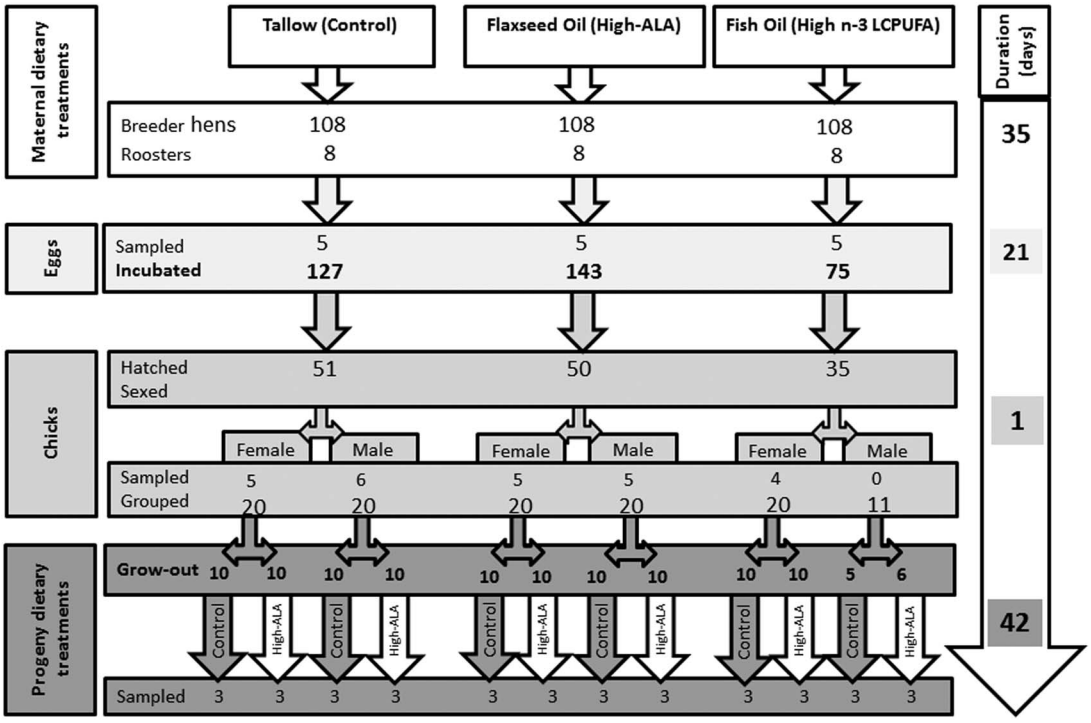

Fig. 1. The methodology and numbers of birds used during different experimental phases.

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Table 1. *Fatty acid composition of the experimental diets*[a]

	Maternal diets[b]			Progeny diets[c]	
Fatty acid group	Control	Flaxseed oil	Fish oil	Control	Flaxseed oil
Crude fat %[d]	7.5	7.7	7.8	6.3	6.6
Total SFA[e]	33.0	15.3	25.4	36.7	19.3
Total *trans*	0.6	0.1	0.3	1.7	0.3
Total *n* − 9 MUFA[f]	37.1	25.9	27.1	30.5	18.2
Total *n* − 7 MUFA[g]	4.2	2.0	7.4	3.7	1.6
Total *n* − 6 PUFA[h]	19.8	25.2	18.9	24.4	30.5
Total *n* − 3 PUFA[i]	3.4	30.6	19.4	2.7	29.9
ALA[j]	3.2	30.6	3.3	2.4	29.8
Total *n* − 3 LCPUFA[k]	0.2	0.1	16.1	0.2	0.0
n − 6:*n* − 3	5.8	0.8	1.0	9.0	1.0

[a]Values are fatty acid group percentage of the total fatty acid.
[b]By mixing basal breeder hens diet with (4% w/w) beef tallow (Control), flaxseed oil-based diet or fish oil ($n = 3$).
[c]By mixing finisher basal diet with (4% w/w) beef tallow (Control), flaxseed oil ($n = 6$).
[d]Percentages are based on the wet weight.
[e]SFA, Saturated fatty acid.
[f]*n* -9 MUFA, Omega 9 monounsaturated fatty acid.
[g]*n* -7 MUFA, Omega 7 monounsaturated fatty acid.
[h]*n* -6 PUFA, Omega 6 polyunsaturated fatty acid.
[i]*n* -3 PUFA, Omega 3 polyunsaturated fatty acid.
[j]ALA, α-linolenic acid ALA.
[k]*n* -3 LCPUFA, Omega 3 long chain polyunsaturated fatty acid.

(Control: $n = 26$ females, $n = 25$ males; High-ALA: $n = 25$ females, $n = 25$ males; Fish-Oil: $n = 24$ females, $n = 11$ males). In total, 20 male and 20 female chicks in each maternal dietary group were then allocated to two separate raised floor pens (1.2 × 0.9 m each; $n = 10$ chicks/pen), except for the Fish-Oil group males where the pens held six and five chicks due to the smaller number of hatchlings (Fig. 1).

The unallocated 1-day old chicks (Control: six females and five males; High-ALA: five of each sex; and Fish-Oil: four females only) were euthanized by cervical dislocation and ~1–2 g of breast and leg meat were collected in plastic vials and immediately placed on dry ice. Samples then were transferred to the laboratory where they stored at −18°C until subsequent determination of crude fat content and fatty acid composition.

Housing of broilers

A complete factorial randomized block design (3 × 2 × 2) was implemented such that one pen of male and one pen of female chicks in each of the three maternal dietary treatment were fed either the control (4% w/w beef tallow) or the High-ALA (4% w/w flaxseed oil) progeny diets for the entire 6 weeks of grow-out. The two experimental progeny diets were nutritionally identical, met requirements for healthy growth and all vitamins and minerals met or exceeded the recommended levels.[25] Broilers were reared under controlled environmental conditions with free access to feed from hoppers and water from a nipple drinker line. The room temperature was 27°C for the first 4 days then gradually decreased to 20°C and maintained until harvest, with pens heated by infrared lamps (175 W) during the first 3 weeks. Feed intake and final body weights (BW) of broilers were recorded and feed conversion rate (FCR) during the final week before slaughter was calculated. The numbers of birds that were culled or died was recorded on daily basis.

Tissue sampling

On day 42 of grow-out, three birds from each pen ($n = 36$) were randomly selected and euthanized by cervical dislocation. Breast and leg meat tissues were sampled, frozen on dry ice and stored at −18°C for subsequent fatty acid profiling.

Fatty acid analysis

Crude lipid was extracted from a representative sample of homogenized feed, egg yolk and lean meat samples.[27] The gravimetric approach was utilized to estimate total crude lipid (% of wet weight). Fatty acid profiling was performed after transmethylation of the extracted crude lipids with 1% H_2SO_4 in methanol at 70°C for 3 hours. Briefly, after cooling to room temperature, the resulting fatty acid methyl esters (FAME) were extracted with *n*-heptane (2 ml) and transferred into gas chromatography (GC) vials containing about 30 mg of anhydrous sodium sulfate and stored at −18°C until GC

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analysis. The FAMEs were separated using a Hewlett-Packard 6890 GC (Hewlett-Packard, CA, USA) equipped with a flame ionization detector, a split injector and a BPX-70 capillary column (50 m × 0.32 mm internal diameter) with a 0.25 µm film thickness (SGE, Victoria, Australia). The operating conditions of the GC, fatty acid identification using the GLC 463 external standard (Nu-Chek Prep Inc., MN, USA) and qualitative analysis were as described previously.[28]

Statistical analyses

The effects of dietary treatment on the fatty acid profile were tested by one-way, two-way and three-way analysis of variance (ANOVA) for egg, chick and broiler tissues, respectively, using SPSS version 21 for Windows (IBM Corp., NY, USA). Duncan's multiple comparison test was implemented where the ANOVA showed significant differences between groups ($P < 0.05$). Due to the uneven number of broilers in each pen, it was not possible to reliably assess the impact of the dietary treatments on growth performance.

Results

Fatty acid composition of the experimental diets

There was no difference in the crude fat percentages between the three breeder diets or the two progeny diets. The crude fat percentage of the progeny finisher diet (fed in the last 3 weeks of broilers grow-out) was lower than the breeder hens' diets by ~1.2%, due to the different nutritional requirements of birds, however, the fatty acid profiles of both the breeder and progeny diets similarly reflected the type of lipid added to the basal feed. Thus, the Control (beef tallow) diet comprised predominately of saturated fatty acid (SFA) and $n-9$ monounsaturated fatty acid (MUFA), the High-ALA diet comprised predominately of $n-3$ PUFA as ALA, whereas the Fish-Oil diet was the only one which contained $n-3$ LCPUFA (Table 1). The ratio of $n-6$:$n-3$ PUFA in the diets decreased from 5.8 in the maternal control diet to 0.8–1.0 in the flaxseed and fish oil diets (Table 1).

Productivity of breeder hens

The laying rate of the breeder hens (number of eggs/breeder) appeared to be lower in hens fed the Fish-Oil diet compared with those fed either the Control or High-ALA diets (Control, 1.22; High-ALA, 1.37; Fish-Oil, 0.74). The ratio of female:male chicks hatched, on the other hand, appeared to be higher in the Fish-Oil group compared with the other dietary treatments (Control, 0.96; High-ALA, 1.00; and Fish-Oil, 2.18). The hatchability of the eggs (number of chicks hatched/egg laid) was similar between groups (Control, 0.39; High-ALA, 0.33; Fish-Oil, 0.44).

Fatty acid composition of egg yolks

The crude fat content of egg yolks ranged from 32–38% of the yolk weight (data not shown), and did not differ between dietary treatments. The fatty acid composition of the yolk

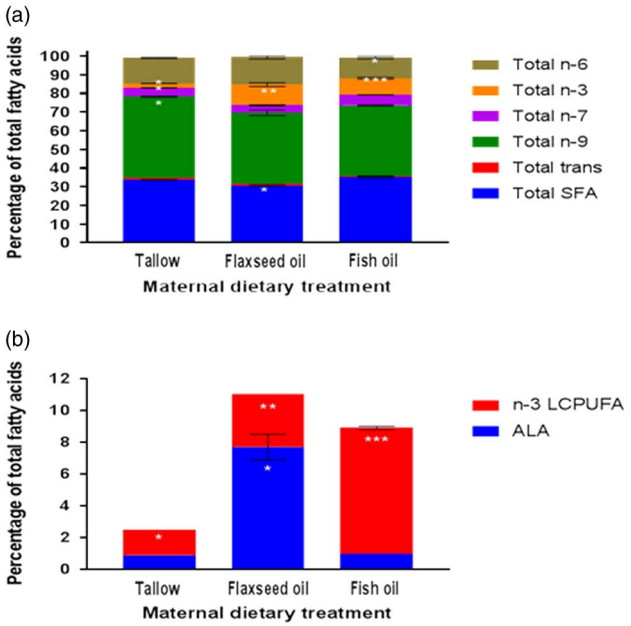

Fig. 2. *Fatty* acid profile (*a*) and omega-3 distribution (*b*) in egg yolk of breeder hens fed three different diets ($n = 5 \pm$ S.E.M.). Different stars within the same fatty acid indicate significant difference ($P < 0.01$ for $n-9$ and $n-6$) or ($P < 0.001$ for other fatty acids) between maternal treatments.

reflected that of the maternal diet (Fig. 2a). Thus, the ALA content of the yolk was higher in the High-ALA dietary group compared with the Control and Fish-Oil groups (Fig. 2b; $P < 0.0001$), whereas the $n-3$ LCPUFA level in the yolk was the highest in the Fish-Oil group compared with the other treatments (Fig. 2b, $P < 0.0001$). The yolk from hens in the Fish-Oil treatment group also contained less $n-6$ PUFA than both other groups ($P < 0.01$). The ratio of $n-6$:$n-3$ PUFA of the egg yolk was 4–5-fold lower in both the High-ALA (1.3) and Fish-Oil (1.2) groups compared with the Control (5.6) group ($P < 0.01$).

Fatty acid composition of day-old chicks

There were significant differences ($P < 0.0001$) in the levels of all major fatty acids in both the breast and leg tissues of the day-old chicks between dietary treatment groups, with the exception of the SFA content, which was similar between treatments. In the breast meat, the MUFA content was lower in the chicks in the High-ALA treatment group compared with both the Fish-Oil and Control treatments. The total $n-3$ PUFA content (ALA + $n-3$ LCPUFA) was highest (13.7%) in chicks in the Fish-Oil group, slightly lower (13.4%) in High-ALA and lowest (5.4%) in the Control group (Fig. 3a; $P < 0.0001$). A similar effect was observed for the $n-3$ LCPUFA content, which was 11.6, 9.0 and 4.9% in the Fish-Oil, High-ALA and Control groups, respectively. The $n-3$ LCPUFA made up a higher proportion of total $n-3$ PUFA in the Fish-Oil group compared with the High-ALA group (Fig. 3b; $P < 0.0001$). The ALA content of the chicks

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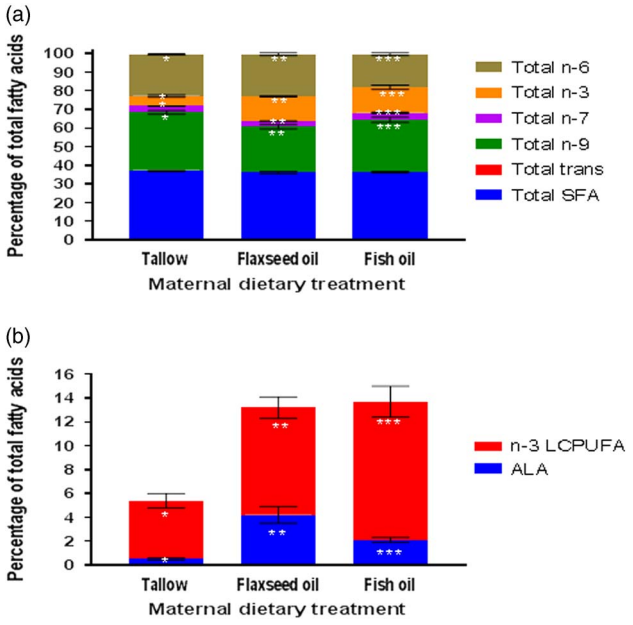

Fig. 3. Fatty acid profile (*a*) and omega-3 distribution (*b*) in breast meat of female day-old chicks from three different maternal dietary treatments ($n = 6.5$ and $4 \pm$ S.E.M. for control, flaxseed and fish oil treatments, respectively). Different stars within the same fatty acid indicate significant difference ($P < 0.0001$) between maternal treatments.

was also different between treatments, and was higher in the High-ALA (4.4%) and Fish-Oil (2.1%) groups compared with the Control group (0.5%) (Fig. 3b; $P < 0.0001$). The $n-6$ PUFA content was ~5% higher in the Control and High-ALA chicks compared with chicks in the Fish-Oil group (Fig. 3a; $P < 0.0001$). However, the $n-6$:$n-3$ PUFA ratio was reduced by 2.5-fold and 3.2-fold ($P < 0.0001$) in the High-ALA and Fish-Oil groups compared with the Control group. Similar effects were observed in the leg meat, and there were no differences between sexes (Control and High-ALA groups) or interactions between chick sex and maternal dietary treatment (males of Fish-Oil group excluded) in either tissue.

Growth of broilers

The final BW of the broilers at 42 days' post-hatch was 2.98 kg in females and 3.64 kg in males, and the FCR in the final week before tissue collection were 1.92 and 1.81 for females and males, respectively. Only two broilers were culled or died before tissue collection throughout the entire 6-week grow-out period.

Fatty acid composition of broilers

Overall, the fatty acid composition of the leg and breast meat tissues was broadly reflective of the diets that the offspring were fed post-hatch, independent of the diet to which they had been

Table 2. P values *at different levels of interaction of three experimental factors (maternal by progeny diets by gender) on 6-week-old broilers (n=3/group)*

		P-value of independent variable interaction (treatment)						
Fatty acid group	Tissue	G[a]	M[b]	P[c]	G × M	G × P	M × P	G × M × P
Total SFA[d]	Breast	<0.001	ns[k]	<0.001	ns	ns	ns	ns
	Leg	<0.001	ns	<0.001	ns	0.004	ns	ns
Total *trans*	Breast	0.001	ns	<0.001	ns	0.023	ns	ns
	Leg	0.004	ns	<0.001	ns	ns	ns	ns
Total *n* – 9 MUFA[e]	Breast	0.008	ns	<0.001	0.025	ns	ns	ns
	Leg	0.047	ns	<0.001	ns	ns	ns	ns
Total *n* – 7 MUFA[f]	Breast	0.033	ns	<0.001	ns	ns	ns	ns
	Leg	0.024	ns	<0.001	ns	ns	0.031	ns
Total *n* – 6 PUFA[g]	Breast	ns	ns	ns	0.022	ns	ns	ns
	Leg	ns	ns	ns	ns	ns	ns	ns
Total *n* – 3 PUFA[h]	Breast	ns	ns	<0.001	ns	0.004	0.015	ns
	Leg	0.002	ns	<0.001	ns	0.001	0.013	ns
ALA[i]	Breast	<0.001	0.028	<0.001	ns	0.000	0.036	ns
	Leg	<0.001	ns	<0.001	ns	0.001	ns	ns
Total *n* – 3 LCPUFA[j]	Breast	<0.001	ns	<0.001	ns	0.004	ns	ns
	Leg	ns	ns	<0.001	ns	0.028	ns	ns

[a]G, gender.
[b]M, maternal diets (birds received 3 different diets).
[c]P, progeny (birds received two different progeny diets).
[d]SFA, saturated fatty acid.
[e]n – 9 MUFA, omega-9 monounsaturated fatty acid.
[f]n – 7 MUFA, omega-7 monounsaturated fatty acid.
[g]n – 6 PUFA, omega-6 polyunsaturated fatty acid.
[h]n – 3 PUFA, omega-3 polyunsaturated fatty acid.
[i]ALA, α-linolenic acid.
[j]n – 3 LCPUFA = omega-3 long-chain polyunsaturated fatty acid.
[k]ns, not significant ($P > 0.05$).

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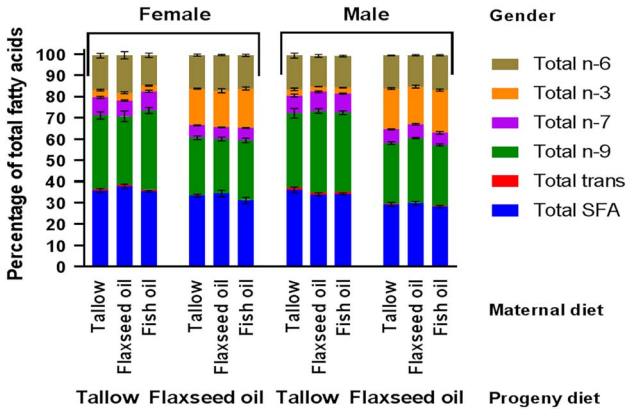

Fig. 4. Fatty acid profile in breast meat of 42-day-old broilers received three maternal by two progeny diets ($n = 3 \pm$ S.E.M.).

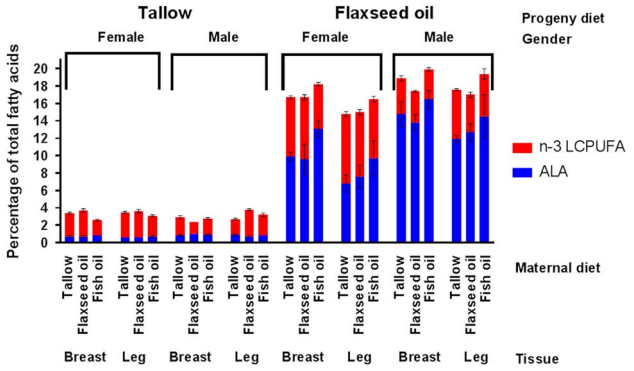

Fig. 5. Omega-3 distribution in meat tissues of 42–day-old broilers received three maternal by two progeny diets ($n = 3 \pm$ S.E.M.).

exposed *in ovo* (Table 2). Therefore, tissues from broilers fed on the High-ALA diet post-hatch had higher levels of total $n-3$ PUFA, ALA and $n-3$ LCPUFA, and lower levels of MUFA and SFA compared with those fed on the control diet, independent of the treatment the chicks had been exposed to *in ovo* (Figs 4 and 5). The percentage of SFA in the tissues ranged from ~28 to ~38% across the different treatments, and was higher in progeny fed the Control diet compared with those fed the High-ALA diet ($P < 0.0001$). Tissue levels of $n-9$ and $n-7$ MUFA were lower in broilers fed the High-ALA diet post-hatch compared with broilers fed a control diet, independent of *in ovo* diet exposure ($P < 0.0001$). The $n-6$ PUFA content of the meat was not different between broilers fed the Control and High-ALA diets. The $n-6$:$n-3$ ratio therefore reflected the variation in $n-3$ PUFA content, and was lower in broilers fed the High-ALA diet (0.8–1.4) compared with those fed the Control diet (4.7–6.4) (Table 2).

Broilers that were exposed to the Fish-Oil treatment *in ovo* had higher ALA levels in the breast meat compared with those exposed *in ovo* to the Control and High-ALA treatments, independent of the diet fed post-hatch, however, the magnitude of this difference was small (Table 2 and Fig. 5, $P < 0.05$). This was accompanied by a reduction in the EPA content of the meat from these broilers. There were no other differences in fatty acid composition of either the breast or leg meat between *in ovo* treatment groups (Table 2 and Fig. 5). There was, however, an interaction between *in ovo* dietary exposure and post-hatch diet on tissue $n-3$ PUFA concentrations (Table 2, $P < 0.05$), such progeny exposed *in ovo* to the Fish-Oil diet that were fed the High-ALA diet post-hatch had significantly higher levels of ALA in breast meat compared with other two maternal treatments fed this same diet (Fig. 5).

The fatty acid profiles of the leg meat tissue of progeny at 42 days of age were largely consistent with the breast meat with the exception that males had higher level of $n-3$ PUFA in their leg meat compared with females (Table 2, $P < 0.01$) and maternal High-ALA diet reduced ALA content only in breast meat (Table 2 and Fig. 5, $P < 0.05$).

Discussion

The reduced number of chicks in the Fish-Oil group appeared to be a result of reduced laying rate in the breeder hens fed the Fish-Oil diet, whereas the proportion of eggs that were laid that produced live chicks did not appear to be adversely affected. It is important to note, however, that the study was not powered to investigate differences in production characteristics between treatments and thus further studies are required to confirm if there are any adverse effects of the Fish-Oil diet on laying performance.

Although our study was not specifically designed to investigate differences in growth in the progeny, there are no suggestions of adverse effects of any of the diets on the productivity of the chicks. The broilers from all treatments grew at a normal rate, and BW, FCR and mortality in the progeny across the different treatments were within the normal standards for this strain[29] and in agreement with our previous findings.[9]

Our finding that feeding breeder hens a High-ALA diet increased not only the ALA content, but also the $n-3$ LCPUFA content of the eggs, supports the capacity of chickens for ALA conversion to $n-3$ LCPUFA, and confirms that ALA-supplementation of feed is an effective strategy for increasing the $n-3$ LCPUFA content of eggs.[20,22,30–32] This is likely to be due to the combined effects of the higher amount of ALA, and lower amount of $n-6$ PUFA in the feed, as the $n-3$ and $n-6$ PUFA compete with each other for both metabolic conversion and accumulation into tissues.[33]

Interestingly, although supplementing the breeder hens directly with $n-3$ LCPUFA, in the form of fish oil, led to the greatest increase in $n-3$ LCPUFA (five-fold of the control eggs), the $n-3$ LCPUFA content of the eggs (8%), was still only half of that of the feed (16.1%). This observation suggests there is a maximum level to which $n-3$ LCPUFA can be incorporated into egg yolk, which may be due to structural limitation of triglycerides and phospholipids. In contrast, the $n-3$ LCPUFA percentage was relatively increased in eggs of the other two treatments in comparison with the levels in the diets. This observation indicates the importance of optimizing the dietary content of $n-3$ LCPUFA in broiler feed, as the

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incorporation of these fatty acids into eggs appears to follow a curvilinear, rather than linear, pattern and reach a plateau at high $n-3$ LCPUFA intakes.

Previous studies have established that >80% of lipids deposited in the egg yolk are consumed by the developing embryo before hatch, and therefore represent a major source of nutrition for supporting chick growth and development.[34] In addition, ~50% of egg total fatty acids are incorporated into the newly hatched chick with embryonic preference to incorporate PUFA at the expense of MUFA.[35] Consistent with the findings of the current study, previous studies[22,32] have demonstrated that the relationship between fatty acid content of the egg yolk and post-hatch chick were closest for the essential fatty acids ($n-3$ and $n-6$ PUFA), compared with MUFA and SFA. Our finding that the $n-3$ and $n-6$ PUFA content in all groups were relatively higher in the meat of the newly hatched chicks than in the eggs provides evidence of continuous synthesis and accumulation of both of PUFA types in the muscle tissue *in ovo*. Hence, the ratio of $n-6$:$n-3$ PUFA in the meat tissues of chicks (especially in the high $n-3$ PUFA treatments) did not shift as much as the levels of the individual PUFA. This was important for the second stage of the experiment, since it ensured that the capacity of the chicks for converting ALA to the $n-3$ LCPUFA was unlikely to be limited by the presence of excessive amounts of $n-6$ PUFA in the tissues.

Interestingly, the contribution of $n-3$ LCPUFA to the total $n-3$ PUFA pool in day-old chicks exposed to the High-ALA diet *in ovo* was ~37% greater than in eggs from High-ALA hens, suggesting that these chicks had the capacity for ALA conversion to LCPUFA during embryogenesis. As with the eggs, however, the ability of the chicks to accumulate $n-3$ LCPUFA appeared to be limited at higher concentrations, as the $n-3$ LCPUFA content of meat tissues in chicks exposed to the Fish-Oil diet *in ovo* was about half that in the yolk. Lin *et al.*[35] found a linear relationship in $n-3$ and $n-6$ PUFA levels between eggs and chicks. However, these authors also suggested that the synthesizing of $n-3$ LCPUFA from ALA was suppressed at higher levels of dietary $n-3$ LCPUFA.

The growth of the 42-day-old broilers (data not shown) in the current study agreed with our previous study[9] and was not affected by dietary fat exposure either *in ovo* or post-hatch.[24,36] At 6 weeks of age, the fatty acid profile of broilers was found to be mainly affected by the post-hatch diet and sex, with minimal influences of dietary exposure *in ovo*. As the post-hatch control diet contained more SFA, *trans*, $n-9$ and $n-7$ MUFA, these fatty acids were predominant in broilers fed this diet. On the other hand, $n-3$ and $n-6$ PUFA were the predominant fatty acid groups in broilers fed High-ALA diet, consistent with our previous findings.[9] Although the High-ALA diet was relatively higher than the Control diet in $n-6$ PUFA content, this did not affect the $n-6$ PUFA level in broiler meat. This is probably related to the preferential utilization of $n-3$ PUFA substrates (ALA) by the enzymes involved in the metabolic conversion of shorter-chain fatty acids to their long-chain derivatives and preferential incorporation of $n-3$ PUFA into tissues.[10,15]

The major finding of this study was that exposure to either a high $n-3$ PUFA (ALA) or $n-3$ LCPUFA (Fish-Oil) diet *in ovo* had very little impact on the capacity of the progeny for converting ALA to the $n-3$ LCPUFA. Indeed, the only effect observed was an apparently inhibitory effect of *in ovo* exposure to maternal fish oil supplementation on ALA conversion to $n-3$ LCPUFA. One possibility is that *in ovo* exposure to a high dietary $n-3$ LCPUFA content may have acted to suppress the expression and/or activity of genes involved $n-3$ PUFA metabolism, specifically the desaturase and elongase enzymes required for the conversion of ALA to $n-3$ LCPUFA. This is supported by previous studies showing that DHA supplementation suppresses endogenous synthesis of $n-3$ LCPUFA from ALA in human subjects[37] and that feeding chickens a diet enriched in fish oil-based diet resulted in an increase in the percentage of both of ALA and linolenic acid in the chicken meat, suggesting reduced conversion.[38] Similarly, in chicken, Ajuyah *et al.*[24] reported no effect of a reserve of yolk $n-3$ LCPUFA from maternal Fish-Oil diet on the $n-3$ PUFA in the offspring cardiac tissue, but showed an adverse effect on the EPA percentage. In contrast, *in ovo* exposure to the High-ALA diet had no effect on the subsequent capacity of the chickens for ALA conversion. Haug *et al.*[39] reported that the concentration of dietary ALA does not affect the gene expression of the elongation and desaturation enzymes in adult chickens, and the results of the current study suggest that this may also be the case during the embryonic stage. Thus, exposing embryos to either High-ALA or High-$n-3$ LCPUFA environments do not enhance their subsequent capacity for depositing more $n-3$ LCPUFA after hatch.

Although there were relatively few differences between the male and female chickens in their response to the diet, we did identify that male broilers fed the High-ALA diet post-hatch accumulated more total $n-3$ PUFA in the leg meat, independent of their *in ovo* exposure. In addition, despite no differences in fatty acid profile of male and female chicks at 1-day post-hatch (Control and High-ALA groups), there were sex differences in the levels of all fatty acids (except $n-6$ PUFA) in the 42-day-old broilers. Thus, tissues from male chickens contained relatively more $n-3$ PUFA and lower $n-3$ LCPUFA, which indicates their lower ALA conversion efficiency, a finding is consistent with our previous study.[14]

In summary, we have shown that exposing broiler chickens to elevated levels of ALA or $n-3$ LCPUFA *in ovo* was effective in increasing $n-3$ LCPUFA deposition into their meat tissues during embryonic development. However, neither of these strategies were effective at increasing the subsequent capacity of the chickens for accumulating $n-3$ LCPUFA in their meat tissues when fed a High-ALA diet post-hatch. In fact, increased *in ovo* $n-3$ LCPUFA exposure appeared to be associated with an impaired capability of the broilers to convert ALA to $n-3$ LPCUFA.

Conclusion

In ovo $n-3$ LCPUFA exposure appeared to be associated with an impaired capability of the broilers to convert ALA to $n-3$

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LPCUFA. Manipulation of dietary fatty acids can affect yolk composition, but on its own it is not an appropriate strategy for enhancing n-3 LCPUFA content in the offspring at market age.

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Conflicts of Interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of animals. This study was approved by the Animal Ethics Committee of the University of Adelaide (approval S-2013-152) and the Department of Primary Industries and Regions South Australia, Australia (approval 15/13).

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