John F. Carragher, Beverly S. Mühlhäusler, Mark S. Geier, James D. House, Robert J. Hughes and Robert A. Gibson

**Effect of dietary ALA on growth rate, feed conversion ratio, mortality rate and breast meat omega-3 LCPUFA content in broiler chickens**


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Effect of dietary ALA on growth rate, feed conversion ratio, mortality rate and breast meat omega-3 LCPUFA content in broiler chickens

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Short title: Omega-3 fats and chicken production

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Summary text for the Table of Contents

In a previous study we significantly increased the long chain omega-3 content of chicken meat by feeding a diet containing short chain omega-3 from flaxseed oil. The present study, using almost 4,000 broiler birds housed under near-commercial conditions, demonstrated the same flaxseed oil diet improved growth rate and feed conversion efficiency from hatch to 6 weeks of age without negative effects on health or mortality. This supports the commercial viability of short-chain omega-3 diets for the chicken industry.
Abstract.

We have previously demonstrated that feeding chickens a diet containing high levels of the n-3 PUFA α-linolenic acid (ALA) significantly increases the content of the principal omega-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in their meat and eggs. However, the effect of the diet on production characteristics of the birds has not been assessed. This study aimed to determine the effect of feeding male and female Cobb 500 broilers (n=3,840) a high ALA diet (containing 2.5% flaxseed oil) compared to a standard commercial control diet (containing 2.5% tallow) on growth, feed conversion ratio (FCR) and mortality until 6 weeks of age. As expected the dietary flaxseed oil significantly increased breast meat levels of omega-3 PUFAs (approximately 4-fold), with most EPA and DHA being deposited in the phospholipid fraction. Both male and female birds fed the high ALA diet were significantly heavier at 6 weeks of age (77g heavier in females, 87g heavier in males). They also had a significantly (10%) lower FCR, and a mortality rate that was not different from the control diet across the 6 week feeding period. These findings indicate that a high ALA diet has the potential to enrich chicken breast meat with EPA and DHA without loss of growth rate or feed efficiency, or increase in fat content of breast meat.

Key words: chicken, omega-3 fats, EPA, DHA, nutrition, growth
Introduction

Dietary lipids play an important role in the health and wellbeing of both humans and animals. Omega-3 (n-3) polyunsaturated fatty acid (PUFA), particularly the long chain (LC) PUFA eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), have been shown to have beneficial effects on infant growth and development (Makrides et al. 2009), cardiovascular (von Schacky 2007), inflammatory and autoimmune diseases (Simopoulos 2002). This has led to recommendations by health agencies to increase dietary n-3 LCPUFA intake by consuming at least two meals of fatty fish per week to achieve a combined EPA+DHA intake of between 250 and 500 mg/day (National Heart Foundation of Australia, 2009; Kris-Etherton et al. 2002). However, despite these recommendations being in place for almost a decade, the majority of Australians continue to consume less than one meal of low fat fish per fortnight, and as a result fail to achieve an adequate dietary intake of n-3 LCPUFA (Howe et al. 2006).

Although n-3 LCPUFA can be obtained most readily from fish, these fatty acids can also be derived via conversion of the precursor n-3 PUFA, α-linolenic acid (ALA, 18:3 n-3), through progressive desaturation and elongation steps in animals including humans, though there is variation between animal species in the efficiency of this conversion (Brenna et al. 2009). It is generally agreed that the synthesis of n-3 LCPUFA in humans is relatively inefficient and a number of studies have demonstrated that the effects of providing pre-formed EPA or DHA in the diet cannot be reproduced by providing the equivalent amount of ALA (Burdge et al. 2002; Burdge and Wootton 2002; Burdge 2004). Thus, an alternative approach to enhance human n-3 LCPUFA consumption without changing existing dietary habits is to increase the level of EPA and DHA in food products.
which are already consumed as part of the typical Western diet (Givens and Gibbs, 2006; Betti et al. 2009).

While the Western diet contains relatively little fish, the popularity of chicken meat has increased significantly in recent years, making this an attractive vehicle for delivering increased n-3 LCPUFA into the diet. Poultry accounts for 30% of global meat consumption and chicken accounts for about 86% of the global poultry industry (Food and Agriculture Organization of the United Nations, 2010) While it has been demonstrated in previous studies (Lopez-Ferrer et al. 1999; Lopez-Ferrer et al. 2001; Schiavone et al. 2004) that it is possible to increase the level of n-3 LCPUFA in poultry meat by supplementing the diet with fish oil, this practice can have negative effects on the shelf life and sensory qualities of the chicken meat (Manilla and Husveth 1999; Gonzalez-Esquerra and Leeson 2000; Schreiner et al. 2005). Thus, an alternative approach is to supplement the diet with vegetable oil rich in ALA to enhance the endogenous synthesis of n-3 LCPUFA. We have recently shown (Kartikasari et al. 2012) that the levels EPA, docosapentaenoic acid (DPA, 22:5 n-3) and DHA in broiler breast and thigh meat were increased by ~4 to 9 fold in chickens fed a diet containing 2.5% flaxseed oil for 42 days compared to chickens that received a commercial diet that was low in n-3 PUFA. In the same study, the level of EPA and DHA in the total lipid fraction of breast meat from broilers fed diets enriched with the highest level of ALA reached levels similar to that observed in a different study using the same strain of broiler fed with 8% fish oil (Lopez-Ferrer et al. 1999).

Our previous study demonstrated that broilers can produce significant amounts of n-3 LCPUFA after being fed a high ALA diet for 42 days, however a larger study was required.
to examine effects on the growth rate, FCR and survival of the birds, and to determine
whether there were sex differences in the efficiency of ALA conversion (Kartikasari *et al.*
2012). The aim of the current study, therefore, was to determine the effect of feeding Cobb
500 broilers a high ALA diet from hatch to 42 days of age on the tissue n-3 LCPUFA
levels, growth rate, FCR and mortality rate when reared under near-commercial conditions
and to compare the responses between male and female birds.

**Material and Methods**

**Animals**

This study was approved by the Animal Ethics Committees of the Department of Primary
Industries South Australia (#06/11) and the University of Adelaide (S-2011-082). The
animal study was undertaken at the Pig and Poultry Production Institute, Roseworthy
Campus, University of Adelaide, South Australia in a controlled environment broiler shed
(36 m × 12 m) divided into 48 floor pens each measuring 2.6 m × 2.6 m. The temperature
in the shed was gradually decreased from ~31°C on the day after hatch to ~18°C in weeks
5 and 6 after hatch, consistent with standard industry practice. Feed and water were
available at all times from feed hoppers and a nipple drinker line, respectively. Chickens
were vaccinated against Marek’s Disease, Newcastle Disease and Infectious Bronchitis
viruses at the hatchery. A total of 3,840 Cobb 500 day-old chicks were used in this study.
The chicks were sexed before transport to the Roseworthy Campus on day of hatch. After
arrival, the 3840 birds were randomly allocated to 48 separate pens, with 80 male or 80
female birds per pen. These pens were then divided into 2 dietary groups (12 male and 12
female pens per diet group; n=1920 birds, 960 males and 960 females) using a randomised
block design.
Two dietary treatments were used: a low ALA diet (Control) with additional dietary fat provided by inclusion of 2.5% w/w tallow and 0.75% macadamia oil (Macoils, NSW, Australia) as required to the pellet mix; and a high ALA diet where the 2.5% tallow was replaced with flaxseed oil (Four Leaf Oils, SA, Australia), with different nutritional formulations for the starter, grower, finisher and withdrawal phases of production to meet breeder recommendations (Ridley Agriproducts, South Australia; Table 1). Thus, the High ALA diet contained 1.31% of ALA and had a LA:ALA ratio of 1.2-1.6:1, compared to 0.20% ALA and a LA:ALA ratio of 6.6-9.4:1 in the Control diet, across the different growth stages (Table 2). The diets had identical ingredients (with the exception of the fat source), were isocaloric and had identical macronutrient and micronutrient profiles.

Feeding, Growth and Mortality

The feeding regime was 0.4 kg/bird of starter crumbles, followed by 1.2 kg/bird of grower, 1.5 kg/bird of finisher and finally withdrawal feed (all in pellet form) as required (until harvest). The feed was weighed into the feed hoppers as required and, at intervals coinciding with measurements of live weight of broilers, all unused feed in the hoppers was weighed to determine total feed consumed in each pen. All birds in each pen were counted and weighed at week 5 (day 34/35) and week 6 (day 41/42). Deaths and culls in each pen were recorded daily.

Feed conversion ratio calculation

Feed Conversion Ratio (FCR) was calculated using three methods; feed intake between days 1 and 35 (5 week FCR) or between days 1 and slaughter (6 week FCR)
divided by (a) body weight gain, (b) body weight adjusted to include the weight of dead or
culled broilers and (c) live weight at 5 or 6 weeks of age.

**Sampling**

When broilers reached 41/42 days of age, one broiler from each pen was killed by
cervical dislocation (n=12 male and 12 female birds per diet group) for sample collection
for fatty acid analysis. Breast meat was collected and lipid extracted for determination of
tissue crude fat content and fatty acid profiles of total lipid, phospholipid and triglyceride
fractions.

**Fatty acid analysis**

Total lipid was extracted from 0.5g of breast meat using chloroform/methanol (2:1, v/v). An aliquot of the extracted total lipid was evaporated in a pre-weighed glass vial and
re-weighed to estimate tissue crude fat. Phospholipid and triglyceride fractions were
separated from another aliquot of extracted total lipid following thin layer chromatography
(TLC) on silica gel plates (Silica gel 60H, Merck, Darmstadt, Germany) with petroleum
spirit/diethyl ether/glacial acetic acid (180:30:2, v/v). The phospholipid and triglyceride
fractions were visualised with fluorescein 5-isothiocyanate against TLC standard 18-5
(NuChek Prep Inc, MN, USA) and scraped off the TLC plates into separate vials. All three
fractions (total lipid, phospholipid and triglycerides) were methylated in 1% H₂SO₄ in
methanol at 70°C for 3 hours. When cooled, the resulting methyl esters were extracted into
n-heptane and transferred to vials containing anhydrous Na₂SO₄ as the dehydrating agent.
Fatty acid methyl esters were separated and quantified using a Hewlett-Packard 6890 gas
chromatograph (Hewlett Packard, CA, USA) equipped with a 50 m capillary column (0.32
mm ID) coated with BPX-70 (0.25 μm film thickness, SGE Pty Ltd, Victoria, Australia).
The injector temperature was set at 250°C and the detector (flame ionisation) temperature at 300°C. The initial oven temperature was 140°C and was programmed to rise to 220°C at 5°C per minute. Helium was used as the carrier gas at a velocity of 35 cm per second. Fatty acid methyl esters were identified based on the retention time relative to authentic lipid standards (Nu-Chek GLC 463) obtained from NuChek Prep Inc. (Elysian, MN, USA).

Statistical analysis

The effects of diet and sex and the diet by sex interaction on live weight gain, feed intake and feed conversion were determined by two-way ANOVA using the general linear models (GLM) procedure in SAS 9.3 for Windows. The separate effects of diet and sex on losses due to mortality and culls were determined by non-parametric analyses using the NPAR1WAY procedure in SAS, as data were not normally distributed, even when subjected to square root or logarithmic transformations. One data point from a female bird given the control diet was identified as an outlier by the UNIVAR procedure in SAS, and subsequently omitted from all statistical analyses. A probability level of 0.05 (P<0.05) was considered to be statistically significant in all analyses. Where changes in the proportions of fatty acid types or individual fatty acids as a percentage of total fatty acids are described in the Results the values are absolute, not relative, terms.

Results

Growth, feed consumption and FCR

There were significant effects of both sex and diet on broiler live weight and weight gain to 5 and 6 weeks of age (Fig. 1). As expected, male birds were significantly
heavier than females at both time points (P<0.001). In addition, birds fed the high ALA diet were significantly heavier than their control counterparts at 5 and 6 weeks of age in both males and females (P<0.001). Female broilers fed the high ALA diet were an average of 135g (6.7%) heavier than the controls at 5 weeks and 77g (2.8%) heavier at 6 weeks of age, while males fed the high ALA diet were an average of 180g (8.3%) and 87g (2.0%) heavier than controls at 5 and 6 weeks of age, respectively.

Independent of dietary treatment, male broilers consumed a significantly greater amount of feed compared to females (P<0.01), consuming on average 250g and 550g more feed than the females at the 5 and 6 week time points respectively. There was, however, no significant difference in feed consumption between the high ALA and control dietary groups of either sex during the study (data not shown). Irrespective of the calculation method, FCR was significantly lower (by ~10%) in the broilers fed the high ALA diet compared to controls in both males and females (P<0.001 and P<0.01 for 5 and 6 weeks of age respectively; Table 3).

Mortality rate

Male birds had a significantly higher mortality rate than females (P<0.001), but there was no effect of dietary treatment on the mortality of the broilers during the trial. Overall mortalities were 6.0% in the control diet fed broilers and 6.7% in the high ALA fed broilers (P>0.05).
Effect of diet and sex on breast meat crude fat content and fatty acid profile

The level of crude fat in the breast meat was significantly higher in males than in females (1.61 ± 0.12% and 1.07 ± 0.05%, respectively; P<0.05), and there was no effect of dietary ALA treatment (P>0.05).

In the breast meat total lipid fraction the proportions (% of total fatty acids) of total saturated, trans, n-9 and n-7 acids were each decreased (P<0.001) by 2-5% in broilers fed the high ALA diet compared to those in the Control group, while total n-3 PUFA was increased from 2.7% to 11.0% (P<0.001) and there was no difference in total n-6 PUFA (Fig. 2a). The increase in n-3 PUFA in the total lipid fraction was predominantly accounted for by an increase in the level of ALA (~8 fold), with more modest (~1.7 to 3.5 fold) increases in the levels of EPA, DPA and DHA (all P<0.001; Fig. 2b). The most prominent effects of sex on total lipid PUFA profile in breast meat were observed in male birds fed on the high ALA diet which had significantly higher LA and ALA levels (by 0.9% and 1.5%, respectively), and lower (by 0.5% to 0.6%) EPA, DPA and DHA levels, compared to female broilers (all P<0.001; Fig. 2b).

The phospholipid composition of breast meat is shown in Figure 3. The proportions of total saturated fatty acids were similar in broilers fed both types of diet. However, in the high ALA diet birds the levels of trans, total n-9, n-7 and n-6 were 1.3 to 3.9% lower compared to the birds in the Control group, while total n-3 PUFA content was significantly increased by 7.5% (all P<0.001; Fig. 3a). LA levels were not significantly affected by the dietary treatment, but AA values were 1.4% lower in the birds fed the high ALA diet (P<0.001; Fig. 3b). There was a low proportion (< 0.3%) of ALA in the phospholipid fraction of breast meat in Control birds that was increased to between 1.0 and 1.1% in the
birds fed the high ALA diet (P<0.001). Interestingly, in the Control birds the proportions of EPA, DPA and DHA were greater (at 1.0 to 2.1% of total fatty acids) than that of ALA, and in the high ALA fed birds they increased again by 0.8 to 2.3 fold (all P<0.001; Fig. 3b). There was a small but significant difference in phospholipid EPA between males and females, with males being 0.3% lower than females, independent of dietary group. The most pronounced difference in breast meat phospholipid fatty acid composition between the sexes was in the level of LA, which was 2.3% higher in males compared to females in both the control and high ALA groups (P<0.01; Fig. 3b).

The composition of the triglyceride fraction of breast meat was quite different to the phospholipid fraction with higher levels of n-9 and lower levels of n-6 PUFA (Fig. 4a). The high ALA diet reduced the proportion of total saturates, *trans*, n-9 and n-7 fatty acid classes by 2 to 4%, with corresponding increases in total n-6 (by 2.2%) and n-3 (by 8.1%) (all P<0.001; Fig. 4a). There were very low levels (< 0.3%) of AA, EPA, DPA and DHA in the triglyceride fraction, even in birds fed the high ALA diet, and the high ALA diet increased the proportions of the n-3 PUFA (all P<0.001), but not AA (P>0.05; Fig. 4b). LA levels were 2.4% higher in the high ALA fed birds (P<0.001), and 1.0% higher in males than females in both diet groups (P<0.05). Levels of ALA were increased from 0.95% to 8.5% (~8 fold) by the high ALA diet (P<0.001), and were not significantly different between males and females (Fig. 4b).

**Discussion**

This study has demonstrated that consuming a diet which contains ~6-fold more ALA than current commercial feed from the time of hatch not only results in an increase in n-3 LCPUFA content in the breast meat, but is also associated with improved FCR and
increased body weight at 5 and 6 weeks of age. Importantly, this increased growth was achieved without any associated increases in mortality, suggesting that increasing the ALA content of chicken feeds may be a commercially viable strategy for improving production.

The heavier body weight at 5 and 6 weeks of age in broilers fed on the high ALA treatment provides evidence that the high ALA diet may have advantages over the current commercial feed for promoting growth in broiler chickens. Importantly, the increased growth was achieved in the absence of an increase in feed consumption and was thus associated with a significant reduction in the FCR, a critical factor in calculating the costs of broiler production. Similar findings were reported by others (Lopez-Ferrer et al. 2001; Zelenka et al. 2006) albeit with smaller numbers of birds. While the high ALA diet is about 30% more expensive to purchase than the current standard commercial feed, this may be off-set by the higher growth rate and 10% lower FCR of birds fed this diet, and it will be important to undertake cost-benefit analysis to assess its commercial viability. Our estimates indicate that the increased cost of the high ALA feed is unlikely to be offset by the increased growth rate of the birds. However, we are currently evaluating whether it is possible to feed the birds on the high ALA feed for shorter periods before slaughter and achieve the same benefits on growth/meat n-3 LCPUFA content. This could potentially mitigate the impact of the higher feed costs and make the use of these feeds more economically viable. In addition, it will be important to compare other properties of the high ALA feed with current commercial diets, in particular the shelf life and whether there is a need to add other ingredients, for example preservatives or anti-oxidants, to improve the stability of the ALA during storage.
In previous studies, rapid growth rates and increases in bird weight in the commercial setting have been associated with an increased incidence of musculoskeletal and cardiovascular diseases and associated mortality in meat poultry (Riddell 1992). It is significant, therefore, that in the present study the higher growth rates in the broilers maintained on the high ALA diet were achieved without an increase in mortality rate. The biological mechanisms through which the high ALA diet increased growth in comparison to the commercial diet remain to be determined. However, previous studies in both humans and animals have shown that n-3 LCPUFA supplementation increases the activation of anabolic signalling proteins in muscle during administration of insulin and amino acids and increases whole-body protein synthesis (Gingras et al. 2007) and the rate of muscle synthesis (Smith et al. 2011a). Other studies have reported that n-3 LCPUFA improved insulin action in insulin sensitive tissues, thus enhancing anabolic growth (Smith et al. 2011b; Kamolrat et al. 2013). It is also possible that other differences between the two feeds, in particular the absence of animal tallow, may have led to improved digestibility and/or adaptations in whole-body metabolism which translated into positive effects on the FCR. The effects on relative growth rate and other production parameters in this study were not influenced by the sex of the birds, indicating that the growth of both male and female broilers could potentially be increased by feeding a diet with a higher ALA content.

The results of the present study confirmed our previous findings that it is possible to significantly increase the n-3 LCPUFA content of the breast meat in broiler chickens by increasing dietary ALA content (Kartikasari et al. 2012). Importantly, the current study shows that these increases in tissue n-3 LPCUFA can be replicated on a near-to-commercial scale. While the chickens fed on the Control diet appeared to be able to convert the majority of the ALA provided into EPA, DPA and DHA, the levels of these n-
3 LCPUFA were still substantially lower than in birds fed the high ALA diet. The total amount of n-3 LCPUFA that was present in the breast meat of the chickens fed the high ALA diet equated to ~30 mg of EPA + DHA/100 g, compared with only ~14 mg in the Control birds, independent of sex. This corresponds to ~64% of the total lipid EPA+DHA level (46.8 mg/100 g) of chicken white meat obtained by feeding broilers diets containing 40-50 g/kg fish meal (Ratnayake et al. 1989) and 21% of the n-3 LCPUFA found in barramundi fillet (271 mg/100 g) (Soltan and Gibson 2008). Based on this, one serve of high ALA chicken meat (150g) would provide ~10% of daily recommended n-3 LCPUFA intake by the National Heart Foundation of Australia of 500mg/day twice the amount provided by meat from birds fed the Control diet. Importantly, we have previously shown that, unlike feeding chickens with fish oil, feeding chickens a high ALA diet does not have any negative effects on the textual or sensory properties of the breast meat, making this a viable option for commercialisation (Kartikasari et al., unpublished observations).

In the current study the ALA, EPA, DPA and DHA content of the breast meat were all significantly increased in broilers fed the high ALA diet, however the fatty acid composition was dependent on the lipid fraction. For total lipids and triglycerides (which make up ~43% of the total lipids in the breast meat), the increase in n-3 PUFA content was predominately in the form of ALA, whereas in the phospholipids the elongated and desaturated EPA, DPA and DHA made up the majority of the fatty acid content. These findings are consistent with previous studies (González-Esquerra and Leeson 2001; Betti et al. 2009).

Overall, the effects of sex on the fatty acid composition of the breast meat were relatively modest, and mostly confined to the total lipid fraction. Thus, male broilers fed the high
ALA diet had higher level of breast meat LA and ALA and lower levels of EPA, DPA and DHA compared to female broilers – suggesting that the males have a reduced capacity for conversion compared to females. The marginal effect of sex on fatty acids in chickens is consistent with the results of a previous study (Poureslami et al. 2010), which reported that male chickens had significantly greater LA and ALA intakes and ALA oxidation rate compared to females, whereas females showed a higher bioconversion of ALA to its desaturation product 18:4n-3 (stearidonic acid). However, other studies reported no difference between the sexes of chicken in their response to dietary PUFA (Leskanich and Noble 1997), although this may be a reflection of the physiological immaturity of the broilers that were assessed in those studies.

In conclusion, this study confirms that it is possible to substantially increase n-3 LCPUFA content of breast meat in chickens without increasing the total fat content by providing a high ALA diet, and that this can be achieved on a near-commercial scale. Moreover, the high ALA diet resulted in increased growth rate and improved FCR with respect to the current standard commercial diet, without any associated increases in mortality. This high ALA diet has the potential to provide a direct commercial benefit to broiler producers, and further studies to assess the economic viability of this are warranted. In addition, metabolic studies to determine the mechanisms through which ALA increases growth are required.

Acknowledgements

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The University of Adelaide. The authors would like to thank all those Pig and Poultry Production Institute (SARDI, Roseworthy Campus, The University of Adelaide) and FOODplus staff involved in the project. BSM is supported by a Career Development Award from the National Health and Medical Research Council of Australia (NHMRC). RG is supported by a Senior Research Fellowship from the NHMRC.
**Figure Legends**

**Figure 1.** Body weight (g) at weeks 5 and 6 of male and female broilers fed a low ALA (Control) and high ALA diet. Data are mean ± SE; n=12. Significant differences between groups are shown by asterisks, ** P<0.01; *** P<0.001.

**Figure 2.** Breast meat total lipid (a) fatty acid classes, and (b) key n-6 and n-3 fatty acids (as a percentage of all fatty acids) of male and female broilers fed a low ALA (Control) and high ALA diet for 42 days. Data are mean ± SE; n=12. Significant differences between diet treatments are indicated by asterisks, *** P<0.001. Significant differences between sexes are indicated by the hash symbol, ## P<0.01; ### P<0.001.

**Figure 3.** Breast meat phospholipid (a) fatty acid classes, and (b) key n-6 and n-3 fatty acids (as a percentage of all fatty acids) of male and female broilers fed a low ALA (Control) and high ALA diet for 42 days. Data are mean ± SE; n=12. Significant differences between diet treatments are indicated by asterisks, *** P<0.001. Significant differences between sexes are indicated by the hash symbol, ## P<0.01.

**Figure 4.** Breast meat triglyceride (a) fatty acid classes, and (b) key n-6 and n-3 fatty acids (as a percentage of all fatty acids) of male and female broilers fed a low ALA (Control) and high ALA diet for 42 days. Data are mean ± SE; n=12. Significant differences between diet treatments are indicated by asterisks, *** P<0.001. Significant differences between sexes are indicated by the hash symbol, # P<0.05; ### P<0.001.
References


Table 1 Tallow and oil content (in g/kg) of trial diets.

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1 A constant amount of tallow was sprayed onto the outer surface of all pellets to maintain integrity during transport and storage.
Table 2. Composition of the experimental diets.

Metabolizable energy (ME), crude protein (CP) and total fat shown as a percentage of wet weight of the feed. Proportion of fatty acids is shown as a percentage of total lipid fatty acids. Total n-6 and n-3 values include other minor fatty acids not shown in the Table. All values are the mean of triplicate samples.

<table>
<thead>
<tr>
<th></th>
<th>Starter</th>
<th>Grower</th>
<th>Finisher</th>
<th>Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME (kcal/kg)</td>
<td>2937</td>
<td>2964</td>
<td>3003</td>
<td>3007</td>
</tr>
<tr>
<td>CP (%)</td>
<td>22.5</td>
<td>20.3</td>
<td>20.0</td>
<td>19.0</td>
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<tr>
<td>Total fat (%)</td>
<td>6.0</td>
<td>7.3</td>
<td>7.0</td>
<td>6.2</td>
</tr>
<tr>
<td>18:2 n-6 (LA)</td>
<td>23.7</td>
<td>26.8</td>
<td>24.8</td>
<td>23.1</td>
</tr>
<tr>
<td>20:4 n-6 (AA)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Total n-6</td>
<td>24.1</td>
<td>27.1</td>
<td>25.0</td>
<td>23.2</td>
</tr>
<tr>
<td>18:3 n-3 (ALA)</td>
<td>3.3</td>
<td>20.5</td>
<td>3.8</td>
<td>14.3</td>
</tr>
<tr>
<td>20:5 n-3 (EPA)</td>
<td>ND¹</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>22:5 n-3 (DPA)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>22:6 n-3 (DHA)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total n-3</td>
<td>3.5</td>
<td>20.6</td>
<td>3.9</td>
<td>14.4</td>
</tr>
<tr>
<td>LA:ALA ratio</td>
<td>7.1</td>
<td>1.3</td>
<td>6.6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

¹ND = Not detected
Table 3. Feed intake (g/bird) and feed conversion ratio (FCR) at 5 and 6 weeks. FCR was calculated by three methods – feed intake divided by (a) body weight gain, (b) body weight adjusted to include the weight of dead or culled birds, or (c) live weight of male and female birds. All values are the mean of 12 replicate pens, each containing 16-18 birds. Values that are significantly different from each other within feed intake and FCR equation and within each week are indicated by different superscripts (P<0.001 at 5 weeks for all values, and at 6 weeks P<0.001 for feed intake and P<0.01 for FCR.

<table>
<thead>
<tr>
<th>Feed Intake (g/bird)</th>
<th>5 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
<td>Pooled SEM</td>
<td>Females</td>
<td>Males</td>
<td>Pooled SEM</td>
</tr>
<tr>
<td>Control</td>
<td>3,745&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3,996&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.1</td>
<td>5,272&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5,756&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.3</td>
</tr>
<tr>
<td>High ALA</td>
<td>3,734&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4,061&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FCR Equation</th>
<th>5 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>Control</td>
<td>1.891&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.894&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.014</td>
<td>1.974&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.995&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>High ALA</td>
<td>1.775&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.774&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>1.904&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.936&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>(b)</td>
<td>Control</td>
<td>1.873&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.854&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.013</td>
<td>1.944&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.934&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>High ALA</td>
<td>1.754&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.703&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>1.877&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.852&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>(c)</td>
<td>Control</td>
<td>1.846&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.850&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.013</td>
<td>1.938&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.961&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>High ALA</td>
<td>1.734&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.736&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>1.871&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.903&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
</tbody>
</table>
Figure 1.

![Graph showing body weight (g) for control and high ALA groups for male and female participants in Week 5 and Week 6.](http://www.publish.csiro.au/nid/72.htm)
Figure 2.

(a)

(b)
Figure 3.

(a) Phospholipid Fatty Acid (%)

(b) Phospholipid Fatty Acid (%)

Legend:
- Control-Female
- Control-Male
- High ALA-Female
- High ALA-Male

**Note:** The chart shows the percentage of total saturates, trans, n-9, n-7, n-6, and n-3 fatty acids in different groups. Significant differences are indicated by *** and ##.
Figure 4.

(a) Triglyceride Fatty Acid (%)

(b) Triglyceride Fatty Acid (%)

- Control - Female
- Control - Male
- High ALA - Female
- High ALA - Male

Legend:
- 18:2n-6
- 20:4n-6
- 18:3n-3
- 20:5n-3
- 22:5n-3
- 22:6n-3

Significance levels:
- # p < 0.1
- ** p < 0.01
- *** p < 0.001
- ### p < 0.0001