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

Animal Production Science, 2016; 56(5):815-823

Originally Published at: http://dx.doi.org/10.1071/AN14743

ANIMAL PRODUCTION SCIENCE

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

Journal:	*Animal Production Science*
Manuscript ID:	AN14743.R2
Manuscript Type:	Research paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Carragher, John; The University of Adelaide, FOODplus Research Centre, School of Agriculture, Food & Wine Muhlhausler, Beverly; The University of Adelaide, FOODplus Research Centre, School of Agriculture, Food and Wine Geier, Mark; South Australian Research and Development Institute, Poultry House, John; University of Manitoba, Department of Human Nutritional Sciences Hughes, Bob; South Australian Research and Development institute, Poultry Gibson, Robert; The University of Adelaide, FOODplus Research Centre, School of Agriculture, Food & Wine
Keyword:	Poultry nutrition, Feed conversion efficiency, Fatty acids, Poultry

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

John F. Carragher[a], Beverly S. Mühlhäusler[a], Mark S. Geier[b,c], James D. House[d], Robert J. Hughes[b,c] and Robert A. Gibson[a*]

[a]FOODplus Research Centre, School of Agriculture, Food and Wine, University of Adelaide, South Australia, Australia

[b]South Australian Research and Development Institute, South Australia, Australia

[c]School of Animal and Veterinary Sciences, University of Adelaide, South Australia, Australia

[d]Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

Short title: Omega-3 fats and chicken production

*To whom correspondence should be addressed:

Prof Robert A. Gibson

FOODplus Research Centre, School of Agriculture, Food and Wine,

Waite Campus, University of Adelaide, South Australia, 5064, Australia

Phone +618 8313 4333, Fax +618 8313 7135, e-mail: robert.gibson@adelaide.edu.au

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 Husvéth 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.

129

130 Diets

131 Two dietary treatments were used: a low ALA diet (Control) with additional
132 dietary fat provided by inclusion of 2.5% w/w tallow and 0.75% macadamia oil (Macoils,
133 NSW, Australia) as required to the pellet mix; and a high ALA diet where the 2.5% tallow
134 was replaced with flaxseed oil (Four Leaf Oils, SA, Australia), with different nutritional
135 formulations for the starter, grower, finisher and withdrawal phases of production to meet
136 breeder recommendations (Ridley Agriproducts, South Australia; Table 1). Thus, the High
137 ALA diet contained 1.31% of ALA and had a LA:ALA ratio of 1.2-1.6:1, compared to
138 0.20% ALA and a LA:ALA ratio of 6.6-9.4:1 in the Control diet, across the different
139 growth stages (Table 2). The diets had identical ingredients (with the exception of the fat
140 source), were isocaloric and had identical macronutrient and micronutrient profiles.

141

142 Feeding, Growth and Mortality

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

150

151 Feed conversion ratio calculation

152 Feed Conversion Ratio (FCR) was calculated using three methods; feed intake
153 between days 1 and 35 (5 week FCR) or between days 1 and slaughter (6 week FCR)

154 divided by (a) body weight gain, (b) body weight adjusted to include the weight of dead or
155 culled broilers and (c) live weight at 5 or 6 weeks of age.
156
157 Sampling
158 When broilers reached 41/42 days of age, one broiler from each pen was killed by
159 cervical dislocation (n=12 male and 12 female birds per diet group) for sample collection
160 for fatty acid analysis. Breast meat was collected and lipid extracted for determination of
161 tissue crude fat content and fatty acid profiles of total lipid, phospholipid and triglyceride
162 fractions.
163
164 Fatty acid analysis
165 Total lipid was extracted from 0.5g of breast meat using chloroform/methanol (2:1,
166 v/v). An aliquot of the extracted total lipid was evaporated in a pre-weighed glass vial and
167 re-weighed to estimate tissue crude fat. Phospholipid and triglyceride fractions were
168 separated from another aliquot of extracted total lipid following thin layer chromatography
169 (TLC) on silica gel plates (Silica gel 60H, Merck, Darmstadt, Germany) with petroleum
170 spirit/diethyl ether/glacial acetic acid (180:30:2, v/v). The phospholipid and triglyceride
171 fractions were visualised with fluorescein 5-isothiocyanate against TLC standard 18-5
172 (NuChek Prep Inc, MN, USA) and scraped off the TLC plates into separate vials. All three
173 fractions (total lipid, phospholipid and triglycerides) were methylated in 1% H_2SO_4 in
174 methanol at 70°C for 3 hours. When cooled, the resulting methyl esters were extracted into
175 n-heptane and transferred to vials containing anhydrous Na_2SO_4 as the dehydrating agent.
176 Fatty acid methyl esters were separated and quantified using a Hewlett-Packard 6890 gas
177 chromatograph (Hewlett Packard, CA, USA) equipped with a 50 m capillary column (0.32
178 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-

328 3 LCPUFA were still substantially lower than in birds fed the high ALA diet. The total
329 amount of n-3 LCPUFA that was present in the breast meat of the chickens fed the high
330 ALA diet equated to ~30 mg of EPA + DHA/100 g, compared with only ~14 mg in the
331 Control birds, independent of sex. This corresponds to ~64% of the total lipid EPA+DHA
332 level (46.8 mg/100 g) of chicken white meat obtained by feeding broilers diets containing
333 40-50 g/kg fish meal (Ratnayake *et al.* 1989) and 21% of the n-3 LCPUFA found in
334 barramundi fillet (271 mg/100 g) (Soltan and Gibson 2008). Based on this, one serve of
335 high ALA chicken meat (150g) would provide ~10% of daily recommended n-3 LCPUFA
336 intake by the National Heart Foundation of Australia of 500mg/day twice the amount
337 provided by meat from birds fed the Control diet. Importantly, we have previously shown
338 that, unlike feeding chickens with fish oil, feeding chickens a high ALA diet does not have
339 any negative effects on the textual or sensory properties of the breast meat, making this a
340 viable option for commercialisation *(*Kartikasari et al., unpublished observations*)*.
341
342 In the current study the ALA, EPA, DPA and DHA content of the breast meat were all
343 significantly increased in broilers fed the high ALA diet, however the fatty acid
344 composition was dependent on the lipid fraction. For total lipids and triglycerides (which
345 make up ~43% of the total lipids in the breast meat), the increase in n-3 PUFA content was
346 predominately in the form of ALA, whereas in the phospholipids the elongated and
347 desaturated EPA, DPA and DHA made up the majority of the fatty acid content. These
348 findings are consistent with previous studies (González-Esquerra and Leeson 2001; Betti *et*
349 *al.* 2009).
350
351 Overall, the effects of sex on the fatty acid composition of the breast meat were relatively
352 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

The work was supported by a project grant from the South Australian Department of Further Education, Employment, Science and Technology (DFEEST) Constellation SA programme and the FOODplus Research Centre, School of Agriculture Food and Wine,

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.

384 **Figure Legends**

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

388

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

394

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

400

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

406

407

References

National Heart Foundation of Australia (2009) Position statement: Fish, fish oils, n-3 polyunsaturated fatty acids and cardiovascular health. *National Heart Foundation of Australia*

Food and Agriculture Organization of the United Nations (2010) Agribusiness Handbook Poultry Meat and Eggs *Food and Agriculture Organization of the United Nations(FAO)*

Betti M, Schneider BL, Wismer WV, Carney VL, Zuidhof MJ, Renema RA (2009) Omega-3-enriched broiler meat: 2. Functional properties, oxidative stability, and consumer acceptance. *Poult Sci* **88**, 1085-95.

Brenna JT, Salem Jr N, Sinclair AJ, Cunnane SC (2009) A-linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins Leukot Essent Fatty Acids* **80**, 85-91.

Burdge G (2004) [alpha]-linolenic acid metabolism in men and women: Nutritional and biological implications. *Curr Opin Clin Nutr Metab Care* **7**, 137-144.

Burdge GC, Jones AE, Wootton SA (2002) Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. *Br J Nutr* **88**, 355-363.

Burdge GC, Wootton SA (2002) Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr* **88**, 411-20.

Gingras AA, White PJ, Chouinard PY, Julien P, Davis TA, Dombrowski L, Couture Y, Dubreuil P, Myre A, Bergeron K, Marette A, Thivierge MC (2007) Long-chain omega-3 fatty acids regulate bovine whole-body protein metabolism by promoting

muscle insulin signalling to the akt-mtor-s6k1 pathway and insulin sensitivity. *J Physiol* **579**, 269-84.

Givens DI, Gibbs RA (2006) Very long chain n-3 polyunsaturated fatty acids in the food chain in the UK and the potential of animal-derived foods to increase intake. *Nutr Bull* **31**, 104-110.

Gonzalez-Esquerra R, Leeson S (2000) Effects of menhaden oil and flaxseed in broiler diets on sensory quality and lipid composition of poultry meat. *Br Poult Sci* **41**, 481-8.

González-Esquerra R, Leeson S (2001) Alternatives for enrichment of eggs and chicken meat with omega-3 fatty acids. *Can J Anim Sci* **81**, 295-305.

Howe P, Meyer B, Record S, Baghurst K (2006) Dietary intake of long-chain ω-3 polyunsaturated fatty acids: Contribution of meat sources. *Nutrition* **22**, 47-53.

Kamolrat T, Gray SR, Thivierge MC (2013) Fish oil positively regulates anabolic signalling alongside an increase in whole-body gluconeogenesis in ageing skeletal muscle. *Eur J Nutr* **52**, 647-57.

Kartikasari LR, Hughes RJ, Geier MS, Makrides M, Gibson RA (2012) Dietary alpha-linolenic acid enhances omega-3 long chain polyunsaturated fatty acid levels in chicken tissues. *Prostaglandins Leukot Essent Fatty Acids* **87**, 103-9.

Kris-Etherton PM, Harris WS, Appel LJ, Committee ftN (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* **106**, 2747-2757.

Leskanich CO, Noble RC (1997) Manipulation of the n-3 polyunsaturated fatty acid composition of avian egg and meat. *World Poult Sci* **53**, 155-183.

Lopez-Ferrer S, Baucells M, Barroeta A, Grashorn M (1999) N-3 enrichment of chicken meat using fish oil: Alternative substitution with rapeseed and linseed oils. *Poult Sci* **78**, 356-365.

457 Lopez-Ferrer S, Baucells MD, Barroeta AC, Galobart J, Grashorn MA (2001) N-3
458 enrichment of chicken meat. 2. Use of precursors of long-chain polyunsaturated
459 fatty acids: Linseed oil. *Poult Sci* **80**, 753-61.
460 Makrides M, Gibson RA, McPhee AJ, Collins CT, Davis PG, Doyle LW, Simmer K,
461 Colditz PB, Morris S, Smithers LG, Willson K, Ryan P (2009)
462 Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic
463 acid: A randomized controlled trial. *JAMA* **301**, 175-182.
464 Manilla HA, Husvéth F (1999) N-3 fatty acid enrichment and oxidative stability of broiler
465 chicken (a review). *Acta Aliment* **28**, 235-249.
466 Poureslami R, Raes K, Turchini GM, Huyghebaert G, De Smet S (2010) Effect of diet, sex
467 and age on fatty acid metabolism in broiler chickens: N-3 and n-6 pufa. *Br J Nutr*
468 **104**, 189-97.
469 Ratnayake WMN, Ackman RG, Hulan HW (1989) Effect of redfish meal enriched diets on
470 the taste and n-3 pufa of 42-day-old broiler chickens. *J Sci Food Agric* **49**, 59-74.
471 Riddell C (1992) Non-infectious skeletal disorders of poultry: An overview. In 'Bone
472 biology and skeletal disorders in poultry.' (Ed. BCC Whitehead.) pp. 119–145.
473 (Carfax Publishing Co.: Abingdon, UK.)
474 Schiavone A, Romboli I, Chiarini R, Marzoni M (2004) Influence of dietary lipid source
475 and strain on fatty acid composition of muscovy duck meat. *J Anim Physiol Anim*
476 *Nutr* **88**, 88-93.

477 **S**chreiner M, Hulan HW, Razzazi-Fazeli E, Böhm J, Moreira RG (2005) Effect of different
478 sources of dietary omega-3 fatty acids on general performance and fatty acid profiles
479 of thigh, breast, liver and portal blood of broilers. *J Sci Food Agri* 85**, 219-226.**
480 Simopoulos AP (2002) The importance of the ratio of omega-6/omega-3 essential fatty
481 acids. *Biomed Pharmacother* **56**, 365-379.

Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ, Mittendorfer B (2011a) Dietary omega-3 fatty acid supplementation increases the rate of muscle protein synthesis in older adults: A randomized controlled trial. *Am J Clin Nutr* **93**, 402-12.

Smith GI, Atherton P, Reeds DN, Mohammed BS, Rankin D, Rennie MJ, Mittendorfer B (2011b) Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women. *Clin Sci* **121**, 267-78.

Soltan SS, Gibson RA (2008) Levels of omega 3 fatty acids in australian seafood. *Asia Pac J Clin Nutr* **17**, 385-90.

von Schacky C (2007) Omega-3 fatty acids and cardiovascular disease. *Curr Opin Clin Nutr Metab Care* **10**, 129-35.

Zelenka J, Schneiderova D, Mrkvicova E (2006) Linseed oils with different fatty acid patterns in the diet of broiler chickens. *Czech Anim Sci* **51**, 117-121.

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

Ingredient (in g/kg)	Starter		Grower		Finisher		Withdrawal	
	Control	High ALA	Control	High ALA	Control	High ALA	Control	High ALA
Tallow (mixer)	25.1	0	25.5	0	25.4	0	25.2	0
Tallow (coater)[1]	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Flaxseed oil	0	25.1	0	25.5	0	25.4	0	25.2
Macadamia oil	0	0	7.0	7.0	7.5	7.5	4.0	4.0

[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.

	Starter		Grower		Finisher		Withdrawal	
ME (kcal/kg)	2937		2964		3003		3007	
CP (%)	22.5		20.3		20.0		19.0	
	Control	**High ALA**	**Control**	**High ALA**	**Control**	**High ALA**	**Control**	**High ALA**
Total fat (%)	6.0	7.3	7.0	6.2	8.0	8.1	7.4	7.4
18:2 n-6 (LA)	23.7	26.8	24.8	23.1	21.0	21.3	20.8	22.1
20:4 n-6 (AA)	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1
Total n-6	24.1	27.1	25.0	23.2	21.4	21.4	21.1	22.3
18:3 n-3 (ALA)	3.3	20.5	3.8	14.3	2.8	17.9	2.2	18.9
20:5 n-3 (EPA)	ND[1]	ND	ND	ND	ND	ND	ND	ND
22:5 n-3 (DPA)	ND	ND	ND	ND	ND	ND	ND	ND
22:6 n-3 (DHA)	ND	ND	ND	ND	ND	ND	ND	ND
Total n-3	3.5	20.6	3.9	14.4	3.0	17.9	2.4	19.0
LA:ALA ratio	7.1	1.3	6.6	1.6	7.5	1.2	9.4	1.2

[1]ND = 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.

		5 weeks			6 weeks		
Feed Intake (g/bird)		**Females**	**Males**	**Pooled SEM**	**Females**	**Males**	**Pooled SEM**
	Control	3,745[b]	3,996[a]	26.1	5,272[b]	5,756[a]	30.3
	High ALA	3,734[b]	4,061[a]		5,199[b]	5,749[a]	
FCR Equation							
(a)	Control	1.891[a]	1.894[a]	0.014	1.974[c]	1.995[c]	0.012
	High ALA	1.775[b]	1.774[b]		1.904[d]	1.936[d]	
(b)	Control	1.873[a]	1.854[a]	0.013	1.944[a]	1.934[a]	0.011
	High ALA	1.754[b]	1.703[b]		1.877[b]	1.852[b]	
(c)	Control	1.846[a]	1.850[a]	0.013	1.938[a]	1.961[a]	0.011
	High ALA	1.734[b]	1.736[b]		1.871[b]	1.903[b]	

Figure 1.

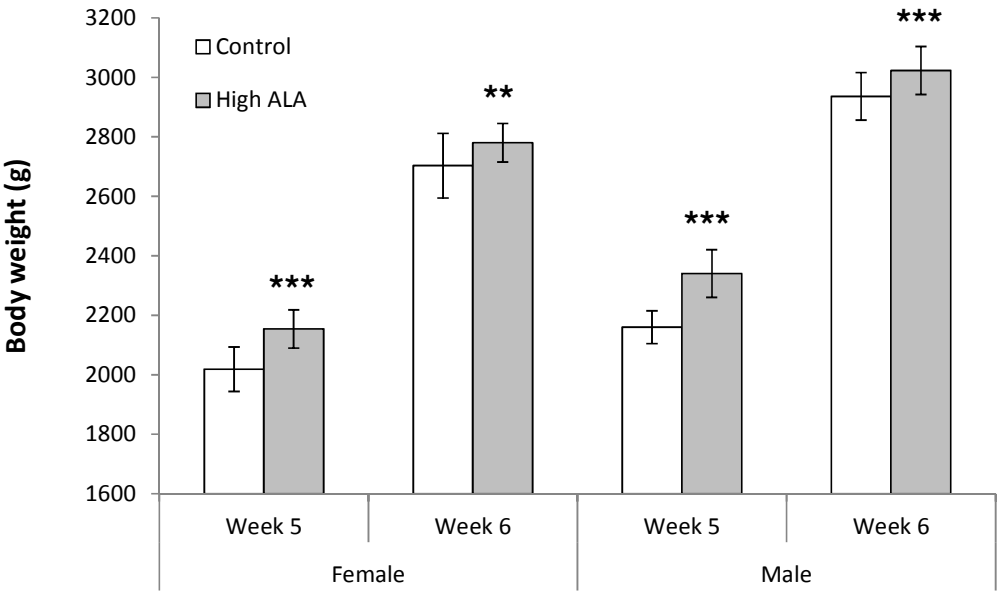


Figure 2.

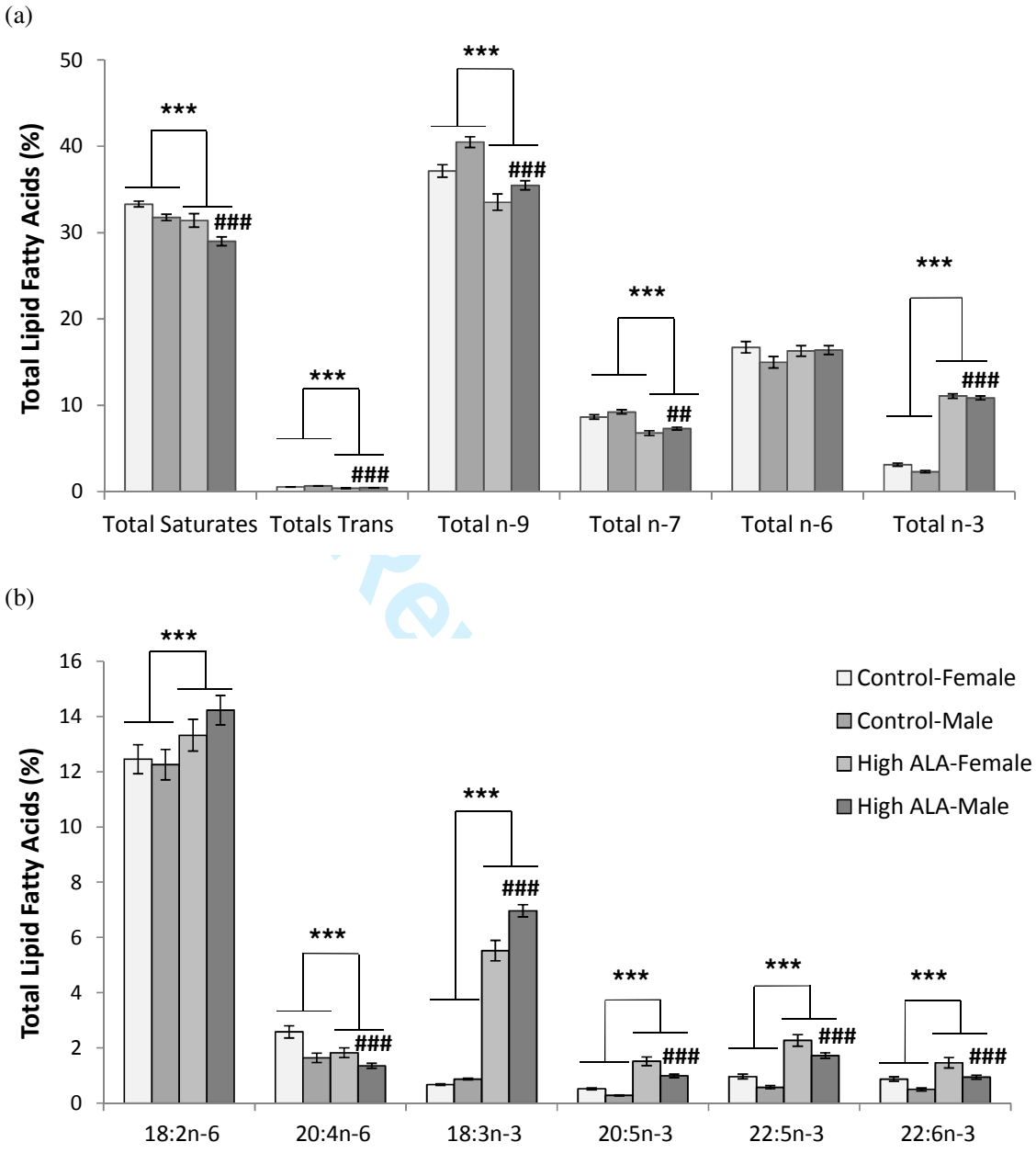
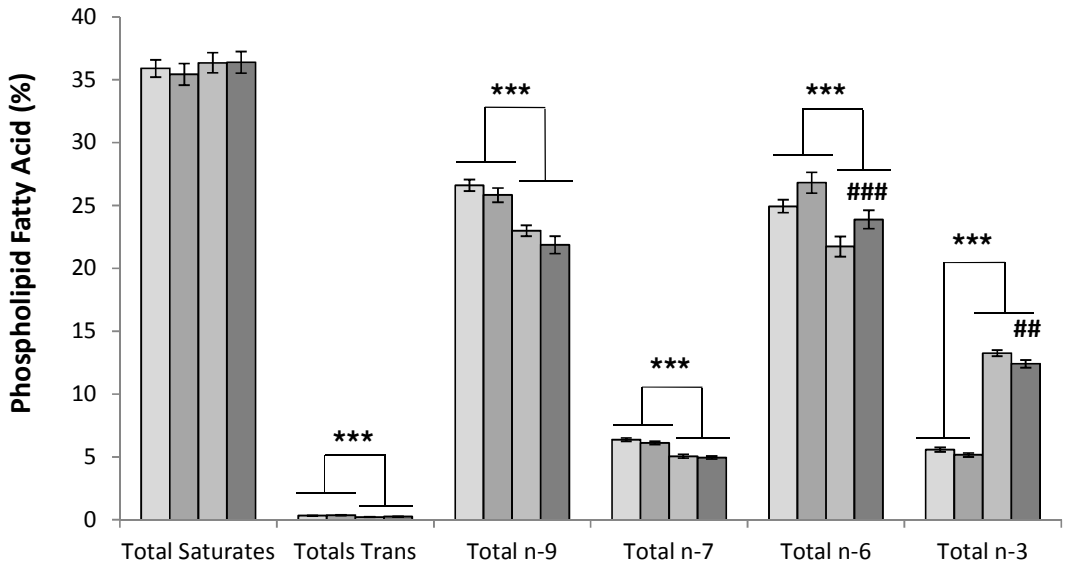

(a)
Total Lipid Fatty Acids (%)
50
40
30
20
10
0
***
###
***
***
###
***
##
***
###
Total Saturates
Totals Trans
Total n-9
Total n-7
Total n-6
Total n-3
(b)
Total Lipid Fatty Acids (%)
16
14
12
10
8
6
4
2
0
***
***
***
###
***
###
***
###
***
###
18:2n-6
20:4n-6
18:3n-3
20:5n-3
22:5n-3
22:6n-3
Control-Female
Control-Male
High ALA-Female
High ALA-Male

Figure 3.

(a)


Phospholipid Fatty Acid (%)
40
35
30
25
20
15
10
5
0
***
***
***
***
###
***
##
Total Saturates
Totals Trans
Total n-9
Total n-7
Total n-6
Total n-3

(b)

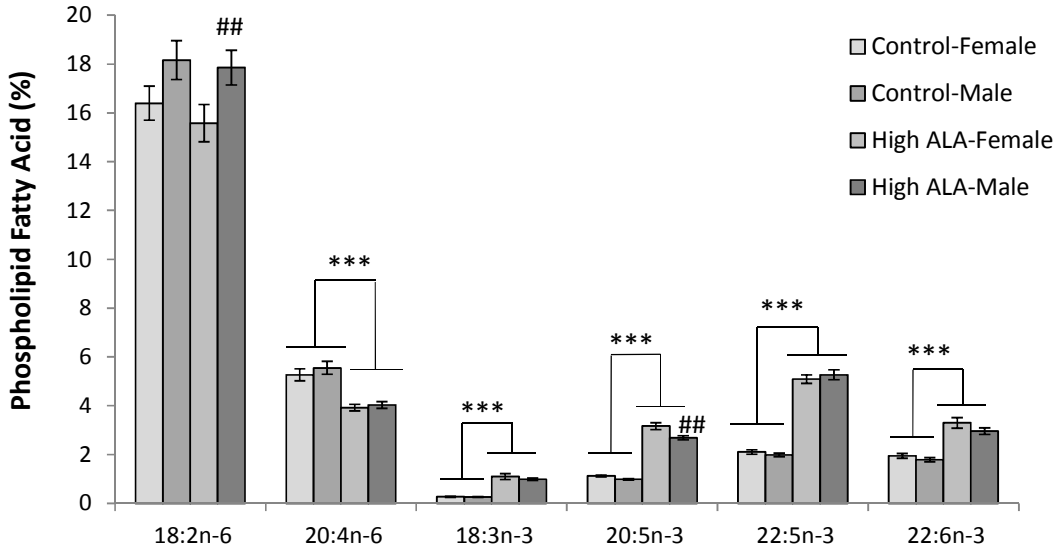
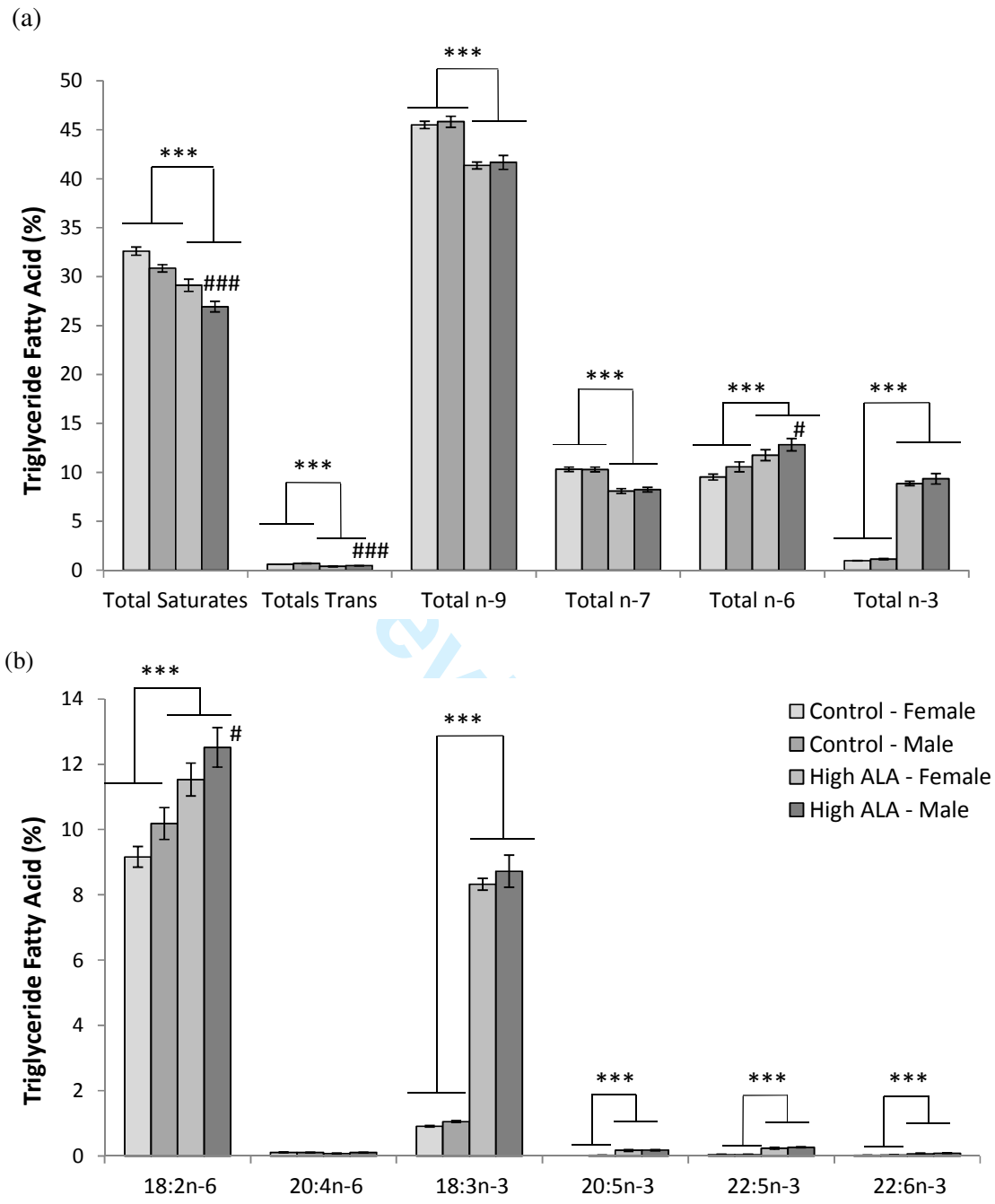

Phospholipid Fatty Acid (%)
20
18
16
14
12
10
8
6
4
2
0
##
***
***
***
##
***
***
Control-Female
Control-Male
High ALA-Female
High ALA-Male
18:2n-6
20:4n-6
18:3n-3
20:5n-3
22:5n-3
22:6n-3

Figure 4.


(a)
Triglyceride Fatty Acid (%)
50
45
40
35
30
25
20
15
10
5
0
***
***
***
***
***
***
###
###
#
Total Saturates
Totals Trans
Total n-9
Total n-7
Total n-6
Total n-3
(b)
Triglyceride Fatty Acid (%)
14
12
10
8
6
4
2
0
***
***
***
***
***
#
#
Control - Female
Control - Male
High ALA - Female
High ALA - Male
18:2n-6
20:4n-6
18:3n-3
20:5n-3
22:5n-3
22:6n-3