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# Effect of diets containing *n*-3 fatty acids on muscle long-chain *n*-3 fatty acid content in lambs fed low- and medium-quality roughage diets<sup>1,2</sup>

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**ABSTRACT:** In two experiments, each with 32 cross-bred ([Merino × Border Leicester] × Poll Dorset) wether lambs (26 to 33 kg weight range), animals were randomly assigned to one of four treatments. A mixture of lucerne chaff:oaten chaff was used as a basal diet, offered in different ratios. Animals were allowed to consume on a free-access basis in Exp. 1 or 90% of ad libitum intake in Exp. 2 in order to provide a low- (6.5 MJ ME/d) and medium- (9.5 MJ ME/d) quality basal diet, respectively. Isoenergetic amounts of lipid supplements, fish meal (80 g DM), canola meal (84 g DM), and soy meal (75 g DM) were tested in Exp. 1. In Exp. 2, fish meal (9% DM), unprotected rapeseed (7% DM), and protected canola seed (6% DM) were fed as supplements. At the end of 53-d (Exp. 1) or 46-d (Exp. 2) experimental periods, lambs were slaughtered at a commercial abattoir and at 24 h postmortem longissimus thoracis (LT) muscle was collected for the analysis of fatty acid (FA) composition of structural phospholipid and storage triglyceride fractions. Fish meal diet increased LT muscle long-chain *n*-3 FA content by 27% ( $P < 0.02$ ) in Exp. 1 and 30% ( $P < 0.001$ ) in Exp. 2

compared with lambs fed the basal diet, but fish meal decreased ( $P < 0.01$ ) the *n*-6 FA content only in Exp. 1. Soy meal and protected canola seed diets increased ( $P < 0.01$ ) LT muscle *n*-6 FA content but did not affect long-chain *n*-3 FA content. Longissimus thoracis muscle long-chain *n*-3 FA were mainly deposited in structural phospholipid, rather than in storage triglyceride. In both Exp. 1 and Exp. 2, the ratio of *n*-6:*n*-3 FA in LT muscle was lowest ( $P < 0.01$ ) in lambs fed fish meal supplement compared with all other treatments. Protected canola seed diet increased the ratio of *n*-6:*n*-3 FA ( $P < 0.01$ ) and PUFA:saturated fatty acid ( $P < 0.03$ ) content from those animals fed the basal, fish meal, and unprotected rapeseed diets in Exp. 2. This was due to an increase in muscle *n*-6 FA content, mainly linoleic acid, of both phospholipid ( $P < 0.001$ ) and triglyceride ( $P < 0.01$ ) fractions and not to an increase in muscle *n*-3 FA content. The results indicate that by feeding fish meal supplement, the essential *n*-3 FA can be increased while lowering the ratio of *n*-6:*n*-3 content in lamb meat to an extent that could affect nutritional value, attractiveness, and the economic value of meat.

Key Words: Food Supplements, Lambs, Muscles, Phospholipids, Roughage, Unsaturated Fatty Acids

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## Introduction

Long-chain *n*-3 fatty acids (FA), especially eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic

acid (DHA, 22:6*n*-3), play an important role in development of brain and retinal tissues and progression and prevention of human diseases, including heart disease and some cancers (Simopoulos, 1991). In Australia it is recommended that individuals should increase their intake of *n*-3 FA in the diet (NHMRC, 1992; Sinclair, 1994). Only fish, eggs, and, to a lesser extent, meat contain sufficient *n*-3 FA, particularly EPA and DHA, to be a reasonable dietary source of these nutrients (Mann et al., 1995). For many people, however, meat is the only source of *n*-3 FA in the diet, and red meat enriched with long-chain *n*-3 FA could make a significant contribution to *n*-3 FA consumption for people consuming little or no fish and(or) eggs.

Ruminant animals grazing pasture may have the ability to synthesize the long-chain *n*-3 FA (EPA and DHA) from their precursor of  $\alpha$ -linolenic acid (18:3*n*-

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3), although the conversion efficiency is relatively low (Howe, 1997). Hydrogenation of  $\alpha$ -linolenic acid in the fermentation process in ruminant digestion further reduces the conversion efficiency. However, modification of dietary 20- and 22-carbon PUFA in the rumen is partial because of limited hydrolysis of these fats (Byers and Schelling, 1988).

Previous work in poultry (Ratnayake et al., 1989), pigs (Morgan et al., 1992), and cattle (Mandell et al., 1997) has shown that feeding natural oils and oil seed meals altered the essential *n*-3 FA content of meat. The objective of this study was to establish whether a single lipid supplement is sufficient to enrich muscle long-chain *n*-3 FA in lambs. The hypothesis tested was that the essential 20- and 22-carbon *n*-3 FA would bypass ruminal hydrogenation and be selectively incorporated into muscle structural phospholipids. Several lipid supplements were tested on a basal diet that provided intakes of ME at a low (Exp. 1) and medium level (Exp. 2).

## Materials and Methods

**Animals and Treatments.** Thirty-two 6-mo-old cross-bred ([Merino  $\times$  Border Leicester]  $\times$  Poll Dorset) wether lambs with live weights ranging from 26 to 33 kg (Exp. 1) or 26 to 32 kg (Exp. 2) were randomly assigned to four dietary treatment groups in each experiment. Lambs were randomly allocated to individual pens and housed indoors for a 53-d (Exp. 1) or a 46-d (Exp. 2) experimental period at the Mount Derrimut Field Station, Deer Park, Victoria, Australia. All lambs were brought into the experiment after being ear-tagged and treated for internal parasites (4 mL of Levamisole; active constituent 80 g/L Levamisole hydrochloride).

In Exp. 1, after 7 d of adaptation, the groups were allocated to one of four treatments: 1) Basal diet (**Basal**), oaten chaff:lucerne chaff (80:20) offered for ad libitum consumption; 2) Basal + fish meal (80 g DM) = **FM**; 3) Basal + canola meal (84 g DM) = **CM**; or 4) Basal + soy meal (75 g DM) = **SM**. Lambs in all treatments were given ad libitum access to the basal diet. All supplements were given in separate containers. Fish meal was also mixed with approximately 100 g of lucerne chaff.

In Exp. 2, after 7 d of adaptation, the groups were allocated to one of four treatments consisting of: 1) Basal diet (Basal), oaten chaff:lucerne (40:60) offered at 90% of ad libitum intake; 2) Basal (50:50) + fish meal (9% DM) = **FM**; 3) Basal (50:50) + unprotected rapeseed (7% DM) = **UPRS**; or 4) Basal (50:50) + protected canola seed (6% DM) = **PCS**. Lucerne chaff was increased to 60% in the Basal diet in order to maintain the daily intake of ME at 9.5 MJ/d. All lambs in Exp. 2 were offered oaten chaff:lucerne chaff at 90% of ad libitum intake in the ratios given above. Ad libitum intake was estimated from intake in the adaptation period. With the exception of FM, all supplements were fed as for Exp. 1. Fish meal was given in a pellet form by mixing ground lucerne hay and FM (50:50) with 3 g of molasses

for every 100 g of FM. In Exp. 1, natural lipid supplements were tested on a basal diet that provided daily intakes of ME at a relatively low level (6.5 MJ ME/d) to support a slow growth rate, whereas in Exp. 2, natural and protected lipid supplements were tested on a basal diet that provided daily intakes of ME at a medium level (9.5 MJ ME/d) to support a moderate growth rate.

Rapeseed contains high amounts of erucic acid, but this is unlikely to cause problems for ruminants (McDonald et al., 1996). Lipids and proteins in the ground canola seed were treated with formaldehyde to protect them from ruminal microbial degradation (Rumentek Ltd., Australia; Scott et al., 1995). Supplements were fed in isoenergetic amounts in Exp. 1 with ad libitum access to roughage, whereas in Exp. 2 the supplements and roughage intake were constructed to give total intake isoenergetic (i.e., isoenergetic substitution). Water was freely available throughout the experimental period.

All animal procedures were reviewed and approved by the Animal Experimentation Ethics Committee, University of Melbourne, Parkville, Australia.

**Measurements and Slaughter Procedure.** Daily feed intake of roughage and supplements and weekly live weight gain were recorded over the 7- and 6-wk experimental periods in Exp. 1 and 2, respectively. Samples of feed materials were collected twice per week for the determination of feed chemical composition, as given in Table 1. In each experiment, half of the lambs from each treatment were randomly selected and transported 10 km to a commercial abattoir. There they were electrically stunned and slaughtered with standard procedures and the carcasses were inspected according to the guidelines given by the Australian Government Meat Authority (AUSMEAT). The remaining lambs in the experiment continued to be fed with the same diet for one additional week before they were slaughtered in the same way for measurement of carcass characteristics and sampling for subsequent evaluation of lipid analysis and meat quality. The 1-wk difference allowed completion of dissection and sampling of muscles within 24 to 30 h postmortem.

**Muscle Sample Collection.** At 24 h postmortem, chilled carcasses were cut into halves along the midline using an electric saw, and the right sides were taken to the Food Science and Technology Laboratory, Victoria University of Technology, Werribee, Australia, and stored at 1°C until dissection and muscle sampling were completed in the next 6 h. The longissimus thoracis (**LT**) and longissimus lumborum muscles were dissected and muscle samples were immediately taken for measurement of FA composition, color, and lipid oxidative substances. The LT sections were then sliced into ~2.0-cm-thick chops and samples (two slices) were collected for FA analyses of fresh meat, which is reported in this paper. These LT samples were immediately frozen at -20°C and after 24 h were stored at -70°C until further analysis.

**Table 1.** Chemical composition of basal diet and supplements used in Exp. 1 and Exp. 2<sup>a</sup>

| Item                   | DM, % | CP, % | Lipid, % | Ash, % | ME, <sup>b</sup> MJ/kg |
|------------------------|-------|-------|----------|--------|------------------------|
| Experiment 1           |       |       |          |        |                        |
| Oaten chaff            | 87    | 6     | 3        | 7.8    | 6.8                    |
| Lucerne chaff          | 86    | 18    | 2.8      | 10.6   | 8.2                    |
| Fish meal <sup>c</sup> | 88    | 61    | 8.6      | 14.7   | 10                     |
| Canola meal            | 90    | 40    | 10.5     | 6.4    | 9.8                    |
| Soy meal               | 88.2  | 44    | 3        | 8      | 10.8                   |
| Experiment 2           |       |       |          |        |                        |
| Oaten chaff            | 90    | 6     | 2        | 5.4    | 6.8                    |
| Lucerne chaff          | 90    | 16    | 2.9      | 9.5    | 8.2                    |
| Fish meal <sup>d</sup> | 93    | 65    | 5.4      | 19     | 13                     |
| Unprotected rapeseed   | 94    | 21    | 37       | 4      | 21                     |
| Protected canola seed  | 95    | 26    | 28       | 5.3    | 23                     |

<sup>a</sup>All values expressed on DM basis and means are an average of two observations.

<sup>b</sup>Reported values; others were determined values.

<sup>c</sup>Fish meal was imported from Peru.

<sup>d</sup>Fish meal was a product of Australia.

**Fatty Acid Analysis.** Eight lambs per treatment were required for the measurement of performance, and these data will be reported elsewhere. However, because of the high cost of the FA analyses, six muscle samples were randomly selected from among the eight chop samples collected in each treatment in Exp. 1 and Exp. 2 for analysis. This number was sufficient for statistical comparisons. Ten grams of each muscle LT sample collected at 24 h postmortem was minced and homogenized with 100 mL of a 2:1 chloroform:methanol (vol/vol) mixture (Folch et al., 1957) containing 10 mg/L (wt/vol) of butylated hydroxy toluene (Labco, Victoria, Australia). The prepared extraction solution was refrigerated overnight (12 h) at 4°C and filtered, and then 20% of an aqueous NaCl solution (9 g/L distilled water [wt/vol]) was added to the homogenate, mixed well, and kept for 12 h at 4°C. The lipid-containing CHCl<sub>3</sub> phase was carefully separated into a round-bottomed flask and the solvent was removed using a roto-evaporator (R-114 Basic, Buchi, Switzerland) as described previously by Mann et al. (1995). The lipid extract was transferred to screw-cap glass tubes using 20 mL of CHCl<sub>3</sub> and immediately stored at -20°C for subsequent FA analysis.

One milliliter of lipid sample was added with 1 mL of C17:0 internal standards of both triglyceride (triheptadecanoic acid [1 mg/mL]; NU-Check-Prep, MN) and phospholipid (diheptadecanoyl [0.25 mg/mL]; NU-Check-Prep, MN). Neutral lipids (triglycerides) and phospholipids of muscle extract were separated by TLC (by streaking three to four samples on a 20- × 20- × 0.25-mm TLC plate) using petroleum ether:diethyl ether:acetic acid, 85:15:2 (vol/vol/vol) as the solvent, as reported previously (Sinclair and O'Dea, 1987). Fatty acid methyl esters of lipid fractions were prepared by saponification using KOH (0.68 M in methanol) followed by transesterification with 20% boron trifluoride (BF<sub>3</sub>) in methanol. Fatty acid composition of phospholipids and triglycerides was determined by gas chroma-

tography using a 50-m × 0.32-mm BPX70 fused silica capillary column as described previously (Sinclair and O'Dea, 1987). Fatty acid composition of dietary chaff and supplements was analyzed from the total lipid fraction. The lipids were extracted as described for muscle tissues. One milliliter of lipid solution was added to 1 mL of heptadecanoic acid (internal standard, 4 mg/mL) and the solvent was evaporated under nitrogen gas. After evaporation to dryness, the FA were saponified and fatty acid methyl esters were prepared as described above. The FA composition of dietary materials was determined by gas chromatography as for muscle tissues. The daily intakes of *n*-3 and *n*-6 FA of lambs fed the basal diet and supplements in Exp. 1 and 2 are given in Table 2.

**Statistical Analysis.** Data were analyzed using the Minitab Statistical Package (Minitab, State College, PA). In both experiments, results for muscle FA composition of triglyceride and phospholipid fractions were analyzed by ANOVA using the GLM procedure. The main effect tested was diet. When indicated by ANOVA, means were separated using LSD, with *P* < 0.05 considered statistically significant. Results are reported as means and pooled SEM values from the indicated number of samples used on each treatment analysis.

## Results

In Exp. 1, the level of dietary *n*-3 FA consumed by FM, CM, and SM treatments was high, intermediate, and similar, respectively, compared with the Basal diet, whereas *n*-6 FA intake was higher with the CM treatment. The other treatments had *n*-6 FA intakes similar to the Basal diet. In Exp. 2, the dietary *n*-3 FA consumed by the FM and PCS groups were similar and were higher with the UPRS treatments than with the Basal diet. Lambs on the UPRS and PCS treatments had higher, and those on the SM treatment had similar,

**Table 2.** Mean daily intake of *n*-3, *n*-6, and long-chain (LC) *n*-3 fatty acids (FA) of roughage and supplements for basal and supplemented lambs in Exp. 1 and Exp. 2<sup>a</sup>

| Item                               | Basal <sup>b</sup> | FM <sup>b</sup> | CM <sup>b</sup>   | SM <sup>b</sup>  |
|------------------------------------|--------------------|-----------------|-------------------|------------------|
| Experiment 1                       |                    |                 |                   |                  |
| Chaff <i>n</i> -3 FA intake        | 706                | 817             | 768               | 754              |
| Supplement <i>n</i> -3 FA intake   | 0                  | 1,550           | 636               | 61               |
| Chaff <i>n</i> -6 FA intake        | 4,814              | 5,575           | 5,234             | 5,140            |
| Supplement <i>n</i> -6 FA intake   | 0                  | 156             | 1,975             | 454              |
| Total <i>n</i> -3 FA intake/day    | 706                | 2,367           | 1,404             | 815              |
| Total <i>n</i> -6 FA intake/day    | 4,814              | 5,731           | 7,209             | 5,594            |
| Total LC <i>n</i> -3 FA intake/day | 0                  | 1,525           | 0                 | 0                |
|                                    | Basal <sup>b</sup> | FM <sup>b</sup> | UPRS <sup>b</sup> | PCS <sup>b</sup> |
| Experiment 2                       |                    |                 |                   |                  |
| Chaff <i>n</i> -3 FA intake        | 1,423              | 1,278           | 1,208             | 1,251            |
| Supplement <i>n</i> -3 FA intake   | 0                  | 140             | 2,251             | 862              |
| Chaff <i>n</i> -6 FA intake        | 3,454              | 4,157           | 3,928             | 4,070            |
| Supplement <i>n</i> -6 FA intake   | 0                  | 65              | 3,964             | 2,602            |
| Total <i>n</i> -3 FA intake/day    | 1,423              | 1,418           | 3,459             | 2,113            |
| Total <i>n</i> -6 FA intake/day    | 3,454              | 4,222           | 7,892             | 6,672            |
| Total LC <i>n</i> -3 FA intake/day | 0                  | 120             | 0                 | 0                |

<sup>a</sup>Intakes are expressed in mg·lamb<sup>-1</sup>·d<sup>-1</sup>.

<sup>b</sup>Diet abbreviations: Basal = basal diet, FM = fish meal diet, CM = canola meal diet, SM = soy meal diet, UPRS = unprotected rapeseed diet, PCS = protected canola seed diet.

*n*-6 intakes compared with lambs fed the Basal diet. For both experiments, only FM-supplemented lambs received dietary long-chain *n*-3 FA (EPA and DHA). However, there was a considerable difference in the amount of long-chain *n*-3 intake between Exp.1 and Exp. 2. The lower intake in Exp. 2 may have been due to the different source of FM used (Table 2). This FM had a lower EPA and DHA content (21 vs 864 and 75 vs 880 mg/100g DM for Exp. 2 and Exp. 1, respectively). Supplementary feeding did not significantly affect the i.m. fat content of LT muscle in Exp. 1 or Exp. 2. However, diet had a significant effect on the FA composition of phospholipid (Tables 3 and 6) and triglyceride (Tables 4 and 7) fractions and essential FA components (Tables 5 and 8) of LT muscle in Exp. 1 and 2.

*Experiment 1.* In the phospholipid fraction of muscle (Table 3), the SM diet increased ( $P < 0.03$ ) stearic and

arachidonic acid (20:4*n*-6) concentrations. The FM diet increased ( $P < 0.001$ ) the *n*-3 FA (DHA) concentration compared with the Basal diet. In the triglyceride fraction (Table 4), LT muscle linoleic acid (18:2*n*-6) was lower ( $P < 0.04$ ) in lambs fed the FM diet than in lambs fed the other treatments, whereas lambs fed the CM and SM diets had lower ( $P < 0.04$ ) levels of EPA than lambs fed the Basal or FM diets. For the major FA classes of LT muscle total fat (aggregate of phospholipids and triglycerides, Table 5), the FM diet increased ( $P < 0.02$ ) long-chain *n*-3 FA and decreased ( $P < 0.01$ ) *n*-6 FA content compared with lambs fed the Basal diet. The ratio of *n*-6:*n*-3 FA in LT muscle was only lower ( $P < 0.01$ ) in FM-fed lambs, compared with those fed the Basal, CM, or SM diets.

*Experiment 2.* Lambs that were supplemented with FM had a greater ( $P < 0.001$ ) level of DHA in the phos-

**Table 3.** Effect of dietary fish meal (FM), canola meal (CM), and soy meal (SM) on individual fatty acid composition<sup>a</sup> of the phospholipid fraction of longissimus thoracis muscle (Exp. 1)

| Fatty acid       | Basal           | FM              | CM              | SM              | SEM <sup>b</sup> | <i>P</i> |
|------------------|-----------------|-----------------|-----------------|-----------------|------------------|----------|
| 16:0             | 63              | 66              | 57              | 87              | 11.8             | 0.12     |
| 16:1             | 4               | 4               | 2.5             | 4.5             | 0.94             | 0.24     |
| 18:0             | 59 <sup>c</sup> | 52 <sup>c</sup> | 58 <sup>c</sup> | 87 <sup>d</sup> | 11.6             | 0.03     |
| 18:1             | 110             | 105             | 94              | 157             | 23.6             | 0.09     |
| 18:2 <i>n</i> -6 | 61              | 50              | 60              | 77              | 10.1             | 0.09     |
| 18:3 <i>n</i> -3 | 13              | 11              | 12              | 16              | 2.0              | 0.17     |
| 20:4 <i>n</i> -6 | 29 <sup>c</sup> | 23 <sup>c</sup> | 24 <sup>c</sup> | 36 <sup>d</sup> | 3.8              | 0.01     |
| 20:5 <i>n</i> -3 | 17              | 20              | 15              | 21              | 2.6              | 0.12     |
| 22:4 <i>n</i> -6 | 1               | 1               | 1               | 1               | 0.1              | 0.19     |
| 22:5 <i>n</i> -3 | 16              | 17              | 14              | 21              | 2.4              | 0.08     |
| 22:6 <i>n</i> -3 | 7 <sup>c</sup>  | 13 <sup>d</sup> | 5 <sup>c</sup>  | 8 <sup>c</sup>  | 1.1              | 0.001    |

<sup>a</sup>All values are expressed in mg/100 g of meat sample and are an average of six (lambs) observations.

<sup>b</sup>Values for pooled SEM are shown.

<sup>c,d</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

**Table 4.** Effect of dietary fish meal (FM), canola meal (CM), and soy meal (SM) on individual fatty acid composition<sup>a</sup> of the triglyceride fraction of longissimus thoracis muscle (Exp. 1)

| Fatty acid  | Basal           | FM              | CM               | SM               | SEM <sup>b</sup> | <i>P</i> |
|-------------|-----------------|-----------------|------------------|------------------|------------------|----------|
| 14:0        | 66              | 48              | 65               | 60               | 11.3             | 0.40     |
| 14:1        | 5.9             | 4.1             | 6.4              | 6.0              | 1.1              | 0.19     |
| 16:0        | 677             | 518             | 588              | 642              | 89               | 0.33     |
| 16:1        | 27              | 21              | 24               | 24               | 4.9              | 0.68     |
| 18:0        | 580             | 428             | 589              | 637              | 79               | 0.08     |
| 18:1        | 976             | 672             | 900              | 898              | 117              | 0.09     |
| 18:2 $n$ -6 | 50 <sup>d</sup> | 33 <sup>e</sup> | 53 <sup>d</sup>  | 50 <sup>d</sup>  | 7.1              | 0.04     |
| 18:3 $n$ -3 | 27              | 17              | 28               | 26               | 4.2              | 0.07     |
| 20:4 $n$ -6 | 2               | 1               | 1                | 1                | 0.76             | 0.19     |
| 20:5 $n$ -3 | 1 <sup>d</sup>  | 1 <sup>d</sup>  | 0.2 <sup>e</sup> | 0.2 <sup>e</sup> | 0.40             | 0.04     |
| 22:5 $n$ -3 | 3               | 4               | 3                | 3                | 0.74             | NS       |
| 22:6 $n$ -3 | 0.5             | 2               | ND <sup>c</sup>  | ND <sup>c</sup>  | 0.44             | 0.001    |

<sup>a</sup>Values are expressed in mg/100 g of meat sample and are an average of six (lambs) observations.

<sup>b</sup>Values for pooled SEM are shown.

<sup>c</sup>ND = not detectable.

<sup>d,e</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

pholipid fraction (Table 6) of LT muscle than lambs fed the Basal, UPRS, or PCS diets. The PCS supplement decreased ( $P < 0.02$ ) LT muscle oleic acid (18:1) and increased ( $P < 0.001$ ) linoleic acid concentration compared with concentrations in lambs fed the Basal, FM, or UPRS. The LT muscle linoleic ( $P < 0.01$ ) and  $\alpha$ -linolenic ( $P < 0.05$ ) concentrations in the triglyceride fraction (Table 7) were higher in lambs fed the PCS diet than in the other groups, whereas the level of EPA was lower ( $P < 0.03$ ) only in lambs fed the UPRS diet. Overall, for the major FA classes (Table 8), LT muscle long-chain  $n$ -3 FA (EPA + docosapentaenoic acid [22:5 $n$ -3] + DHA) content in total fat was higher ( $P < 0.001$ ) in lambs fed the FM supplement, whereas lambs fed the PCS supplement had greater ( $P < 0.01$ ) levels of PUFA and  $n$ -6 FA than lambs receiving Basal, UPRS, or FM treatments. The ratio of  $n$ -6: $n$ -3 was decreased by FM and increased by the PCS diet com-

pared with the Basal or UPRS diet, whereas the PUFA:saturated FA (SFA) ratio was only increased by the PCS diet.

## Discussion

The aim of the experiments was to investigate the use and effectiveness of natural FM along with natural and technologically protected oilseed meals and oilseeds in manipulating the long-chain  $n$ -3 FA content of lamb muscle. Among the supplements used in Exp. 1 and Exp. 2, only FM significantly increased LT muscle  $n$ -3 FA concentration while decreasing the ratio of  $n$ -6: $n$ -3 FA compared with lambs fed the Basal diet, either on a poor- (Exp. 1) or medium- (Exp. 2) quality roughage-based diet.

Lucerne chaff was added in either a low ratio (20%) or higher ratios (50% and 60%) to oaten chaff to provide

**Table 5.** Effect of dietary fish meal (FM), canola meal (CM), and soy meal (SM) on fatty acid classes and essential fatty acid components of total (phospholipid and triglyceride) fats<sup>a</sup> of longissimus thoracis muscle (Exp. 1)

| Variable                          | Basal              | FM               | CM                  | SM                 | SEM <sup>b</sup> | <i>P</i> |
|-----------------------------------|--------------------|------------------|---------------------|--------------------|------------------|----------|
| MUFA <sup>c</sup>                 | 1,148 <sup>g</sup> | 825 <sup>f</sup> | 1,042 <sup>fg</sup> | 1,155 <sup>g</sup> | 119              | 0.04     |
| PUFA <sup>c</sup>                 | 239 <sup>fg</sup>  | 201 <sup>f</sup> | 227 <sup>fg</sup>   | 279 <sup>g</sup>   | 23               | 0.03     |
| SFA <sup>c</sup>                  | 1,478              | 1,136            | 1,383               | 1,601              | 165              | 0.07     |
| $n$ -3 FA                         | 85                 | 85               | 78                  | 96                 | 8.2              | 0.26     |
| $n$ -6 FA                         | 144 <sup>fg</sup>  | 107 <sup>h</sup> | 139 <sup>f</sup>    | 169 <sup>g</sup>   | 14               | 0.004    |
| Long-chain $n$ -3 FA <sup>d</sup> | 45 <sup>fg</sup>   | 57 <sup>h</sup>  | 38 <sup>f</sup>     | 53 <sup>gh</sup>   | 5.9              | 0.02     |
| $n$ -6: $n$ -3 FA                 | 1.8 <sup>f</sup>   | 1.2 <sup>g</sup> | 1.8 <sup>f</sup>    | 1.8 <sup>f</sup>   | 0.07             | 0.001    |
| PUFA:SFA                          | 0.17               | 0.18             | 0.17                | 0.18               | 0.02             | 0.93     |
| I.M. fat content, %               | 4.1                | 3.5              | 4.0                 | 4.1                | 0.36             | 0.08     |

<sup>a</sup>Values are expressed in mg/100 g of meat sample and are an average of six (lambs) observations.

<sup>b</sup>Values for pooled SEM are shown.

<sup>c</sup>MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids.

<sup>d</sup>Long-chain  $n$ -3 fatty acids = 20:5 $n$ -3 + 22:5 $n$ -3 + 22:6 $n$ -3.

<sup>f,g,h</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

**Table 6.** Effect of dietary fish meal (FM), unprotected rapeseed (UPRS), and protected canola seed (PCS) on individual fatty acid composition<sup>a</sup> of the phospholipid fraction of longissimus thoracis muscle (Exp. 2)

| Fatty acid       | Basal            | FM                | UPRS              | PCS              | SEM <sup>b</sup> | <i>P</i> |
|------------------|------------------|-------------------|-------------------|------------------|------------------|----------|
| 16:0             | 61               | 62                | 58                | 59               | 5.1              | 0.75     |
| 16:1             | 5.1 <sup>c</sup> | 5.3 <sup>c</sup>  | 3.4 <sup>cd</sup> | 2.0 <sup>d</sup> | 1.2              | 0.001    |
| 18:0             | 48               | 46                | 54                | 53               | 4.3              | 0.28     |
| 18:1             | 121 <sup>c</sup> | 114 <sup>c</sup>  | 125 <sup>c</sup>  | 92 <sup>d</sup>  | 10               | 0.02     |
| 18:2 <i>n</i> -6 | 53 <sup>c</sup>  | 44 <sup>c</sup>   | 52 <sup>c</sup>   | 78 <sup>d</sup>  | 5.3              | 0.001    |
| 18:3 <i>n</i> -3 | 11.5             | 10.2              | 11.3              | 13.3             | 1.3              | 0.16     |
| 20:4 <i>n</i> -6 | 30.2             | 26.3              | 29.7              | 31.6             | 3.0              | 0.38     |
| 20:5 <i>n</i> -3 | 12.8             | 16.0              | 13.1              | 12.8             | 1.5              | 0.13     |
| 22:4 <i>n</i> -6 | 1.5              | 1.1               | 1.2               | 1.4              | 0.12             | 0.04     |
| 22:5 <i>n</i> -3 | 14.5             | 14.7              | 14.1              | 13.8             | 1.4              | 0.89     |
| 22:6 <i>n</i> -3 | 4.1 <sup>c</sup> | 10.2 <sup>d</sup> | 4.5 <sup>c</sup>  | 4.8 <sup>c</sup> | 0.71             | 0.001    |

<sup>a</sup>Values are expressed in mg/100 g of meat sample and are an average of six (lambs) observations.

<sup>b</sup>Values for pooled SEM are shown.

<sup>c,d</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

**Table 7.** Effect of dietary fish meal (FM), unprotected rapeseed (UPRS), and protected canola seed (PCS) lipid supplements on individual fatty acid composition<sup>a</sup> of the triglyceride fraction of longissimus thoracis muscle (Exp. 2)

| Fatty acid       | Basal             | FM                | UPRS               | PCS               | SEM <sup>b</sup> | <i>P</i> |
|------------------|-------------------|-------------------|--------------------|-------------------|------------------|----------|
| 14:0             | 49                | 60                | 67                 | 48                | 12               | 0.35     |
| 16:0             | 555               | 705               | 709                | 576               | 124              | 0.47     |
| 16:1             | 23                | 30                | 28                 | 24                | 5.5              | 0.59     |
| 18:0             | 486               | 564               | 678                | 488               | 119              | 0.36     |
| 18:1             | 832               | 1,049             | 1,051              | 954               | 196              | 0.65     |
| 18:2 <i>n</i> -6 | 28 <sup>c</sup>   | 31 <sup>c</sup>   | 39 <sup>c</sup>    | 54 <sup>d</sup>   | 7.0              | 0.006    |
| 18:3 <i>n</i> -3 | 12.7 <sup>c</sup> | 13.8 <sup>c</sup> | 17.6 <sup>cd</sup> | 21.3 <sup>d</sup> | 3.2              | 0.05     |
| 22:5 <i>n</i> -3 | 1.5 <sup>c</sup>  | 2.1 <sup>c</sup>  | 0.0 <sup>d</sup>   | 1.2 <sup>c</sup>  | 0.7              | 0.03     |

<sup>a</sup>Values are expressed in mg/100 g of meat sample and are an average of six (lambs) observations.

<sup>b</sup>Values for pooled SEM are shown.

<sup>c,d</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

**Table 8.** Effect of fish meal (FM), unprotected rapeseed (UPRS), and protected canola seed (PCS) on major fatty acid classes and essential fatty acid components of total (phospholipid and triglyceride) fats<sup>a</sup> of longissimus thoracis muscle (Exp. 2)

| Item                                   | Basal             | FM               | UPRS             | PCS              | SEM <sup>b</sup> | <i>P</i> |
|--|-------------------|------------------|------------------|------------------|------------------|----------|
| MUFA <sup>c</sup>                      | 980               | 1,198            | 1,207            | 1,072            | 206              | 0.65     |
| PUFA <sup>c</sup>                      | 172 <sup>e</sup>  | 172 <sup>e</sup> | 185 <sup>e</sup> | 235 <sup>f</sup> | 13               | 0.001    |
| SFA <sup>c</sup>                       | 1,200             | 1,437            | 1,565            | 1,224            | 252              | 0.43     |
| <i>n</i> -3 FA                         | 57                | 67               | 61               | 67               | 4.8              | 0.13     |
| <i>n</i> -6 FA                         | 116 <sup>ef</sup> | 105 <sup>e</sup> | 124 <sup>f</sup> | 168 <sup>g</sup> | 8.7              | 0.001    |
| Long-chain <i>n</i> -3 FA <sup>d</sup> | 33 <sup>e</sup>   | 43 <sup>f</sup>  | 32 <sup>e</sup>  | 33 <sup>e</sup>  | 3.2              | 0.006    |
| <i>n</i> -6: <i>n</i> -3 FA            | 2.1 <sup>f</sup>  | 1.5 <sup>e</sup> | 2.1 <sup>f</sup> | 2.6 <sup>g</sup> | 0.08             | 0.001    |
| PUFA:SFA                               | 0.16              | 0.13             | 0.13             | 0.20             | 0.02             | 0.03     |
| I.M. fat content, %                    | 3.4               | 3.5              | 4.2              | 3.6              | 0.56             | 0.46     |

<sup>a</sup>Values are expressed in mg/100 g of meat sample and are an average of six (lambs) observations.

<sup>b</sup>Values for pooled SEM are shown.

<sup>c</sup>MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids.

<sup>d</sup>Long-chain *n*-3 polyunsaturated fatty acids = 20:5*n*-3 + 22:5*n*-3 + 22:6*n*-3.

<sup>e,f,g</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

a low- (6.5 MJ ME/d) and medium- (9.5 MJ ME/day) quality basal diet in Exp. 1 and Exp. 2, respectively. These energy levels represent a feed quality pattern similar to the dry seasonal conditions encountered in Australia during summer/autumn, when pasture availability and nutritional quality is insufficient to support rapid growth (Feedtest, PVI, 1997). Under these conditions, cereal supplementation with additional hay and crop residues is practiced to improve the growth rate of sheep grazing poor- to medium-quality pasture or forages (Lee et al., 1987; Valentine and Bartsch, 1995). The most commonly fed cereal and legume grain supplements are poor sources of  $\alpha$ -linolenic acid and have high  $n-6$  relative to  $n-3$  FA content. Thus, a single dietary supplement that contains high amounts of  $n-3$  FA was used to evaluate the effectiveness of manipulating FA composition of lamb muscle. Such supplements can be fitted into practical feeding methods applicable to Australian livestock feeding systems. The potential biological role of 20- and 22-carbon long-chain  $n-3$  FA intake (EPA and DHA) on the prevention of human diseases and enhancement of physiological performance have been supported by research in the last decade (BNF, 1992; NHMRC, 1992; Weisinger et al., 1996). The feeding strategy, if successful, would thus increase the nutritional quality of meat and potentially performance to achieve market target weights with lambs on pastures in seasons of decreasing herbage quality.

The lamb performance data from Exp. 1 and Exp. 2 were not reported in this paper. Although there were some improvement in BW gain and carcass weights for the supplemented treatments compared with the Basal diet in both experiments, there was no significant effect on the percentage of i.m. fat in LT muscle or evidence that this was associated with any variation in the rate or extent of incorporation of long-chain  $n-3$  FA into muscle phospholipid or triglyceride.

Research published in the early 1990s in lambs reported only the dietary manipulation of PUFA content, especially linoleic acid, an  $n-6$  FA, and  $\alpha$ -linolenic acid, an  $n-3$  FA, in muscle and(or) subcutaneous fat (Solomon et al., 1991; Lough et al., 1992). There has been little work on the dietary modification of muscle long-chain  $n-3$  FA composition in lambs. One study reported that feeding technologically protected lipid supplements containing high levels (8% and 12%) of fish oil in feedlot rations increased muscle EPA and DHA content (Ashes et al., 1992). This was conducted in aged sheep (18 to 24 mo old) with a limited number of animals (two sheep/treatment). At the time of commencement of this study (March 1996), there were no published data on the manipulation of muscle long-chain  $n-3$  FA content in growing lambs using natural lipid supplements. Since the commencement of this research program there have been two independent reports that feeding FM (Mandell et al., 1997) or fish oil (Vatansever et al., 1998) increased muscle total  $n-3$  FA in cattle fed concentrated feedlot rations. A point to consider is that basing expectations of effects on lamb meat lipids from

results with cattle may not be valid if differences exist in the extent of ruminal escape of dietary constituents such as FA.

Isoenergetic oilseed or meal supplements used in Exp. 1 and Exp. 2 had no effect on i.m. fat content of LT muscle, which is similar to results reported in lambs supplemented with canola seed, soy lecithin, or a combination of both (Lough et al., 1992), or with rapeseed meal, soy meal, or rape seed-soy meal (Solomon et al., 1991), or in cattle with FM fed at 5% or 10% for 56, 112 or 168 d (Mandell et al., 1997). However, in the present study dietary supplements significantly altered the muscle  $n-6$  and long-chain  $n-3$  FA content.

*n-3 Fatty Acids.* The twofold increase in DHA concentration of LT muscle with FM feeding was consistent with the results reported in cattle by Mandell et al. (1997). However, the level of DHA in muscle from cattle fed the control diet (1.8 mg/100 g fresh weight) for 56 d was smaller than the level reported in the present study. Thus, differences in  $n-3$  FA in the control diet may influence the response to FM supplement. Docosapentaenoic acid is a metabolite of EPA. The docosapentaenoic acid content of muscle lipid was not affected by FM, oilseed meals, or oilseeds in Exp. 1 or Exp. 2. This is consistent with the findings of Mandell et al. (1997) in cattle and Leskanich et al. (1997) in pigs, when fish products were fed. In contrast, feeding FM significantly increased muscle docosapentaenoic acid content in poultry (Ratanayake et al., 1989). There were no data reported on muscle DHA and EPA content when lambs were fed oilseed meals and(or) oil seed as a supplement (Solomon et al., 1991; Lough et al., 1992). An increased concentration of EPA (30 mg) in longissimus muscle of cattle fed 10% FM after 168 d of feeding (Mandell et al., 1997) was greater than in the present study in Exp. 1 (21 mg) or Exp. 2 (16 mg), in which lambs were fed 8 to 9% FM for 53 or 46 d, respectively.

$\alpha$ -Linolenic acid is a precursor of long-chain  $n-3$  FA such as EPA and DHA. The decrease in muscle  $\alpha$ -linolenic acid content with FM in Exp. 1 was mainly because of a low concentration in muscle triglyceride compared with that of lambs fed the Basal diet. This, however, was not found in Exp. 2. In contrast, cattle fed FM at 5% or 10% for 56 or 112 d were reported to have levels of  $\alpha$ -linolenic acid in longissimus muscle similar to those in the control animals. The increase in LT muscle total  $\alpha$ -linolenic content with the PCS diet was due to a significant increase in the muscle triglyceride fraction. Ashes et al. (1993) and Scott et al. (1995) found an increase in  $\alpha$ -linolenic acid content in the triglyceride fraction of s.c. fat of cattle fed PCS alone or with protected sunflower meal; however, muscle levels were not reported. Feeding canola seed in a natural form to lambs reduced the  $\alpha$ -linolenic acid content of longissimus muscle (Lough et al., 1992). This is in contrast to the results reported here that feeding CM or SM in Exp. 1 or UPRS in Exp. 2 had no significant effect on  $\alpha$ -linolenic acid concentration in either muscle phospholipid or triglyceride fractions.

*n*-6 Fatty Acids. The trend was different for *n*-6 FA deposition; the FM diet decreased LT muscle *n*-6 FA content, mainly due to a decrease in muscle linoleic acid concentration rather than arachidonic acid. This was more prominent when animals were supplemented with a poor-quality basal diet (Exp. 1) rather than with the medium-quality basal diet (Exp. 2). In contrast, in cattle the linoleic acid concentration was not changed by a FM diet (Mills et al., 1992; Mandell et al., 1997). The significant increase in LT muscle total *n*-6 FA with SM and PCS diets in the present study was mainly due to an increase in muscle linoleic acid content. An increase in muscle linoleic acid content in lambs with soy lecithin (Lough et al., 1992) and in cattle with PCS or protected sunflower seed (Ashes et al., 1993; Scott et al., 1995) has also been shown.

Arachidonic acid is the metabolite of linoleic acid produced by an enzymatic desaturation and elongation process (Cave, 1991; Clandinin et al., 1991; Storlien et al., 1995). Synthesis of arachidonic acid from linoleic acid involves a rate-limiting step at the  $\Delta$ -6 desaturase and there is competition between *n*-3 and *n*-6 PUFA for the  $\Delta$ -6 desaturase enzyme (Hayek and Reinhart, 1997). The decrease ( $P < 0.01$ ) in LT muscle total linoleic acid content (data not shown) with the FM diet (Exp. 1) may have led to the reduced production of muscle arachidonic acid ( $P < 0.01$ ) content. When FM was offered with the medium-quality basal diet (Exp. 2), the arachidonic acid content in muscle was not affected by the marginal decrease in muscle linoleic acid content. The increase in muscle total linoleic acid concentration with SM (Exp. 1) and PCS (Exp. 2) diets did not change muscle arachidonic acid content (data not shown). This suggests that the conversion efficiency of 18-carbon FA to 20- and 22-carbon FA may be low in ruminants.

*Ratio of n-6:n-3 Fatty Acids.* The current ratio of *n*-6:*n*-3 FA in the Western diet is 10 to 20:1, and the recommendation is to reduce it to less than 4:1 in diets for humans (Simopoulos, 1991; BNF, 1992; Sim, 1997). Among the supplements fed in Exp. 1 and Exp. 2, FM diet was beneficial in reducing the ratio of *n*-6:*n*-3 in meat. In contrast, the increase in the ratio of *n*-6:*n*-3 FA in LT muscle with the PCS diet was mainly due to an increase in muscle linoleic acid content, which in turn increased the ratio of PUFA:SFA of meat. Increasing the ratio of PUFA:SFA in the human diet is considered a priority for reducing plasma cholesterol (Lough et al., 1992; Morgan et al., 1992). However, it would be beneficial that long-chain *n*-3 FA are increased rather than *n*-6 FA in meat.

*Effect of Diet Type on Muscle n-3 Fatty Acid Content.* Ashes et al. (1992) reported that ruminal microflora could not hydrogenate 20:5*n*-3 and 22:6*n*-3 to any significant extent when fish oil preparations were incubated with strained rumen liquor. Others have reported that changes in dietary PUFA from fish oil in the rumen is partial because of limited hydrolysis of fats (Byers and Schelling, 1988). The greater incorporation of long-chain (EPA and DHA) *n*-3 FA into muscle phospholipid

with the FM diet in the present study (26% and 30% higher than Basal diets for Exp. 1 and Exp. 2, respectively) indicates that the *n*-3 FA from FM were not completely hydrolyzed and hydrogenated by microbial activity in the rumen. In Exp. 2 this enhanced incorporation was achieved despite a considerably lower intake of dietary long-chain *n*-3 FA compared with Exp. 1, such that the absolute amount of long-chain *n*-3 FA in muscle in Exp. 1 (57 mg/100 g of meat) and Exp. 2 (43 mg/100 g of meat) were not greatly different. An explanation for this is not readily apparent. Moreover, the relationship between dietary intake level of long-chain *n*-3 FA and the subsequent rate of incorporation into muscle (phospholipid and triglyceride) is poorly understood. However, it is possible that the way in which FM was given to lambs in the two experiments (Exp. 1, fed loose; Exp. 2, incorporated into a pellet) altered the efficiency of absorption, transfer and/or incorporation of long-chain *n*-3 FA into muscle.

The increase in *n*-3 and *n*-6 FA in phospholipid fraction with the SM diet may have been due to the effect of soy lecithin, which can play an important role as an emulsifying agent in lipid hydrolysis and subsequent absorption in the duodenum. Doreau and Chilliard (1996) have reported that the differences in FA digestion could depend on the production of biliary salts, which is related to the nature of FA reaching the intestine. The SM diet may have increased bile secretion, which contributed to more phospholipid in micelle formation and subsequent absorption of these phospholipids into intestinal mucosal cells (Merchen, 1988).

When FA were protected from ruminal microbial degradation (PCS supplement), the 62 and 46% increase in muscle linoleic acid and  $\alpha$ -linolenic acid content, respectively, did not affect their longer-chain metabolites arachidonic acid, EPA, or DHA, either in the phospholipid or triglyceride fraction. This suggests that the synthesis of longer-chain *n*-3 and *n*-6 FA from their 18-carbon precursors is not only inefficient in humans (Enser et al., 1996; Abril and Barclay, 1997) but also in ruminants. The failure of the CM and UPRS supplements to increase the long-chain *n*-3 FA content of muscle, even though the level of  $\alpha$ -linolenic acid was greater than with the other supplements, may have been due to the insufficient amount of FA escaping ruminal hydrogenation.

In conclusion, dietary FM resulted in an increase in muscle long-chain *n*-3 FA content and decreased the ratio of *n*-6:*n*-3 in lamb meat. Feeding SM modestly increased both the long-chain *n*-3 and *n*-6 FA content of meat, resulting in no difference in the *n*-6:*n*-3 ratio of meat. The PCS diet did not have a major effect on muscle *n*-3 FA but resulted in an increase in *n*-6 and the *n*-6:*n*-3 ratio of meat.

## Implications

The fatty acid composition of meat of lambs fed low- or medium-quality pasture or roughage diet can be al-

tered by feeding supplements such as fish meal or soy meal for 6 to 7 wk before slaughter. The alteration in the essential fatty acid classes of intramuscular fat of lamb may improve the nutritional quality of lamb meat and offer an alternative choice of meat products available to nutrition- and health-conscious meat eaters in Australia. The results also support our hypothesis that the essential 20- and 22-carbon *n*-3 fatty acids reaching the small intestine from natural supplements were preferentially deposited in muscle membrane phospholipid fraction because these fatty acids are the structural and functional components of muscle membranes that play a major role in maintaining viable cell membranes. Further research is needed to investigate the optimum level of muscle long-chain *n*-3 fatty acid enrichment on growth performance, carcass traits, fat deposition, and eating quality of lamb meat.

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