



## Sources of variation of health claimable long chain omega-3 fatty acids in meat from Australian lamb slaughtered at similar weights<sup>☆</sup>

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### ABSTRACT

The sources of variation of health claimable omega-3 polyunsaturated fatty acids (eicosapentaenoic acid, EPA + docosahexaenoic acid, DHA) in 2000 Australian lambs were investigated using 98 sires (Merino, maternal or terminal breeds) that were mated to about 5000 Merino or crossbred (Border Leicester × Merino) ewes. Pasture was supplemented with feedlot pellets, grains or hay as necessary, when the availability of quality green pasture was limited. Lambs were grown at 8 sites across Australia and when slaughtered the *longissimus lumborum* muscle was collected. Site and kills within sites were the major sources of variation for health claimable fatty acids. These environmental effects are likely to be driven by dietary background. The sire variance differed from about one twentieth to a half of the residual lamb within dam variation, depending on site and kill. This is the first comprehensive study to investigate on-farm sources of variation of long chain omega-3 polyunsaturated fatty acid content of lamb meat.

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### 1. Introduction

Fats in the human and animal body provide structure and energy for work, act as a media for transporting nutrients (vitamins and carotenoids), and act to regulate reproduction and health (Mattos, Staples, & Thatcher, 2000; Palmquist, 2009; Sampath & Ntambi, 2005). Research from the last two decades has shown that long chain omega-3 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have beneficial effects over other fatty acids present in diets for the maintenance of long term health (McAfee et al., 2010; Simopoulos, 1999). In some countries, such as Australia and New Zealand, it is legal to make claims that foods with higher levels of EPA and DHA offer health benefits (Anonymous, 2012; Food and Drug Authority, 2011). Red meat, including lamb, is a dietary source of EPA and DHA, but there is

considerable variability in the reported concentration of these fatty acids in red meat (Droulez, Williams, Levy, Stobaus, & Sinclair, 2006; Enser et al., 1998; French et al., 2000; Ponnampalam et al., 2010; Scerra et al., 2011; Scollan et al., 2006). Thus, there is a need to identify those on-farm factors that cause variation in the levels of EPA and DHA in lamb.

The Australian Sheep Industry Co-operative Research Centre is currently running the sheep Information Nucleus Flock programme, with the aim to estimate genetic parameters for new traits, to undertake a large-scale whole-genome study and to enhance the breeding values of animals in commercial studs (van der Werf, Kinghorn, & Banks, 2010). In a preliminary report, the large differences in EPA + DHA between flocks (sites) and kills (different slaughter dates) at the same site, for the 2007/2008 cohort were reported, although this reporting did not include a full consideration of all sources of variation (Pannier et al., 2010). The present study describes an analysis of all detectable sources of variation using the 2008/2009 cohort.

In brief, this study investigated the sources of variation for the health claimable long chain omega-3 polyunsaturated fatty acid content of Australian lamb, at similar carcass weights. Approximately 2000 lambs from 8 sites across Australia, covering a wide range of sheep genetics and production environments, were included.

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## 2. Materials and methods

### 2.1. Details of animals and management

The data were recorded on animals born in the second year (2008/2009 cohort) of the Information Nucleus Flock programme of the Australian Sheep Industry Cooperative Research Centre. The detailed analysis of all observable sources was restricted to one year so as to reduce the complexity of the statistical analysis, although preliminary analyses of other years indicate similar outcomes. The 2008/2009 cohort was chosen as this was the first year of the study to include all study sites.

Details of sire types, dam breeds, experimental locations, breeding and measurement programmes for this flock are given elsewhere (van der Werf et al., 2010). At each site lambs were slaughtered across several slaughter dates so as to achieve a target carcass weight of approximately 21.5 kg. As is the common practice in Australia, lambs were grown under extensive grazing systems with the provision of supplementary feeds during times when the availability of quality pasture is low (Table 1). Post weaning, with the exception of the Katanning site at some periods, all lambs at a site were managed as a single mob. Large differences in environment and pasture/feeding management led to large differences between sites in the slaughter ages of lambs (~150 to 500 days, Table 2). The target carcass weight was approximately achieved at most slaughters across all sites (flocks) (Table 2).

All sites had Merino×Merino and Maternal (mainly Border Leicester)×Merino lambs. All sites had lambs from terminal sires joined to Merino dams (Terminal×Merino) or terminal sires joined

to crossbred dams (Terminal×Border Leicester Merino), or both (Table 2). Ninety one out of the 98 sires in the current study were of the Poll Dorset (a terminal breed), Border Leicester (a maternal breed), Merino, White Suffolk (a terminal breed) or Poll Merino breeds. Other breeds represented were Southdown, Texel, Suffolk, Booroola and Ile de France. Pure Merinos were mostly slaughtered at much later ages, due to their slower growth rates.

About 2000 of the lambs were slaughtered at 5 abattoirs across Australia. At 24 h post-slaughter, a sample of 40 g (loin; m. *longissimus lumborum*) was collected for individual fatty acid and total fatty acid determination. Each muscle sample was cut into small square (1 cm) pieces, freeze dried and used for the analysis.

### 2.2. Fatty acid analysis

Samples collected from the 8 sites were systematically allocated in order of sample to two laboratories for sample processing and fatty acid determination. Each laboratory followed the same procedures, columns and temperature setup. Calibration was achieved by testing the same pool sample 10 times each year. Variation less than 5% between laboratories was maintained in the current study. A homogeneous sample of freeze dried ground material (0.5 g) was used for the determination of fatty acid composition using a rapid modified procedure developed from the method reported by O'Fallon, Busboom, Nelson, and Gaskins (2007). One hundred µL of nonadecanoic acid methyl ester (C19:0, Sigma Aldrich Pty Ltd, Castle Hill, NSW 2154, Australia) was added to muscle samples as an internal standard dissolved in chloroform (10 mg C19:0/mL CHCl<sub>3</sub>). The contents were hydrolysed using 0.7 mL of

**Table 1**  
The nutritional history of 2008/2009 cohort lamb progeny used in the study.

Site	Pasture type early post-weaning	Pasture type late post-weaning	Concentrate early post-weaning	Concentrate late post-weaning
Kirby				
Kill 1	Mixture of dried native grass/improved perennial grass/white clover	Dried pasture	No supplement	Finisher pellets
Kill 2				
Kill 3				
Kill 4	Dried pasture	Dried pasture	Finisher pellets plus barley	Finisher pellets plus barley
Trangie				
Kill 1	Mainly native pasture-windmill grass, spear grass, barley grass/some sown pasture (lucerne)	Green 80%/dry 20% Green 20%/dry 80% Green/dry 100% 0%	Lucerne hay + oats/pellets	Pellets
Kill 2				
Kill 3				
Cowra				
Kill 1	Temperate perennials-phalaris, sub clover/Lucerne-winter and summer active	Desiccating lucerne and other native pasture	No supplement	No supplement
Kill 2				
Kill 3				
Rutherglen				
Kill 1	Annual green pasture-annual ryegrass, sub clover/lucerne, phalaris	Annual pasture/lucerne Annual pasture/lucerne Lucerne/clover 90/10% Lucerne/clover 90/10%	Cereal/canola hay	Cereal/canola hay
Kill 2				
Kill 3				
Kill 4				
Hamilton				
Kill 1	Perennial pasture-ryegrass	Rape and millet Green pasture Ryegrass and tall fescue	Barley 3 kg/wk/head	Barley 4 kg/wk/head
Kill 2				
Kill 3		Green pasture ryegrass and tall fescue	Barley 3 kg/wk/head	Barley 4 kg/wk/head
Kill 4				
Struan				
Kill 1	Green pasture followed by pasture senescence and then irrigated pasture (14/11/08)	Irrigated pasture (kills 1 & 2)	No supplement (kills 1 & 2)	No supplement (kills 1 & 2)
Kill 2				
Kill 3		Dry pasture 23/3/09 to 4/5/09 Feedlot 4/5/09	No supplement	Silage/lentils Lentil/barley/silage
Turretfield				
Kill 1	Windrowed ryegrass & wild oats	Dry pasture and barley stubble Dry pasture and barley stubble Feedlot	0.25 kg/hd/day barley/pea mix	0.50 kg/hd/day barley/pea mix
Kill 2			0.25 kg/hd/day barley/pea mix	0.75 kg/hd/day barley/pea mix
Kill 3			0.4 kg/hd/day barley/pea mix	Ad-lib/hd/day barley/pea mix plus oaten hay
Katanning				
Kill 1	Annual grass and subclover	Mostly green Senesced pasture	Lupins, oats grains for 1–3 months	Lupins, oats grains for 1–3 months
Kill 2				
Kill 3				
Kill 4				
Kill 5	Annual grass, subclover		No supplement	No supplement

**Table 2**

Kill dates, average kill age (days), median carcass weight (kg) and the number of lambs of different genotypes, slaughtered within each site and kill group over the eight sites. Numbers only include lambs that had known sire, dam, gender and rearing type and had measured fatty acid profiles.

Site	Kill date	Kill age	Carcass weight	Merino sire		Terminal sire		Maternal sire
				Merino dam	Merino dam	Crossbred dam	Merino dam	
Kirby	25/5/09	269	19.7	0	26	66	6	
	10/8/09	345	27.9	0	32	51	16	
	20/10/09	408	31.8	0	45	27	27	
Trangie	26/10/09	420	27.8	67	11	7	11	
	13/1/09	193	24.9	0	35	33	19	
	28/1/09	208	24.5	0	35	30	23	
Cowra	4/6/09	333	23.3	43	0	0	0	
	4/12/08	138	22.0	0	13	41	11	
	15/1/09	180	25.4	0	35	6	11	
Rutherglen	4/6/09	320	22.5	32	0	0	0	
	3/3/09	215	22.0	0	0	49	9	
	21/4/09	265	22.1	0	0	47	10	
Hamilton	19/5/09	292	23.5	0	0	43	13	
	26/8/09	387	21.3	42	0	0	0	
	28/5/09	271	22.4	0	53	0	10	
Struan	24/6/09	298	21.0	0	42	0	11	
	1/7/09	305	19.0	0	37	0	10	
	24/11/09	449	19.2	28	0	0	0	
Turretfield	13/1/09	219	21.8	0	17	19	13	
	10/2/09	247	19.3	0	17	18	14	
	11/8/09	426	20.3	23	0	0	0	
Katanning	17/2/09	188	20.3	0	45	17	26	
	17/3/09	216	19.6	0	37	19	32	
	11/8/09	361	22.2	45	0	0	0	
Katanning	4/2/09	222	23.8	0	60	0	15	
	17/2/09	235	23.0	0	57	0	19	
	24/2/09	242	21.9	0	64	0	18	
	12/5/09	319	21.6	0	56	0	21	
	10/11/09	499	17.2	90	0	0	0	

10 N KOH in water and 5.3 mL of methanol to form free fatty acids. After mixing well with a vortex, the contents were incubated at 55 °C for 1.5 h, with vigorous mixing at 20 min intervals and then cooled to room temperature using tap water. Upon cooling, the contents were mixed with 0.6 mL of 24 N sulphuric acid in water and the mixing, incubation and cooling process occurred as above. After cooling the tubes to room temperature, the fatty acid methyl ester (FAME) was separated with 1 mL of hexane solvent by mixing for 5 min and centrifuging at 2000 rpm for 10 min. Two hundred µL of hexane containing FAME was collected into a Gas Chromatograph (GC) vial and fatty acid fractions were quantified by capillary GC (HP INNOWAX 60 m×0.25 mm, 0.5 µm, Agilent J&W Scientific, Santa Clara, CA, USA).

Fatty acid peaks were identified using a reference standard (Supelco C4-C24 mix, Sigma Aldrich Pty Ltd, NSW 2154, Australia), which was run in each batch. Fatty acid levels in the muscles are reported in mg/100 g meat. The total amount of EPA and DHA (EPA + DHA) was calculated as the sum from the fatty acid profiles of GC quantification. Total muscle fatty acid content was determined by addition of all the individual fatty acids that were identified in GC quantification and muscle fat content was determined using near infrared reflectance (NIR, as described by Perry, Shorthose, Ferguson, & Thompson, 2001).

### 2.3. Statistical analysis

A parsimonious restricted maximum likelihood (REML) model was developed for the logarithm of EPA + DHA across sites. Effects examined included sire identity, dam identity, site, kill within site, sire type, sire breed, dam breed, birth type, rearing type, age at kill, age of dam, lamb gender, calculated total fatty acid content, NIR measured total fat content, separate residual variation of lambs between sites and kills, and various fixed and random effect interactions. Terms were excluded or included in the model using chi-squared change in deviance tests for random effects and Wald F tests for fixed effects.

The chosen final model had random effects for sire identity, dam identity and a residual (lamb within dam) variance that differed for each kill of each site (Table 3). The fixed effects in the model were of the form outlined below;

$$\text{SiteKill} + \text{Site} * \text{SingleRT} + \text{Site} * \text{SireBreed} + \text{SireBreed} * \text{DamBreed} + \text{Gender}.$$

SiteKill is a 27 level factor indicating the 27 site and kill combinations; Site is an 8 level factor representing different sites, that are Kirby, Trangie, Cowra, Rutherglen, Hamilton, Struan, Turretfield, and Katanning; SingleRT is a 2 level factor indicating whether a lamb was raised as a single or multiple; SireBreed is a 10 level factor with levels for Border Leicester, Southdown, Poll Dorset, Texel, Suffolk, White Suffolk, Booroola, Merino, Poll Merino and Ile de France sires; DamBreed is a 2 level factor with levels for Merino and Crossbred dams; Gender is a 2 level factor with levels for male and female; and the \* symbol indicates main effects and an interaction between two terms.

Analysing EPA + DHA after a logarithmic transformation, rather than analysing the raw untransformed data, greatly simplified the final model because it ensured that the between sire variance was similar between sites ( $P=0.76$  for comparing the final model with a model that allowed sire effects to have different variances in each site, Table 3). If the data were not transformed, the between sire standard deviation for sites was roughly proportional to the typical predicted means for those sites.

Predicted values were determined on the logarithmic scale, after adjusting for other terms in the model, and then by back-transforming. Except where specified in the results, equal weighting of category levels is used for the adjustment of fixed effect categorical factors. Deviation from equal weighting was used when corresponding predictions were not estimable, or had poorer precision. All statistical analysis was carried out using the REML model facilities of GenStat 13 (Payne, 2010), which

**Table 3**  
Tests for terms included and excluded in final model for muscle EPA + DHA. Change in deviance chi-squared tests are used for random effect terms and residual variance terms, while Wald F tests are used for fixed effect terms.

Terms	Type of test	Degrees of freedom	X <sup>2</sup> /F	P-value
<i>Terms included</i>				
Sire	Deviance	1	54.75	<0.0001
Dam	Deviance	1	4.08	0.043
Residual variance differs between kill groups within each site	Deviance	21	83.08	<0.0001
Kill within site (i.e. kill effects that cannot be described by overall site effects)	Wald F	14, 515.3	32.47	<0.0001
Single rearing effect differs with site	Wald F	7, 526.0	4.27	0.0001
Sire breed effect differs with site	Wald F	39, 819.2	2.03	0.0003
Dam breed by sire breed interaction	Wald F	5, 325.9	3.26	0.007
Gender	Wald F	1, 872.0	6.87	0.009
<i>Terms excluded</i>				
Sire effect variance differs with each site, but with correlation equal to 1	Deviance	7	4.90	0.67
Sire effect differs with site	Deviance	1	0.00	1.00
Dam effect differs with site	Deviance	7	4.30	0.74
Single rearing effect differs with kill within site	Wald F	21, 508.6	0.95	0.52
Dam breed effect differs with site	Wald F	4, 326.6	0.66	0.62
Sire breed effect differs with kill within site <sup>a</sup>	Wald X <sup>2</sup>	70	70.19	0.47
Gender effect differs with site	Wald F	7, 445.5	1.37	0.22
Sire breed effect differs with single rearing	Wald F	9, 764.9	1.55	0.13
Dam breed effect differs with single rearing	Wald F	1, 630.0	0.25	0.62
Single rearing effect differs with gender	Wald F	1, 1137.0	0.08	0.77
Sire Breed effect differs with gender	Wald F	6, 330.5	1.15	0.33
Dam breed effect differs with gender	Wald F	1, 824.1	1.21	0.27
Single rearing effect differs with sire	Deviance	1	1.18	0.28
Between sire variance is different for different breeds	Deviance	6	4.53	0.61
Sire effect differs with Merino ewes	Deviance	1	0.19	0.66
Sire effect variance differs with Merino ewes, but with correlation equal to 1	Deviance	1	1.92	0.17
Sire effect differs with gender	Deviance	1	2.67	0.10
Dam effect differs with gender	Deviance	1	0.01	0.92
Dam variance is different for different sire breeds	Deviance	9	3.49	0.94
Dam variance is different for Merino ewes	Deviance	1	0.91	0.34
Twin vs triple vs quadruple rearing type	Wald F	2, 157.7	0.18	0.83
Birth type	Wald F	3, 314.0	1.85	0.14
Age at slaughter	Wald F	1, 953.0	0.33	0.57
Age of dam	Wald F	1, 924.5	0.16	0.69
Total fatty acids (calculated)	Wald F	1, 1108.4	1.31	0.25
Total intramuscular fat (NIR)	Wald F	1, 1152.1	1.35	0.25

<sup>a</sup> Used Wald X<sup>2</sup> due to numerical failure of the algorithm calculating the denominator degrees of freedom for Wald F test.

uses the same algorithms as the statistical package ASREML (Gilmour, Gogel, Cullis, & Thompson, 2006).

### 3. Results

Both the median of the raw EPA + DHA data, and the range of the raw EPA + DHA data, differed greatly between sites and kills within site (Fig. 1). Meat from nearly all lambs from the Cowra, Rutherglen and Hamilton sites, and from lambs slaughtered at the first two kills from the Struan site had a muscle EPA + DHA content of more than 30 mg/135 g portion. Meat from nearly all lambs from the Katanning site, Turretfield site and the last kill of the Struan site had EPA + DHA contents less than 30 mg/135 g portion. At other sites and kills, meat from some of the animals had values more than 30 while others had less than 30 mg/135 g portion (Fig. 1).

The adjusted values of EPA + DHA for each site and kill combination are similar to the median values of the raw EPA + DHA measurements (Fig. 2). This reflects the fact that, with the exception of final kills at each site mainly consisting of pure Merinos, the effects of site and kill have relatively low levels of confounding with other terms in the model.

The effect of dam breed is only reported for the two most common terminal sire breeds, namely Poll Dorset and White Suffolk, because the precision of the dam breed effect is lower for other sire breeds. Lambs from Merino dams had about 7% (95% confidence interval (3%, 11%) using asymptotic normal approximation logarithmic scale) greater EPA + DHA levels than lambs from crossbred dams when their sire was Poll Dorset, but not when their sire was White Suffolk (3% increase with 95% confidence interval (−1%, 8%). This

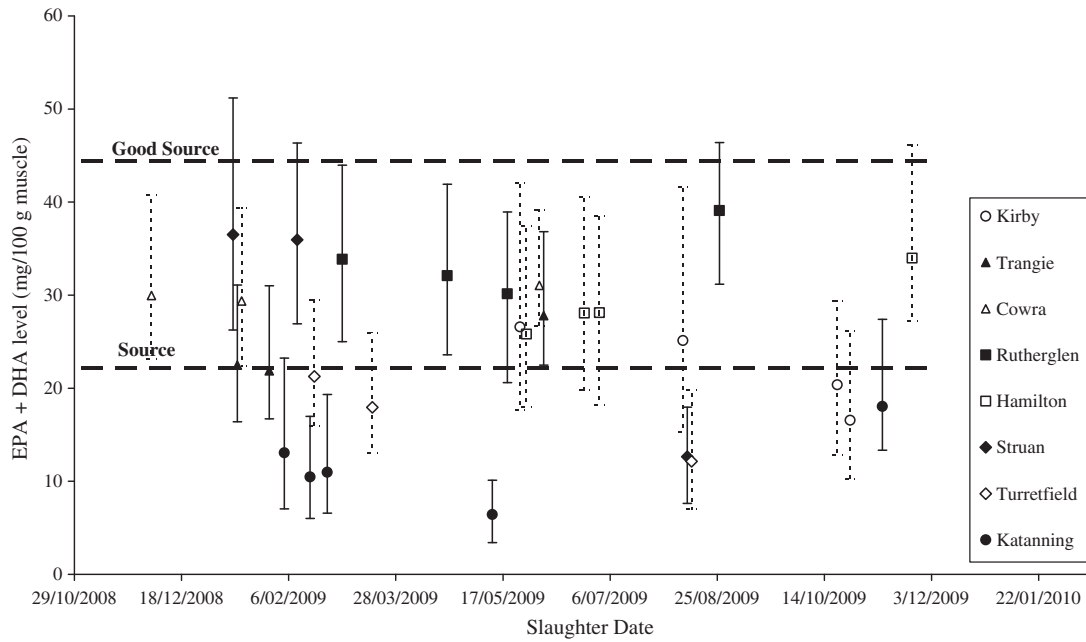
7% corresponds to approximately 2 mg/100 g when EPA + DHA is 25 mg/100 g.

The site by sire breed interaction (P=0.0003, Table 3) indicates that there are differences in breeds, but that these differences differ with site. For instance, lambs from Border Leicester sires have lower EPA + DHA than lambs from Poll Dorset and White Suffolk sires at Cowra and Kirby, but not within most other sites (Table 4). Although there was only one Booroola sire, lambs from this sire appeared to have a greater EPA + DHA levels than lambs from sires of other breeds, for a number of sites (Table 4).

Single reared lambs had up to 2 mg/100 g higher EPA + DHA than multiple reared lambs at some, but not all, sites (Table 5). Female lambs had greater EPA + DHA levels than male lambs (P=0.009), but the magnitude of the effect was only about 0.5 mg/100 g for lambs with moderate EPA + DHA levels.

The largest form of random variation was the variation in lambs (residual) within dams (that is the effect of individual lambs that cannot be explained for by sire, dam, and fixed model terms). However, the variance of the lamb within dam effect differed substantially between combinations of sites and kills (Table 6). For instance there was a fourfold difference in the lamb variance between the 21/4/09 and 26/8/09 kills for lambs from the Rutherglen site.

The sire variance differed from about one twentieth to a half of the residual lamb within dam variation, depending on site and kill (Table 6). This corresponds to the heritability for the logarithm of EPA + DHA ranging from about 0.2 to about 1, depending on site and kill. Although estimated with lower precision, as indicated by the larger P value (P=1×10<sup>−13</sup> for sires, P=0.04 for dams;

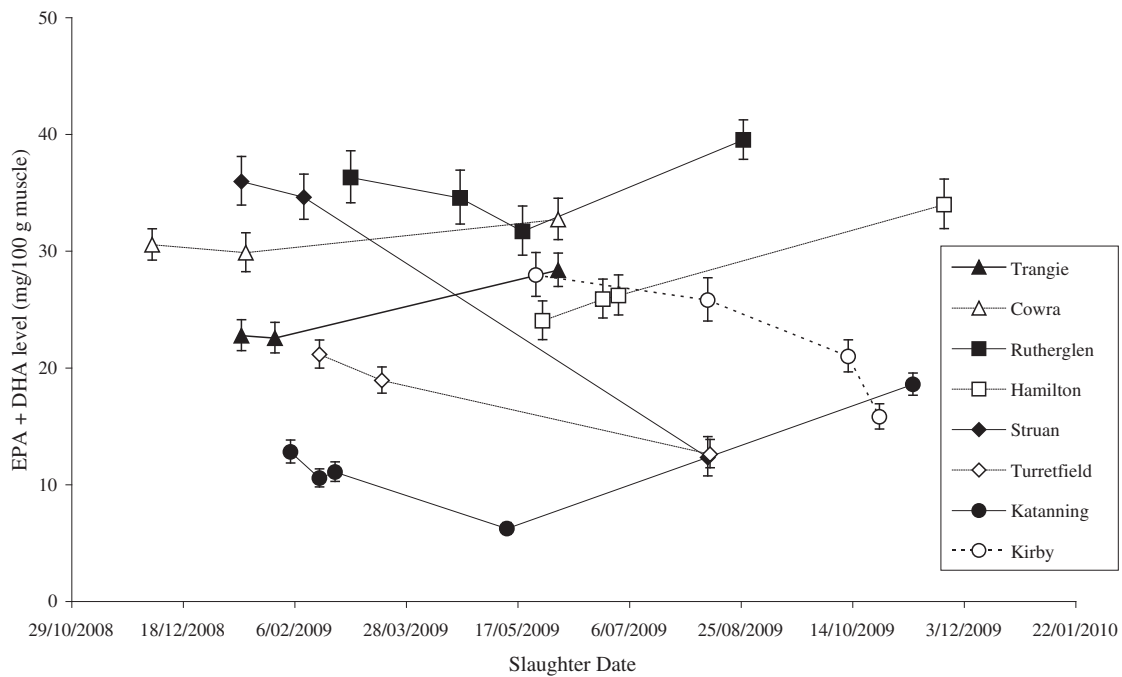


**Fig. 1.** Median values of raw data of muscle EPA + DHA content for each kill at each site. Bars represent 2.5% and 97.5% quantiles, so that the bars represent 95% of lambs. Horizontal dashed lines indicate minimum values for meat to be classified as a source of omega-3 fatty acids (30 mg/135 g serve = 23 mg/100 g muscle) or a good source (60 mg/135 g serve = 46 mg/100 g muscle), according to food standards of Australia and New Zealand.

Table 3), the dam variance was estimated to be similar to the sire variance.

There was no evidence of any additional effect of total fatty acid content on EPA + DHA when calculated from GC ( $P = 0.25$ , Table 3) or total fat content when obtained from NIR ( $P = 0.25$ , Table 3). When all effects of the current study are examined on a comparable basis (Table 7), the largest contributions are due to differences between sites and kills within

sites. The only other effects that are within an order of magnitude are the variation of lamb within dam (residual). These three effects were considerably greater than the variation due to any other sources. The next largest source of variation is due to differences between sires and dams of the same breed (Table 7). The sire variance of the logarithm of EPA + DHA was 0.00054, implying a standard deviation of 0.023. This corresponds to an increase of 6%, which corresponds to about 1 mg/100 g muscle



**Fig. 2.** Predicted means with 95% confidence intervals for muscle EPA + DHA levels across sites and kill groups. Except for the last kill at each site, predicted means are presented for Poll Dorset sires with Merino dams (this being the most numerous breed combination). At the last kill of each site, predicted means are presented for Merino sires with Merino dams.

**Table 4**  
Effect of three most common maternal and terminal sire breeds (Border Leicester, Poll Dorset and White Suffolk) and Booroola sire breed on muscle EPA + DHA, for each site. Predictions are averaged over all combinations of kill and site, except for combinations that are the last kill of a site, on the logarithmic scale.

Site	Log <sub>10</sub> transformed				Back transformed (mg/100 g)					
	Border Leicester (a)	Poll Dorset (a)	White Suffolk (a)	Booroola (b)	Sed (a) vs (a)	Sed (a) vs (b)	Border Leicester	Poll Dorset	White Suffolk	Booroola
Kirby	1.34	1.39	1.38	1.41	0.018–0.021	0.041–0.042	22	25	24	26
Trangie	1.39	1.36	1.35	–	0.018–0.020	–	24	23	23	–
Cowra	1.46	1.48	1.49	1.54	0.018–0.025	0.035–0.039	29	30	31	35
Rutherglen	1.54	1.53	1.51	1.49	0.020–0.022	0.035–0.036	34	34	32	31
Hamilton	1.43	1.40	1.44	1.50	0.019–0.024	0.037–0.039	27	25	28	32
Struan	1.56	1.55	1.58	–	0.021–0.023	–	37	35	38	–
Turretfield	1.31	1.30	1.27	1.34	0.017–0.020	0.033–0.035	20	20	19	22
Katanning	0.98	0.99	0.99	1.10	0.020–0.022	0.063	10	10	10	13

when the median value of EPA + DHA is 20 mg/100 g muscle. This implies that it is possible to select sires, from within present populations, whose median progeny are 2 mg/100 g muscle greater than the typical sire of the same breed type.

#### 4. Discussion

##### 4.1. Environmental effects on the levels of EPA + DHA in lamb

Similar to the preliminary results of Pannier et al. (2010), the effects of site and kill within sites are very large (Fig. 2), and the dominant source of variation (Table 7). Other factors investigated, such as genetics, gender, rearing and age of dam, were relatively small. An immediate corollary is that this dominant source of variation must be environmental or age related. However, there was no systematic trend with age, a finding consistent at all sites. We suggest that the most likely difference is nutrition, and in particular, the type of feed the lambs are consuming.

The levels of EPA + DHA in lamb from Cowra, Rutherglen, Struan, Turretfield and Katanning were generally similar to those found at these sites in the previous year (Pannier et al., 2010), reflecting a degree of year to year consistency in climate and feeding management at each site. Values for Kirby, Trangie and Hamilton were similar to those reported by Ponnampalam et al. (2010) and those for Katanning were similar to those reported by Kiteessa et al. (2010). The EPA + DHA level at Hamilton was observed to be considerably higher than in the previous year, most likely due to much more green pasture associated with more rainfall (Ponnampalam et al., 2012 within this edition).

Meat from nearly all lambs from the Cowra, Rutherglen and Hamilton sites, and from lambs slaughtered at the first two kills from the Struan site, could be considered as a source (>30 mg/135 g portion), but not a good source (>60 mg/135 g portion), of omega-3 polyunsaturated fatty acids according to Australian New Zealand Food Standards (Anonymous, 2012). Meat from nearly all lambs from the Katanning, and Turretfield sites and the last kill from the Struan site, could not be considered as a source of omega-3. At other sites and kills, some of the

**Table 5**  
Effect of rearing type on muscle EPA + DHA, at each site. For each site, predictions are averaged over all kills of that site, except the last kill, on the logarithmic scale. Predictions are presented for lambs with Poll Dorset sires and Merino dams.

Site	Log <sub>10</sub> transformed			Back transformed (mg/100 g)	
	Single	Multiple	sed	Single	Multiple
Kirby	1.40	1.39	0.012	25	25
Trangie	1.35	1.36	0.010	22	23
Cowra	1.49	1.47	0.009	31	30
Rutherglen	1.53	1.53	0.009	34	34
Hamilton	1.42	1.39	0.013	27	24
Struan	1.56	1.54	0.016	36	34
Turretfield	1.33	1.28	0.011	21	19
Katanning	1.00	0.99	0.011	10	10

meat could be considered as a source of omega-3 polyunsaturated fatty acids, and some could not.

Green pasture or grass provides higher levels of  $\alpha$ -linolenic acid (ALA, 18:3 n-3) and grain feeds/concentrates provide higher levels of linoleic acid (LA, 18:2 n-6) and lower ALA, which are the precursors for the production of their longer chain omega-3 (EPA and DHA) and omega-6 fatty acids, respectively (Sinclair, 2007). Previous reports have shown that replacing grain based silage or concentrate (pellets) diets rich in 18:2 n-6 with pasture rich in 18:3 n-3 improved the fatty acid composition of muscle by increasing omega-3 or reducing the omega-6 or the omega-6/omega-3 ratios (French et al., 2000; Nuernberg et al., 2005), which is beneficial for human consumption (Palmquist, 2009; Scollan et al., 2006).

The nutritional quality of the lamb diet during the post-weaning period in the present study is likely to explain the major portion of the variation on the health claimable EPA + DHA in the meat (refer Table 1 and

**Table 6**

Sources of random variation due to sires, dams of the same sire and individual lambs of the same dam in the logarithm of muscle EPA + DHA. An estimated heritability of the logarithm of muscle EPA + DHA is presented for each site, based on the standard formula using the variance of the sire and the lamb within dam effects ( $h^2 = 4\sigma_s^2 / (2\sigma_s^2 + \sigma_l^2)$ ).

Sources	Variance	Standard error of variance	Estimated heritability
Sire effect	0.00054	0.00014	
Dam effect	0.00064	0.00027	
Lamb within dam effects (residual)			
Kirby 25/5/09	0.0093	0.00150	0.21
Kirby 10/8/09	0.0122	0.0019	0.16
Kirby 20/10/09	0.00812	0.00132	0.23
Kirby 26/10/09	0.00798	0.00134	0.24
Trangie 13/1/09	0.00375	0.00072	0.45
Trangie 28/1/09	0.00364	0.00070	0.46
Trangie 4/6/09	0.00128	0.00053	0.91
Cowra, 4/12/08	0.00123	0.00039	0.93
Cowra, 15/1/09	0.00371	0.00088	0.45
Cowra, 4/6/09	0.00101	0.00051	1.03
Rutherglen, 3/3/09	0.00200	0.00058	0.70
Rutherglen, 21/4/09	0.00399	0.00091	0.42
Rutherglen, 19/5/09	0.00302	0.00075	0.53
Rutherglen, 26/8/09	0.00086	0.00043	1.11
Hamilton, 28/5/09	0.00703	0.00145	0.27
Hamilton, 24/6/09	0.00462	0.00110	0.38
Hamilton, 1/7/098	0.00441	0.00115	0.39
Hamilton 24/11/09	0.00225	0.00092	0.65
Struan, 13/1/09	0.00370	0.00099	0.45
Struan, 10/2/09	0.00305	0.00085	0.52
Struan, 11/8/09	0.00994	0.00336	0.20
Turretfield, 17/2/09	0.00358	0.00071	0.46
Turretfield, 17/3/09	0.00359	0.00070	0.46
Turretfield, 11/8/09	0.00954	0.00224	0.20
Katanning 4/2/09	0.0134	0.0024	0.15
Katanning, 17/2/09	0.0109	0.0020	0.18
Katanning, 24/2/09	0.0128	0.0022	0.16
Katanning, 12/5/098	0.0123	0.0022	0.16
Katanning, 10/11/09	0.00591	0.00106	0.31

**Table 7**

Relative contribution of different sources of variation of the logarithm (base 10) of muscle EPA + DHA, on a roughly comparable basis. The larger the relative contribution, the more important the source of variation.

Source of variation	Relative contribution	Method calculated
<i>Total variation in data</i>		
95% data range	0.97	97.5% quantile minus 2.5% quantile of raw data
<i>Components of variation</i>		
Sites and kills (excluding last kills)	0.76	Difference between largest and smallest predicted mean
Sites at last kill (with predominantly pure Merinos)	0.51	Difference between largest and smallest predicted mean
<i>Lamb within dam</i>		
Largest	0.46	4 times square root of variance at Katanning on 4/2/09
Smallest	0.14	4 times square root of variance at Cowra on 4/12/08
Sire	0.09	4 times square root of variance
Dam	0.10	4 times square root of variance
<i>Dam breed</i>		
Poll Dorset sires	0.03	Merino minus crossbred effect
White Suffolk sires	0.01	Merino minus crossbred effect
<i>Sire breed</i>		
Booroola-Poll Dorset	0.05	Effect averaged over sites with Booroola progeny
White Suffolk-Poll Dorset	0.00	Effect averaged over sites
Border Leicester-Poll Dorset	0.00	Effect averaged over sites
Rearing type	0.02	Single minus multiple effect averaged over sites
Gender	0.01	Female minus male effect

Fig. 2). The general trend was for EPA + DHA to be much higher when the sheep were mostly fed high quality green pasture, and much lower when pellets or grain with dry pasture was a large part of their diets pre-slaughter. For example, when lambs were grazing green pasture continuously until slaughter, the EPA + DHA levels were maintained above 30 mg/100 g muscle in all slaughter groups (Rutherglen and Cowra sites). At Struan, where the feeding of animals was changed from green pasture to dry pasture supplemented with grains, the EPA + DHA levels were lowered from 33 mg/100 g at the first kill to 10 mg/100 g muscle at the last kill. When the animals were initially grazing lower quality pasture supplemented with lower amounts of grains (250 g/day/head), but later were fed greater amounts of grain (750 g/day/head) and ultimately fed ad libitum grains or pellets, the levels of EPA + DHA were continually lowered over time (Turretfield and Kirby). At Hamilton and Katanning sites the last kill groups were elevated, compared to earlier kills, after a full spring season when good quality green pasture was available.

#### 4.2. Environmental effects on the between lamb variation of EPA + DHA

In addition to the median (or average) levels of EPA + DHA differing greatly with site and kill, the between lamb (residual) variation differed greatly between site and kill. We suggest that this most likely reflects differences in the between animal variation of the diet actually consumed by lambs. However, in the current study the diet of individual lambs was not measured, and thus we cannot directly attribute which aspect of diet is associated with between animal variation in EPA + DHA levels. Sheep are highly selective when grazing (Kyriazakis & Oldham, 1993), and thus it is expected that this would manifest itself in the composition of the fat. The quantity eaten, and the composition of diet selected, reflects the sheep's requirements for crude protein for maintenance and growth (Kyriazakis & Oldham, 1993) or the palatability of feed related to senescent stage (Colebrook, Black, Purser, Collins, & Rossiter, 1990). Cooper and Kyriazakis (1995) have reported that sheep make short-term changes in diet selection to promote effective rumen conditions for achieving a feed intake which allows for nutrient and energy needs to be met.

The main message is that, to maintain higher levels of health claimable fatty acids in lamb, it is likely that both the overall nutritional status of a flock and the nutritional status of individual animals are important. Thus we suggest that both the feed available and the feed consumed by

individual lambs are likely to be important in maintaining high omega-3 polyunsaturated fatty acid levels in meat.

#### 4.3. Meeting source of omega-3 guidelines

In the current study the combination of site and kill, together with the residual lamb within dam variance were found to be the greatest sources of variation for EPA + DHA (Table 7). These two sources of variation (site and kill) will largely determine the proportion of lambs that will meet Australian and New Zealand standards as a source of omega-3 (>30 g/135 mg serve). Since these two sources are environmental, our results suggest that if the system used in the current study represented lamb production systems in the wider population in Australia, the proportion of lambs meeting standards will largely be determined by environment (Fig. 1). Meat from lambs finished in some regions consistently fails to meet the claimable levels with current production systems when finished at certain times of the year. In this context, almost all lamb meat from Cowra, Rutherglen, Struan (except the last kill) and Hamilton can be considered as a source. Katanning and Turretfield sites did not satisfy the levels required to claim lamb meat as a source of omega-3. Meat from very few lambs met the higher Australian standard as a good source of omega-3 (>60 mg/135 g serve) or the European standard for source (40 mg/100 g) and good source (80 mg/100 g) of EPA + DHA (Commission Regulation of European Union, 2010).

Further research is needed to examine if the effect of site and kill date seen in this study represents any trends in the wider population and whether this is likely to jeopardise the legality of claims for omega-3 content of Australian lamb in a generic sense. If the trends account for large proportion of lamb produced then further appropriate interventions to production systems in the regions where natural levels of omega-3 concentrations are low could be developed mainly through changes in lamb genetics or finishing diet.

#### 4.4. Breed type effects on the levels of EPA + DHA in lamb

Lambs from Merino dams had about 2 mg/100 g higher levels of EPA + DHA than lambs from crossbred dams, when the sire breed was Poll Dorset. This is similar to Ponnampalam et al. (2009), who found that the ratio of polyunsaturated fatty acids to saturated fatty acids in meat increased from second cross Poll Dorset to first cross Poll Dorset and from first cross Poll Dorset to pure Merino. Ponnampalam et al. (2009) found that this appeared to be due to

an increase in polyunsaturated fats and not due to a decrease in saturated fats.

Sire breed effects on EPA + DHA were detected, but these effects were not consistent between sites. For instance, at Cowra and Kirby, but not at other sites, progeny from Border Leicester sires had lower EPA + DHA levels than progeny from some other sire breeds.

#### 4.5. Rearing type and gender effects on the levels of EPA + DHA in lamb

For several sites, lambs raised as singles had slightly greater levels of EPA + DHA than multiple reared lambs (about 2 mg/100 g muscle). The reason for this effect may be heavier birth weight or growth rate. However, single lambs are also likely to consume more milk during suckling (Gardner, Hogue, & Bensadoun, 1964), which may be a good source of pre-cursors for EPA + DHA. It is not surprising that the effect of single rearing is larger at some sites than others, because dam and lamb nutrition are likely to differ between sites.

Although very small in magnitude, there was a gender effect found on health claimable fatty acid content in lamb such that females showed greater levels than males. Human studies show that the conversion of short chain omega-3 fatty acid (ALA) to long chain omega-3 fatty acids (EPA and DHA) in women and men differ and is influenced by the composition of the diet as well as other factors. The ALA is converted to EPA and DHA in humans at ranges from 8 to 20% and 0.5 to 9%, respectively for women and men and women can convert better than men (Burdge, Jones, & Wootton, 2002; Stark, Crawford, & Reifen, 2008). As female lambs approach their reproductive stage, it is possible that they synthesise more long chain omega-3 fatty acid in the body for the production of series-3 eicosanoids that is associated with the ovulation process, conception and pregnancy. Others have reported that dietary fats favour reproductive function by supplying energy and by action on the reproductive process such as increased steroid and eicosanoid secretion, which could alter ovarian and uterine functions and affects pregnancy rates (Mattos et al., 2000; Santos, Bilby, Thatcher, Staples, & Silvestre, 2008).

#### 4.6. Inheritance of EPA + DHA in lamb

Our results show that sire variation is definitely present, although the present population differences between sires are several times less than the differences between sites and kills and, for the majority of kills, differences between the lamb within dam (residual) effects (Table 7). Dam variance was also detected to be different from 0, and its best estimate was similar to the sire variance. To aid in interpretation we have presented the random sources of variation of the logarithm of EPA + DHA, both as variances and an estimate of heritability. However, these estimates of heritability are based on only 1 cohort of lambs, do not utilise pedigree information, do not utilise between dam variance and are calculated on the logarithm of EPA + DHA. Estimates of heritability, that utilise pedigree information and 3 cohorts of lambs (including the present cohort), for selected fatty acids and other meat quality traits are reported by Mortimer et al. (2014–this issue).

## 5. Conclusions

The level of health claimable fatty acids, EPA + DHA, were mainly dependent on site and kill date. These environmental effects are at least an order of magnitude greater than the differences between sires or breeds in the present population. The level of health claimable long chain polyunsaturated omega-3 fatty acid (EPA + DHA) was highest in lambs grazing green pasture (Cowra, Rutherglen and Struan) and decreased when grain supplementation was applied to lambs during periods of low availability of quality pasture. The results indicate that EPA + DHA levels in muscle are sensitive to the diet available to lambs.

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