Final report

Project Code: A.TEC.0096

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Date published: November 2014

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 1961
NORTH SYDNEY NSW 2059

Using Computed Tomography to describe intramuscular fat content within a range of muscles in lamb, and genetic and non-genetic factors impacting this content

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

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Executive Summary

The aims of this project were:

1. Determine whether a relationship can be found between computed tomography (CT) scan image characteristics and intramuscular fat in lamb meat.
2. Determine whether this relationship is consistent within a range of muscles within the carcass
3. Determine how consistently intramuscular fat is distributed throughout other muscles of the carcass
4. Determine whether this distribution is influenced by carcass Australian Sheep Breeding Values.

In this experiment 400 lambs which were part of the Sheep CRC Information Nucleus Flock were used. These lambs were born in 2011 at the Katanning site in Western Australia. They were slaughtered and a number of carcass measurements made prior to CT scanning. Five muscles were dissected from the carcass (*M. longissimus lumborum*, *M. supraspinatus, M. infraspinatus,* and *M. semimembranosus*) and individually CT scanned and their IMF% subsequently determined. An association between the average pixel density of the pixels identified as being fat or lean was determined. Additionally, the genetic and non- genetic factors that impact IMF% across muscles was determined.

IMF% Prediction

- Correlations exist between the *M. longissimus lumborum* and the *M. supraspinatus* (0.34), and *M. infraspinatus* (0.34) in the fore-section, and the *M. semimembranosus* (0.4), and *M. semitendinosus* (0.4) in the hind section demonstrating that the IMF% in the *M. longissimus lumborum* can be extrapolated to other muscles within the carcase.
- Simple correlations are only slightly stronger than partial correlations indicating that between muscle correlations will not be excessively inflated when on-farm production factors such as sex, breed, birth type-rear type etc are unknown.

- CT density demonstrated the expected relationship with IMF%, increasing as IMF% decreased, but as a “stand-alone” measurement it was a poor predictor of IMF% (R^2 0.07 and RMSE 1.05).
- Cut described a large proportion of variation in IMF%, and when included in the model predicting IMF% this markedly improved the precision of prediction (R^2 0.25 and RMSE 0.93).
- The inclusion of additional measures in the model predicting IMF% such as hot carcase weight, GR tissue depth, carcase fat percentage and other on-farm production factors (sex, sire type, birth type-rear-type) further improved the precision of prediction (R^2 0.38 and RMSE 0.86).

IMF% Phenotypic Description

- The lowest IMF% was found in the *M. semimembranosus* (3.58%) and *M. infraspinatus* (3.86%), while the *M. supraspinatus* (4.87%) and the *M. semitendinosus* (4.54%) had the highest IMF%. The *M. longissimus lumborum* (4.21%) had mid-range IMF%.
- Merinos demonstrated lower IMF% than Maternal or Terminal sire breeds, but only in the *M. longissimus lumborum*, and *M. semitendinosus*. These differences were partly accounted for (in the *M. longissimus lumborum*) by differences in hot carcase weight.
- Decreasing post-weaning c-site fat depth breeding value reduced IMF% most strongly in the *M. longissimus lumborum*, having less impact in the hind section muscles, and no effect in the muscles of the fore section.
- Increasing post-weaning c-site eye muscle depth breeding value only had an effect in multiple born and single raised lambs, reducing IMF% across all muscles. This group represented only 10% of the lambs used in this study.
- For the majority of lambs in this study there was no effect of post-weaning weight breeding value on IMF%. The only impact evident was in the multiple born and single raised lambs, increasing IMF% across all muscles.
- Increasing lean meat yield % and decreasing hot carcase weight were both associated with marked reductions in IMF% across all muscles analysed

The use of computed tomography to predict intramuscular fat percentage in lambs.

Introduction

Intramuscular fat (IMF) has been identified as an important factor influencing the eating quality of lamb. In beef it accounts for up to 15 % of the variation in palatability (Dikeman 1987) and in lamb it is thought that a minimum of 4-5% IMF is required for consumer satisfaction with regard to palatability (Hopkins, Hegarty et al. 2006). It has been suggested that loin IMF% is a useful predictor of eating quality and therefore be used to maintain premium eating quality of lamb (Pannier, Gardner et al. 2013; Pannier, Pethick et al. 2013). Therefore it would be useful to be able to determine the IMF % of a cut or muscle in a rapid, non-destructive manner prior to sale, allowing for quality control, targeted marketing or value adding to cuts and feedback to suppliers with regards to meat quality.

A study by Brackebush *et al* (1991*)* revealed considerable variation in IMF% of muscles throughout the carcass. It also demonstrated a strong linear relationship with the IMF% of the *M. longissimus lumborum* on other muscle depots within the carcass. Therefore in cattle, measurement of IMF% in the loin can be used to predict IMF in other muscles. In sheep, the loin musculature is considered of premium quality and research in Australia has focused on the eating quality and intramuscular fat levels of this muscle (Pannier, Gardner et al. 2013; Pannier, Pethick et al. 2013). The IMF% of muscles other than the *M. longissimus lumborum* has not been well described. In lamb, a high correlation between the *M. longissimus lumborum* and other muscle depots would indicate measurement in this muscle alone would be useful in predicting IMF% elsewhere. This would be advantageous as a single site measurement may enable grading of eating quality and the development of an IMF% breeding value. Conversely, if there is poor correlation between muscles then IMF% may need to be predicted and assessed from sites other than the *M. longissimus lumborum*.

X-ray computed tomography (CT) has been used to accurately determine carcass composition of fat, muscle and bone in sheep and pigs (Kolstad, Jopson et al. 1996;

Simm, Lewis et al. 2001; Gardner, Williams et al. 2010). The use of CT to predict eating quality through assessment of IMF% and shear-force of the *M. longissimus lumborum* has been investigated (Lambe, Navajas et al. 2009). Karamichou *et al* (2006) demonstrated muscle CT density was correlated to meat quality traits. They showed the potential for CT muscle density to predict IMF%, juiciness, overall liking and flavour in the *M. longissimus lumborum* of lamb. Computed tomography may become an important tool to aid carcass quality control and carcass sorting prior to boning and sale. If this technology could demonstrate adequate prediction of IMF% in musculature then it may be used for the prediction of intramuscular fat in more muscles and cuts than just the loin (*M. longissimus lumborum)* so it is important to test the robustness of CT prediction across other muscles.

The Australian Cooperative Research Centre for Sheep Industry Innovation established an Information Nucleus Flock commencing in 2007 (Fogarty, Banks et al. 2007). In addition to the standard carcass measurements described by Fogarty (2007), 2000 animals underwent computed tomography (CT) scanning to determine proportion of fat, lean and bone. Of these CT scanned animals, 400 lambs born in Katanning in 2011 had additional samples collected to investigate the IMF% of multiple muscle depots. We hypothesise that the IMF% of the *M. longissimus lumborum* muscle will be correlated with the IMF% of other muscles examined. Additionally, we hypothesise that CT pixel density will adequately predict the IMF% of CT scanned muscles, allowing non-destructive rapid determination of IMF%.

Materials and Methods

Experimental design and slaughter details

Details of the design of the Sheep CRC's Information Nucleus Flock (INF) were presented by Fogarty *et al.* (2007). The lambs used in this experiment for the progeny of sires representative of a wide range of traits, including LMY% but all lambs were born and raised at Katanning in Western Australia, in 2011.

Lambs were yarded the day prior to slaughter and transported to a commercial abattoir in Katanning, held in lairage overnight and slaughtered the following day at a target average carcase weight of 23 kg. Carcasses were subjected to a medium voltage electrical stimulation. Carcasses were measured and sampled the day after

slaughter for a wide range of carcass and meat quality traits after being chilled overnight (4°C). Measurements taken immediately post slaughter were: hot carcass weight (HCWT); GR tissue depth at the 12th rib, 11cm from the midline and taken as the total tissue depth above the surface of the rib; C-site fat depth and eye muscle depth at the 12th rib, 45 mm from midline.

The carcasss were split into halves and then divided into their three primal components: fore-section, saddle and hind-section as a requirement for other components of the experiment not reported in this paper. The fore-section was separated from the saddle by a cut between the fourth and fifth ribs. The hind-section was separated from the saddle by a cut through the mid-length of the sixth lumbar vertebrae. The saddle was cut between the 12th and 13th ribs and the eye muscle depth measured in millimetres.

Samples

There were a total of 400 lambs born and raised at Katanning in 2011. Whole carcass CT scanning was performed on 382 lambs due to significant carcase imperfections in 19 carcases. Due to carcass imperfections all muscle types could not be obtained from each carcase (numbers of each muscle are reported in Table 1).

Computed tomography scanning

Carcases were transported for computed tomography (CT) scanning to Murdoch University (Picker PQ 5000 spiral CT scanner) within 72 hours of slaughter. Following CT scanning of the whole carcase the individual were dissected from fore and hind sections of the carcase, weighed and CT scanned individually: from the fore section the *M. supraspinatus* and *M. infraspinatus*; and from the hind section, *M. semimembranosus* and *M. semitendinosus*. The *M. longissimus lumborum* was not dissected from the saddle section and its weight calculated from the loin weight of the contralateral side of the carcase which was used for determination of IMF% and other carcase measurements required in other studies.

The spiral abdomen protocol was selected with settings: pilot scan length of 512mm, field of view set at 480, Index 20, mA 150, revs 40, pitch 1.5 and standard algorithm.

The carcase and individual muscles were scanned in 10mm slice widths, with each slice taken 10 mm apart. The muscles were analysed by taking a 'core' through the centre of each muscle extending for its length. The *M. longissimus lumborum* was analysed in situ within the saddle section in a similar fashion. The images produced from the CT scan were edited to remove non-carcase image artefacts and partitioned into bone, muscle and fat components (Image J version 1.37v, National Institutes of Health, Bethesda, MD, USA, used in conjunction with Microsoft Excel). The discrimination point to identify the hounsfield barriers for associating pixels with bone, muscle or fat were: –235 to 2.3 for fat, 2.4 to 164.3 for lean and greater than 164.3 for bone. Any of the pixels considered by the CT to be bone were excluded from the models with the average pixel density of the fat and muscle components subsequently determined. There were three methods used to determine average CT pixel density: average of fat only pixels; average of muscle only pixels and average of the combined fat and muscle pixels.

Intramuscular fat measurement

Approximately 40 g of diced loin muscle was collected in 50 ml tubes. Samples were stored at -20°C until subsequent freeze drying. **Samples were commercially freeze-dried using a Cuddon FD 1015 freeze dryer (**Cuddon Freeze Dry**, NZ).** The IMF % of each muscle was determined using a near infrared procedure (NIR) in a Spectro Star 2400. Calibration of the Spectro Star 2400 was achieved by the development of an equation for the estimation of percentage chemical fat from 160 muscle samples. Readings were validated with chemical fat determinations using chloroform solvent extraction. Weighed samples (approximately 6g) were packaged in filter paper and placed in a multi-sample Soxhlet chamber for 48 hours with chloroform used as the extracting solvent. Samples were dried for 72 hours at 80 °C prior to weighting ensuring all chloroform was evaporated from the samples. Intramuscular fat percentage was calculated as the difference between the sample weight before and after solvent extraction multiplied by the percentage dry matter of the sample. Approximately 10% of the samples analysed using the NIR were routinely monitored using chloroform solvent extraction.

Statistical analyses

The correlation between the IMF% in the five muscles (*M. longissimus lumborum, M. semimembranosus, M. semitendinosus, M. supraspinatus, M. infraspinatus*) was determined using a multivariate analysis (SAS Version 9.1, SAS Institute, Cary, NC, USA). The model included the fixed effects: sex within sire type, birth-type rear-type, sire type, kill group and dam breed within sire type and their first order interactions. Simple correlations between IMF% of each muscle were also determined using PROC CORR in SAS (SAS Version 9.1, SAS Institute, Cary, NC, USA).

A general linear model was used to predict IMF% (SAS Version 9.1, SAS Institute, Cary, NC, USA). A number of models were tested to reflect scenarios where varying amounts of information was available for predicting IMF%. These models included varying combinations of the following terms:

1. CT pixel density and muscle type
2. CT pixel density, muscle type and basic carcass measurements (HCWT, GR tissue depth)
3. CT pixel density and muscle type plus more detailed carcass measurements including eye muscle depth and C site fat depth.
4. CT pixel density and muscle type plus percentage of fat or lean tissue determined using CT scan.
5. CT pixel density and muscle type plus the inclusion of known production factors including sex, sire type, birth type-rear type, kill group, and dam breed. Sex and dambreed were both fitted within sire type, and in this experiment kill group in part described age, given that the average age for kill groups 1 to 4 was 167, 238, 280 and 355 days.

Results

The average IMF% of all muscles was 4.4±1.1 (Table 1). The muscles with the greatest range in IMF% were the *M. semimembranosus* and *M. supraspinatus* (Table 1), with these muscles also having the highest standard deviation. However for average CT pixel density the greatest range and standard deviation was seen in the *M.* i*nfraspinatus* and *M. s*e*mitendinosus.* The range of IMF% and average CT pixel density by muscle type is shown in Figure 1 The highest precision was obtained using the average of the pixels classified as fat and muscle, with raw data reported in Table 1.

Table 1. Sample numbers, intramuscular fat % and CT pixel density for the *M. semimembranosus, M. semitendinosus, M. supraspinatus, M. infraspinatus* and *M. longissimus lumborum*.

	n	**IMF% ± SD (max, min)**	**Average CT pixel density ± SD (max, min)**
All muscles	1908	4.4 ± 1.1 (2.2, 9.9)	46 ± 11.4 (5, 71)
M. semitendinosus	390	4.8 ± 1.2 (2.6, 9.1)	41 ± 6.4 (15, 63)
M. semimembranosus	391	3.7 ± 0.8 (2.2, 6.1)	55 ± 3.8 (40, 64)
M. supraspinatus	374	5 ± 1.1 (2.9, 9.9)	39 ± 6.2 (21, 60)
M. infraspinatus	374	4 ± 0.9 (2.2, 7.9)	36 ± 8.3 (5, 62)
M. longissimus lumborum	379	4.3 ± 0.8 (2.5, 8.1)	61 ± 3.7 (44, 71)

Average CT pixel density: average of the pixels classified as muscle and fat.

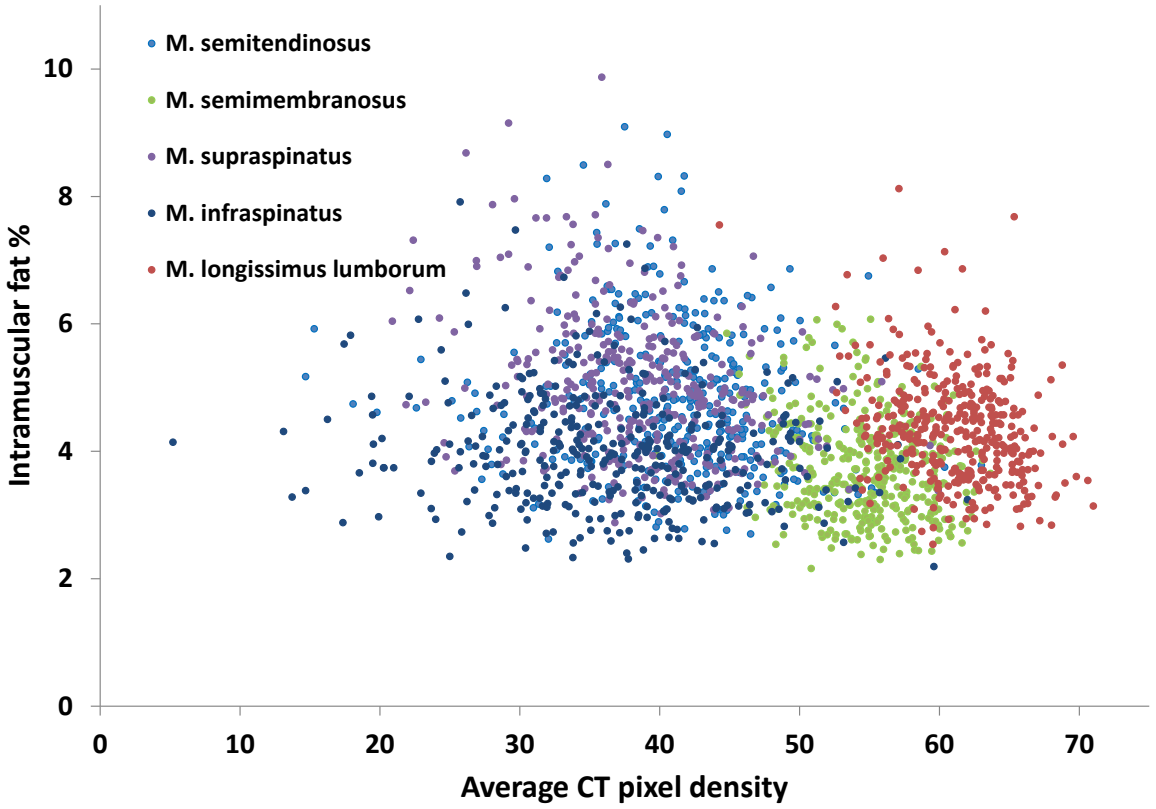

Figure 1. Raw data showing the intramuscular fat % of the *M. semimembranosus, M. semitendinosus, M. supraspinatus, M. infraspinatus* and *M. longissimus lumborum* as it relates to average CT pixel density of the fat and muscle pixels.

The correlation coefficients of IMF% between the five muscles were variable (Table 2), with the highest correlation coefficients evident between the *M. supraspinatus* and the *M. infraspinatus* (Table 2) in the fore section of the carcass. The correlation between the *M. longissimus lumborum* to the other muscles was fairly consistent with values ranging between 0.34 to 0.40 (partial correlation coefficient), and the weakest correlations were those between the forequarter and hindquarter muscles. Simple correlations demonstrated similar trends to the partial correlation coefficients, and were only mildly inflated above the partial correlation coefficient values.

Table 2. Partial correlation coefficients (above the diagonal) and simple correlation coefficients (below the diagonal) of the IMF% between the *M. semimembranosus, M. semitendinosus, M. supraspinatus M. infraspinatus* and *M. longissimus lumborum.*

	M. semi-membranosus	***M. semi-tendinosus***	***M. supra-spinatus***	***M. infra-spinatus***	***M. longissimus lumborum***
M. semimembranosus	1	0.43	0.34	0.41	0.39
M. semitendinosus	0.42	1	0.27	0.29	0.4
M. supraspinatus	0.41	0.3	1	0.67	0.36
M. infraspinatus	0.48	0.34	0.75	1	0.34
M. longissimus lumborum	0.45	0.47	0.45	0.45	1

Prediction of IMF% using average CT pixel density and muscle type.

There was a negative linear relationship ($P<0.01$) between IMF% and average CT pixel density which varied between muscles (Figure 2). As such, a combination of average CT pixel density and knowledge of muscle type provides adequate precision for predicting IMF% describing 25% ($R^2 = 0.25$) of the variation in IMF% with 2/3 of the data falling within 0.93 IMF% units (RMSE = 0.93) of the predicted value (see Model 3, Table 3). However most of the variation is explained by muscle alone. Therefore when the muscle is unknown the precision falls (R^2 0.07 and RMSE 1.05; Model 2, Table 3). This is further evidenced by the model which only contains the muscle type yet still predicts IMF% with a similar degree of precision to that also including average CT pixel density ($R^2 = 0.21$ and RMSE of 0.97; Model 1, Table 3).

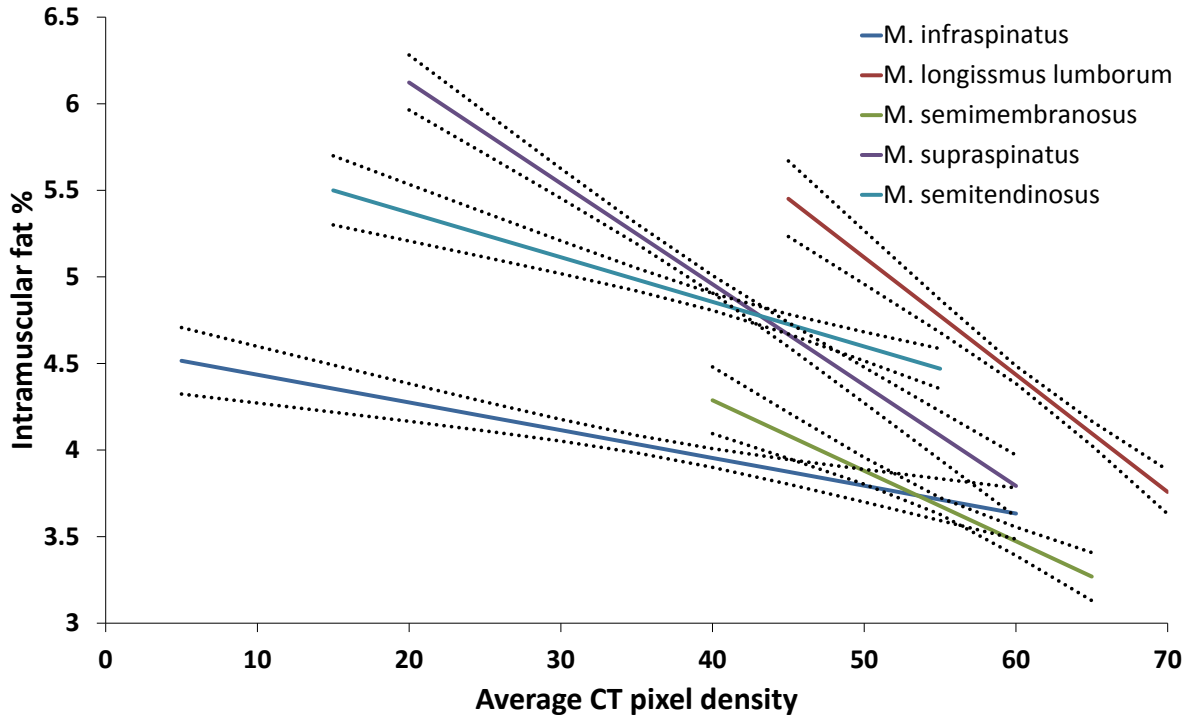

Figure 2. The prediction of intramuscular fat % using average CT pixel density and muscle type.

Prediction of IMF% using average CT pixel density, muscle type, and other carcase measures.

Including hot carcass weight (HCWT), and GR tissue depth (mm) with average CT pixel density and muscle, only marginally improved the precision of prediction (R^2 0.28 and RMSE 0.93; Model 7, Table 3) compared to CT pixel density and muscle type alone. Additional carcass tissue depth measures (Eye muscle depth (mm), and C-site fat depth (mm)) did not deliver any further improvements in precision (R^2 0.28 and RMSE 0.93; Model 11, Table 3). However whole body estimates of fat% and lean% derived from CT scans of the entire carcase provided a further improvement in precision for predicting IMF% (R^2 0.34 and RMSE 0.89, Model 17, Table 4).

Prediction of IMF% using average CT pixel density, muscle type, and on-farm information.

The inclusion of on-farm information also improved the prediction of IMF%. When all significant terms were incorporated as well as hot carcase weight and GR tissue depth the prediction of IMF% exceeded that demonstrated by the model containing CT fat% (R^2 0.38 and RMSE 0.86, Model 33, Table 5). Of the production factors tested, kill group (age) described the largest portion of variance (Model 33, Table 5)

Table 3. Table showing the prediction of IMF% using muscle type, CT density, Hot Carcass Weight, GR Tissue depth (mm), eye muscle depth (mm) and C-site fat depth (mm).

	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**	**Model 6**	**Model 7**	**Model 8**	**Model 9**	**Model 10**	**Model 11**
Muscle	125**	-	131**	-	125.8**	-	14.8**	-	123.86**	-	14.37**
CT Density	-	131**	82**	-	-	134.3**	87.2**	-	-	132.7**	86.01**
CT Density*muscle	-	-	6.59**	-	-	-	6.1**	-	-	-	5.91**
Hot carcass weight	-	-	-	5.59*	6.0*	3.52	3.81	5.24*	9.68*	4.21*	4.81*
GR tissue depth (mm)	-	-	-	32.3**	125.8**	32.9**	38.65**	22.02**	29.19**	22.42**	24.98**
Eye muscle depth (mm)	-	-	-	-	-	-	-	1.19	1.74	1.34	1.75
C-FAT (mm)	-	-	-	-	-	-	-	0.31	0.37	0.38	0.99
R-Square	0.21	0.07	0.25	0.03	0.23	0.1	0.28	0.025	0.24	0.09	0.28
Root MSE	0.97	1.05	0.94	1.08	0.96	1.04	0.93	1.08	0.96	1.04	0.929

*P<0.05, **P<0.01

Table 4. Table showing prediction of IMF% using percentage of fat and/or lean tissue, Hot Carcass Weight, GR Tissue depth (mm), muscle, CT density, eye muscle depth (mm) and C-site fat depth (mm).

	Model 12	**Model 13**	**Model 14**	**Model 15**	**Model 16**	**Model 17**	**Model 18**	**Model 19**	**Model 20**
Muscle	-	-	-	138.01**	-	11.88**	13.04**	11.88**	11.3**
CT Density	-	-	-	-	113.73**	43.18**	60.27**	43.18**	36.6**
CT Density*muscle	-	-	-	-	-	4.39**	5.19**	4.39**	4.1**
Hot carcass weight	0.94	1.03	1.19	1.45	0.66	0.73	0.48	0.73	0.89
GR tissue depth (mm)	1.35	5.57	6.34*	8.51**	3.89*	4.46*	2.53	4.46	2.16
Eye muscle depth (mm)	-	-	-	-	-	-	-	-	6.2*
C-FAT (mm)	-	-	-	-	-	-	-	-	6.1*
% Lean	97.97**	-	0.88	1.32	0.57	0.42	98.68**	0.42	0.27
% Fat	-	148.83**	49.13**	64.99**	41.1**	46.28**	-	46.28**	38.3**
R-Square	0.08	0.1	0.1	0.31	0.16	0.34	0.32	0.34	0.34
Root MSE	1.05	1.04	1.04	0.91	1.01	0.89	0.91	0.89	0.89

*P<0.05, **P<0.01

Table 5. Table showing prediction of IMF% using muscle type, CT density, on-farm information and carcass measurements.

	Model 21	**Model 22**	**Model 23**	**Model 24**	**Model 25**	**Model 26**	**Model 27**	**Model 28**	**Model 29**	**Model 30**	**Model 31**	**Model 32**	**Model 33**
Muscle	140.0**	-	-	11.5**	13.1**	15.1**	14.1**	14.4**	13.9**	14.1**	13.5**	13.8**	13.8**
CT Density	-	113.2**	-	35.7**	63.7**	73.2**	37.3**	67.3**	36.8**	37.3**	36.3**	42.4**	42.4**
CT Density*muscle	-	-	-	5.8**	6.3**	6.3**	5.8**	5.7**	5.6**	5.8**	5.3**	5.33**	5.33**
sex(sire type)	-	-	-	-	12.8**	14.7**	17.2**	24.4**	18.7**	17.2**	15.5**	8.57**	8.57**
Sire type	-	-	-	-	-	11.5**	32.3**	12.7**	21.2**	32.3**	23.3**	2.4	-
Kill group	76.8**	51.1**	59.4**	59.2**	-	-	72.8**	-	52.8**	72.8**	57*	56.7**	56.7**
Birth-type rear-type	-	-	-	-	-	-	-	35.5**	7.7**	-	4.3*	5.2**	5.2**
Dam breed(sire type)	-	-	-	-	-	-	-	-	-	-	14.1**	4.58*	4.58*
Hot carcass weight	-	-	-	-	-	-	-	-	-	-	-	0.75	0.75
GR tissue depth (mm)	-	-	-	-	-	-	-	-	-	-	-	34.4**	34.4**
R-Square	0.29	0.14	0.09	0.32	0.27	0.27	0.35	0.3	0.35	0.35	0.36	0.38	0.38
Root MSE	0.92	1	1	0.9	0.93	0.93	0.88	0.92	0.88	0.88	0.88	0.86	0.86

*P<0.05, **P<0.01

Discussion

Correlation of IMF% between muscles.

Aligning with our initial hypothesis, the IMF% within lamb muscles from the fore-section and hind-section were consistently correlated with the IMF% in the *M. longissimus lumborum*. This indicates that a measurement taken in the *M. longissimus lumborum* will be a useful predictor of IMF in other muscles of the carcass. Furthermore, the relatively similar values demonstrated through the partial versus simple correlation coefficients suggests that a single measurement taken on one muscle can be extrapolated to other muscles with little bias by production factors such as sex, sire type, etc. This demonstrates improved robustness of any method focused just on the *M. longissimus lumborum*. Correlations between other muscles of the carcase appeared to be more dependent upon co-location rather than absolute amount of IMF. This is evidenced by the fact that the highest correlations were seen between the muscles of the fore-section (*M. supraspinatus* and *M. infraspinatus*) and the hind-section (*M. semimembranosus,* and *M. semitendinosus*). These high correlations are in spite of the fact that these muscles have different functions, for example the *M. supraspinatus* is considered a stabilising muscle and the *M. infraspinatus*is used for extension and flexion of the shoulder joint. Also aligning with this general theme of correlations between co-localised muscles, the poorest correlation of IMF% was between the muscles of the fore and hind sections, and the *M. longissimus lumborum* had a moderate and similar correlation for IMF% with all other muscles. In beef, the correlations that exist between these muscles is higher, where the R^2 between the *M. longissimus lumborum* and the *M. supraspinatus, infraspinatus, M. semimembranosus,* and *M. semitendinosus* are 0.63, 0.69, 0.83 and 0.77 (Brackebrush, McKeith et al. 1991). This may be a reflection of a greater range in IMF% across which these R^2 were estimated. In conclusion, a single measurement of IMF% taken from the *M. longissimus lumborum* in lamb carcases is likely to be an adequate predictor of IMF% within the rest of the carcase.

Prediction of IMF% based on CT density and muscle.

In support of our initial hypothesis the average CT pixel density based on the Hounsfield units within each image was negatively associated with increasing IMF%.

As such average CT pixel density could be used to predict IMF%, albeit with relatively poor precision across muscles. Alternatively, if muscle is known (and used in the prediction model) then the precision of prediction of IMF% improved markedly, indicating that there are factors more influential than IMF% that elicit differences in density between muscles. This is further evidenced by the poor association of IMF% and average CT pixel density between muscles, an example being the *M.longissimus lumborum* which despite having the highest average CT pixel density did not have the lowest IMF%. The reason for these discrepancies is likely attributed to the fact that some muscles (for example the *M. longissimus lumborum*) appear homogeneous on cut section where as other muscles, such as the *M. infraspinatus* has a heterogeneous appearance based on their muscle structure and function. The amount of collagen may influence the average CT pixel density of the muscles and therefore interfere with the prediction of IMF between muscles. Another alternative that may contribute to muscle appearance on CT scan could be muscle fibre orientation or the wide range in muscle types examined. Previous studies in beef used sirloins (Prieto, Navajas et al. 2010) and sheep (ref) used loin. The *M. longissimus lumborum* muscle appears to be quite homogenous with the majority of the pixels classified as being muscle with fibres predominantly running parallel to each other. In contrast the *M. supraspinatus*, *M. infraspinatus* and *M. semimembranosus* have more multidirectional fibres and this may influence how they appear following CT.

The ability to predict IMF% using average CT pixel density alone within this study is less than that of previous studies. Lamb et al (2010) showed that using one CT scanner they were able to predict loin IMF% with similar precision in both live animals and dissected loins on the basis of muscle density alone (R^2 0.36 and 0.33). However, their precision of prediction diminished when data from multiple CT scanners was used, with R^2 similar to those obtained in our study.

Incorporating additional information for predicting IMF%

Given that industry routinely measures hot carcase weight and fat score at the GR site we tested the potential for CT to predict IMF% in the presence of these terms. This led to only a marginal improvement in the prediction of IMF%, with the R^2 increasing from 0.25 to 0.28. None-the-less, this aligns well with the work of Pannier

et al (2014) who demonstrated an association between IMF% and both hot carcase weight and GR tissue depth. Further work is currently underway in Australia to develop additional carcase measurements of eye muscle depth (mm) and C-site fat depth (mm) to improve the precision of predicting lean meat yield. However, these additional measurements provided no further improvement in prediction precision for IMF% and would therefore not be of specific benefit for inclusion in a prediction equation. Alternatively, using whole carcase measurements of CT fat% did provide a marked improvement in yield prediction. Furthermore, if CT measurements were available, then IMF% could just as readily be predicted from CT fat%, with relatively little further information provided by CT pixel density.

Finally the Australian industry is moving towards improved individual animal tracking. Therefore information regarding individual animal production factors such as sex, birth type-rear type, etc may become available. As such we also tested the addition of pre-slaughter information into the prediction equation and saw improved accuracy of prediction of IMF%. In particular there was a marked improvement in the prediction of IMF% when kill group was included in the model. Although potentially confounded by specific day effects kill group is likely to largely reflect the impact of age/maturity which has previously been demonstrated to increase IMF% (Pannier, Pethick et al. 2013). As such knowledge of the animals' age offers the most potential for improving the prediction of IMF%. This improvement in the prediction of IMF% using pre-slaughter production information is in contrast to work by Prieto (Prieto, Navajas et al. 2010) who used the CT pixel density only as additional information did not further improve the precision of IMF% prediction. They predicted IMF% in two breeds of cattle with R^2 values of 0.76 and 0.71 based on CT pixel density only. One possibility to explain this discrepancy may be associated with larger cell size in the muscle of cattle compared to lamb (Brackebrush, McKeith et al. 1991; Greenwood, Gardner et al. 2006; Greenwood, Tomkins et al. 2009). Given they pixel resolution of the CT images, this may result in better tissue/density differentiation between pixels, potentially amplifying the density differences between high and low IMF samples.

Conclusion

The IMF of the *M. longissimus lumborum* correlated with each of the other muscles in this study, with the strength of this correlation similar for all muscles. However, the strongest correlations in IMF% existed between muscles located within the same region of the carcase. The scanning of lamb carcasses using a CT scanner has the ability to predict IMF% within individual muscles of the carcass, however the precision was relatively poor. The majority of the variation in IMF% between muscles was described by knowledge of the muscle type alone. However, if used in conjunction with pre-slaughter information and carcass measurements, particularly CT fat%, the prediction of IMF% was greatly improved.

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The impact of selection for improved lean meat yield% on the intramuscular fat% of lambs.

Introduction

Consumers have an increasing desire for lamb which produces large cuts of meat that are also lean, in both domestic and international markets (Pethick, Banks et al. 2006). The Australian lamb industry has responded to market drivers too select for larger, lean lambs (Hall, Kelf et al. 2000; Banks 2002; Laville, Bouix et al. 2004). Health characteristics such as low levels of fat will remain important (Harper and Pethick 2004), however eating quality has also been shown to be important to consumers (Harper and Pethick 2004). Intramuscular fat % (IMF%) is a key determinant of eating quality in red meat and it is well accepted that IMF% has a positive impact on flavour, juiciness and tenderness (Shorthose and Harris 1991; Thompson 2004; Hopkins, Hegarty et al. 2006). In beef, IMF% accounts for between 10 to 15% of the variation in palatability (Dikeman 1987) mainly through its contribution to juiciness and flavour. In lamb it is thought that a minimum of 4-5% IMF is required for consumer satisfaction with regard to palatability (Hopkins, Hegarty et al. 2006). As such, loin IMF% has been identified as a key factor for maintaining premium eating quality of lamb (Pannier, Gardner et al. 2013; Pannier, Pethick et al. 2013).

Given that the loin musculature contains the highest value cuts in the carcase previous research has focused on the eating quality and intramuscular fat levels of this muscle (McPhee, Hopkins et al. 2008; Pannier, Gardner et al. 2013; Pannier, Pethick et al. 2013). As such, the IMF% of muscles other than the *M.longissimus lumborum* has not been well described in lamb. These levels are likely to vary between muscles in part as a consequence of function variation in muscle fibre type (Hocquette, Gondret et al. 2010). Within a muscle, more oxidative fibres contain more phospholipids and triglycerides (Hocquette, Gondret et al. 2010). Muscles responsible for maintenance of posture tend to be more oxidative and are predominantly comprised of Type 1 fibres with a propensity for higher IMF% (Picard, Lefaucheur et al. 2002). Therefore postural muscles that have an increase in oxidative capacity are likely to have greater levels of IMF%.

A key factor driving reduced IMF% in the *M. longissimus lumborum* is selection for lean growth. In particular, Pannier et al (2013) demonstrated an association between various carcass indicators of fatness and IMF% in the *M. longissimus lumborum*, and in this same muscle Gardner et al. (Gardner, Williams et al. 2010) reported a negative phenotypic correlation (-0.24) and genetic correlation (-0.46) between IMF% and lean meat yield percentage (LMY%). Selection for improved LMY% in Australian sheep is performed indirectly through the use of Australian Sheep Breeding Values (ASBVs) for post weaning weight (PWWT), and c-site eye muscle depth (PEMD) and reduced c-site fat depth (PFAT; c-site defined as a measure 45 mm from the midline over the 12th rib). Pannier et al (2013) demonstrated that both PFAT and PEMD reduced IMF% in the *M. longissimus lumborum*. It is likely that the impact of these breeding values may in part be delivered through their impact on muscle hypertrophy, in effect diluting the intramuscular fat content (Hocquette, Gondret et al. 2010). Previous work suggests that this muscle hypertrophy effect is likely to be more focused on muscles in the saddle section of the carcass. Lambs from high PEMD sires had increased weight of the *M. longissimus lumborum*, which is located in the saddle section (Gardner, Williams et al. 2010), plus Anderson *et al* (2013) demonstrated that PEMD increased lean weight in the saddle proportionately more than in other regions of the carcass. Likewise, Anderson (2013) demonstrated a similar effect of PFAT, which also caused a greater increase in saddle lean than in other regions of the carcass. Therefore it seems likely that the impact of these breeding values, delivered through their effects on muscle hypertrophy, will be greater in the saddle musculature.

Lambs from sires selected for high PWWT may also have reduced IMF%. These lambs are faster growing due to their larger mature size (Huisman and Brown 2008), thus when compared at the same carcass weight they will be leaner as they are less mature and subsequently have reduced IMF%. However, in the study of Pannier (2013) there was no effect of PWWT on IMF% in the *M. longissimus lumborum*, suggesting that any maturity linked effect may be too subtle to impact. This is likely to extend to other muscles of the carcass.

Therefore, given the negative phenotypic correlation of LMY% and IMF% we hypothesise that as LMY% increases IMF% will decrease. Based on the impact of ASBVs used to improve LMY% on carcass composition, we hypothesise that lambs from high PEMD sires or from low PFAT sires will have reduced IMF% in the saddle and to a lesser extent in the hind and fore sections, and that increasing sire PWWT will have no impact on IMF% in the carcass. Lastly, we hypothesise that the IMF% of muscles in postural regions of the carcase will have greater IMF% than muscles in locomotive regions.

Materials and methods.

Experimental design and slaughter details

The Australian Cooperative Research Centre for Sheep Industry Innovation established an Information Nucleus Flock commencing in 2007 (Fogarty, Banks et al. 2007). This paper examines data from 400 lambs born at Katanning, Western Australia in 2011. The lambs were progeny of sires representative of a wide range of traits, including PEMD, PFAT and PWWT (Table 6). In addition to the standard carcass measurements described by Fogarty (2007), the lambs underwent computed tomography (CT) scanning to determine proportion of fat, lean and bone and had additional samples collected to investigate the IMF% of muscle depots in the fore, saddle and hind sections of the carcass.

Table 6. Number of sires and mean (min, max) of Australian Sheep Breeding Values for each sire type.

Sire type	No. of sires	PWWT (kg)	PFAT (mm)	PEMD (mm)
Maternal	16	5.9 (-3.1, 12.4)	-0.8 (-2.1, 0.6)	-0.1 (-1.6, 1.8)
Merino	35	2.7 (-3.6, 10.8)	-0.1 (-1.4, 1.9)	0.1 (-2.6, 2)
Terminal	46	13.3 (7.3, 18.6)	-0.44 (-1.7, 1.3)	1.5 (-0.7, 3.8)

PWWT: post weaning weight; PFAT: Post weaning c-site fat depth; PEMD:Post weaning c-site eye muscle depth

Lambs were yarded the day prior to slaughter and transported to a commercial abattoir in Katanning, held in lairage overnight and slaughtered the following day at a target average carcass weight of 21kg. Carcasses were subjected to medium voltage electrical stimulation (Pearce, Van de Ven et al. 2010), and then sampled the

day after slaughter for a wide range of carcass and meat quality traits after being chilled overnight (4°C).

Sample collection and measurement

Hot carcass weight (HCWT) was measured after slaughter and GR tissue depth (11cm from the midline to the lateral surface of the 12th rib) and eye muscle area (between 12th and 13th rib) were measured on the carcass post slaughter. Carcasses were transported to Murdoch University to undergo computed tomography (CT) scanning (see Computed Tomography). Following CT scanning of the carcass, the individual muscles were dissected from each carcass and weighed: from the fore section, the *M. supraspinatus* and *M. infraspinatus*; and from the hind section, *M. semimembranosus* and *M. semitendinosus*. The *M. longissimus lumborum* was not dissected from the saddle section and its weight calculated from the loin weight of the contralateral side of the carcass which was also used for determination of IMF% and other carcass measurements required for other studies. Due to carcass imperfections all muscles could not be obtained from each carcass.

Computed tomography scanning

Carcasses were transported for computed tomography (CT) scanning to Murdoch University within 72 hours of slaughter. Whole carcass CT scanning was performed using a Picker PQ 5000 spiral CT scanner on 382 lambs. There were 19 carcasses that underwent significant post-slaughter trimming and were therefore excluded. The spiral abdomen protocol was selected with settings: pilot scan length of 512mm, field of view set at 480, Index 20, mA 150, revs 40, pitch 1.5 and standard algorithm. Each carcass was scanned in 10mm slice widths, with each slice taken 10 mm apart. Prior to scanning the carcasses were split into three primal components: fore-section, saddle and hind-section. The fore-section was separated from the saddle by a cut between the fourth and fifth ribs. The hind-section was separated from the saddle by a cut through the mid-length of the sixth lumbar vertebrae.

The images produced from the CT scan were edited to remove non-carcass image artefacts and partitioned into bone, muscle and fat components (Image J version 1.37v, National Institutes of Health, Bethesda, MD, USA, used in conjunction with Microsoft Excel). The discrimination point to identify the hounsfield barriers for

associating pixels with bone, muscle or fat were –235–2.3 for fat, 2.4–164.3 for lean and >164.3 for bone. An estimate of volume using cavalieri's (Gundersen and Jensen 1987; Gundersen, Bendtsen et al. 1988) was calculated as follows:

$$\text{Volume}_{Cav} = d \times \sum_{g=1}^{m} \text{area}_g - t \times \text{area}_{max}$$

where m is the number of CT scans taken and d is the distance the CT scans are apart, in this case 1 cm. The value of t is the thickness of each slice (g), in this example 1 cm, and area max is the maximum area of any of the m scans.

The average of the hounsfield units of the pixels of each component was then determined and converted into density (kg/L) using a linear transformation (Mull 1984). This was then used along with the volume of each component to determine the weight of fat, lean and bone, which was then expressed as a percentage of total carcass weight at the time of scanning. Given the density of the marrow tissue, it is classified as either fat or lean using the boundary discrimination method described above. Additional editing within Image J enabled the isolation of the marrow component of bone within all images. Thus the above procedures could be repeated on the 'marrow only' images. This enabled back correction for these pixels, reallocating them as bone and removing their associated volumes from the lean and fat components of the first iteration of image analysis. Thus using the CT scans we were able to determine the percentage of fat, lean and bone within each carcass and therefore LMY% of the carcass as a whole and within each section.

Intramuscular fat measurement

Approximately 40 g of diced muscle was collected from each of the 5 muscles in 50 ml tubes. Samples were stored at -20°C until subsequent freeze drying. Samples were commercially freeze-dried using a Cuddon FD 1015 freeze dryer (Cuddon Freeze Dry**,** NZ). The IMF % of each muscle was determined using a near infrared procedure in a Spectro Star 2400. Calibration of the Spectro Star 2400 was achieved by the development of an equation for the estimation of percentage chemical fat from 160 muscle samples. Readings were validated with chemical fat determinations using chloroform solvent extraction. Weighed samples (approximately 6g) were

packaged in filter paper and placed in a multi-sample Soxhlet chamber for 48 hours with chloroform used as the extracting solvent. Samples were dried for 72 hours at 80 °C prior to weighing ensuring all chloroform was evaporated from the samples. Intramuscular fat percentage was calculated as the difference between the sample weight before and after solvent extraction multiplied by the percentage dry matter of the sample. Approximately 10% of the samples analysed using the near infrared spectroscopy were routinely monitored using chloroform solvent extraction.

Statistical analyses

The IMF levels were analysed using linear mixed effect models (SAS Version 9.1, SAS Institute, Cary, NC, USA). The base model included fixed effects for muscle type (*M. semimembranosus, M. semitendinosus, M. longissimus lumborum, M. supraspinatus, M. infraspinatus*), sex within sire type (Merino wether, Maternal wether, Terminal female and Terminal wether), birthing and rearing type (term representing if lamb was born and reared as a single, born as multiple and raised as single or born and raised as a multiple), sire type (Merino, Maternal, Terminal), kill group (named with a prefix of the average age in days of the lambs at slaughter: 167K11, 238K11, 280K11 and 355K11) and dam breed within sire type (Merino x Merino, Maternal x Merino, Terminal x Merino and Terminal x Border Leicester-Merino). Sire identification, dam identification and animal identification were included as random terms. All relevant first order interactions between fixed effects were tested and non-significant (P>0.1) terms were removed in a stepwise manner.

The association between IMF% and sire ASBVs for PWWT, PEMD and PFAT were tested in the derived base model. These ASBVs were initially included concurrently as covariates along with linear and quadratic interactions with the fixed effects. Non-significant (P>0.1) terms were removed in a stepwise manner. Correlations exist between the three ASBVs, therefore this process was repeated with ASBVs included one at a time to determine the independence of their effects.

The base and ASBV models described above were additionally tested with HCWT included in the model as a covariate to determine if the effects are associated with their correlated impacts on HCWT. The impact of LMY% was also assessed by including these terms in the base and ASBV models. This was analysed with whole

carcass lean %, corrected for whole carcass CT weight (kg) included in the base and ASBV models. The relevant linear and quadratic interactions with these covariates was also included.

Descriptive Statistics

The data analysed in the base model has been summarised in Table 7 and Table 9. Of the total of 400 animals available for sampling, there were 1908 muscles obtained. The raw mean IMF% of all muscles (n = 1908) was 4.4±1.1 (Table 7). The muscles with the greatest range in IMF% were the *semimembranosus and semitendinosus* (Table 7), with these muscles also having the highest standard deviation. The raw mean data for hot carcase weight, carcase lean percentage, and age are shown in Table 2.

Table 7. Intramuscular fat % of the 5 muscle types **±** SD (max, min)

	n	**IMF% ± SD (max, min)**
All muscles	1908	4.4 ± 1.1 (2.2, 9.9)
M. semitendinosus	390	4.8 ± 1.2 (2.6, 9.1)
M. semimebranosus	391	3.7 ± 0.8 (2.2, 6.1)
M. supraspinatus	374	5 ± 1.1 (2.9, 9.9)
M. infraspinatus	374	4 ± 0.9 (2.2, 7.9)
M. longissimus lumborum	379	4.3 ± 0.8 (2.5, 8.1)

Table 8. Raw mean **±** s.d. (min, max) for intramuscular fat and hot carcass weight (kg), carcass lean percentage and age (days).

		Hot carcass weight (kg) (min, max)	**Percentage of lean in carcass (min, max)**	**Age (days) (min, max)**
Birth type-rear type				
Born and raised single		20.9 ± 2.8 (15.3, 29.0)	59 ± 3 (52, 66)	234 ± 72 (162, 364)
Born multiple-raised single		21.4 ± 2.5 (17.0, 27.3)	59 ± 3 (54, 65)	261 ± 61 (166, 361)
Born and raised as multiple		21.9 ± 2.9 (13.5, 27.8)	58 ± 3 (51, 65)	281 ± 58 (165, 362)
Dam breed (sire type)	***Sex***			
Maternal x Merino	Wether	21.1 ± 2.5 (13.9, 26.4)	58 ± 3 (51, 66)	296 ± 57 (164, 364)
Merino x Merino	Wether	19.6 ± 2.3 (13.5, 24.6)	59 ± 2 (54, 0.64)	339 ± 33 (232, 361)
Terminal x Merino	Wether	22.5 ± 2.8 (17.0, 29.0)	60 ± 2 (55, 66)	237 ± 41 (162, 352)
Terminal x Merino	Female	21.8 ± 2.6 (17.0, 27.1)	58 ± 3 (53, 63)	246 ± 48 (168, 352)
Terminal x Border Leicester-Merino	Wether	22.0 ± 3.1 (17.5, 27.7)	60 ± 3 (54, 65)	207 ± 47 (162, 287)
Terminal x Border Leicester-Merino	Female	22.2 ± 2.9 (16.5, 28.4)	58 ± 3 (52, 65)	212 ± 52 (162, 362)
Kill group				
167 Katanning 2011		19.6 ± 2.2 (16.5, 26.6)	61 ± 2 (56, 66)	167 ± 3 (162, 175)
238 Katanning 2011		24.6 ± 1.7 (20.8, 29.0)	58 ± 2 (52, 63)	238 ± 3 (230, 244)
280 Katanning 2011		21.1 ± 2.0 (16.2, 25.8)	57 ± 2 (51, 63)	280 ± 4 (271, 287)
355 Katanning 2011		20.7 ± 2.8 (13.5, 27.4)	58 ± 2 (52, 64)	355 ± 4 (346, 364)

Kill group: average age of lambs at slaughter, location , birth year.

Table 9. Number of progeny analysed in the base model according to sex, sire type, birthing and rearing type, dam breed and kill group.

	Sex		**Birth-rearing type**			**Dam breed**		**Kill group**			
	Female	**Wether**	**Single born and raised**	**Born as multiple-raised as single**	**Born and raised as multiple**	**Merino**	**BLM**	**167K11**	**238K11**	**280K11**	**355K11**
Maternal	0	92	34	6	52	92	0	6	16	32	38
Merino	0	70	32	10	28	70	0	0	1	13	56
Terminal	111	127	96	24	117	140	95	95	83	55	5
Total	111	289	162	40	197	302	95	101	100	100	99

BLM: Border Leicester-Merino

Kill group: average age of lambs at slaughter; K= Katanning; birth year (2011)

Results

Effect of non-genetic factors

The base model used 1900 of the 2005 observations available, after excluding animals with missing data and described 52% of the total variance in IMF%. The IMF% varied between all muscles (*P*<0.01, Table 10) by as much as 1.3%. The overall ranking of IMF% from highest to lowest was *M. supraspinatus* (4.87±0.1), *M. semitendinosus* (4.54±0.1), *M. longissimus lumborum* (4.21±0.1), *M. infraspinatus* (3.86±0.1), *M. semimembranosus* (3.58±0.1). Within the Terminal sired lambs, females (4.59±0.08) had on average 0.2% more IMF (*P*<0.01, Table 10) than wether lambs (4.38±0.08). Birth type-rear type impacted on IMF%, however this was only evident in the Merino sired lambs (*P*<0.05, Table 10), with the multiple born and raised lambs having 0.5 IMF% higher than that of the single born and raised or multiple born and single raised lambs. The IMF increased with each successive kill group (*P*<0.01, Table 10), and on average there was an increase of 1.3 IMF% between the first and last kill groups (Table 11). This increase varied between muscles (*P*<0.01, Table 10), with the greatest increase seen in the *M. supraspinatus*, of 1.8 IMF%, and the smallest increase seen in the *M. semimembranosus,* with a 0.9 unit increase in IMF% (Table 11).

When the model was corrected for HCWT the impact of kill group was reduced, with IMF% differing by only 1% over the four kill groups. The impact of sex and birth type-rear type, and the differences between muscles remained unchanged. Including CT Lean % and carcase weight at scanning had no effect on any of these terms described.

Effect of sire type and dam breed

In the base model, sire was significant at *P*= 0.08. Comparison of IMF% between sire types was possible only in the male progeny of Merino dams. The Merino sired lambs had on average 0.15 and 0.32 less IMF% than the Maternal and Terminal sired lambs (*P*<0.01, Table 10). The greatest difference in IMF% was seen in the *semitendinosus* where the Merino sired lambs (3.95± 0.14) had 0.71 and 0.65 IMF% units less than both the Terminal and Maternal sired animals. In the *M. longissimus lumborum,* the Merino sired lambs had 0.19 IMF% units less than the Maternal sired lambs, but were not different compared to Terminal sired lambs. There were no differences between sire types in the other muscles. The impact of dam breed on IMF% was assessed in the Terminal sired lambs where it varied between muscles (*P*<0.01, Table 10). The Border Leicester-Merino dams produced lambs that had 0.25, 0.37 and 0.52 more IMF% in the *M. infraspinatus*, *M. supraspinatus* and *M. semitendinosus* than lambs of Merino dams (Table 11). A similar trend was seen in the *M. longissimus lumborum* although this only bordered on significance (*P*<0.1, Table 10).

Correcting the model for HCWT accounted for the difference between sire types in the *M. longissimus lumborum*, and partly accounted for the difference in the *M. semitendinosus*, with the Merinos having only 0.5 IMF% less in the *M. semitendinosus* than the Maternal and Terminal sired lambs. The difference between dam

Table 10. F values plus numerator and denominator degrees of freedom for the effects of the base linear mixed effects model plus corrected for hot carcass weight, % CT Lean and Australian Sheep Breeding Values on intramuscular fat (%) of the *M. semimembranosus, M. semitendinosus, M. supraspinatus, M. infraspinatus* and *M. longissimus lumborum*

Effect	Model not corrected for HCWT NDF, DDF	F-value	Model corrected for HCWT NDF, DDF	F-value	Model corrected for CTLean % NDF, DDF	F-value	Model corrected for ASBVs NDF, DDF	F-value
Muscle	4, 1476	153.31***	4, 1423	0.47	4, 1446	150.98***	4, 1407	104.14***
Sex(sire type)	1, 1476	7.58***	1, 1423	5.8**	-	-	1, 1407	7.43***
Birth-type rear-type	2, 1476	3.45**	2, 1423	3.93**	2, 1446	5.83***	2, 1407	5.12***
Sire type	2, 1476	7.8***	2, 1423	3.07**	2, 1446	3.24**	2, 1407	3.12**
Kill group	3, 1476	29.77***	3, 1423	23.85***	3, 1446	25.06***	3, 1407	25.87***
Dam breed(sire type)	1, 1476	7.46***	1, 1423	4.51**	1, 1446	2.37	1, 1407	6.06**
Muscle*sire type	8, 1476	3.52***	8, 1423	1.88*	8, 1446	3.68***	8, 1407	4.1***
Muscle* kill group	12, 1476	5.69***	12, 1423	4.43***	12, 1446	5.35***	12, 1407	5.19***
Muscle*dam breed(sire type)	4, 1476	6.54***	4, 1423	5.39***	4, 1446	7.11***	4, 1407	6.72***
Birth-type rear-type*sire type	4, 1476	3.21**	4, 1423	3.01**	4, 1446	2.92**	4, 1407	2.69**
HCWT	-	-	1, 1423	13.25***	-	-	-	-
HCWT*muscle	-	-	4, 1423	2.57**	-	-	-	-
HCWT*sire type	-	-	2, 1423	2.22	-	-	-	-
HCWT*muscle*sire type	-	-	8, 1423	2.14**	-	-	-	-
CT Weight (kg)	-	-	-	-	1, 1446	7.55***	-	-
CT Weight (kg)*CT Weight (kg)	-	-	-	-	1, 1446	6.73***	-	-
CT Lean %	-	-	-	-	1, 1446	44.77***	-	-
PWWT	-	-	-	-	-	-	1, 1407	0.64
PWWT*birth-type-rear-type	-	-	-	-	-	-	2, 1407	4.63***
PFAT	-	-	-	-	-	-	1, 1407	0.65
PFAT*muscle	-	-	-	-	-	-	4, 1407	2.31*
PFAT*PFAT	-	-	-	-	-	-	1, 1407	0.06
PFAT*PFAT*muscle	-	-	-	-	-	-	4, 1407	2.13*
PEMD	-	-	-	-	-	-	1, 1407	2.5
PEMD*birth-type-rear-type	-	-	-	-	-	-	2, 1407	3.05**
PEMD*PEMD	-	-	-	-	-	-	1, 1407	0.38
PEMD*PEMD*birth-type-rear-type	-	-	-	-	-	-	2, 1407	3.42**

NDF, DDF: numerator and denominator degrees of freedom.
* $P < 0.1$
** $P < 0.05$

*** $P < 0.01$

Table 11. . Least squared means ± standard error for the base model (not corrected for hot carcass weight)

Sex	Dam breed	Sire type	*M. infraspinatus*	*M. longissimus lumborum*	*M. semimembranosus*	*M. supraspinatus*	*M. semitendinosus*
			Least Squared Means ± SE				
Wether	Merino	Maternal	3.78± 0.13	4.33± 0.13	3.63± 0.13	4.83± 0.13	4.60± 0.13
Wether	Merino	Merino	3.71± 0.15	3.89± 0.15	3.36± 0.15	4.66± 0.15	3.95± 0.15
Female	BLM	Terminal	4.24± 0.23	4.54± 0.23	3.74± 0.23	5.29± 0.23	5.54± 0.23
Female	Merino	Terminal	4.02± 0.15	4.45± 0.15	4.00± 0.15	5.05± 0.15	4.98± 0.15
Wether	BLM	Terminal	4.18± 0.15	4.45± 0.15	3.62± 0.15	5.31± 0.15	5.12± 0.15
Wether	Merino	Terminal	3.88± 0.14	4.15± 0.13	3.69± 0.13	4.80± 0.14	4.66± 0.13
Kill group							
167K11			3.18± 0.14	3.59± 0.14	3.39± 0.14	4.04± 0.14	3.97± 0.14
238K11			3.51± 0.12	4.09± 0.11	3.30± 0.11	4.60± 0.12	4.41± 0.12
280K11			4.08± 0.10	4.39± 0.10	3.37± 0.10	4.98± 0.10	4.62± 0.10
355K11			4.67± 0.11	4.75± 0.11	4.28± 0.11	5.86± 0.11	5.17± 0.11

Kill group: average age of lambs at slaughter; K= Katanning; birth year (2011)

breeds was relatively unchanged. Including CT Lean % and carcase weight at scanning had no additional effect on either the sire type or dam breed differences.

Effect of Australian Sheep Breeding Values

When the sire ASBVs for PWWT, PFAT and PEMD were included at the same time in the base linear mixed effects model, all three demonstrated a significant effect (P<0.1, Table 10). The impact of sire PWWT on IMF% varied with different birthing and rearing types (P<0.01, Table 10). As sire PWWT increased, lambs that were born as a multiple and raised as a single had an increase in IMF% of 1.5% across the range of PWWT ASBVs or 0.08% per unit increase in PWWT ASBV (Figure 3). There was no effect in the other birth type-rear type groups.

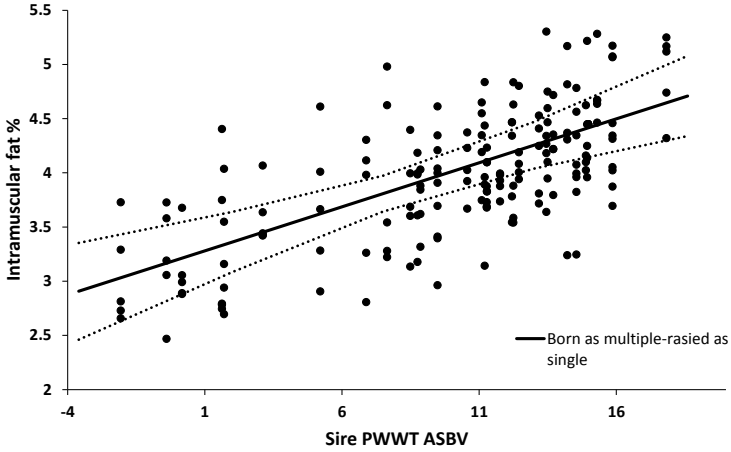

Figure 3. The association between sire post weaning weight (PWWT) Australian Sheep Breeding Value (ASBV) and intramuscular fat for the lambs born as multiples and raised as singles..

The impact of sire PFAT ASBV differed between muscles (P<0.05, Table 10). Across a 2.75 unit range (1.0 to -1.75) of decreasing PFAT values, IMF% in the *M. semitendinosus* and *M. semimembranosus* decreased by 0.4% units and the *M. longissimus lumborum* decreased by 0.3% units. The fore section muscles showed no change in IMF% in response to decreasing sire PFAT (Figure 4 a, b and c). There was only one lamb with a sire PFAT value greater than 1.5mm, however when this animal was removed from the analysis there was no change to the magnitude of the responses.

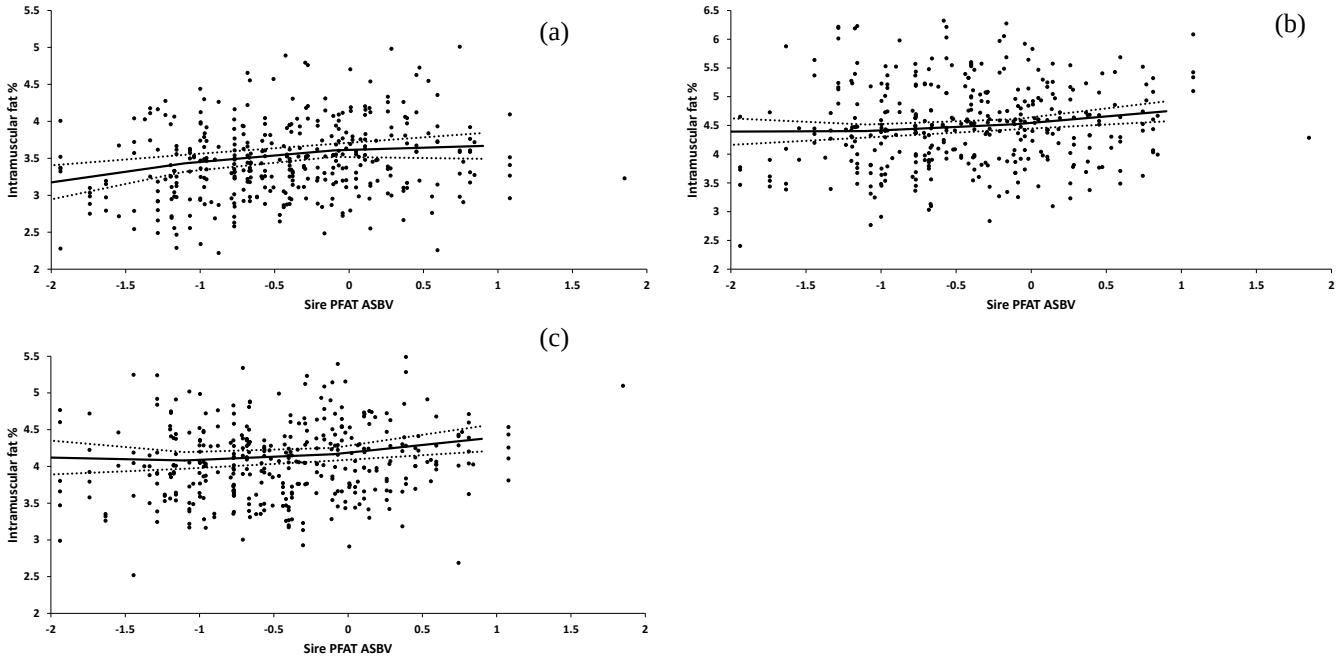

Figure 4. The association between sire Post Weaning C-Site Fat depth (PFAT) Australian Sheep Breeding Value (ASBV) and intramuscular fat for the a) *M. semimembranosus* b) *M. semitendinosus* and c) *M. longissimus lumborum.*

An impact of increased sire PEMD was observed only in the lambs born as multiples and raised as singles (*P*<0.05, Table 10). Across a 3 unit range of sire PEMD (-1.1 to 2.25) IMF% decreased in single raised lambs by 0.9 IMF% units (Figure 5).

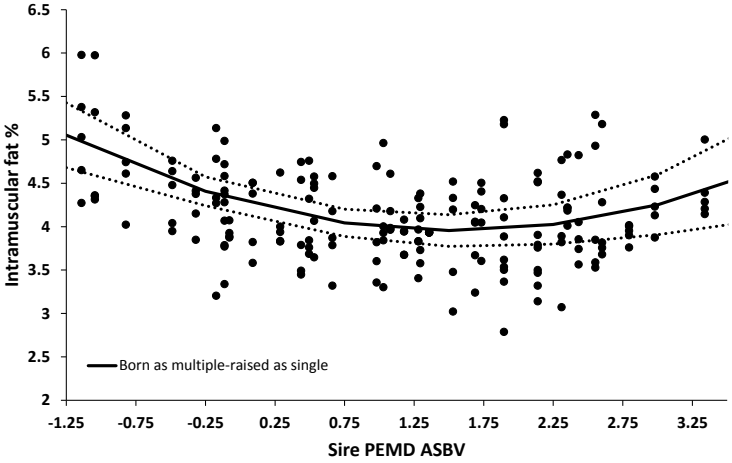

Figure 5. Relationship between sire post weaning eye muscle depth (PEMD) Australian Sheep Breeding Value (ASBV) and intramuscular fat for the lambs born as multiples and raised as singles.

The impact of the ASBVs on IMF did not alter when they were included individually in the model. When HCWT was included in the ASBV model, the significant effects did not change except for the magnitude of the PWWT effect which was reduced by a quarter. As such there was only a 1.1 IMF% increase in the lambs born as a multiple and raised as a single from PWWT -2 to 17. Including LMY% and carcase weight at scanning in the ASBV model reduced the magnitude of the PEMD and PWWT effects in the lambs born as multiples and raised as singles by 0.2 IMF% units. The impact of PFAT was reduced, with the effect only significant in the *M. semimembranosus*. The magnitude of the impact of PFAT on the *M. semimembranosus* and *M. semitendinosus* was halved compared to the ASBV model.

Effect of hot carcase weight and lean meat yield percentage

When HCWT was included in the model as a covariate, heavier carcasses contained more IMF although this varied between muscles and sire types (*P*<0.01, Table 10). On average IMF% increased by 0.82 IMF% over a range of HCWT from 15 to 28kg, which is equivalent to 0.06 IMF%/kg of HCWT (Figure 6). Most muscles followed this trend, with the outlier to this being the *M. supraspinatus* in the Merino sired lambs where IMF went up by 0.27 IMF%/kg of HCWT (Figure 7). Excluding the *M. supraspinatus*, the Merinos increased at 0.07 IMF% per kg HCWT compared to the Maternal and Terminal sired lambs which increased on average by 0.04 IMF% per kg HCWT. When comparing muscles, the *M. semitendinosus* increased at the greatest rate per unit HCWT (0.07 IMF%/kg HCWT), followed by the *M. infraspinatus* (0.05 IMF%/kg HCWT), *M. longissimus lumborum* (0.04 IMF%/kg HCWT), *M. semimembranosus* (0.04 IMF%/kg HCWT) (Table 12).

Table 12. Coefficients (±SE) for the effect of hot carcass weight (kg) on intramuscular fat percentage in 5 muscles of 3 different sire types.

	Coefficient ± SE		
Muscle	**Maternal**	**Merino**	**Terminal**
M. infraspinatus	0.03±0.04	0.10±0.05	0.01±0.03
M. longissimus lumborum	0.03±0.04	0.06±0.05	0.04±0.03
M. semimembranosus	0.04±0.04	0.04±0.05	0.04±0.03
M. supraspinatus	0.06±0.04	0.27±0.05	0.03±0.03
M. semitendinosus	0.07±0.04	0.09±0.05	0.04±0.03

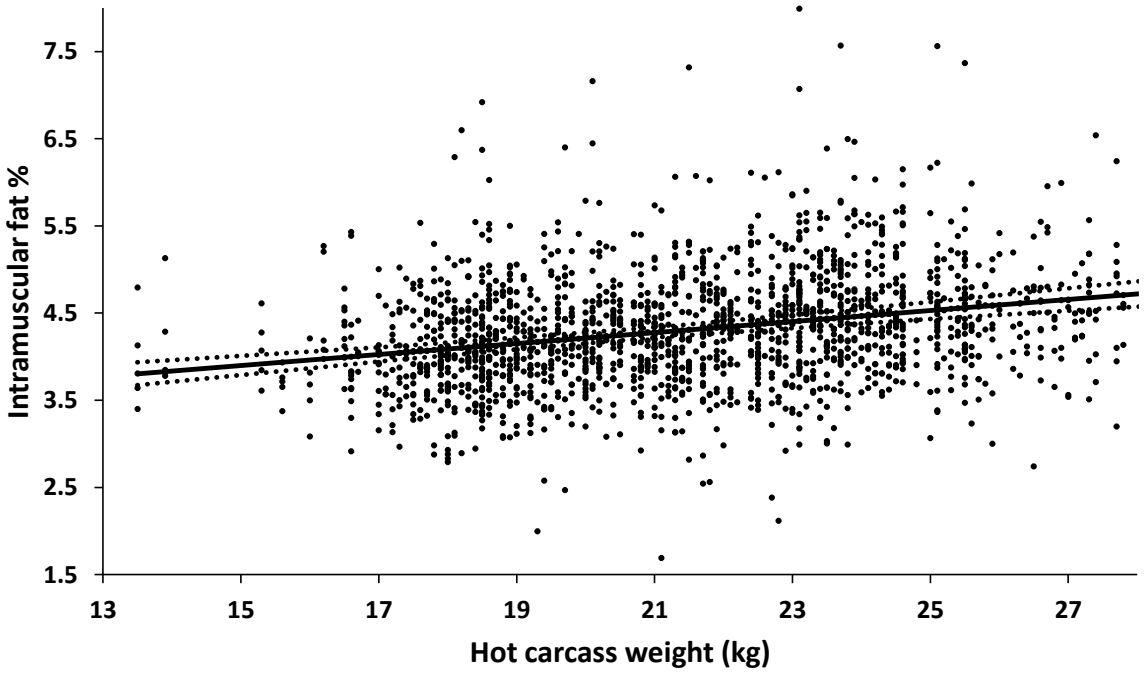

Figure 6. The association between intramuscular fat percentage and hot carcass weight (kg).

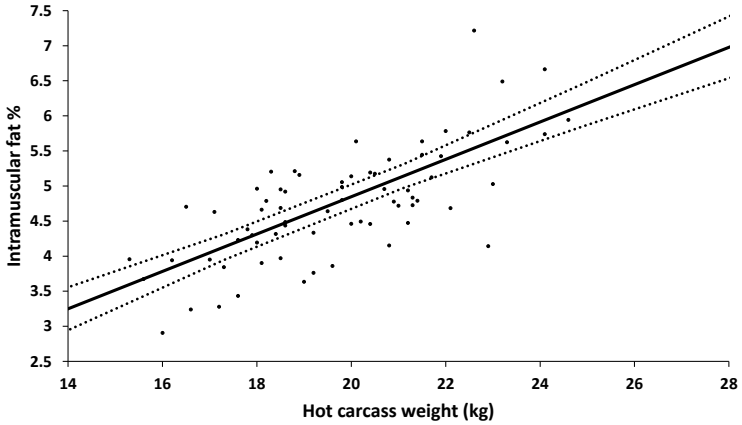

Figure 7. The association between intramuscular fat percentage and hot carcass weight (kg) in the *M. supraspinatus* of the Merino sired lambs.

When LMY% was included in the base model along with carcass weight at scanning it was associated with a decrease in IMF% (P<0.01, Table 10). On average IMF% decreased by 1.3 IMF% units as whole carcass LMY% increased from 52% to 66% (Figure 8).

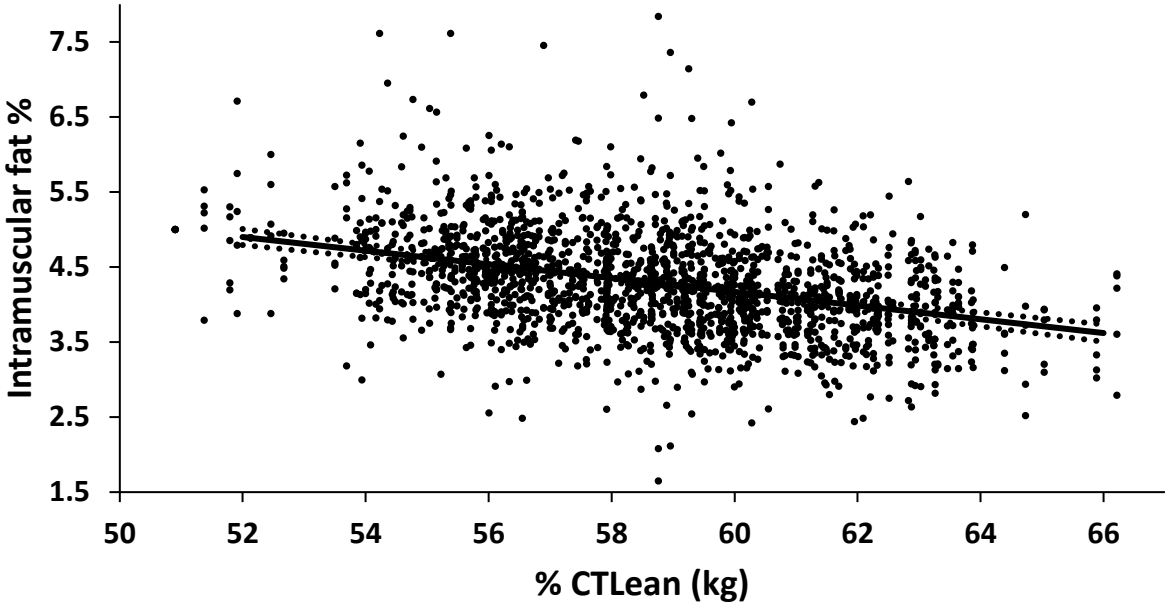

Figure 8. The effect of lean meat yield% on intramuscular fat.

Discussion

Difference between muscles

There was variation in IMF% of the 5 muscles sampled, however in contrast to our hypothesis IMF% was not related strictly to postural versus locomotive function. Variation in IMF% can be related to species, breed of animal and muscle fibre type (Hocquette, Gondret et al. 2010). Fibre type is related to the function of the muscle, therefore muscle location and function is likely to impact on IMF%. Muscles responsible for maintenance of posture are more oxidative and are predominantly comprised of Type 1 fibres with a propensity for higher IMF% (Picard, Lefaucheur et al. 2002). Within a muscle, more oxidative fibres contain more phospholipids and triglycerides and conversely muscles with high glycolytic activity have lower IMF% (Hocquette, Gondret et al. 2010). On this basis it would be expected that the *M. longissimus lumborum* would have the highest IMF% of the 5 muscles examined,

being a postural muscle, however this was not observed in our results. Alternatively, the *M. supraspinatus* could be considered a postural muscle as it acts as a stabilizer of the shoulder in lambs and helps to bear body weight. It had greater IMF% than the *M. infraspinatus* which can be considered a locomotive muscle as it is used for extension and flexion of the shoulder joint in sheep (Suzuki 1995). Therefore when comparing these two muscles it would be expected that the *M. supraspinatus* would have higher IMF% than the *M. infraspinatus.* A study examining the metabolic characteristics in sheep classified both the *supra*- and *M. infraspinatus* muscles as being more oxidative relative to the *M. semimembranosus*, *M. semitendinosus* and the *M. longissimus lumborum* (Briand, Talmant et al. 1981). Based on a fibre type and metabolic activity it is unclear why the *M. infraspinatus* has less IMF% than the *M. semitendinosus* and the *M. longissimus lumborum*. The *semitendinosus* is considered a fast glycolytic muscle (Briand, Talmant et al. 1981; Gardner, Hopkins et al. 2007; Hocquette, Cassar-Malek et al. 2012) especially in comparison with the *M. semimembranosus* and *M. longissimus lumborum.* Therefore it would be expected for the *M. semitendinosus* to have less intramuscular fat than these muscles, which was contrary to our findings. It has been shown that oxidative metabolism is not always well correlated with IMF% as it has been shown that bison with low oxidative muscles had more IMF% than *Bos Taurus* with highly oxidative musculature (Agabriel 1998). Our findings would support their findings that indicate that muscle function/fibre type alone cannot be used to predict IMF% of a muscle.

There are no previously published comparisons of IMF% over a range of carcass regions in Australian lamb, making this study unique. Recent Australian work by Pannier *et al* (2013) has shown the IMF% of the *M. longissimus lumborum* in lamb to be 4.2% which aligns well with the results for the *M. longissimus lumborum* in this study (IMF% 4.2±0.1). Other Australian work by Warner *et al* (2010) showed mean IMF% of the *M. semitendinosus* in to be 3.11±0.3 which is lower than the IMF% in this experiment (3.6±0.1) but does reflect that the IMF% of this muscle is lower than that of the *M. longissimus lumborum*. It is difficult to make comparisons of IMF% in studies from other countries as lambs are from vastly different genotypes and slaughtered at greatly varying ages. The variation in the IMF content of the 5 muscles examined (1.28%) was not as large as has been observed in other species such as beef (Brackebrush, McKeith et al. 1991). The ranking of IMF% between

muscles in our experiment and cattle from the Brackebrush (1991) study are vastly different. In our study the ranking of IMF% from highest to lowest was *M. supraspinatus, M. semitendinosus, M. longissimus lumborum, M. infraspinatus* and *M. semimembranosus*. In contrast Brackebrush (1991) found the highest IMF% in the *M. infraspinatus,* followed by the *M. longissimus lumborum, M. supraspinatus, M. semimembranosus* and *M. semitendinosus.* The reasons for this re-ranking is difficult to explain, although the comparison is imperfect given that the animals were older in the study of Brackebrush (1991), and this may be influenced by variation in the maturation patterns of the individual muscles and therefore IMF%.

Effect of Australian Sheep Breeding Values

In support of our hypothesis decreasing sire PFAT ASBV decreased IMF%, with the magnitude of this effect varying between muscles. As expected there was a marked impact in the *M. longissimus lumborum*, with a 0.3 unit decrease in IMF% over a 2.75 unit range of PFAT. However, there was a marginally greater effect in the *M. semimembranosus* and *M. semitendinosus*, and no effect in the muscles of the fore section. Assuming that this impact is associated with increase muscle hypertrophy (Hocquette, Gondret et al. 2010), this aligns well with the hypertrophy effects induced by PFAT which Anderson *et al* (2013)demonstrated were focused on the saddle section of the carcass, with least impact in the fore section. The reduced IMF% in the *M. longissimus lumborum* was slightly less than that observed by Pannier *et al* (2013) where IMF% decreased by 0.17% per mm reduction in PFAT, however Pannier's results were only evident for the Terminal sired animals.

Contrary to our hypothesis, increasing sire PEMD-ASBV had no effect in the majority of lambs studied in this experiment. The only effect was seen in multiple born lambs raised as singles, where IMF% was reduced in all of the 5 muscles examined. The basis for our PEMD hypothesis was associated with muscle hypertrophy in the saddle section, with previous studies demonstrating that PEMD increased the weight of the loin muscle (Gardner, Williams et al. 2010), and increased the proportion of lean in the saddle section (Anderson, Williams et al. 2013). However, a recent analysis of the composition data derived from CT scanning of the 400 animals in this study showed that PEMD did not increase lean saddle weight, so the lack of PEMD impact is not unexpected. These results contrast with work by Pannier *et al* (2013)

who demonstrated that increasing PEMD reduced IMF% in the *M. longissimus lumborum.* However, this effect was only evident in the Terminal sired lambs, and appeared to be driven by a small number of sires that were extreme for this ASBV. Indeed, the lack of impact of PEMD in this analysis may be due to the absence of these extreme sires which had PEMD values of between 4 – 5 in the Pannier *et al* (2013) study, contrasting with a maximum of only 3.8 in this study. Alternatively, the marked effect of PEMD on IMF in the multiple born and single raised lambs is difficult to explain, and has not been previously documented.

As expected, increasing sire PWWT ASBV did not impact on IMF% in the majority of lambs used in this experiment. Although increasing PWWT has been shown to impact on mature size (Huisman, Brown et al. 2008), and therefore maturity when lambs are compared at the same age, previously analyses of this trait have shown no PWWT/maturity-linked impact on IMF% (Pannier, Pethick et al. 2013). Alternatively, there was a substantial effect of PWWT on IMF% in the multiple born and single raised lambs, increasing it across all muscles. This is the same sub-group of lambs where the PEMD effect was identified, representing only 10% (40 lambs) of the population used in this study, and like the PEMD effect has not been documented previously. Furthermore, in an analysis of a much larger data set Pannier *et al* (2013) found no such interaction of PWWT with birth or rear type, and therefore more work is required before attributing confidence to this effect.

These results demonstrate the need to carefully manage the potentially negative impact of PFAT on IMF%. Alternatively, they also highlight that some monitoring of the hind section muscles may be required, given that the impact of PFAT was greater in this region of the carcase. PEMD and PWWT largely had no effect, although the unusual result found in the multiple born and single raised lambs may require future investigation.

Differences between sire types and dam breeds

In contrast to our hypothesis the Maternal sired lambs did not have more IMF% than the other sire types. The only differences were for the Merino sired lambs which had less IMF% than both the Maternal and Terminal sired lambs in the *M. semitendinosus,* and less IMF% compared to the Terminal sired lambs in the *M.*

longissimus lumborum. These differences in the *M. semitendinosus* were present even after correcting the model for HCWT. These results are in contrast to those of Pannier *et* al (2013) who found no differences in IMF% between sire types in the *M. longissimus lumborum* and that Merinos had the highest IMF% in this muscle when compared at the same HCWT.

The Border Leicester-Merino dams produced lambs with more IMF% in 3 of the 5 muscles, although not in the *M. longissimus lumborum* as had been previously reported (McPhee, Hopkins et al. 2008; Pannier, Pethick et al. 2013). The magnitude of the dam breed effect was not accounted for by the difference in HCWT. The increased IMF% in Border Leicester-Merino dams aligns well with previous work by Hopkins *et al* (2007) and McPhee *et al* (2008), which describe greater levels of adiposity in these genotypes.

In conclusion, the generally lower IMF% levels of Merino lambs can only partly be attributed to differences in weight. More importantly, this highlights that IMF% is unlikely to account for the superior eating quality of Merino lambs as demonstrated by Pannier *et al* (2013).

Lean meat yield percentage and hot carcass weight

In support of our hypothesis increasing LMY% led to a decrease in IMF%. This effect was consistent across all sire types and all muscles. Increasing muscularity is thought to dilute the final fat content in muscle (Hocquette, Gondret et al. 2010) and therefore reduce IMF%. When LMY% was included in the ASBV model the impact of the breeding values was reduced however still significant which indicates that LMY% does not account for all the variation in IMF%. As with previous studies, increasing HCWT was associated with an increase in IMF% of the *M. longissimus lumborum* (McPhee, Hopkins et al. 2008; Pannier, Pethick et al. 2013), however the variation in the association across muscles and between sire types has not been previously reported in Australian sheep. In particular, the large increase in IMF in the *M. supraspinatus* of the Merino sired lambs is a unique finding, the reason for which is not currently known. These results, particularly those in response to phenotypic increase in LMY%, highlight the importance of maintaining IMF% as the lamb industry continues to select for lean growth. Furthermore this demonstrates that this

impact is not restricted to the loin, affecting muscles in both the fore section and hind section of the carcase.

Effect of birth-rearing type, kill group, and sex

The increase in IMF% with each successive kill group is likely to be a reflection of age and weight. The average age in days of these kill groups was 167, 238, 280 and 355, therefore the linear increase in IMF% with age aligns well with the impact of maturity on adiposity as has previously been observed in the *M. longissimus lumborum* (McPhee, Hopkins et al. 2008; Pannier, Pethick et al. 2013). None-the-less we cannot completely discount other factors that may also have impacted such as changing nutrition/pasture quality across this period. When corrected for HCWT, the magnitude of the kill group effect was reduced, though still significant, indicating that increasing animal size contributes to IMF% but that there are likely to be effects of age or maturity that impact beyond their simple correlation with weight. The increase in IMF% present across all muscle types varied in magnitude between muscles, with the *M. supraspinatus* showing the greatest increase in IMF% and the *M. semimembranosus* showing the least. These differences are likely to reflect development towards differing IMF% at maturity (ie higher in the *M. supraspinatus*, and lower in the *M. semimembranosus*), although we can't completely discount the possibility of differential maturation rates to the same IMF% at maturity.

The impact of birthing and rearing types was only evident within the Merino sired lambs. The IMF% of the multiple born and raised lambs was higher than that of the multiple born-single raised and singleton born and raised lambs. This does not appear to be the result of an impact of HCWT as its inclusion in the core model did not alter the magnitude of the effect. One explanation may be associated with Maternal nutritional restriction. During the early gestation period in sheep restriction has been shown to increase the IMF% of the *M. M. longissimus lumborum* muscle of their offspring at 8 months of age (Zhu, Ford et al. 2006). The mechanism for the increased IMF% is thought to be related to a down regulation of catabolic enzymes. If the IMF% is related to gestational nutritional restriction it is unclear why the lambs born as multiples and then raised as singletons also have low IMF, though it is possible that the postnatal growth of musculature in the single raised lambs results in a dilution effect on IMF%. Another explanation may be associated with fibre type as

rearing type has been shown to impact on the percentage and size of the type 1 and 2A myofibres in the *M. semitendinosus* (Greenwood, Harden et al. 2007). The muscle of multiple raised lambs was metabolically more oxidative and less glycolytic than those reared as singletons. Given that oxidative muscle types have been shown to associate positively with IMF% ((Hocquette 2010), this may account for the higher IMF% in this group, although this was only observed in the *M. semitendinosus*. Other studies have shown increased oxidative metabolism in the *M. longissimus lumborum*, however the impact of rearing type in the other muscles in unknown. In the study of Greenwood the effect appeared early in the lambs postnatal development and did not persist to 22 months of age so it is possible if the lambs were slaughtered at an older age then the birth-rear type effect may disappear. It is unclear why the birth-rear type effect was only observed in the Merino sired lambs in our study.

Female lambs had higher IMF% than the wethers, which was consistent across all muscle types. This result was not affected by the inclusion of HCWT in the model, indicating that it was not simply a reflection of differences in weight. None-the-less this difference is likely associated with the earlier maturation of females at lighter live weights compared to males (Butterfield 1988). The sex differences between females and wethers has previously been investigated in the *M. longissimus lumborum* by Pannier *et al* (2013) who similarly found ewes to have 0.2% more IMF%, though the information regarding the other 4 muscles in our study has not previously been reported. Craigie *et al* (2012) has also shown greater IMF% in ewes, however this comparison was made restricted to Texel rams and only reported on the *M. longissimus lumborum*. One other study has shown no effect of sex on IMF%, (Tejeda, Peña et al. 2008) however this was a small study (n=48) consisting only of Merinos at low live weights.

Conclusion

The ranking of IMF% varied to those observed in studies in beef, and the *M. longissimus lumborum* did not have the highest IMF% levels of the muscles assessed. PFAT breeding value had a negative impact on IMF%, however this effect was greater in the muscles of the hind section indicating that additional monitoring may be required in these muscles for managing the broader impact of PFAT. The

PEMD and PWWT breeding values largely had no impact, although the effect identified in the multiple born – single raised lambs may require further investigation. Lastly, the marked effect of phenotypic LMY% across all muscles of the carcase further emphasises the need to manage the potential impact of this selection goal on eating quality.

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