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# **Technical Report**



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# Abstract

This project analysed data collected at 2 commercial abattoirs (sites) that have installed dual energy x-ray absorptiometry (DEXA) systems used to drive carcass cutting devices in plant and concurrently predict carcass composition of fat, lean and bone. Carcasses were subsequently used in eating quality experiments, enabling the relationship between DEXA and eating quality to be explored. An algorithm has recently been established which better identify bone pixels within the DEXA images allowing better determination of all carcass bone DEXA R values. The all carcass bone DEXA R values and those from individual bones that were manually isolated from DEXA images (humerus, lumbar vertebra and femur) were used to predict eating quality from cuts across the lamb carcass. Data from both sites was analysed independently due to the inability to calibrate DEXA images between the two sites, however future data acquired will utilise phantoms to allow bone DEXA values to be compared. An increase in all carcass and some individual bone DEXA R Mean terms demonstrated an association with decreasing eating quality (overall liking, tenderness, juiciness and flavour). The best prediction was of the loin grill where a decrease of 10.5 and 9 overall liking scores was observed across the range of all carcass bone DEXA R Mean at site 1 and site 2. The other cut with relatively strong associations with bone DEXA was the shoulder roast, however the prediction of eating quality in other cuts was more tenuous and lacked consistency between sites. This experiment also used whole carcass lean % and loin intramuscular fat % to investigate relationships with eating quality. In this experiment the bone DEXA R Mean terms were generally independent predictors of eating quality to those of loin IMF % and carcass lean %. The biology underpinning the relationship between DEXA and eating guality has not been identified, though is likely associated with an index of maturity. Bone mineral content did not directly relate to eating quality, however there were some relationships of bone minerals (magnesium, calcium and phosphorus) with bone DEXA R and carcass composition. The isolation of bone pixels from the carcass during routine DEXA scanning at abattoirs may be able to provide input into a multi-trait eating quality model in the future, however future research is necessary.

## **Executive Summary**

- Data was obtained from 2 sites (abattoirs) representing lambs from an earlier eating quality experiment (site 1 new season v old season, n = 120) and site 2 which represented predominantly mutton (n = 108), with a small number of lamb (n= 10) slaughtered on two consecutive days at the same abattoir.
- DEXA images were collected from lambs during routine processing along with a number of phenotypic measures. All images from both sites were analysed using a new algorithm for identifying bone pixels thus although this experiment utilised historical data it was regenerated to determine new values for each pixel's bone DEXA R value. Therefore, the lean % and bone R data from site 1 was regenerated along with data from the additional site. From each DEXA image, values were determined for all carcass bone DEXA R along with specific regions (humerus, vertebra, femur).
- The historical nature of the site 1 data meant that the bone DEXA values from site 1 and site 2 were unable to be analysed together as they did not share a common phantom to calibrate the images. Additionally, the ageing times of the eating quality cuts differed (site 1 = 5 days, site 2 = 10 days).
- The most consistent relationship between eating quality was between an increase in all carcass bone DEXA R and a decrease in the loin grill as well as shoulder roast eating quality. This relationship was evident at both sites with a similar magnitude of effect. In this analysis the relationship between bone DEXA R and eating quality appears to be somewhat independent to the relationship that exist between the eating quality of this cut and both loin intramuscular fat (IMF) % and carcass lean % as the bone DEXA R term remained significant when these terms were included in models predicting eating quality.
- There was not a strong or consistent relationship between bone DEXA R Mean and the other eating quality cuts analysed.
- Investigations into the biology underpinning the relationships between DEXA and eating quality did not find a direct relationship between bone mineral concentration and eating quality. Additionally, the bone DEXA R Mean values generated from individual bones and the entire carcass do not do not have a strong relationship with bone mineral content. However, the magnesium of the bone mineral content of certain bones demonstrated a decrease in concentration as bone DEXA R Mean increased eluding to possible links with maturity.
- The results of this experiment and analysis demonstrate a promising relationship between the bone DEXA R Mean values and the eating quality of cuts across the carcass. The benefit of using bone DEXA is the independence of this prediction method from loin grill's relationship with IMF %. Additionally, the low correlation of bone DEXA R with lean % make the DEXA prediction independent to the relationship of loin grill and carcass lean %.

# Contents

Cit	ation			2
Ac	know	ledge	ments	2
Ab	stract			3
Ex	ecutiv	ve Su	mmary	4
Co	ntent	s		5
1	Intro	oduct	ion	7
2	Met	hods		8
2	2.1	Exp	erimental design and slaughter details	8
2	2.2	Dua	I energy x-ray absorptiometry scanning and image analysis	9
	2.2.	1	DEXA scanning and determination of R value	9
	2.2.	2	DEXA detection of bone	9
	2.2.	3	DEXA bone regions	10
2	2.3	Bon	e mineral analysis	10
2	2.4	Eati	ng quality samples and assessment	11
2	2.5	Stat	istical analysis	12
3	Res	ults.		13
3	3.1	Raw	<i>i</i> data	13
3	3.2	Cori	elations	17
	3.2.	1	Carcass phenotypic traits and bone DEXA values	17
	3.2.	2	Correlation of overall liking within roast and grill cuts	17
3	3.3	DEX	A prediction of eating quality	18
	3.3.	1	Loin grill	18
	3.3. rum	2 ip an	Prediction of eating quality of grill cuts of the hind section (knuckle, outside, d topside)	22
	3.3.	1	Roast cuts from the lamb carcass	24
3	3.4	Bon	e minerals and their relationship with DEXA R Mean and eating quality	28
	3.4.	1	Mineral content of bones and correlations with carcass composition and	
	mea	asure	·S	28
	3.4.	2	Bone mineral differences between age.	30
	3.4.	3	Relationship between bone DEXA R Mean, mineral content and eating qualit 30	y
3	3.5	Disc	sussion	33
	3.5.	1	Prediction of eating quality across the carcass	33
				5

	3.5.2	Bone mineral content	35
	3.5.3	Limitations	37
	3.5.4	Industry significance and future work	37
	3.6 Cor	nclusion	38
4	Bibliogra	aphy	40
	-		

# **1** Introduction

Eating quality remains an important attribute of lamb and is an important profit driver in the industry (Pethick, Banks, Hales et al. 2006). There are many production, genotypic and environmental factors that contribute to the variation in meat quality of beef and lamb. For beef, these factors have largely been incorporated in the Meat Standards Australia (MSA) eating quality model to predict cuts prepared using different cooking methods. Ossification in the beef MAS model refers to the calcification of the sacral and dorsal vertebrae and an increase in ossification is associated with reduced eating quality, especially in younger animals (Watson, Polkinghorne and Thompson 2008). Australian studies show that sheep sensory scores for eating quality decrease as animal age increases (Pethick, Hopkins, D'Souza et al. 2005, Hopkins, Hegarty, Walker et al. 2006). In cattle, ossification has been shown to be a better indicator of eating quality than age prior to skeletal maturity (Bonny, Pethick, Legrand et al. 2016). Therefore measurement of skeletal maturity in lamb may be a good indicator of sheep eating quality.

Dual energy x-ray absorptiometry (DEXA) has been used for the accurate determination of body composition in in production animals including sheep (Mercier, Pomar, Marcoux et al. 2006, Dunshea, Suster, Eason et al. 2007, Pearce, Ferguson, Gardner et al. 2009). Dual energy x-ray absorptiometry (DEXA) has been developed for use in Australian abattoirs to predict lamb carcass composition of fat, lean and bone (Connaughton, Williams, Anderson et al. 2020). Knowledge of carcass composition, including lean %, has the potential to influence price payments to producers and allow sorting of carcasses based on composition. Images captured using DEXA are at two different energy levels, with the pixels captured at low energy expressed as a ratio (R value) to those captured at a high energy (Peppler and Mazess 1981), and reflect the atomic mass of a tissue (Pietrobelli, Formica, Wang et al. 1996). It is on this basis, DEXA scanning in humans is used to assess bone density (Pouilles, Tremollieres, Todorovsky et al. 1991). This principle has been applied to calves using a human DEXA scanner (López-Campos, Juárez, Larsen et al. 2018) to assess age.

DEXA images obtained from abattoirs have demonstrated isolated bones such as the lumbar vertebra and the carcass skeleton have been shown to have an association with eating quality (Anderson, Payne, Pannier et al. 2021). In this study the strongest association was demonstrated in the loin grill, where overall liking was shown to decrease across an increasing DEXA R value of the vertebrae. The result was somewhat independent of carcass lean % and intramuscular fat % of the loin however these terms were correlated with bone DEXA R values. This study utilised a relatively small number of lamb across a limited age range, however demonstrated that DEXA may be a useful tool for predicting carcass eating quality.

The association between bone DEXA R values and eating quality may be related to animal maturity and therefore may reflect differences in bone structure and mineral content. Bone mineral concentrations have been shown to change as an animal matures, with magnesium (Mg) shown to decrease as animals age and is replaced by calcium as hydroxypapatite (Ravaglioli, Krajewski, Celotti et al. 1996) with the molar ratio of Ca:P increasing. This has been demonstrated in lambs by Cake, Gardner, Boyce et al. (2006) where cortical magnesium of the metacarpal decreased over a 40 month time period. A preliminary study has demonstrated small but inconsistent changes in lumbar vertebra magnesium in lambs

(Payne, Anderson, Pannier et al. 2022). These R-values positively correlate with the atomic mass of the particles within each pixel (Pietrobelli et al. 1996), and can therefore be used to measure differences in bone mineral density, although associations with mineral concentration are not well identified in humans or animals. An abattoir based scanner used to show an association between DEXA images of the lumbar vertebrae and lumbar mineral content of the lumbar vertebra in lambs (Payne et al. 2022), however in this study was not independent of other carcass factors such as lean %.

This report describes a data set which includes sheep of a range of ages and uses advanced DEXA image processing to investigate the link between DEXA images of lambs, eating quality and bone mineral content. We hypothesis that sheep with increased bone density as detected by increased bone DEXA R values will be associated a decrease in eating quality. Furthermore, we hypothesise that as bone DEXA R increases it will represent increased maturity and therefore a decrease in bone magnesium and an increase in bone calcium, with these minerals directly linked to decreased eating quality.

# 2 Methods

#### 2.1 Experimental design and slaughter details

Animals originated from 2 separate experiments that were conducted in different locations and used 3 separate abattoirs and DEXA scanners. Site 1 was at Bordertown in South Australia, where 120 lambs were slaughtered that were of two age classes: 'new season', which were between 209 and 252 days of age and 'old seasons' which were 298 to 308 days of age. These lambs were from 2 separate properties where 30 new and 30 old seasons lambs were finished under the same environmental conditions and lambs from each property slaughtered on the same day. Lambs from each property were genetically related to each other and were White Suffolk on one property and Border Leicester Merino x Poll Dorset on the other.

Site 2 were Merino ewes were sourced from an existing trial run by Charles Sturt University and Scibus, NSW. Approval for that study was obtained by the SBScibus Animal Ethics Committee (number 1014-1220). These animals were born in 2014 at the Camden research site and slaughtered in 2020 at Gundagai over 2 consecutive days along with 20 unrelated lambs which were also included in the analysis of site 2 sheep.

Following slaughter at their respective abattoirs, all carcasses were trimmed according to AUSMEAT standards (AUSMEAT 2005) with hot standard carcass weight (HCWT) (kg) recorded. Carcasses were chilled overnight (3-4 °C) before further sampling. Vernier callipers were used to record GR tissue depth (total tissue depth (mm) above the surface of the 12<sup>th</sup> rib 110mm from the midline), C-site fat depth (mm), eye muscle depth and width (mm).

A 40 g sample of the left loin cut (AUS-MEAT code 5150) was collected for the determination of intramuscular fat (IMF) % at the same time that cuts were collected for eating quality sampling. IMF samples were freeze-dried in a Coolsafe 95-15 Pro (Scanvac, Lillerød, Denmark) and chemical fat determined after analysed using near-infrared technology (Technicon InfrAlyser 450 (19 wavelengths)) (Perry, Shorthose, Ferguson et al. 2001).

#### 2.2 Dual energy x-ray absorptiometry scanning and image analysis

#### 2.2.1 DEXA scanning and determination of R value

Animals underwent dual energy x-ray absorptiometry (DEXA) scanning at a commercial abattoir following slaughter as described by . Briefly, the two x-ray images were generated using 2 photodiodes separated by a copper filter (Scott Automation and Robotics). The first photodiode used ZnSe as the scintillant and the second used CsI as the scintillant. These scintillants were selected due to their specificity for low and high energy photons (Ryzhikov, Opolonin, Pashko et al. 2005). For each carcass there were two images captured: a low energy image (ZnSe photodiode) and high energy image (CsI photodiode). Each image was calibrated using a method which accounts for detector drift throughout the day, this process was repeated with the inclusion of an adjustment based on the ratio of the unattenuated pixel values recorded in each current image and those captured in a calibration scan at the start of the day. This allows images within and between days at an abattoir to be compared. From the two images collected from each carcass the ratio of the photon attenuation for corresponding pixels within the low and high energy images was then used to calculate an R value for each pixel (Pietrobelli et al. 1996):

R = In ( $I_{Low}$ /AirAtten)/ In ( $I_{High}$ /AirAtten)) where:  $I_{Low}$  represents the pixel value in the low energy image (ZnSe Photodiode);  $I_{High}$  represents the pixel value in the high energy image (CsIPhotodiode) and AirAttan respresents the pixel value corresponding to the un-attenuated photons within each image that have passed through air only.

The lean and fat weights of each carcass were determined as described by Gardner et al. (2018) which provides a measure of lean tissue as a proportion of carcass weight (DEXA lean %) for each carcass.

#### 2.2.2 DEXA detection of bone

The previous algorithm for detecting bone was based on the technique described above, however without the bone edge detection. Without this bone edge detection, the soft tissue R-value was not calculated at the neighbour pixels, but rather over the entire carcass. Through rearranging of this equation, the mean R-value of all pixels was used as the new zero, with pixels greater than that of the mean classified as containing bone, and those less than the mean classified as soft tissue.

As tissue composition is a function of R-value and the thickness of the tissue, this method is prone to error in areas at the extremes of thickness. In general, the R-value of a tissue will decrease with an increase in thickness, despite the same composition. Therefore, very thin areas will have high R-values, and potentially be classified incorrectly as bone, while areas of greater thickness, despite containing bone, may fall under the mean R-value of the entire carcass, and therefore remain classified as not containing bone.

The new technique attempts to incorporate tissue thickness, to avoid the incorrect classification of pixels. As there is still no effective bone edge detection technique in such a rapid system, a placeholder threshold is still required for the effective determination of bone containing pixels.

Each tissue-containing pixel is evaluated by calculating the R-value, and a proxy for thickness by the following equation:

$$thickness = \ln(I_L)$$

Where  $I_L$  is the attenuation value for the low-energy image.

To extend the range of the R-values that would normally exist over a whole lamb carcass, this value is multiplied by itself. This squared R-value is divided by the thickness proxy of the pixel, with the natural log of this result used as the final value, following this equation:

$$\ln\left(\frac{R^2}{thickness}\right)$$

This value is compared to the determined threshold for each DXA system, which is informed using the Scott Automation and Robotics phantom. The calculation of the predicted bone % is expanded upon from its previous form, using the ratio of bone containing pixels to the total number of carcass pixels, and the mean R-value of the bone containing pixels.

#### 2.2.3 DEXA bone regions

Individual regions of the carcass were isolated from the DEXA images using Image J (version 1.44p), with these regions located over specific bones (femur, humerus, lumbar vertebrae, proximal lumbar vertebra). The bones were chosen based on their ease of collection of the bones at slaughter and their close locality to the various cuts collected from across the carcass which were subsequently assessed for eating quality. In an earlier analysis the entire lumbar vertebra was traced around, however in this analysis an additional variable was included which was the proximal lumbar vertebra which is the location of the vertebra used for bone mineral analysis was conducted. The R value of pixels within the bone regions was determined and the mean and standard deviation of the R values (termed DEXA R Mean and DEXA R SDev). Additionally, all bones of the carcass were isolated using the newly developed bone algorithm described above and the mean and standard deviation of the R Mean and SDev.

#### 2.3 Bone mineral analysis

In all animals, the humerus, lumbar vertebrae and femur were collected within 24 hours of slaughter and frozen until mineral analysis was performed. For the lumbar spine, the second lumbar vertebra was isolated using a bandsaw and a longitudinal cross section sample of approximately 1.5 cm was removed from the centre of the isolated vertebra for mineral analysis. The spinal cord, any remaining muscle and tissue were removed from each sample using scalpels, tweezers and forceps. A 1 cm cross section of the humerus and femur was removed using a bandsaw and samples cleaned of any excess tissue.

The mineral content of all bones was performed according to those in Payne et al. (2022) but are briefly described below. Cleaned samples were weighed to obtain the wet weight and soaked in diethyl ether overnight. Samples were dried overnight in a 100 °C oven and weighed again to obtain a dry weight. Samples were ashed in a furnace at 600 °C for 24 h. After ashing, the final ash weight of the samples was recorded. Ashed samples were ground

and digested using aqua regia (3:1 hydrochloric/nitric acid) at 95 °C based on method AS4479.2. Measurement of digest solutions was performed using a glass expansion method on an Agilent 720 ICP-AES with Seaspray nebulizer, glass-cyclonic Tracey spray chamber and Niagara Plus (Glass Expansion). Bone ash was measured for calcium, phosphorus and magnesium concentration, which was calculated back to per gram of bone.

#### 2.4 Eating quality samples and assessment

Eating quality samples were collected from all lambs post slaughter however the range of cuts collected from each site and ageing times differed (Table 1).

	Site 1	Site 2
Days of ageing	5	10
Cut tested for eating quality (overall liking, tenderness, juiciness and flavour)	n	n
Shoulder (roast)	82	116
Rack (roast)	82	-
Rack cutlet (roast)	-	116
Leg (roast)	82	-
Knuckle (roast)	-	69
Topside (roast)	31	116
Knuckle (grill)	115	118
Topside (grill)	80	118
Loin (grill)	81	115
Outside (grill)	79	118
Rump (grill)	81	118

Table 1. Cut types and cook method, days of ageing and number of samples tested at sites 1 and 2

Sensory testing for grilled lamb cuts was conducted according to (Thompson, Gee, Hopkins et al. 2005), with cuts cooked on a Silex grill (S-tronic steaker, Silex, Hamburg, Germany) and the top plate set to 185 °C and bottom plate to 190 °C. Steaks were grilled to an approximate internal temperature of 65 °C as measured by a thermometer, rested for 90 seconds and halved before serving.

The collection of cuts and their preparation for eating quality assessment at site 1 is described in detail by Payne, Pannier, Anderson et al. (2020) and reported briefly here. Sheep were dissected to extract 8 cuts for eating quality assessment: roasts (rack (site 1), rack cutlet (site2), shoulder, leg, knuckle (site 1), topside) and grill (rump, loin, outside, topside, knuckle). Additional carcasses were also used to collect samples which were used as links within a tasting session and served as starter samples. All grill cuts were sliced into 5 steaks of 15 mm thickness and trimmed of subcutaneous fat and epimysium (silver skin) with the topside prepared with the cap off and the adductor removed. The leg and shoulder cuts were netted whole and the rack cuts had the cap removed. All cuts were vacuum packed and stored at 2°C for 5 days of aging before being frozen at -20°C. Cuts were later thawed for subsequent sensory testing. Leg and shoulder roast cuts were trimmed into a 15cm x 15 cm block, rolled and bound in butchers string prior to cooking. These roast cuts were cooked in an Electrolux 10 tray dry oven and set to a temperature of 160 °C. In order to cook to an internal temperature of 65 °C, the roasts were removed from the oven at an

internal temperature of 60 °C and rested for 10 minutes. Roasts were then sliced into 4 mm samples and ten suitable slices from each cut were selected for consumer testing. External fat and connective tissue seams were removed and slices trimmed to 50 mm x 50 mm x 4 mm thick. The 10 consumer samples were placed in steel pans which were maintained at a temperature of 50 °C until serving. The exception to this was the rack cutlet at site 2 which were sliced into cutlets approximately 2.5 cm wide and served on the bone.

Untrained consumers were used to score each sample on a scale from 0 to 100, for tenderness, juiciness, liking of flavour and overall liking. A score of 0 indicates a tough, dry, unliked sample. The study was conducted in accordance with the Declaration of Helsinki, and the protocol as approved by the Ethics Committee of Murdoch University on 11 October 2018 (2018/129).

#### 2.5 Statistical analysis

Simple correlations were determined using PROC. CORR in SAS (Version 9.1, SA Institute, Cary, NC, USA) between all carcass bone DEXA R Mean and DEXA R SDev, the individual bone DEXA R Mean and SDev (humerus, lumbar and femur), DEXA lean %, GR tissue depth (mm), IMF % and HCWT. These correlations were made within a site (site 1 or site 2).

The DEXA R Mean and DEXA R SDev for all carcass bone and bone regions were included in general linear models (SAS Version 9.1, SAS Institute, Cary, NC, USA) to predict eating quality (tenderness, juiciness, flavour and overall liking) of the individual cuts. The range of Bone DEXA R values was much greater at site 2 (0.137) compared to site 1 (0.024). As such the data sets were analysed independently as there is not currently a method for calibrating bone DEXA R values between sites in these two data sets. Additionally, the ageing and types of cuts and cook methods differed between the sites and it was not always possible to analyse the same cuts at each site. DEXA Lean % and IMF % were also included as covariates in general linear models separately and along with significant DEXA R Mean and DEXA R SDev to assess if DEXA bone image components were merely reflecting the influence of lean % and IMF % on eating quality. To demonstrate the precision of each model, R<sup>2</sup> and RMSE values are reported, with coefficients and F-values reported to reflect the relative importance of each term in the model. Lastly, to reflect the descriptive power, each model was used to predict the values of the population upon which it was trained. The magnitude of effect for prediction of eating quality traits is reported across the mean of the covariate used in the model plus and minus twice the standard deviation of the mean, thus effectively reporting the magnitude of effect across 4 times the standard deviation of each covariate, representing the range across which 95.4% of the predicted values are found. As an additional test, where terms were significant predictors of eating quality, a linear mixed effect model was run with the same terms in the model, however also including eating quality session (pick) in the model.

Simple correlations (PROC CORR SAS) were made within a site (site 1 or 2) for the mineral content (Ca, Mg, P) between bones (humerus, lumbar vertebra and femur). Simple correlations are also reported between lean % and loin IMF % and the mineral content of the humerus, lumbar vertebra and femur.

For mineral content of bones (calcium, magnesium and phosphorus), comparisons were made between lambs (site 1) and mutton (site 2) for the humerus and femur. Site differences

between the lumbar vertebra were unable to be made due to differences in the processing of the lumbar vertebra at site 1. Additionally, to remove the influence of the small number of lambs that were included at site 2, these 8 animals were excluded from the mineral analysis.

At site 1, differences between new season and old season lambs were compared for bone mineral content of the lumbar vertebra, humerus and femur. At site 1, each kill group represented lambs that were of similar genetics within a kill group, finished under similar conditions and slaughtered on the same day but of differing ages.

Within a site (1 or 2), bone mineral content was used in separate general linear models (SAS) to predict eating quality scores (overall liking, tenderness, juiciness and flavour) of the various cuts at each site. Similarly, bone DEXA R Mean values for all carcass and individual bones were used to predict bone minerals content of magnesium, calcium, phosphorus using general linear models (SAS). For any significant terms, lean % and IMF % were included as covariates in the model.

# 3 Results

#### 3.1 Raw data

The mean  $\pm$  SD, minimum and maximum, HCWT (kg), DEXA lean %, DEXA Fat %, DEXA Bone %, GR tissue depth (mm), c-site fat depth (mm), eye muscle area (cm<sup>2</sup>), loin intramuscular fat (%) from site 1 and site 2 are shown in Table 2.

The raw mean  $\pm$  SD, minimum and maximum for overall liking for roast cuts (shoulder, leg, rack, topside) and grill cuts (knuckle, topside, outside, rump and loin) for all lambs, new season and old season lambs are shown in Table 3. The raw mean  $\pm$  SD, minimum and maximum for DEXA R Mean values for the selected regions (all carcass bone, all lumbar vertebra, anterior lumbar vertebra, femur and humerus) are shown in Table 4

	_	Site 1			Site 2	
	n	Mean ± SD	Min, Max	n	Mean ± SD	Min, Max
Hot carcass weight	118	22.5 ± 2.2	14.9, 28.7	118	20.1 ± 4.3	13.5, 34.4
Lean %	118	54.3 ± 2.1	49.9 <i>,</i> 60.6	118	59.3 ± 3.6	46.8, 66.6
Fat %	118	24.1 ± 3.7	13.3, 32.0	118	17.1 ± 6.6	5.3 <i>,</i> 39.0
Bone %	118	18.1 ± 1.3	15.2, 22.8	118	21.8 ± 3.6	14.5, 30.1
GR tissue depth (mm)	117	13.7 ± 4.4	3.0, 26.0	118	7.7 ± 6.3	1.0, 25.0
C-site fat depth (mm)	117	2.9 ± 1.4	0.4, 7.5	118	2.3 ± 2.0	0.0, 10.0
Eye muscle area (cm <sup>2</sup> )	117	14.8 ± 2.0	9.2, 20.1	118	10.8 ± 3.2	1.4, 23.5
Intramuscular fat of loin (%)	117	4.15 ± 1.0	2.4, 7.2	117	5.8 ± 2.3	1.7, 12.7

Table 2. Raw Mean  $\pm$  SD for hot carcass weight (kg), lean %, fat %, bone%, GR tissue depth (mm), c-site fat depth (mm), eye muscle area (cm<sup>2</sup>), loin intramuscular fat (%) for sheep from sites 1 and 2.

Table 3. Raw Mean ± SD for average consumer overall liking for roast cuts (shoulder, leg, rack, rack cutlet, topside, knuckle) and grill cuts (knuckle, topside, outside, rump and loin) for sheep from sites 1 and 2.

		Site 1		_	Site 2				
Eating quality (overall liking)	n	Mean ± SD	Min, Max	n	Mean ± SD	Min, Max			
Shoulder (roast)	82	62.5 ± 10.3	34.1, 86.9	116	55.1 ± 10.0	36.6, 78.7			
Rack (roast)	82	67.7 ± 9.1	38.0, 84.3	-	-	-			
Rack cutlet (roast)	-	-	-	116	60.0 ± 10.1	36.4, 83.0			
Leg (roast)	82	55.4 ± 9.3	35.5, 78.3	-	-	-			
Knuckle (roast)	-	-	-	69	59.7 ± 8.0	44.0, 76.9			
Topside (roast)	31	49.6 ± 7.2	31.1, 65.8	116	55.0 ± 9.5	30.0, 79.5			
Knuckle (grill)	115	66.5 ± 8.0	45.9 <i>,</i> 88.7	118	66.1 ± 9.0	42.4, 87.2			
Topside (grill)	80	53.5 ± 9.4	32.9, 84.2	118	52.3 ± 10.1	26.8, 74.2			
Loin (grill)	81	65.7 ± 8.8	42.1, 85.1	115	67.7 ± 9.1	45.6, 92.0			
Outside (grill)	79	59.4 ± 9.4	34.3, 75.7	118	55.9 ± 9.7	29.4, 81.0			
Rump (grill)	81	70.4 ± 8.0	52.0, 88.4	118	59.9 ± 10.2	34.4, 85.7			

Table 4. Raw Mean ± SD (minimum, maximum) for DEXA R Mean for all bone, all lumbar vertebra, anterior lumbar vertebra, femur and humerus regions for sheep from sites 1 and 2.

		Site 1			Site 2			
	n	Mean ± SD	Min, Max	n	Mean ± SD	Min, Max		
All carcass bone DEXA R Mean	118	$1.28 \pm 0.01$	1.27, 1.29	118	$1.31 \pm 0.03$	1.22, 1.36		
Lumbar vertebra (all) DEXA R Mean	118	$1.30 \pm 0.01$	1.28, 1.32	118	$1.44 \pm 0.02$	1.36, 1.48		
Lumbar vertebra (single) DEXA R Mean	118	$1.30 \pm 0.01$	1.27, 1.33	118	$1.45 \pm 0.03$	1.37, 1.53		
Femur DEXA R Mean	118	$1.26 \pm 0.00$	1.25, 1.27	118	$1.37 \pm 0.01$	1.32, 1.40		
Humerus DEXA R Mean	116	$1.27 \pm 0.01$	1.24, 1.30	118	$1.39 \pm 0.03$	1.31, 1.49		

This project is supported by funding from the Australian Government Department of Agriculture, Fisheries and Forestry as part of its Rural R&D for Profit programme in partnership with Research & Development Corporations, Commercial Companies, State Departments & Universities.

Table 5. Site 1 simple correlation coefficients between carcass measures of hot carcass weight (kg), GR tissue depth (mm), c-site fat depth (mm), eye muscle area (cm<sup>2</sup>) with DEXA determined carcass composition (lean, fat and bone %) and bone DEXA R Mean (all carcass, humerus, lumbar vertebra and femur) and their mean standard deviation (SDev)

	Hot carcass weight (kg)	DEXA lean %	DEXA fat %	DEXA bone %	GR tissue depth (mm)	C-site fat depth (mm)	Eye muscle area (cm²)	Loin intramuscular fat %	All carcass DEXA R Mean	All Carcass DEXA R SDev	Lumbar vertebra DEXA R Mean	Lumbar vertebra DEXA R SDev	Lumbar vertebra (single) DEXA R Mean	Lumbar vertebra (single) DEXA R SDev	Humerus DEXA R Mean	Humerus DEXA R SDev	Femur DEXA R Mean	Femur DEXA R SDev
Hot carcass weight (kg)	1	0.08	0.46	-0.54	0.53	0.21	0.10	0.28	-0.66	-0.12	-0.31	-0.28	-0.21	-0.29	-0.21	-0.21	-0.52	0.08
DEXA lean %	-	1	-0.74	0.48	-0.47	-0.26	0.18	-0.58	0.01	-0.06	0.04	-0.20	0.01	-0.25	0.06	0.05	-0.20	-0.16
DEXA fat %	-	-	1	-0.90	0.85	0.42	-0.07	0.76	-0.52	0.08	-0.34	0.01	-0.23	0.00	-0.35	-0.26	-0.15	0.16
DEXA bone %	-	-	-	1	-0.85	-0.42	0.03	-0.71	0.70	-0.06	0.50	0.11	0.38	0.14	0.42	0.33	0.27	-0.08
GR tissue depth (mm)	-	-	-	-	1	0.47	0.07	0.67	-0.66	0.05	-0.44	-0.08	-0.32	-0.07	-0.43	-0.40	-0.25	0.25
C-site fat depth (mm)	-	-	-	-	-	1	-0.06	0.38	-0.31	0.18	-0.27	-0.08	-0.23	-0.09	-0.20	-0.16	-0.25	0.08
Eye muscle area (cm²)	-	-	-	-	-	-	1	-0.05	-0.03	0.05	0.04	-0.12	-0.03	-0.12	-0.01	-0.06	-0.09	0.11
Loin intramuscular fat %	-	-	-	-	-	-	-	1	-0.43	0.12	-0.36	-0.04	-0.34	-0.04	-0.25	-0.14	-0.14	0.18
All carcass DEXA R Mean	-	-	-	-	-	-	-	-	1	0.05	0.71	0.32	0.66	0.41	0.56	0.44	0.44	-0.25
All Carcass DEXA R SDev	-	-	-	-	-	-	-	-	-	1	-0.08	-0.15	-0.09	-0.13	-0.06	-0.14	0.11	0.05
Lumbar vertebra DEXA R Mean	-	-	-	-	-	-	-	-	-	-	1	0.47	0.89	0.53	0.28	0.25	0.17	-0.20
Lumbar vertebra DEXA R SDev	-	-	-	-	-	-	-	-	-	-	-	1	0.41	0.74	-0.01	-0.01	0.17	-0.02
Lumbar vertebra (single) DEXA R Mean	-	-	-	-	-	-	-	-	-	-	-	-	1	0.56	0.24	0.19	0.11	-0.24
Lumbar vertebra (single) DEXA R SDev	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.02	-0.07	0.20	-0.01
Humerus DEXA R Mean	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.76	0.17	-0.19
Humerus DEXA R SDev	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.11	-0.22
Femur DEXA R Mean	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.11
Femur DEXA R SDev	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Bold indicates P < 0.05

15

Table 6. Site 2 simple correlation coefficients between carcass measures of hot carcass weight (kg), GR tissue depth (mm), c-site fat depth (mm), eye muscle area (cm<sup>2</sup>) with DEXA determined carcass composition (lean, fat and bone %) and bone DEXA R Mean (all carcass, humerus, lumbar vertebra and femur) and their mean standard deviation (SDev)

	Hot carcass weight (kg)	DEXA lean %	DEXA fat %	DEXA bone %	GR tissue depth (mm)	C-site fat depth (mm)	Eye muscle area (cm²)	Loin intramuscular fat %	All carcass DEXA R Mean	All Carcass DEXA R SDev	Lumbar vertebra DEXA R Mean	Lumbar vertebra DEXA R SDev	Lumbar vertebra (single) DEXA R Mean	Lumbar vertebra (single) DEXA R SDev	Humerus DEXA R Mean	Humerus DEXA R SDev	Femur DEXA R Mean	Femur DEXA R SDev
Hot carcass weight (kg)	1	-0.07	0.58	-0.76	0.84	0.72	0.75	0.10	-0.86	-0.16	-0.72	-0.02	-0.39	-0.19	-0.63	-0.13	-0.88	0.15
DEXA lean %	-	1	-0.79	0.45	-0.23	-0.44	0.08	-0.37	0.28	0.00	0.20	-0.01	0.17	0.09	0.11	0.04	0.05	-0.05
DEXA fat %	-	-	1	-0.89	0.61	0.71	0.36	0.43	-0.75	-0.10	-0.59	-0.02	-0.37	-0.20	-0.49	-0.13	-0.55	0.06
DEXA bone %	-	-	-	1	-0.71	-0.70	-0.57	-0.38	0.91	0.15	0.73	0.05	0.45	0.26	0.64	0.15	0.75	-0.04
GR tissue depth (mm)	-	-	-	-	1	0.73	0.61	0.23	-0.78	-0.15	-0.72	-0.01	-0.45	-0.24	-0.50	-0.18	-0.73	0.14
C-site fat depth (mm)	-	-	-	-	-	1	0.52	0.23	-0.73	-0.08	-0.65	-0.05	-0.45	-0.20	-0.49	-0.14	-0.68	0.04
Eye muscle area (cm <sup>2</sup> )	-	-	-	-	-	-	1	-0.04	-0.71	-0.07	-0.60	0.08	-0.28	-0.18	-0.57	-0.19	-0.76	0.20
Loin intramuscular fat %	-	-	-	-	-	-	-	1	-0.17	-0.01	-0.13	0.03	-0.08	0.02	-0.11	-0.05	-0.04	-0.09
All carcass DEXA R Mean	-	-	-	-	-	-	-	-	1	0.19	0.85	0.09	0.59	0.31	0.71	0.26	0.87	-0.10
All Carcass DEXA R SDev	-	-	-	-	-	-	-	-	-	1	0.16	0.13	0.13	0.19	0.06	0.01	0.17	0.05
Lumbar vertebra DEXA R Mean	-	-	-	-	-	-	-	-	-	-	1	0.31	0.72	0.48	0.47	0.27	0.72	-0.06
Lumbar vertebra DEXA R SDev	-	-	-	-	-	-	-	-	-	-	-	1	0.56	0.49	-0.06	0.05	-0.02	0.08
Lumbar vertebra (single) DEXA R Mean	-	-	-	-	-	-	-	-	-	-	-	-	1	0.52	0.26	0.36	0.40	0.04
Lumbar vertebra (single) DEXA R SDev	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.12	0.25	0.14	0.04
Humerus DEXA R Mean	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.39	0.66	0.09
Humerus DEXA R SDev	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.12	0.10
Femur DEXA R Mean	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.10
Femur DEXA R SDev	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

Bold indicates P < 0.05

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#### 3.2 Correlations

#### 3.2.1 Carcass phenotypic traits and bone DEXA values

At sites 1 and 2 lean % had a negative correlation with GR tissue depth (mm), c-site fat depth (mm) and IMF % (P < 0.05) and at site 1 there was a positive correlation with EMA (mm<sup>2</sup>) (P< 0.5, Table 5 and Table 6). At site 1 there was a positive correlation between C-site fat depth (mm) and IMF % (P < 0.05, simple correlation 0.38) and at site 2 there was a positive correlation of c-site fat depth (mm) and both EMA (cm<sup>2</sup>) (P < 0.05, 0.52 and 0.23)

There was little correlation of lean % with the bone DEXA R terms, although at site 1, lean % had a negative correlation with the femur DEXA R Mean (P < 0.05, -0.20; Table 5) and at site 2, a positive correlation with all carcass bone DEXA R mean and all lumbar vertebra bone DEXA R (P < 0.05, 0.28 and 0.20; Table 6). For IMF % there was a negative correlation of this term with bone DEXA R Mean terms at site 1 only for all carcass bone, all lumbar vertebra, anterior vertebra and humerus (P < 0.05; -0.43, -0.36 -0.34).

At sites 1 and 2 there was moderate positive correlation between a number of the bone DEXA R Mean values (whole vertebra, anterior vertebra, humerus) with their standard deviation (P<0.05, range simple correlation coefficient 0.47 to 0.76). The exception the femur where there was no correlation between the 2 variables. For all carcass bone DEXA R value there was no correlation between this value and all carcass bone DEXA R SDev at site 1. In contrast at site 2 there was a small but significant correlation between all carcass bone DEXA R and the all carcass bone DEXA R SDev (P < 0.05, simple correlation coefficient 0.19).

#### 3.2.2 Correlation of overall liking within roast and grill cuts

At site 1, there was significant correlation of overall liking between roast cuts (Table 5, P < 0.05). The strength of these correlations was relatively poor, with simple correlation coefficients of shoulder to the rack and hind leg roasts 0.28 and 0.30. A similar correlation was observed between the hind leg and rack roast (simple correlation coefficient 0.25). For grill cuts, the loin was correlated to the outside and topside (Table 5, P < 0.05, simple correlation coefficient 0.28). The grill cuts of the hind section (rump, knuckle, outside and topside) were all similarly correlated to one another (P < 0.05), with the highest correlation between the outside and knuckle (simple correlation coefficient 0.45) and the lowest between the topside and rump (0.21).

For site 2 there were also correlations between overall liking of some of the roast cuts (P < 0.05). Similar to site 1, they were relatively poor, with simple correlations existing between the shoulder roast and both rack cutlet (0.29) and topside roast (0.23). Additionally, there was a simple correlation of 0.20 between the rack cutlet and topside roast. Within the grill cuts at site 2, the loin was correlated with the rump, knuckle, outside and topside grill cuts with similar strength (simple correlations 0.40, 0.42, 0.47 and 0.55). All grill cuts of the hind section were correlated to each other (P < 0.05).

17

#### 3.3 DEXA prediction of eating quality

#### 3.3.1 Loin grill

#### 3.3.1.1 Site 1

For the bone DEXA values, all carcass DEXA R Mean demonstrated a significant relationship with loin grill overall liking (Table 7, Model 1: P<0.01, R<sup>2</sup> 0.10). As all carcass DEXA R Mean increased there was a decrease in loin grill overall liking of 10.5 eating quality scores, with this relationship demonstrated in Figure 1. DEXA lean % showed a negative relationship with loin grill overall liking (Table 7, Model 2: P<0.05 R<sup>2</sup> 0.06 RMSE 8.59). Lean % also demonstrated a negative relationship with the other eating quality traits (tenderness, juiciness and flavour) although individual model results are not shown for these traits. In contrast, loin IMF% showed a positive relationship with loin grill overall liking (Table 7, Model 3: P<0.01 R<sup>2</sup> 0.10 RMSE 8.34), with a similar effect demonstrated with tenderness, juiciness and overall liking (individual model results not shown).

The all carcass DEXA R Mean R term remained significant when included in a model containing either DEXA Lean % (Table 7, Model 4) and Ioin IMF % (Table 7, Model 5). When all three terms were included in a model the only All carcass bone DEXA R Mean remained significant. When these model were tested as linear mixed effect models and the eating quality "pick" included as a random term the significance of the terms did not alter.

All carcass bone DEXA R was also a predictor of tenderness, juiciness, and flavour of the loin grill (P <0.05). When either IMF % or lean % or both IMF % and lean % were included in these models along with Bone R, the Bone DEXA R term remained significant (individual model results not shown).

Both anterior vertebra DEXA R Mean (P < 0.01) and whole vertebra DEXA R Mean (P < 0.05) showed a negative relationship with loin grill overall liking. Whole vertebra DEXA R Mean only remained significant when included in a model with lean % (P < 0.05), compared with the anterior vertebra DEXA R Mean which remained significant when included in a model with either IMF % or lean % or both terms. Similarly, for prediction of loin grill tenderness, anterior vertebra DEXA R Mean and whole vertebra DEXA R Mean, however these DEXA terms only remained significant when included in models with lean % (P < 0.05, individual models not shown).

#### 3.3.1.2 Site 2

All carcass DEXA R Mean demonstrated a negative relationship with loin grill overall liking (P < 0.05; Table 7, Model 7: R<sup>2</sup> 0.08 RMSE 9.79), with overall liking decreasing by 9.79 eating quality scores across an increasing bone DEXA R Mean (Figure 2). Similar to lambs at site 1, loin IMF% had a positive association with loin grill overall liking (Table 7, Model 9: P<0.01 R<sup>2</sup> 0.06 RMSE 8.78), increasing loin grill overall liking by 9.1 points across the range of IMF%. IMF % also demonstrated a positive relationship with loin grill tenderness, juiciness and flavour.

When IMF % was included in a model with All carcass bone DEXA R Mean to predict overall liking (Table 7, Model 11) juiciness, and flavour both terms remained significant. When the younger lambs were removed from the analysis this did not alter the significance of lean %, IMF % or all carcass bone DEXA R, although the magnitude of effect of the DEXA term

decreased slightly to 8.8 eating quality units. Lean % did not have a significant relationship with eating quality of the loin grill (overall liking, flavour, juiciness, tenderness). When included along with any of the bone DEXA R Mean values that were significant it continued to remain insignificant.

For vertebra DEXA R Mean there was a negative relationship with loin grill overall liking, tenderness, juiciness and flavour (P<0.05). When loin IMF % was included in these models both terms remined significant. Proximal vertebra DEXA R Mean had a negative association with loin grill overall liking, juiciness and flavour (P<0.05). When loin IMF % was included with the DEXA term remained significant.

Table 7. F-values, coefficient, intercept,	coefficient of determination (R-	-square), and root mean	square error (RMSE) f	for models predicting overall	liking for the loin, grill using
All carcass bone DEXA R Mean, lean %	6 and loin intramuscular fat % fo	or sheep from site 1 and	site 2.		

			Sit	:e 1			Site 2							
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8	Model 9	Model 10	Model 11	Model 12		
Dependent variable						F-'	Values							
All bone DEXA R Mean	8.97**	-	-	10.02**	4.03*	5.48*	8.63**	-	-	7.05**	6.82*	6.94**		
Lean %	-	4.7*	-	5.74*	-	1.9	-	1.61	-	0.18	-	0.23		
Loin IMF %	-	-	9.65**	-	4.66*	0.88	-	-	7.66**	-	5.76**	5.77*		
		Coefficients and intercepts												
Intercept	724.59	127.04	53.22	806.6	523.46	675.33	184.27	85.44	61.84	184.86	164.9	162.66		
All bone DEXA R Mean	-515.99	-	-	-3.17	-365.69	-445.37	-89.28	-	-	-85.14	-78.28	-82.38		
Lean %	-	-1.13	-	-2.4	-	-0.85	-	-0.299	0.98	-0.1	-	0.12		
Loin IMF %	-	-	2.97	-	2.19	1.18	-	-	-	-	0.84	0.9		
					Magnit	ude of effec	t (max - min	predicted)						
	10.5	9.45	11.43	10.81	7.46	9.09	9.79	-	9.11	8.58	8.58	9.03		
						Precisio	on estimates							
R <sup>2</sup>	0.10	0.06	0.1	0.16	0.15	0.17	0.08	0.01	0.06	0.07	0.12	0.12		
RMSE	8.38	8.59	8.34	8.13	8.19	8.14	8.77	9.03	8.78	8.8	8.54	8.57		



Figure 1. Predicted loin grill overall liking for sheep at site 1 using all carcass bone DEXA R Mean (P<0.05, Table 6 model 1). Dots represent residuals from the predicted means (solid line) ± SD (dotted lines).



Figure 2. Predicted loin grill overall liking for sheep at site 2 using all carcass bone DEXA R Mean (P<0.05, table 6, model 7). Dots represent residuals from the predicted means (solid line)  $\pm$  SD (dotted lines).

21

3.3.2 Prediction of eating quality of grill cuts of the hind section (knuckle, outside, rump and topside)

There was not a consistent or strong relationship between carcass bone DEXA R Mean either from the whole carcass or the isolated bones of the carcass with the grill cuts of the hind section.

#### 3.3.2.1 Knuckle grill

There were few predictors of knuckle grill eating quality, however at site 2 there was a positive relationship with loin IMF % and knuckle grill overall liking (P < 0.05, Table 8, Model 1:  $R^2 0.05$  RMSE 8.88), increasing overall liking of this cut by 8 units across the range of loin IMF %. Loin IMF % demonstrated a positive relationship with knuckle grill tenderness, juiciness and flavour (P<0.05, individual model results not shown).

#### 3.3.2.2 Outside grill

There were few predictors of outside grill at either site, however at site 2, all carcass bone DEXA R Mean demonstrated a negative relationship with outside grill overall liking (P < 0.05, Table 8, Model 2:  $R^2 0.03 RMSE 9.56$ ) which resulted in a decrease in eating quality of 6.2 scores across the all carcass DEXA R Mean range. Loin IMF % was a positive predictor of outside grill overall liking at site 2, (P < 0.05, Table 8 Model 3:  $R^2 0.03 RMSE 9.65$ ), with an increase of 7.1 eating quality scores across the increasing range of IMF %. When both IMF % and all carcass bone DEXA R were included in the same models the significance of both terms remained significant (P < 0.1, Table 8, Model 4:  $R^2 0.06 RMSE 9.62$ ). There was a similar relationship between IMF % and all carcass bone DEXA R were included in the same models the significance of both terms remained significant (P < 0.1, Table 8, Model 4:  $R^2 0.06 RMSE 9.62$ ). There was a similar relationship between IMF % and all carcass bone DEXA R with juiciness and flavour (P <0.05). For juiciness, when combined in a model together it demonstrated an 8.5 unit decrease across the all carcass DEXA R Mean (P < 0.05, Table 8, Model 5  $R^2 0.09 RMSE$  9.79). At site 2, in addition to all carcass bone DEXA R Mean, whole vertebra, femur and humerus bone DEXA R demonstrated a negative relationship with outside grill juiciness, which remained significant when IMF % was included in models along with the bone DEXA terms (P < 0.05, individual model results not shown).

#### 3.3.2.3 Rump grill

There were no predictors of rump grill eating quality traits at site 1. However, at site 2, the overall liking of the rump grill decreased as the bone DEXA R Mean from all carcass bone, (P < 0.01, Table 8, Model 6:  $R^2 0.06$  RMSE 9.96) however this effect was largely driven by the small number of younger animals with the lower all carcass bone DEXA R as when these were removed from the analysis the bone DEXA term was no longer significant. Loin IMF % demonstrated a positive association with rump grill overall liking and flavour (P < 0.05 Table 8, Model 7:  $R^2 0.06$  RMSE 9.96). When all carcass bone DEXA R Mean and Ioin IMF % were included in a model together only the bone DEXA term remained significant.

Table 8. F-values, coefficient, intercept, coefficient of determination (R-square), and root mean square error (RMSE) for models predicting eating quality of the knuckle, outside, rump and topside) using all carcass bone DEXA R Mean and loin intramuscular fat %.

						Site 2								
Dependent variable	Knuckle grill overall liking	Outside Outside Outside Outside Rump grill Rump grill Il grill overall grill overall grill overall overall overall liking liking liking juiciness liking liking liking		Rump grill overall liking	Topside grill overall liking	Topside I grill overal liking	Topside I grill overall liking							
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8	Model 9	Model 10	Model 11			
						F-Values								
All bone DEXA R Mean	-	4.14**	-	2.98*	5.34*	7.51**	-	5.95**	6.07*	-	4.69*			
Loin intramuscular fat %	2.77**	-	3.93**	2.79*	4.66*	-	4.05**	2.57	-	5.28*	3.7			
	Coefficients and intercepts													
Intercept	61.1	142.18	51.48	126.11	156.44	180.23	55.11	164.13	160.08	48.9	141.15			
All bone DEXA R Mean	-	-66.06	-	-56.59	511.7	-92.07	-	-82.67	-82.47	-	-			
Loin intramuscular fat %	0.86	-	0.76	0.64	446.5	-	0.81	0.64	-	0.9	0.75			
	Magnitude of effect (max - min predicted)													
	8	7.2	7.1	6.2	8.5	10.1	7.55	9.06	9.04	8.35	7.83			
					Pre	cision estimation	ates							
R <sup>2</sup>	0.05	0.03	0.03	0.06	0.09	0.06	0.03	0.08	0.05	0.04	0.08			
RMSE	8.88	9.62	9.65	9.56	9.79	9.96	10.1	9.89	9.92	9.79	9.64			

23

An increase in lumbar vertebra and femur DEXA R Mean also resulted in an increase in rump grill overall liking (P < 0.01, individual models not shown), with the DEXA terms remaining significant when loin IMF % was included in these models. Tenderness and juiciness of the rump grill decreased as the bone DEXA R Mean of all carcass bone, whole vertebra and femur increased (P < 0.05). The flavour of the rump grill decreased as all carcass DEXA R Mean increased (P < 0.05), with the DEXA term remaining significant when loin IMF % was included in this model.

#### 3.3.2.4 Topside grill

At site 2, all carcass DEXA R Mean had a negative association with the overall liking of the topside grill, (P < 0.05, Table 8, Model 9:  $R^2 0.05$  RMSE 9.92), which resulted in a decrease in eating quality of 9 overall liking scores across the DEXA Mean range. The result in this cut was largely driven by the small number of younger animals that had lower all carcass bone DEXA R values as when they were removed from the model the DEXA term was no longer significant.

An increase in loin IMF % was associated with an increase in the overall liking scores of 8.35 across the increasing range of IMF % (P < 0.05, Table 8, Model 10:  $R^2$  0.04 RMSE 9.79) and a similar magnitude of effect to juiciness and flavour (P < 0.05, individual model results not shown).

Lean % impacted on topside grill flavour (P < 0.01,  $R^2$  0.06 RMSE 8.23, individual model result not shown) and for this eating quality trait resulted in a decrease of 7.9 flavour scores.

In relation to the other eating quality traits, all carcass DEXA R Mean was also associated with a decrease in juiciness and flavour, and when included along with either IMF %, both terms remained significant (P < 0.05, individual model results not shown). In models predicting topside grill flavour, when the bone DEXA term was included along with lean %, neither of the terms were significant (P > 0.05, individual model result not shown).

#### 3.3.1 Roast cuts from the lamb carcass

#### 3.3.1.1 Shoulder Roast

The shoulder roast demonstrated associations with bone DEXA R Mean, Ioin IMF % and Iean % (P < 0.05), however this varied between sites 1 and 2. At site 1, of the eating quality traits, only tenderness was predicted, with humerus DEXA R Mean demonstrating a negative relationship (P < 0.05, Table 9, model 1:  $R^2 0.23$  RMSE 8.8). Across the 4 standard deviation range of increasing humerus DEXA R Mean the tenderness of the shoulder roast decreased by 9.7 points. At site 1, Iean % also demonstrated a negative relationship with shoulder roast tenderness (P < 0.05, Table 9, Model 2), with a similar result observed for juiciness (individual model result for juiciness not shown). Conversely, an increase in Ioin IMF % increased shoulder roast juiciness (P < 0.05, Table 9, Model 3). The relationship between humerus bone DEXA R Mean and shoulder roast tenderness remained significant when Ioin IMF % was included in the model with both terms remaining significant when Iean % was included (P < 0.05, Table 9, Model 4).

At site 2 there both all carcass DEXA R Mean and humerus DEXA R Mean had a significant negative association with should roast overall liking, tenderness, juiciness and flavour (P < 0.05). For brevity of results, only results for overall liking's association with all carcass DEXA R Mean is shown in Table 9, with the associations with humerus DEXA R Mean similar. For overall liking, both these DEXA terms demonstrated a negative relationship with shoulder roast (P < 0.05) with this relationships remaining significant when IMF % was included in the model. For all carcass DEXA R Mean this resulted in a decrease in shoulder roast overall liking of 19.2 across the range of DEXA R Mean values (Table 9, Model 5: R<sup>2</sup> 0.23 RMSE 8.8). Given the high magnitude of effect this model was tested when the small number of younger animals were removed from the analysis the relationship between bone DEXA R Mean and overall liking remained significant, however the magnitude of effect was reduced to 9.3 overall liking scores across the reduced range of all carcass DEXA R Mean of the mutton. When a linear mixed effect model was run with eating quality session (pick) was run on both models the DEXA term remained significant.

At site 2, lean % had a weak negative association with shoulder roast juiciness (P < 0.05), however did not impact the other eating quality traits. Loin IMF % had a positive association (P < 0.05) with shoulder roast overall liking, juiciness and flavour (P < 0.05, Table 9, Model 6:  $R^2 0.06$  RMSE 9.78). Across the 9.3 IMF % range the shoulder roast overall liking increased by 9.6 units and when IMF % and all carcass DEXA R Mean and Ioin IMF % were included in a model concurrently both terms remained significant (Table 9, Model 7).

For shoulder roast tenderness, all of the bone DEXA R Mean variables (all carcass bone, whole vertebra, anterior vertebra, femur and humerus) were significant predictors of this trait for the shoulder roast (P < 0.05), which all remained significant when either lean % or IMF % were included in the models. Similarly for flavour, all bone DEXA R mean terms were significant predictors of this trait (P < 0.05) which remained significant when IMF % was included in the models. The magnitude of effects for tenderness, juiciness and flavour are not reported individually however were similar to those reported for overall liking.

#### 3.3.1.2 Rack roast cuts

The rack cutlet was collected and tested at site 2 only. All carcass DEXA R Mean and whole lumbar vertebra demonstrated a negative relationship with rack overall liking, tenderness, juiciness and flavour (P < 0.05). Across the range of all carcass DEXA at this site, there was a decrease in overall liking of the rack cutlet of 17.2 overall liking scores (Table 9, model 8  $R^2 0.21 RMSE 9.03$ ), however the magnitude of effect was largely driven by the range in all carcass bone R Mean when including the lambs. With these animals excluded the DEXA R Mean term remained significant but the magnitude of effect decreased to 13.1 overall liking scores. The DEXA term remained significant when eating quality session was included in the model. As loin IMF % increased there was an increase in the rack overall liking of 11.0 eating quality units (Table 9, model 9), with a similar magnitude of effect for tenderness, juiciness and flavour (P < 0.05). When all carcass DEXA R Mean and IMF were included in the same model both terms remained significant (Table 9, model 10).

There was no association of lean %, IMF % or bone DEXA R Mean terms with the rack roast at site 1 (P > 0.1).

#### 3.3.1.3 Knuckle roast

At site 2, the knuckle roast was collected and tested for eating quality. There were few predictors of eating quality in this cut, although all carcass bone DEXA R Mean and femur DEXA R Mean, both had a negative association with overall liking and tenderness (P < 0.1). For all carcass bone DEXA R Mean, there was a decrease in overall liking scores of 9.15 across the increasing range of all carcass bone DEXA R Mean (P<0.1,  $R^20.05$ , RMSE 7.77, individual model results not shown)

Table 9. F-values, coefficient, intercept, coefficient of determination (R-square), and root mean square error (RMSE) for models predicting eating quality (tenderness and overall liking) of the roast cuts (shoulder and rack cutlet) using All carcass DEXA R Mean, lean % and loin intramuscular fat % for sheep from site 1 and site 2.

		Sit	e 1		Site 2							
Dependent variable	Shoulder roast tenderness	Shoulder roast tenderness	Shoulder roast tenderness	Shoulder roast tenderness	Shoulder roast overall liking	Shoulder roast overall liking	Shoulder roast overall liking	Rack cutlet overall liking	Rack cutlet overall liking	t Rack cutlet overall liking		
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8	Model 9	Model 10		
					F-Values							
All bone DEXA R Mean	-	-	-	-	34.23**	-	30.35**	30.27**	-	26.52**		
Humerus DEXA R Mean	4.2**	-	-	4.11**	-	-	-	-	-	-		
Lean %	-	3.3*	-	3.21*	-	-	-	-	-	-		
Loin IMF %	-	-	3.32*	-	-	6.77**	4.01**	-	9.01**	6.03**		
				Coeff	icients and in	tercepts						
Intercept	266.45	124.4	55.47	319.7	283.51	49.09	266.78	280.51	53.16	259.71		
All bone DEXA R Mean	-	-	-	-	-174.81	-	-165.22	-186.75	-	-156.76		
Humerus DEXA R Mean	-159.26			-155.33	-	-	-	-	-	-		
Lean %	-	-1.11	-	-1.07	-	-	-	-	-	-		
Loin IMF %	-	-	2.1	-	-	1.03	0.71	-	1.19	0.089		
				Magnitude o	f effect (max -	min predict	ed)					
	9.65	8.98	8.12	9.52	19.2	9.56	18.1	18.5	11	17.2		
				Ρ	recision estim	ates						
R <sup>2</sup>	0.05	0.04	0.04	0.09	0.23	0.06	0.26	0.21	0.07	0.25		
RMSE	10	10.1	10.1	9.87	8.8	9.78	8.72	9.03	9.8	8.85		

\*P < 0.05 \*\* P < 0.01

# 3.4 Bone minerals and their relationship with DEXA R Mean and eating quality

3.4.1 Mineral content of bones and correlations with carcass composition and measures.

The raw mean  $\pm$  SD of the mineral content (calcium, magnesium and phosphorus) for the humerus, lumbar vertebra and femur bones are shown in Table 10.

	S	ite 1	Site	2 (Lamb)	Site 2 Mutton		
	Mean ± SD	(min, max)	Mean ± SD	(min, max)	Mean ± SD	(min, max)	
Humerus (mg/g bone)	n	= 84		n = 10	n = 108		
Calcium	259.12 ±	(243.7, 272.2)	275.24 ±	(247.9, 326.4)	263.08 ±	(152.2, 283.2)	
Magnesium	4.82 ± 0.3	(4.2, 5.4)	5.06 ± 0.7	(4.3, 6.2)	4.41 ± 0.3	(2.5, 5.1)	
Phosphorus	130.77 ±	(123.3, 137.4)	135.84 ±	(122.5, 161.3)	129.36 ±	(77.7, 138.4)	
Lumbar vertebra	n = 79		1	n = 10	n = 108		
Calcium	118.83 ±	(92.0, 144.0)	197.79 ±	(169.6, 225.7)	208.15 ±	(164.5, 290.3)	
Magnesium	2.33 ± 0.2	(1.9, 2.9)	3.76 ± 0.4	(3.3, 4.6)	3.46 ± 0.3	(2.7, 4.5)	
Phosphorus	60.63 ± 4.7	(48.0, 72.4)	102.86 ±	(87.1, 120.3)	107.19 ±	(82.6, 148.4)	
Femur (mg/g bone)	n	= 84	I	n = 10	n	= 108	
Calcium	261.24 ±	(195.3, 283.1)	268.20 ±	(250.3, 324.1)	271.61 ±	(182.5, 349.2)	
Magnesium	4.85 ± 0.4	(3.4, 5.5)	5.05 ± 0.5	(4.5, 6.2)	4.55 ± 0.4	(3.0, 6.7)	
Phosphorus	131.87 ±	(98.4, 140.7)	133.47 ±	(110.0, 162.0)	124.40 ±	(80.1, 175.6)	

Table 10. Raw mean  $\pm$  SD (minimum, maximum) for the mineral content (mg/g bone) of calcium, magnesium and phosphorus of the humerus, lumbar vertebra and femur.

Correlations between the carcass composition (fat, lean and bone %), the carcass measures of GR tissue depth, EMA (mm) and c-site fat depth (mm) with the bone mineral content (Ca, Mg and P) of the humerus, lumbar vertebra and femur are shown in Table 11 and Table 12.

Within a bone region (humerus, lumbar vertebra and femur) there was generally good correlation between the bone minerals. The bone with the highest correlation between minerals was the lumbar vertebra, with both site 1 (lambs) and site 2 (mutton) demonstrating moderate to high correlation between all three minerals in this bone. For the humerus and femur there was also good correlation between the minerals in the other bones (humerus and femur) although in the lambs at site 1 there was no correlation between Ca and Mg (P > 0.05). Of the minerals, Mg had the best correlation between the bones with significant correlations (P < 0.05) between the Mg of the humerus, lumbar vertebra and femur at both sites 1 and 2. For Ca and P there was less consistent correlation of mineral content between the bones.

There was not a consistent relationship between loin IMF %, carcass lean % and the bone mineral content of the humerus, lumbar vertebra and femur at each site. At site 1, IMF % demonstrated a positive relationship with humerus Mg (P < 0.05), lumbar vertebra Ca and Mg (P < 0.05) and femur Mg and P (P < 0.05) (Table 11).

Carcass lean % at site 1 had a negative relationship with lumbar vertebra Ca, Mg and P (P < 0.05) (Table 11). At site 2, lean % demonstrated a positive relationship with femur Ca and P (P < 0.05) Table 12.

Table 11. Simple correlation at site 1 lambs between carcass composition (lean, fat and bone %), carcass measures of GR tissue depth (mm), c-site fat depth (mm), eye muscle area (mm), loin intramuscular fat % and the bone mineral content (Ca, Mg, and P) of the humerus, lumbar vertebra and femur

	DEXA lean % in	Loin tramuscular fat %	Humerus Ca (mg/g bone)	Humerus Mg (mg/g bone)	Humerus P (mg/g bone)	Lumbar Ca (mg/g bone)	Lumbar Mg (mg/g bone)	Lumbar P (mg/g bone)	Femur Ca (mg/g bone)	Femur Mg (mg/g bone)	Femur P (mg/g bone)
DEXA lean %	1.00	-0.57	0.06	-0.10	0.07	-0.35	-0.29	-0.34	-0.03	-0.13	-0.19
Loin intramuscular fat %	-	1.00	-0.11	0.23	-0.10	0.25	0.30	0.20	0.01	0.24	0.19
Humerus Ca (mg/g bone)	-	-	1.00	0.01	0.89	-0.05	-0.25	-0.06	-0.08	-0.26	-0.23
Humerus Mg (mg/g bone)	-	-	-	1.00	0.25	-0.12	0.49	-0.07	-0.27	0.83	0.11
Humerus P (mg/g bone)	-	-	-	-	1.00	-0.12	-0.15	-0.10	-0.19	-0.05	-0.21
Lumbar Ca (mg/g bone)	-	-	-	-	-	1.00	0.65	0.97	0.12	-0.14	0.11
Lumbar Mg (mg/g bone)	-	-	-	-	-	-	1.00	0.67	-0.05	0.48	0.19
Lumbar P (mg/g bone)	-	-	-	-	-	-	-	1.00	0.11	-0.10	0.09
Femur Ca (mg/g bone)	-	-	-	-	-	-	-	-	1.00	0.13	0.81
Femur Mg (mg/g bone)	-	-	-	-	-	-	-	-	-	1.00	0.50
Femur P (mg/g bone)	-	-	-	-	-	-	-	-	-	-	1.00

Bold P < 0.05; EMA eye muscle area (cm<sup>2</sup>); IMF intramuscular fat

Table 12. Simple correlation at site 2 (mutton only) between carcass composition (lean, fat and bone %), carcass measures of GR tissue depth (mm), c-site fat depth (mm), eye muscle area (mm), loin intramuscular fat % and the bone mineral content (Ca, Mg, and P) of the humerus, lumbar vertebra and femur

	DEXA lean % in	Loin tramuscular fat %	Humerus Ca (mg/g bone)	Humerus Mg (mg/g bone)	Humerus P (mg/g bone)	Lumbar Ca (mg/g bone)	Lumbar Mg (mg/g bone)	Lumbar P (mg/g bone)	Femur Ca (mg/g bone)	Femur Mg (mg/g bone)	Femur P (mg/g bone)
DEXA lean %	1.00	-0.36	0.17	-0.04	0.05	-0.02	0.06	-0.01	0.22	0.05	0.24
Loin intramuscular fat %	-	1.00	0.05	0.11	0.13	0.04	-0.02	0.02	0.05	0.05	0.02
Humerus Ca (mg/g bone)	-	-	1.00	0.46	0.66	0.08	0.04	0.06	0.27	0.22	0.58
Humerus Mg (mg/g bone)	-	-	-	1.00	0.56	-0.03	0.47	0.02	0.12	0.70	0.30
Humerus P (mg/g bone)	-	-	-	-	1.00	-0.10	0.03	-0.11	0.04	0.18	0.23
Lumbar Ca (mg/g bone)	-	-	-	-	-	1.00	0.66	0.98	0.25	0.02	-0.13
Lumbar Mg (mg/g bone)	-	-	-	-	-	-	1.00	0.74	0.16	0.44	-0.03
Lumbar P (mg/g bone)	-	-	-	-	-	-	-	1.00	0.28	0.09	-0.12
Femur Ca (mg/g bone)	-	-	-	-	-	-	-	-	1.00	0.53	0.42
Femur Mg (mg/g bone)	-	-	-	-	-	-	-	-	-	1.00	0.50
Femur P (mg/g bone)	-	-	-	-	-	-	-	-	-	-	1.00

Bold P < 0.05; EMA eye muscle area (cm<sup>2</sup>); IMF intramuscular fat

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29

#### 3.4.2 Bone mineral differences between age.

Although this experiment was not designed to examine age differences specifically there was an attempt to compare age with site and also between sites. Within site age comparisons were made at site 1 between new and old season lambs within a kill group, for bone mineral content of the femur, humerus and lumbar vertebra. There was no consistent differences for the bone mineral content between new and old season lambs across both kill groups. Within kill 1 the new season lambs had 8.77 mg/g more and 8.12 mg/g less calcium in the humerus and vertebra (P < 0.05). For magnesium in kill group 1 the new season lambs has less magnesium in the femur (0.14 mg/g) and lumbar vertebra (0.21 mg/g) than old season lambs. The result for magnesium was not consistent across kill groups as in kill group 2 the new season lambs had 0.15 mg/g more magnesium in the humerus. For phosphorus, new season lambs in kill 1 had 3.16 mg/g more and 4.46 mg/g less humerus and vertebra of the season lambs in kill 1 had 3.16 mg/g more and 4.46 mg/g less humerus and vertebra phosphorus.

Comparisons between bones (humerus and femur) were made between site 1 (all lambs) and site 2 (mutton). Comparison of vertebra mineral content was unable to be made due to differences in process of the lumbar vertebra between sites 1 and 2. For calcium, the concentration was higher at site 2 for both femur and humerus (P < 0.05, Table 13). Conversely the magnesium was higher for both femur and humerus at site 1 compared to site 2. Phosphorus concentration was higher at site 2, however this was only evident in the femur as there was no difference between the sites for the humerus.

	Least squared means ± se					
	Site 1	Site 2				
Calcium						
Femur	262.04 ± 1.07ª	271.39 ± 0.95 <sup>b</sup>				
Humerus	259.12 ± 1.06ª	264.12 ± 0.94 <sup>b</sup>				
Lumbar vertebra*	118.83 ± 1.10	207.38 ± 0.94				
Magnesium						
Femur	$4.86 \pm 0.03^{a}$	$4.54 \pm 0.03^{b}$				
Humerus	$4.82 \pm 0.03^{a}$	$4.43 \pm 0.03^{b}$				
Lumbar vertebra	$2.33 \pm 0.03$	$3.46 \pm 0.03$				
Phospohorus						
Femur	132.17 ± 0.65ª	123.27 ± 0.59 <sup>b</sup>				
Humerus	130.77 ± 0.65ª	129.85 ± 0.57ª				
Lumbar vertebra	60.63 ± 0.67	106.80 ± 0.57				

Table 13. Least squared means  $\pm$  s.e. for bone mineral content (mg/g/bone) for the humerus and lumbar vertebra from animals at sites 1 (lambs) and 2 (mutton only).

<sup>a,b</sup> Values within a row with different superscripts differ significantly at P < 0.05

\*no comparison made between sites due to differing laboratory method for lumbar vertebra

# 3.4.3 Relationship between bone DEXA R Mean, mineral content and eating quality

There was no direct relationship between bone mineral content of the humerus, lumbar vertebra and femur with eating quality (overall liking only) (P > 0.05). The other relationships explored was between both all carcass bone DEXA R Mean, the DEXA R Mean values of the individual bones (humerus, lumbar vertebra and femur) and the bone mineral content of the bones. These relationships were tested within a site due to the differences in range in bone DEXA R values and inability to calibrate images between sites.

The most consistent association between bone minerals and DEXA R Mean was seen with Mg. Humerus Mg (mg/g bone) demonstrated a negative relationship with all carcass bone DEXA R Mean at site 1 (Figure 3a:  $R^2 0.07$  RMSE 0.27) and site 2 (Figure 3b:  $R^2 0.07$  RMSE 0.38). A similar result was observed in the femur where femur Mg (mg/g bone) decreased as the all carcass bone DEXA R Mean increased at site 1 (Figure 3c:  $R^2 0.08$  RMSE 0.34) and site 2 (Figure 3d:  $R^2 0.03$  RMSE 0.42).



Figure 3. Relationship between bone magnesium concentration (mg/g bone) in the humerus (a and b) and femur (c and d) with all carcass bone DEXA R Mean.

The magnesium content of the bones also had the most consistent relationship with the individual bone DEXA R Mean values. As the humerus DEXA R Mean values increased the Mg content of the humerus decreased at both site 1 (Figure 4a) and site 2 (Figure 4b). For lumbar DEXA R Mean this relationship was only observed at site 1 (Figure 4c), and for the femur was observed at site 2 (Figure 4d).



Figure 4. Relationship between humerus magnesium concentration (mg/g bone) and humerus DEXA R Mean at site 1 (a) and site 2 (b); lumbar vertebra magnesium concentration (mg/g bone) and lumbar vertebra DEXA R Mean (c) and femur magnesium concentration (mg/g bone) and femur DEXA R Mean (d)

There was less consistency in relationships between the bone mineral content of Ca and P with the bone DEXA R Mean values. At site 2, humerus P (mg/g bone) also had a negative relationship with humerus DEXA R Mean (P < 0.05; R<sup>2</sup> 0.03 RMSE 7.88) and femur P (mg/g bone) showed a similar relationship with femur DEXA R Mean (P < 0.05; R<sup>2</sup> 0.04 RMSE 11.6). For calcium, the bone mineral content of the lumbar vertebra increased as all carcass bone DEXA R Mean increased (P < 0.05; R<sup>2</sup> 0.04 RMSE 16.46), but this was only at site 2.

#### 3.5 Discussion

#### 3.5.1 Prediction of eating quality across the carcass

In support of our hypothesis, bone DEXA R values generated in the scanning of lamb carcasses within an abattoir can predict eating quality (overall liking, tenderness, juiciness and flavour) from across the lamb carcass. The precision of prediction of eating quality was generally poor and there was variation in the cuts that were associated with the bone DEXA R Mean terms. The relationship was most consistent relationship with eating quality was using the all carcass DEXA R Mean term rather than the individually traced bones (humerus, lumbar vertebra and femur) where an increase in the bone DEXA R Mean resulted in a decrease in eating quality.

The cut with the largest and most consistent association with bone DEXA R Mean with eating quality was in the loin grill for both sites 1 and 2. For this cut, the DEXA term was associated with all eating quality traits: overall liking, tenderness, juiciness and flavour. An increase in all carcass DEXA R Mean across 4 standard deviations of its range at site 1 and 2 resulted in a decrease in loin grill overall liking of 10.5 and 9.8 scores. This represents a significant proportion of the overall liking range at site 1 (43) and site 2 (46) overall liking scores. Site 2 did contain a small number of lambs (n=10) in the analysis, however the relationship between carcass bone DEXA R Mean and loin grill eating quality remained in the mutton with similar magnitude when these lambs were removed from the analysis and was not a reflection of the lambs at this site. For the loin grill the relationship between DEXA R Mean appears somewhat independent of carcass lean % and loin IMF % as when these terms were included in the models al terms remained significant. This is in contrast to earlier work, where the bone DEXA terms were often more strongly correlated to lean % and when both terms were included in an eating quality prediction model the DEXA bone terms became insignificant (Anderson et al. 2021). This is important from an industry perspective as predictors of eating quality should ideally be independent of other carcass traits which allows independent selection for carcass improvements. In the previous analysis of animals at site 1, the all carcass bone DEXA R Mean term was moderately correlated to lean % (simple correlation coefficient 0.85) (Anderson et al. 2021). The new algorithm for determining bone DEXA R values resulting in no correlation of lean % and this DEXA term for site 1 and therefore using the new bone method can be considered a more independent term compared to lean %.

The association of bone DEXA R Mean with the other grill cuts across the carcass was variable. All carcass DEXA R Mean had a negative association with the overall liking of grill cuts for the outside, rump grill and topside but at site 2 only. The association of the bone DEXA term at site 2 extended to other eating quality traits (tenderness, juiciness and flavour) in some cuts, but this was somewhat variable. Given a lack of other predictors of hind grill cut eating quality, the use of bone DEXA R Mean may still be useful and its relationship with eating quality should continue to be explored. Especially considering the bone DEXA terms generally remain significant when lean % or loin IMF % was included in models along side the relevant bone DEXA terms.

There prediction of roast cut eating quality was variable with a lack of consistency in the prediction of the shoulder and rack roasts and no effect in the leg roast. The largest effects were observed in the shoulder roast at site 2 where all carcass DEXA R Mean had a large

impact on prediction of overall liking (19 overall liking scores). At this site there were a small number of lambs included in the analysis as they were slaughtered on the same day as the mutton and as such the DEXA images were able to be analysed together. Adding strength to the relationship between shoulder roast and bone DEXA when the younger animals were removed from the analysis, and also when eating quality session was included in the analysis the DEXA term was significant. This relationship still existed when loin IMF % was included in the model.

Although a lack of consistent association of bone DEXA R Mean with cuts across the carcass is somewhat disappointing from an eating quality prediction perspective, it is not entirely unexpected. In the beef MSA system, ossification, which it is thought bone DEXA R Mean may be a proxy for, has a variable impact on the eating quality of cuts across the beef carcass (Watson et al. 2008). Therefore, it is unlikely that different cuts and cook combinations will have the same significance or magnitude of effect. Therefore, the use of bone DEXA R Mean is likely to require modelling with considerations such as muscle, IMF %, lean % and HCWT in mind, requiring further analysis of eating quality data.

The correlation between DEXA R Mean of the whole carcass and DEXA R Mean of individual bones (humerus, lumbar vertebra and femur) was moderate to high. This is consistent with previous studies in cadaver sheep using human DEXA (Turner, Mallinckrodt, Alvis et al. 1995). This study demonstrated some difficulty in the repeatability of isolating certain bones such as the proximal femur, humerus and proximal tibia and highlights the necessity to use regions of the sheep that can be reliably scanned to obtain similar orientation of carcass regions. In this current experiment the femur, lumbar vertebra and all carcass bone DEXA R values were relatively easy to obtain, however those from the humerus were more difficult and therefore possibly subject to increased error and less repeatability. Despite the high correlation of DEXA R Mean between bones, the all carcass DEXA R Mean had the most consistent relationship with the eating quality cuts. The rationale for testing the individual bones for their association with eating guality was that they may demonstrate biology relating to the muscles which were being tested in close proximity. However there appears currently to be no advantage in isolating the individual bones which is beneficial as all carcass DEXA R Mean can be readily acquired during routine scanning in the abattoir without further image processing.

This experiment has demonstrated variability in the association of lean % with eating quality and highlights it is not a reliable predictor of eating quality alone. The largest impact of lean % was in the loin grill at site 1 with a decrease in overall liking of this cut of 9.5 across an increasing range of lean %. This experiment has also demonstrated that increasing lean % also resulted in a decrease in scores for tenderness, juiciness and flavour although the magnitude of these effects have not reported. Carcass lean % had little impact on the eating quality of the hind section grill cuts and in this study was associated only with topside grill flavour at site 2 and for the topside at site 2 with overall liking, juiciness and flavour. Increasing lean % has previously been shown to negatively impact on eating quality using indicator muscles and fat weights (Pannier, Gardner, Pearce et al. 2014) and carcass lean % (Anderson et al. 2021). However, the current experiment demonstrates a relative lack of association of lean % with eating quality in many cuts. The lack of consistent association of lean % with eating quality means that other indicators of eating quality such as maturity would improve a cuts based prediction system of eating quality in lamb. Additionally, it is useful to have an independent predictor of eating quality to that of lean so that selection for lean % and eating quality can be selected for independently.

Loin IMF % had a consistent positive relationship with loin grill eating quality (overall liking, tenderness, juiciness and flavour). This is consistent with previous experiments in the loin grill in lamb (Pannier, Pethick, Geesink et al. 2014, Pannier, Gardner, O'Reilly et al. 2018). Although there is a more consistent relationship between loin IMF % and eating quality than lean %, this effect is variable across the carcass. In particular, the impact of loin IMF % on the eating quality of hind grill cuts was not always consistent between sites. At site 2 an increase in IMF % demonstrated a positive effect on the overall liking of the knuckle, outside, rump and topside with a magnitude of effect 8, 7.1, 7.6 and 5.3 respectively. In general where a bone DEXA term was significant, the inclusion of loin IMF % did not alter the significance of the bone term. This indicates that if objective carcass measurement of IMF % is introduced to abattoirs then it can be used in combination with lean % and carcass bone DEXA R Mean to independently describe eating quality.

In an earlier analysis of lambs at site 1, the DEXA R SDev was a significant predictor of eating quality across the carcass (Anderson et al. 2021), however was not associated with eating quality in this analysis. This difference is likely attributed to the improvements in bone detection using the new algorithm The ability to predict eating quality using all carcass bone DEXA R Mean alone is likely due to the improvements in the bone isolation.

This analysis also identified the impact that bone DEXA R Mean has on tenderness, juiciness and flavour, although has not identified the magnitude of effect for traits other than overall liking. Previous analysis only tested associations with overall liking, given its moderate to high correlation with the other eating quality traits (Thompson et al. 2005, Anderson et al. 2021). Knowledge of the impact of the other eating quality traits is an important finding as with the introduction of a revised lamb MSA meat eating quality system combinations of the four eating quality traits may be used in a similar way to MQ4 in beef.

#### 3.5.2 Bone mineral content

In contrast to our hypothesis, there was no direct relationship between bone mineral content of Ca, Mg or P with the eating quality of the cuts tested. This indicates that the relationship between the bone DEXA R Mean values and eating quality identified does not have a strong association with the mineral concentration of Ca, Mg and P in these bones. Despite the lack of direct association of eating quality and mineral content, the link between the bone DEXA R Mean and eating quality appears to have a biologically sensible link. In humans, a DEXA scan is designed to measure bone mineral density (BMD) which is reported in grams per square centimetre. Increases in DEXA bone values in our study is consistent with the notion that as mammals mature their bones become denser and therefore have an increased R value of bones which is detected using DEXA in growing children (Southard, Morris, Mahan et al. 1991). The rationale for testing bone mineral content was to determine if the identified link between increasing bone DEXA R Mean and decreasing eating quality was linked to markers of maturity such as mineral content of these minerals given previous studies have identifies that magnesium decreases as sheep mature (Ravaglioli et al. 1996, Cake, Gardner, Hegarty et al. 2006). In partial support of our hypothesis there was an association of bone mineral content of Ca, Mg and P with bone DEXA R Mean, with the most consistent relationship demonstrated with magnesium concentration of the bones. For all carcass bone DEXA R Mean and also the individual bone (humerus and femur) DEXA R Mean, an increase in their magnitude was associated with a decrease in bone magnesium concentration (mg/g bone). There is little information relating the Cadaver studies in humans have demonstrated high correlation between the bone mineral content as predicted by DEXA with ash weight and density (Ho, Kim, Schaffler et al. 1990). The study by Ho et al. (1990) examined relationships between ash weight and DEXA images rather than the individual mineral content of Ca, Mg and P, which was the aim of the current experiment. Although magnesium was not a direct predictor of the eating quality traits of the cuts, given an increase in bone DEXA R Mean is also related to a decrease in eating quality of cuts, it adds weight to the argument that bone DEXA R values may be an indicator of maturity in sheep which may influence eating quality. It is unusual that this relationship still exists in the mature sheep at site 2 that would be considered no longer in a bone growth phase, although there are many factors that result in changes in bone density which is why it is used in humans for this purpose. With It was hypothesised that Ca content of bone would increase as the animals matured and bone DEXA R Mean increased. The lack of change in bone calcium may reflect the fact that the increase in density of the bones is not as related to calcium concentration but more so the structure of the calcium and other minerals within the bones.

The results in this study differ to those of an earlier study by Payne et al. (2022) where there was no association between bone mineral content and DEXA R Mean. Although this paper used some of the same animals, the difference in results in not surprising given the methods for determining bone DEXA R values have been refined and there was a larger number of bones analysed.

There was an association of carcass fat % and lean % with calcium, magnesium and phosphorus, although this was not consistent across all bones and sites. At site 1, with an increase in fat % and decrease in lean % there was also an associated with a decrease in the Ca, Mg, and P of the lumbar vertebra. This relationship is difficult to explain, although in children there is an association of obesity with increase in bone mineral content as assessed using DEXA (Ferrer, Castell, Marco et al. 2021). Further data obtained from growing lambs may help to determine if this is a consistent association.

This experiment was not specifically designed to analyse the differences in bone mineral content between sheep of different ages there was some ability to assess this at site 1 as new and old season lambs were finished under similar conditions and slaughtered on the same day. However, it was shown that lamb age did not have a consistent relationship with the bone mineral content. The lumbar vertebra and femur bones in old season lambs had higher Mg concentrations compared in new season lambs for kill group 1. Whereas in kill group 2 there was no difference for the lumbar vertebra or femur, however the Mg concentration of humerus in new season lambs was higher than that of old season lambs. Although it is difficult to draw comparisons between the mineral content of the 2 sites as the lambs were finished under different conditions and were of different genetics, the bone mineral content reflected established theories that magnesium decreased with age and calcium increases (Ravaglioli et al. 1996).

#### 3.5.3 Limitations

In the two data sets used in this report the range of bone DEXA R values are not comparable and as noted in the methods at site 1 the bone DEXA R range was 0.02 (0.27 - 1.29) compared with site 2 which was 0.14 (0.22 - 0.36). Site 1 DEXA measures are from a historical data set and there was not a method for comparing these bone DEXA values to those of site 2 which were taken at a later date at a different abattoir without a common phantom or method to compare bone DEXA across sites. As such the bone DEXA R Mean values were not compared between sites and the eating quality analysis was conducted within a site which limits the strength of the analysis for cuts that were eaten from both sites 1 and 2, such as the loin grill. Recent advances in the DEXA methodology will enable comparisons between sites and kill dates in the future, however the analysis of these historical data sets means that each site (abattoir device) must be analysed independently.

This experiment as a whole was not specifically designed to assess differences in bone mineral content between sheep of different ages. Some associations between Mg and bone DEXA R Mean were identified however it is unclear how this directly relates to differences in eating quality and collection and analysis of a greater number of bones would be required. Additionally, the structure of the bones could be assessed using histological sections to try and determine the biological changes associated with changes in bone DEXA R.

The limited number of animals included in this analysis make strong recommendations about the suitability of DEXA for predicting eating quality difficult. There are other data awaiting eating quality and bone mineral results that will enable further testing of the DEXA and its relationship with eating quality.

#### 3.5.4 Industry significance and future work

On-line DEXA is a commercial reality in an increasing number of abattoirs across Australia. This experiment has demonstrated that there is a relationship between bone DEXA R Mean values with eating quality, with the strongest effect shown in the loin grill cut. The relationship of bone DEXA R Mean extended to the shoulder roast and some grill cuts of the hind leg, however the associations varied in strength. The data collected and reported on is a larger than the initial report that has been published and includes animals from a larger age range (mutton). There is an association of DEXA bone R and eating quality traits that extends across the ages.

An important finding in this experiment was that the all carcass DEXA R Mean using an improved algorithm to previous analysis demonstrated a relationship with eating quality. Advanced image processing such as isolation of individual bones not required to obtain this value. From an industry perspective this potentially enables rapid identification of not only carcass lean % using DEXA, but an indicator of eating quality of some cuts across the carcass. The bone DEXA R Mean may be able to be used in a similar way to ossification is used in beef MSA prediction models, however further data and analysis is required. This has implications for the lamb industry with respect to carcass sorting and marketing based on yield and quality, carcass pricing and feedback to the producers.

A key finding in this experiment was that in many cases where both bone DEXA R Mean and IMF % were predictors of eating quality and that they could be included in prediction models

with the bone DEXA R term remaining significant indicating an independence to the prediction. This is beneficial from an industry perspective as with the development of technologies that can predict IMF %, the use of carcass DEXA scanning, which is installed in a number of abattoirs across Australia the combined use of DEXA bone and IMF % has the potential to provide information about the eating quality of cuts across the carcass prior to bone out. This information can potentially influence boning room decisions to maximise carcass profitability.

The lack of consistent relationship of lean % with eating quality of cuts is an important finding as there are few studies that use direct measures of lean % to assess this relationship with previous studies using indicator muscles and fat weights rather than whole carcass lean %.

This experiment investigated a range of cuts across the carcass and included associations with overall liking, tenderness, juiciness and flavour. The results demonstrate that bone DEXA R values relate not only to overall liking (Anderson et al. 2021) but also the other eating quality traits. It is likely that in the future a lamb MSA model will include these terms in a combined index such as an MQ4 score, however the model and weightings has not been finalised. In future eating quality analysis it will be useful to include the impact of bone DEXA R Mean on the indexed or MQ4 eating quality score.

The biology underpinning the relationship between bone DEXA R Mean and eating quality remains elusive. There was no direct relationship between bone mineral concentration of Ca, Mg and P with eating quality. There was a relationship between the bone DEXA R mean and magnesium of some bones, supporting a notion that increases in bone DEXA R reflects maturity however further investigation is required.

Future work will need to focus on collection and analysis of a larger number of samples with the ability to compare DEXA images so that the eating quality data can be analysed together. The impact of bone DEXA R Mean may then be able to be better related to changes in eating quality allowing it to be used for rapid in plant prediction of eating quality.

#### 3.6 Conclusion

The use of existing DEXA systems installed in abattoirs for the purpose of determining cutting lines and carcass lean % can be used to predict the eating quality of cuts across the carcass. The precision of prediction is relatively poor, and analysis of this larger data set confirms that the impact of bone DEXA R Mean on eating quality of cuts across the carcass is variable.

Although bone DEXA R Mean values were not related to all cuts across the carcass, the fact that either lean %, IMF %, bone DEXA R Mean or combinations of the three terms were related to eating quality indicates an eating quality model that has the potential to measure all three in the abattoir has a flexible way of predicting eating quality. It may be that a lamb MSA model utilises different combinations or weightings of these variables to predict eating quality in different cuts.

The biology underpinning the relationship between eating quality and DEXA remains elusive.

38

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