THE POTENTIAL FOR DUAL ENERGY X-RAY ABSORPTIOMETRY TO PREDICT LAMB AGE

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Executive Summary

This study assessed the ability of an online prototype Dual Energy X-Ray Absorptiometry (DEXA) to predict bone mineral content and lamb age. Lambs from six slaughter groups of known age were scanned using DEXA at a commercial abattoir and mineral content of calcium, magnesium and phosphorus of the 12th rib determined for the extremes of the age range of these lambs. DEXA showed moderate precision to predict lamb age (days) using Bone DEXA R Mean values and standard deviation (R^2 = 0.19, RMSE 29.6) and was superior to using Rib DEXA Mean and SD alone (R^2 = 0.13, RMSE = 30.6). Calcium and phosphorus content could not be predicted using DEXA although magnesium content (mg/g) was predicted, albeit poorly. Improving the ability to predict lamb age will provide more reliable inputs to the Meats Standards Australia eating quality assessments, and may be suitable for eating quality assessment of lamb independently of age. Further evaluation of DEXA across a larger age range of lambs is required.

Key Words – bone mineralisation, eating quality, maturity.

I. INTRODUCTION

Lamb age/maturity has been identified as a factor that contributes to eating quality (Meat Standards Australia 2012), with the current Meat Standards Australia (MSA) lamb model utilising lamb dentition to classify carcasses as lamb or hogget. The accuracy of lamb age prediction using dentition is poor (NSW Department of Primary Industries, 2016), therefore a single categorical description of age such as teeth eruption is not ideally suited to the marketplace. Improvements to the lamb MSA model requires improved measurement technologies for characteristics such as age and ideally would utilise a continuous variable to describe lamb age.

Dual energy x-ray absorptiometry (DEXA) has been used for the accurate determination of body composition in production animals including sheep (Dunshea, Suster, Eason *et al.* 2007, Mercier, Pomar, Marcoux *et al.* 2006, Pearce, Ferguson, Gardner *et al.* 2009). R values are obtained from the analysis of high and low energy DEXA images, and reflect the atomic mass and mineral content of the tissue being scanned (Pietrobelli, Formica, Wang *et al.* 1996). DEXA has been used for the measurement of bone in multiple locations in humans (Gilsanz 1998) and reflects a composite of cancellous and cortical bone. In humans, skeletal growth and mineralization increases through adolescence with these changes detected using DEXA (Del Rio, Carrascosa, Pons *et al.* 1994). In lamb bone mineral content has also been shown to change over time, with older animals having decreased concentrations of cortical bone magnesium (Cake, Gardner, Boyce *et al.* 2006) and an increase in the quantity of mineralization with mineral of higher densities (Grynpas 1993). Therefore DEXA images of lambs are likely to reflect the changing bone mineral content and subsequently lamb age and/or maturity. Thus we hypothesise that DEXA R values will associate with lamb age, reflected through changing bone mineral content.

II. MATERIALS AND METHODS

A total of 595 lambs representing 6 slaughter ages (kill groups) underwent DEXA scanning using a commercially installed online DEXA scanner at an abattoir in Border Town, South Australia. Of these lambs 544 had information regarding sire type, sex, litter size and dam breed **Table 1**.

Table 1. Number of lambs according to sire type, sex, litter size and dam breed.

	Sire type			**Sex**		**Litter size**		**Dam breed**	
	Maternal	**Merino**	**Terminal**	**Wether**	**Female**	**Single**	**Multiple**	**BLM**	**Merino**
Kill group 1	17	29	44	28	62	20	70	30	60
Kill group 2	22	29	41	28	64	23	69	36	56
Kill group 3	23	25	45	37	56	24	69	30	63
Kill group 4	20	28	45	57	36	45	48	29	64
Kill group 5	20	32	39	59	32	51	40	19	72
Kill group 6	20	23	42	41	44	33	52	20	65
	122	166	256	250	294	196	348	164	380

The first and the last kill groups had bone mineral content of the rib determined to examine the extremes of lamb age in the experiment. The 12th rib was collected from these carcasses each carcass for subsequent analysis of bone magnesium, phosphorus and calcium concentrations. The 12th rib was chosen due to the ease of sample collection and the fact that this rib is strategically identified under the existing image analysis protocols for the purposes of robotic dissection. On this basis it provides a realistic application of the DEXA/radiographic technology within a plant.

Bone mineral analysis was carried out with bones defatted in diethyl ether, dried, then ashed at 600◦C for 24 hours. Samples were prepared from 200mg of ash powder by digestion in aqua regia (1 : 3 conc.HNO3 : HCl), prior to further dilution in 1% nitric acid. Analysis was conducted by inductively coupled plasma-atomic emission spectrometry (ICP-AES), using Agilent 720 Simultaneous ICP-AES with a sea spray nebuliser and glass-cyclonic spray chamber, and appropriate standards for calibration.

DEXA images were obtained using a single emission from a 140kV X-ray tube, with a set of 2 images captured by 2 photodiodes separated by a copper filter as described by Gardner *et al*. (2016). The photodiodes used differed due to their specificity for low and high energy photons (Ryzhikov, Opolonin, Pashko *et al.* 2005).

The analysis of the DEXA images was carried out in two separate analyses. A preliminary analysis was carried out by determining R values for every bone pixel based upon a ratio of the attenuation within the low energy versus the high energy image (Gardner *et al.* 2016). The mean of the R values for each carcass image (Bone DEXA R Mean) and standard deviations (Bone DEXA R SD) were calculated. In addition to the analysis of the bone of the entire carcass, the 12^{th} rib was located and isolated from the DEXA images using Image J (version 1.44p) and R Values calculated for these pixels. The mean and standard deviations for both the entire skeleton (Bone DEXA R) and 12th rib (Rib DEXA R) were used in separate general linear models (SAS version 9.2, SAS Institute, Cary, NC, USA) to predict age (days) and bone mineral content (where applicable). In both the Bone DEXA R and Rib DEXA R analysis, fixed effects included sire type, sex, litter size and dam breed, which were included where appropriate in the models.

For the Rib DEXA images an additional method was used to calculate pixel density based on the weighted impact of the surrounding pixel densities (nearest neighbour technique). For example if the pixel was weighted at 100%, then only the pixel information was used, compared to a weighting of 50% where the original pixel information was weighted at 50%, with the surrounding pixel information weighted at 50% to calculate a new pixel density. A range of weightings were used to calculate pixel density: 75, 50, 25 and 11%. The mean and standard deviation of all calculated pixel densities from every slice for each sample was then used in general linear models as previously described to predict lamb age in days.

III. RESULTS AND DISCUSSION

Results

The mean ± SD, minimum, and maximum for lamb age, hot carcass weight Bone DEXA R Mean, Bone DEXA R SD, Rib and bone mineral content are shown in Table 2.

Whole carcass bone DEXA

Bone DEXA R varied between kill groups, sire type, and dam breeds ($P<0.01$). The average Bone DEXA R value in the first kill group was higher than all other kill groups ($P<0.01$). The differences between the other kill groups was more variable with no consistent trend between kill groups (age of slaughter) and Bone DEXA R. Merino sired lambs had the highest Bone DEXA R values, which were 0.02 and 0.03 units greater than the Maternal and Terminal sired lambs ($P<0.01$). Dam breed was only able to be compared within the Terminal sired lambs, and within this group of lambs, the Merino dams had a Bone DEXA R that was 0.01 greater than the Border Leicester x Merino dams ($P<0.05$).

The Bone DEXA R value decreased and Bone DEXA R SD increased as lamb age increased (**Figure 1**a and 1b). The ability to predict age using Bone DEXA R and SD was moderate (Table 3, model 3: $R^2 = 0.19$, RMSE 29.6). As an individual term the Bone DEXA R value was as stronger predictor of lamb age than Bone DEXA SD having less impact (Table 3, models 1 and 2). The effect was driven by the high Bone R of kill group 1 as when this kill group was removed from the model, Bone R was no longer significant, although standard deviation was. If kill group was included in the model then both Bone DEXA R and SD were no longer significant. The inclusion of kill group in the model accounted for most of the variance as kill group in this instance is a proxy for lamb age. If sire type and dam breed were included in model 3 then precision of prediction was improved ($R^2 = 0.24$, RMSE 28.9).

Table 2. Raw Mean ± SD (minimum and maximum) of lamb age (days), hot carcass weight (kg), Bone DEXA R, Bone DEXA R SD, Rib DEXA R, Rib DEXA R SD, calcium, magnesium and phosphorus (mg/g wet weight).

	HCWT (kg) Mean ± SD (Min, Max)	Age (days) Mean ± SD (Min, Max)	Bone R Mean ± SD (Min, Max)	Bone R SD Mean ± SD (Min, Max)	Rib R Mean ± SD (Min, Max)	Rib SD Mean ± SD (Min, Max)	Ca (mg/g) Mean ± SD (Min, Max)	Mg (mg/g) Mean ± SD (Min, Max)	P (mg/g) Mean ± SD (Min, Max)
Kill group 1	20.0 ± 3.3 (13.5, 29.0)	265.3 ± 7.9 (252.0, 276.0)	1.42 ± 0.02 (1.4, 1.5)	0.11 ± 0.01 (0.09, 0.14)	1.56 ± 0.08 (1.5, 1.8)	0.16 ± 0.05 (0.1, 0.3)	367.8 ± 26.1 (198.6, 388.7)	7.86 ± 0.81 (4.4, 10.1)	185.75 ± 13.89 (100.6, 205.8)
Kill group 2	23.86 ± 4.9 (13.5, 35.3)	292.71 ± 7.7 (279.0, 304.0)	1.38 ± 0.01 (1.3, 1.4)	0.10 ± 0.01 (0.09, 0.12)	1.48 ± 0.06 (1.3, 1.7)	0.12 ± 0.03 (0.1, 0.2)	-	-	-
Kill group 3	23.59 ± 4.6 (13.0, 34.2)	300.08 ± 7.9 (287.0, 311.0)	1.38 ± 0.01 (1.4, 1.4)	0.11 ± 0.01 (0.09, 0.13)	1.48 ± 0.04 (1.4, 1.6)	0.12 ± 0.02 (0.1, 0.2)	-	-	-
Kill group 4	21.24 ± 4.9 (12.3, 33.5)	306.47 ± 7.6 (295.0, 318.0)	1.39 ± 0.01 (1.4, 1.4)	0.11 ± 0.01 (0.09, 0.13)	1.47 ± 0.05 (1.4, 1.7)	0.12 ± 0.03 (0.1, 0.2)	-	-	-
Kill group 5	22.11 ± 5.4 (10.9, 37.1)	341.79 ± 7.9 (328.0, 352.0)	1.38 ± 0.01 (1.3, 1.5)	0.11 ± 0.01 (0.09, 0.14)	1.46 ± 0.06 (1.4, 1.8)	0.12 ± 0.04 (0.1, 0.4)	-	-	-
Kill group 6	26.20 ± 6.1 (13.2, 39.3)	363.02 ± 7.5 (349.0, 373.0)	1.38 ± 0.01 (1.4 , 1.4)	0.10 ± 0.01 (0.09, 0.13)	1.48 ± 0.05 (1.4, 1.6)	0.11 ± 0.03 (0.1, 0.2)	377.24 ± 16.45 (232.8, 397.9)	9.14 ± 1.01 (7.1, 13.0)	188.74 ± 8.32 (125.2, 205.2)

HCWT hot carcass weight; DEXA dual energy x-ray absorptiometry

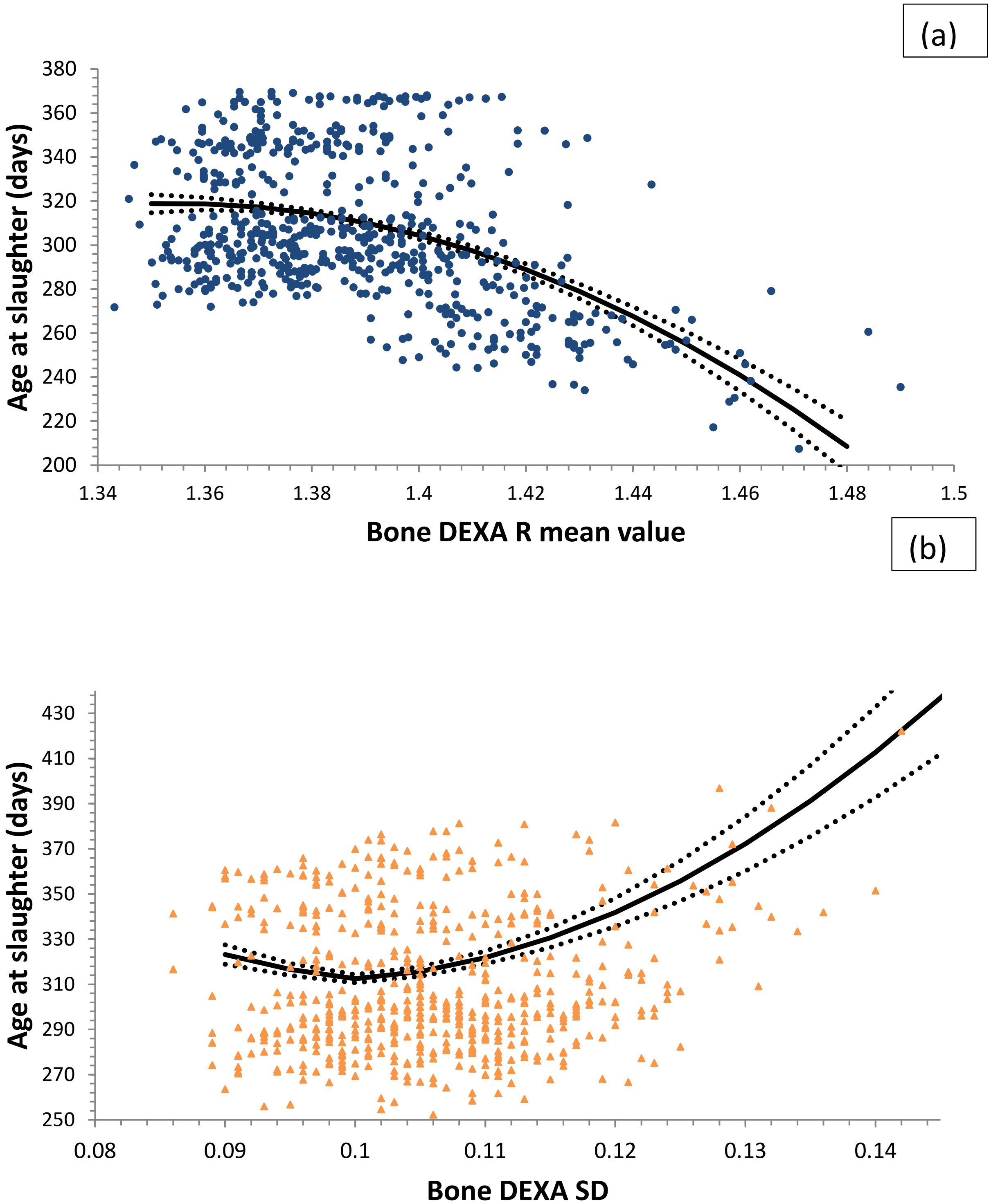

Figure 1 Graph showing the predicted age in days at slaughter using Bone DEXA R (1a) and Bone DEXA SD (1b). Markers represent residuals from the predicted means (solid line) ± SD (dotted line).

Table 3. F-values, coefficient, intercept, coefficient of determination (R-square), and root mean square error (RMSE) for models predicting age (days) using Bone DEXA R and Bone DEXA R SD.

	Model 1	Model 2	Model 3	Model 4
Dependent variable	**Age (days)**	**Age (days)**	**Age (days)**	**Age (days)**
		F Values		
Bone DEXA R	99.94**	-	17.82**	0.05
Bone DEXA R x Bone DEXA R	-	-	18.99**	0.04
Bone DEXA SD	-	19.94**	20.73**	0.06
Bone DEXA SD x Bone DEXA SD	-	-	21.89**	0.06
Kill group	-	-	-	1568.75**
		Co-efficients and intercepts		
Intercept	1028.90	377.300	-11998.61	567.30
Bone DEXA R	-516.80	-	19027.82	-265.97
Bone DEXA R x Bone DEXA R	-	-	-7023.50	90.01
Bone DEXA SD	-	-622.8	-10808.11	-156.56
Bone DEXA SD x Bone DEXA SD	-		51253.36	703.47
Kill group 1	-	-	-	-97.30
Kill group 2	-	-	-	-70.32
Kill group 3	-	-	-	-62.86
Kill group 4	-	-	-	-56.50
Kill group 5	-	-	-	-21.33
Kill group 6	-	-	-	0.00
		Precision estimates		
R2	0.14	0.03	0.19	0.94
RMSE	30.3	32.3	29.6	7.8

Rib DEXA R and SD

The R values for Rib DEXA varied between sex, litter size, kill group, sire types and dam breeds. Kill group had the greatest impact on Rib DEXA R with the first kill group having higher mean Rib DEXA R values than all other groups ($P<0.01$). The relationship between the other kill groups was less well described though in general there was a decrease in R value with increasing slaughter age (kill group number). Merino sired lambs had Rib DEXA R values 0.06 and 0.04 greater than those of the Terminal and Maternal sired lambs ($P<0.01$). There were smaller differences between lambs of different sex and litter size with wether lambs having Rib DEXA R values 0.01 greater than those of the female lambs ($P<0.05$) and lambs born as multiples having 0.01 greater average Rib R values than those born as singles ($P<0.01$).

The Rib DEXA R value decreased and Rib DEXA R SD increased with increasing lamb age (Figure 2 and b). These two values used simultaneously in a model had poor precision for predicting lamb age. The ability of rib DEXA to predict lamb age (days) was relatively poor (Model 7, Table 4: $R^2 = 0.13$, RMSE = 30.6) when both DEXA R Mean value and DEXA R SD were included in the model. The inclusion of kill group in the model resulted in both Rib DEXA R and SD being insignificant. The addition of lamb information such as HCWT alone did not improve precision although, sex and sire type, dam breed lamb improved the precision ($R^2 = 0.35$, RMSE = 26.8). Inclusion of lamb information such as sex, sire type, dam breed and litter size improved the ability of Rib DEXA R and SD ($R^2 = 0.22$, RMSE = 29.3).

The use of a smooth technique did not improve the precision of prediction of lamb age for any of the proportions tested.

Table 4. F-values, coefficient, intercept, coefficient of determination (R-square), and root mean square error (RMSE) for models predicting age (days) and magnesium (mg/g) in lamb rib sections using DEXA R, DEXA standard deviation and magnesium.

	Model 5	Model 6	Model 7	Model 8	Model 9	Model 10
Dependent variable	**Age (days)**	**Age (days)**	**Age (days)**	**Age (days)**	**Mg (mg/g)**	**Mg (mg/g)**
			F Values			
Rib DEXA R	73.73**	-	3.2	-	17.72**	-
Rib DEXA R SD	-	58.8**	3.98**	-	-	16.65**
Rib DEXA R*Rib DEXA R	-	-	12.51**	-	-	-
Rib DEXA R SD*Rib DEXA R SD	-	-	12.98**	-	-	-
Magnesium	-	-	-	61.01**	-	-
			Co-efficients and intercepts			
Intercept	558.57	344.07	-468.72	112.4	8.6	5.39
Rib DEXA R	-166.35	-	1232.9	-	-2.46	-
Rib DEXA R SD	-	-261.55	-453.27	-	-	-4
Rib DEXA R*Rib DEXA R	-	-	-667.66	-	-	-
Rib DEXA R SD*Rib DEXA R SD	-	-	2016.92	-	-	-
Magnesium	-	-	-	42.49	-	-
			Precision estimates			
R2	0.11	0.09	0.13	0.27	0.1	0.09
RMSE	30.9	31.2	30.6	42.7	0.57	0.58

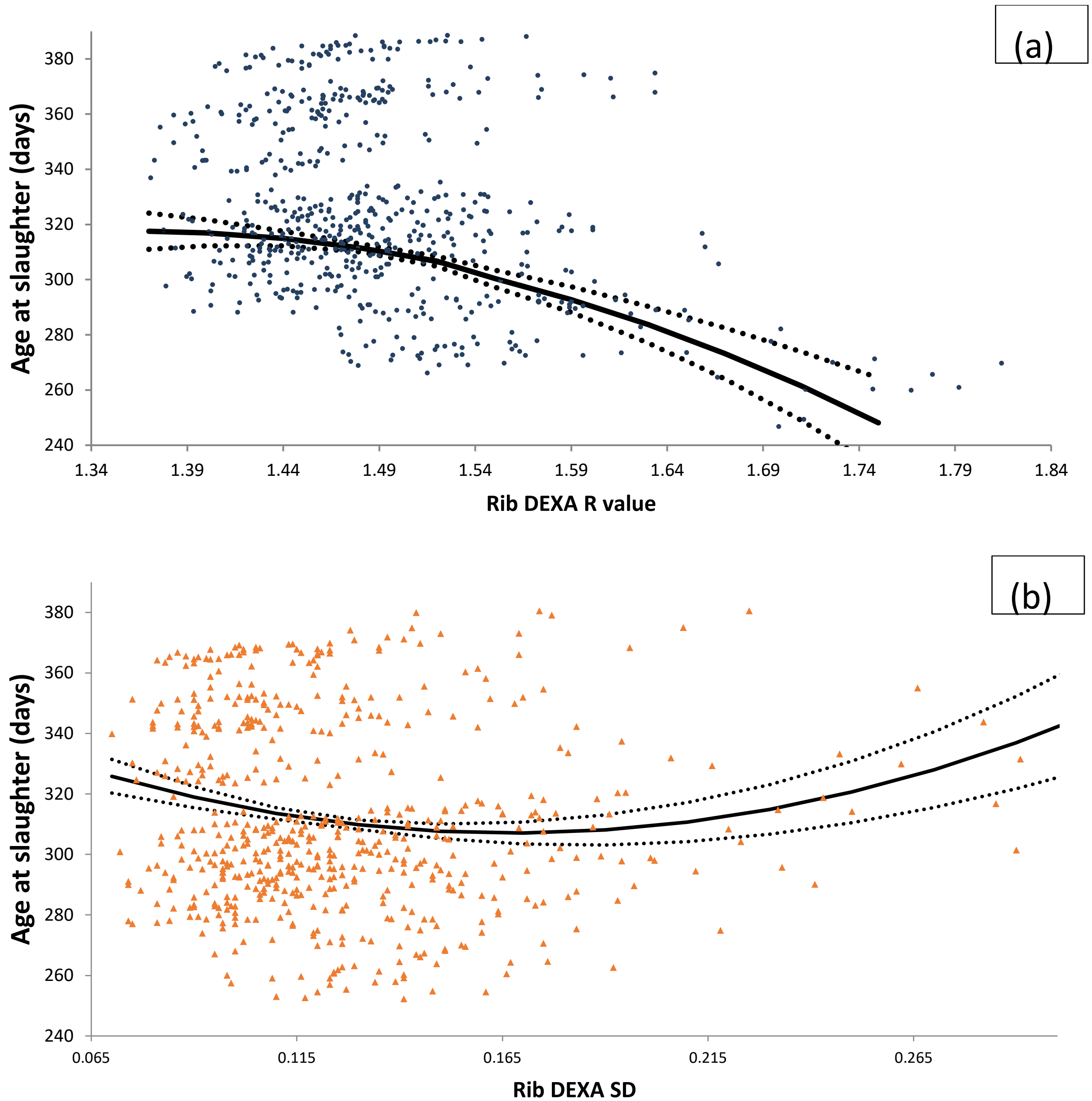

Figure 2. Graph showing the predicted age in days at slaughter using Rib DEXA R (2a) and Rib DEXA SD (2b). Markers represent residuals from the predicted means (solid line) ± SD (dotted line).

Rib mineral content

The magnesium and calcium ash content of the rib varied between kill groups ($P<0.01$) with kill group 1 having 1.27mg/g less magnesium, and 9.43mg/g less calcium than kill group 6. When compared at their adjusted wet weights there was only a significant difference in magnesium content.

Only rib magnesium concentration (mg/g wet weight) demonstrated any association predicting lamb age in days with moderate precision (Model 8, Table 4: R^2 = 0.27, RMSE = 42.7). This result includes mineral data from only the first and last kill groups for which mineral content was determined.

Lamb rib magnesium content was predicted with poor precision by Rib DEXA R Mean (Model 9, Table 4: R^2 = 0.10, RMSE = 0.57) and Rib DEXA R SD (Model 10,

Table 4 4: $R^2 = 0.09$, RMSE = 0.58).

Discussion

In support of our hypothesis DEXA has been shown to differentiate lamb age, albeit with relatively poor precision. In this experiment the relationship between both Bone DEXA R and Rib DEXA R value was negative, which suggests that DEXA R values decrease with lamb age. Given the association of DEXA R value with mineral content and atomic mass (Pietrobelli *et al.* 1996) we were expecting the opposite as bones mineralize with maturity (Grynpas 1993, Ravaglioli, Krajewski, Celotti *et al.* 1996). In a normal paediatric population bone mineral density as measured by DEXA increases continuously from infancy to maturity and is correlated with age, height and weight (Del Rio *et al.* 1994). Given the sigmoid relationship between bone mineral density and age in children (Del Rio *et al.* 1994), it is possible that the decrease in density associated with age seen in this experiment may be in the time period where there is relatively small change in bone mineral content and bone density.

The relationship between bone mineral content (calcium, magnesium and phosphorus) and Rib DEXA R was investigated, but showed limited association, with only magnesium demonstrating an association with DEXA R Mean and SD. In most models tested Rib DEXA R was a stronger descriptor of age and bone magnesium content than the Rib DEXA SD. However care should be taken in interpreting this result as the two are highly correlated (simple correlation coefficient = 0.9).

Contrary to previous studies in lamb, bone magnesium content increased with age (Cake *et al.* 2006, Ravaglioli *et al.* 1996). Therefore in this experiment DEXA showed an association with both lamb age and magnesium however opposite to that expected. The magnesium concentration result is difficult to explain but may reflect environmental, dietary, or health differences between the two kill groups. Alternatively, work in cattle has shown bone magnesium to increase between 3 and 12 months and thereafter remaining constant (Blincoe, Lesperance and Bohman 1973), indicating that further work is required to investigate the relationship between age and bone magnesium. Future experiments could investigate the magnesium content of different indicator bones, their DEXA R values and their relationship with lamb age.

A key limitation of this study was that age was confounded by kill group. Between kill groups, age varied by 99 days (3.3 months), yet within kill groups the age range was only 10 days. Not surprisingly, when all of the above models were adjusted for kill group, DEXA R Mean value and SD were no longer significant. This does not invalidate the associations with age but instead indicates that DEXA cannot discriminate across a 10 day age range – hardly surprising given the RMSE quoted (29 days). None-the-less these results are preliminary, with more studies required to address the confounding between age and kill group in this study.

In this study there was a difference in the Rib DEXA R values between sexes and genotypes. Studies in human children have shown various results of bone density between gender and ethnicity (Del Rio *et al.* 1994, Gilsanz 1998) highlighting that further investigation into genetics and sex is necessary in sheep, and may need to be accounted for when predicting lamb age. Despite the limited study size and the use of only a narrow age range, the results indicate DEXA may be a useful technology to accurately establish lamb age. Future experiments will be designed to slaughter a larger age range of lambs of diverse genotypes. Future investigations should also consider focussing on specific bone regions for determining DEXA R values. Studies in human children use vertebral bones as they are trabecular (cancellous), and more sensitive to bone mineral changes than cortical bone. A similar approach could be applied in sheep.

Another application of DEXA in addition to age determination would be to predict eating quality of lamb meat. Cattle ossification has been shown to be a better indicator of eating quality in beef prior to skeletal maturity (Bonny, Pethick, Legrand *et al.* 2016), therefore similarly bone mineral profile, and DEXA R Mean and SD values may be useful in describing eating quality in lamb.

IV. CONCLUSION

A rapid post slaughter method for determining age would provide assurance that lambs were being correctly classified better satisfying the requirements for inclusion in the MSA pathways program. Future work will focus on improving the relationship between DEXA R value, lamb age and mineral content over an extended lamb age range and diverse genetics. Establishing a link between age, bone mineral content and eating quality has obvious benefits to the lamb industry through its input to the MSA grading system and may lead to better unitisation of older carcasses if eating quality can also be predicted.

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