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

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Abstract – This study assessed the ability of an online Dual Energy X-Ray Absorptiometry (DEXA) to predict bone mineral content and lamb age. Lambs from two 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. DEXA showed moderate precision to predict lamb age (days) using DEXA R Mean values and pixel standard deviation (R^2 0.30, RMSE 41.6). Calcium and phosphorus content could not be predicted using DEXA although magnesium content (mg/g) was predicted, albeit poorly, (R^2 0.09, RMSE 0.57). 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 has been identified as a factor that contributes to eating quality[1], 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 [2], 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 [3-5]. 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 [6]. Bone mineral content has been shown to change over time with older lambs have decreased concentrations of cortical bone magnesium [7] and an increase in mineralization with mineral of higher densities [8]. Therefore DEXA images of lambs are likely to reflect the changing bone mineral content of lambs 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 172 lambs representing 2 slaughter ages (kill groups) underwent DEXA scanning using a commercially installed online DEXA scanner at an abattoir in Border Town, South Australia. The 12th rib was collected from 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 h. 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 a using a 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 using a single emission from a 140kV X-ray tube, with a set of 2 images captured using 2 photodiodes separated by a copper filter as described by Gardner *et al*. 2016 [9]. These photodiodes differed due to their specificity for low and high energy photons [10]. The 12th rib was located and isolated from the DEXA images using Image J (version 1.44p) and R Values calculated for every pixel based upon a ratio of the attenuation within the low energy versus the high energy image [9]. The mean (DEXA R Mean) and standard deviation (DEXA R SD) of these R Values within each image was then calculated. DEXA R Mean and DEXA R SD were subsequently used in general linear models (SAS version 9.2, SAS Institute, Cary, NC, USA) to predict age (days) and bone mineral content.

III. RESULTS AND DISCUSSION

The mean ± SD, minimum, and maximum for lamb age, hot carcass weight DEXA R Mean, DEXA R SD and bone mineral content are shown in Table 1.

Table 1. Mean ± SD (minimum and maximum) of lamb age (days), hot carcass weight (kg), and rib DEXA R Mean, DEXA standard deviation, calcium, magnesium and phosphorus (mg/g wet weight).

	Age (days) ± SD (min, max)	HCWT (kg) ± SD (min, max)	DEXA R ± SD (min, max)	DEXA R SD ± SD (min, max)	Ca (mg/g) ± SD (min, max)	Mg (mg/g) ± SD (min, max)	P (mg/g) ± SD (min, max)
Kill group 1	264.8 ± 8.1 (252, 276)	19.9 ± 3.2 (13.5, 29)	1.54 ± 0.08 (1.4, 1.8)	0.16 ± 0.05 (0.1, 0.3)	367.81 ± 26.13 (198.6, 388.7)	7.86 ± 0.81 (4.4, 10.1)	185.75 ± 13.89 (100.6, 205.8)
Kill group 2	363.0 ± 7.5 (349, 373)	26.2 ± 6.1 (13.2, 39.3)	1.47 ± 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

The ability of DEXA to predict lamb age (days) was moderate when both DEXA R Mean value and DEXA R SD were included in the model (Model 1, Table 2: $R^2 = 0.30$, RMSE = 41.6). Thus in support of our hypothesis DEXA has been shown to differentiate lamb age. In this experiment the relationship between DEXA R value was negative which suggests that it decreases with lamb age. Given the association of DEXA R value with mineral content and atomic mass [6] we were expecting the opposite as bones mineralize with maturity [8, 11].

Table 2. 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 using DEXA R, DEXA standard deviation and magnesium.

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Dependent variable	Age (days)	Age (days)	Age (days)	Age (days)	Mg (mg/g)	Mg (mg/g)
F Values						
DEXA R Mean	0.7	47.5*	-	-	13.0*	-
DEXA R SD	19.8*	-	72.2*	-	-	16.6*
Magnesium	-	-	-	61.0*	-	-
Coefficients and intercepts						
Intercept	289.4	810.8	398.6	112.4	8.3	5.4
DEXA R Mean	83.3	-	-	-	-2.3	-
DEXA R SD	-723.0	-328.2	-604.8	-	-	-4.0
Magnesium	-	-	-	42.5	-	-
Precision estimates						
R^2	0.30	0.22	0.30	0.27	0.07	0.09
RMSE	41.6	43.9	41.6	42.5	0.58	0.57

*P<0.01
DEXA dual energy x-ray absorptiometry

On this basis we investigated the direct association between bone mineral content (calcium, magnesium and phosphorus) and age, however in this case only rib magnesium concentration (mg/g wet weight) demonstrated any association predicting lamb age in days with moderate precision (Model 4, Table 2: $R^2 = 0.27$, RMSE = 42.5). Contrary to previous studies in lamb, bone magnesium content increased with age [7, 11], a result that 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 [12], indicating that further work is required to investigate the relationship between age and bone magnesium.

The relationship between bone mineral content (calcium, magnesium and phosphorus) and DEXA was investigated, but showed limited associations, with only magnesium demonstrating an association with DEXA R Mean and SD.

Rib magnesium content was predicted with poor precision by DEXA R Mean (Model 5, Table 2: R^2 = 0.07, RMSE = 0.58) and DEXA R SD (Model 6, Table 2: R^2 = 0.09, RMSE = 0.57). When both DEXA R Mean and DEXA R SD were fitted in the model then DEXA R Mean was not significant due to the greater impact of DEXA R SD. Similar to the results of DEXA association with age, the association with magnesium was in the opposite direction to that expected. This expectation was based upon increasing age associated with mineralisation and a corresponding higher atomic mass [8], which would be reflected in an increase in DEXA R Mean [6]. Therefore in this experiment DEXA showed an association with both lamb age and magnesium however opposite to that expected.

In most models tested DEXA R SD was a stronger descriptor of age and bone magnesium content than the DEXA R Mean value. However care should be taken in interpreting this result as the two are highly correlated (simple correlation coefficient = 0.9).

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 about. None-the-less these results are preliminary, with more studies required to address the confounding between age and kill group in this study. Despite the limited study size and the use of only 2 lamb ages, 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 lamb ages, of diverse genetics.

In addition to providing an accurate estimate of lamb age, DEXA may be better able to predict eating quality in lamb than age alone. Cattle ossification has been shown to be a better indicator of eating quality in cattle prior to skeletal maturity [13], 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. Better 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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