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Validation of single photon absorptiometry for on-farm measurement of density and mineral content of tail bone in cattle

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Abstract. A validation study examined the accuracy of a purpose-built single photon absorptiometry (SPA) instrument for making on-farm *in vivo* measurements of bone mineral density (BMD) in tail bones of cattle. *In vivo* measurements were made at the proximal end of the ninth coccygeal vertebra (Cy9) in steers of two age groups (each $n = 10$) in adequate or low phosphorus status. The tails of the steers were then resected and the BMD of the Cy9 bone was measured in the laboratory with SPA on the resected tails and then with established laboratory procedures on defleshed bone. Specific gravity and ash density were measured on the isolated Cy9 vertebrae and on 5-mm^2 dorso-ventral cores of bone cut from each defleshed Cy9. Calculated BMD determined by SPA required a measure of tail bone thickness and this was estimated as a fraction of total tail thickness. Actual tail bone thickness was also measured on the isolated Cy9 vertebrae. The accuracy of measurement of BMD by SPA was evaluated by comparison with the ash density of the bone cores measured in the laboratory. *In vivo* SPA measurements of BMD were closely correlated with laboratory measurements of core ash density ($r = 0.92$). Ash density and specific gravity of cores, and all SPA measures of BMD, were affected by phosphorus status of the steers, but the effect of steer age was only significant ($P < 0.05$) for steers in adequate phosphorus status. The accuracy of SPA to determine BMD of tail bone may be improved by reducing error associated with *in vivo* estimation of tail bone thickness, and also by adjusting for displacement of soft tissue by bone mineral. In conclusion a purpose-built SPA instrument could be used to make on-farm sequential non-invasive *in vivo* measurements of the BMD of tailbone in cattle with accuracy acceptable for many animal studies.

Additional keywords: bone density, phosphorus status.

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Introduction

Radiation absorption techniques for measuring bone mineral in humans and domestic animals include X-ray absorptiometry (Siemon *et al*. 1974), monochromatic γ-radiation or single photon absorptiometry (SPA) (Cameron and Sorenson 1963; Sorenson and Cameron 1967; Cameron *et al*. 1968) and dichromatic photon absorptiometry (Zetterholm 1978; Zetterholm and Dalen 1978). Dual-energy X-ray absorptiometry appears to have become the preferred approach for human medicine and smaller domestic animals such as pigs and sheep (Mazess *et al*. 1990; Zotti *et al*. 2010; Ryan *et al*. 2011) but requires high cost fixed instrumentation and poses difficulties for use on-farm or for large untrained animals such as cattle. It is thus not suitable for on-farm measurement of bone minerals in cattle such as is needed for studies of phosphorus (P) and calcium nutrition and physiology of cattle grazing extensive rangelands. A purpose-built portable SPA bone densitometer suitable for use on-farm to measure bone mineral density (BMD) of tail bones in cattle has been developed and described (Murray 1989; Murray *et al*. 1994), and good discrimination in BMD of tailbone was observed between groups of steers in P-adequate or P-deficient status (Coates and Murray 1994). However, the accuracy of the BMD measurements was not determined. The present study was undertaken to further evaluate and validate the SPA instrument used by these research workers. On-farm *in vivo* measurements of tail bone BMD were made on cattle restrained in a cattle crush or

cattle race, and compared with BMD measurements made in the laboratory on the resected tails of the same animals.

Materials and methods

Cattle and pasture

Two age groups of Droughtmaster steers (each $n = 10$) grazed acutely P-deficient pasture for 12 months at the CSIRO Lansdown Pasture Research Station (19°41′S, 146°51′E) near Townsville, Australia. Ten 3.8-ha paddocks were each stocked with two steers, one from each age group. Tail bone BMD and other bone measurements were made at the end of the grazing period when the cohorts of steers were on average ~32 months and ~20 months old. The pasture was a mixture of tropical grasses and stylo legume (*Stylosanthes hamata* cv. Verano). The soil was very low in available P with bicarbonate extractable P (Colwell 1963) of <4 ppm in the top 10 cm. Steers in five of these paddocks, designated P adequate (P_{adeq}), were supplemented with 5–7 g P per day in the drinking water. Steers in the other five paddocks, designated P deficient (P_{defic}), received no P supplement. Salt blocks were provided in all paddocks. The steers were weighed at monthly intervals.

Bone mineral density in tail bone of the cattle

In vivo *measurement*

At the end of the 12-month grazing period BMD at the proximal end of the ninth coccygeal vertebra (Cy9) was measured *in vivo* using a purpose-built single SPA γ-ray densitometer and the procedures described by Murray (1989) and Murray *et al*. (1994). The primary components of the densitometer were a removable 3600 MBq (100 mCi) radioactive source of ^{241}Americium, a sodium iodide detector and photomultiplier tube together with a counter and rate-meter linked to a computer. The radioactive source and detector were mounted on a U-shaped frame, which was clamped on the tail at the measurement site, and the source was collimated to direct a 3-mm-diameter γ-ray beam through the tail. A linear potentiometer was attached to measure tail thickness at the measurement site. In addition, a moving stage allowed the source and detector to be racked along the tail while maintaining the clamp in position so that the measurement site could be accurately located from the graphical display of counts. The intervertebral space between coccygeal vertebrae was identified by maximum count rate. By racking the stage distally from the Cy8 to Cy9 intervertebral space, the measurement site was identified by minimum count rate. This coincides with the thickest dorso-ventral section of bone at the proximal end of Cy9. The average of the lowest three counts, each recorded over 30 s and moving the stage 1 mm between readings, was used for the calculation of BMD.

Cattle were restrained in a covered veterinary crush with a sliding head bail at the front and a half-gate at the rear to protect the operator. The densitometer frame with γ-ray source, detector and potentiometer was slung from the roof of the crush with computer and other electronic equipment positioned at the side of the crush. Most of the cattle stood quietly and did not have to be restrained in the head bail.

The BMD was calculated (Murray *et al*. 1994) as: $BMD = [\mathrm{Ln}(I_O/I_t) - {}^{um}{}_{ST}P_{ST}{}^{x}{}_{ST}]/({}^{um}{}_{B}{}^{x}{}_{B})$.

Where: I_O = counts/second through air;

I_t = counts/second through tail;

${}^{um}{}_{ST}$ = mass attenuation coefficient of soft tissue = 0.198 counts/s.cm;

P_{ST} = density of soft tissue = 1 g/cm^3;

${}^{x}{}_{ST}$ = thickness (cm) of soft tissue = tail thickness;

${}^{um}{}_{B}$ = mass attenuation of bone mineral = 0.404 counts/s.cm; and

${}^{x}{}_{B}$ = bone thickness (cm).

Measurement of BMD on resected tails

The day following the *in vivo* measurement of BMD, the tails of the 20 steers were surgically removed by a veterinary surgeon. Resection was between Cy7 and Cy8 using epidural anaesthesia. Resected tails were then severed between Cy12 and Cy13, and the Cy8 to Cy12 tail sections were retained; these were placed on crushed ice in an insulated container for immediate storage and for transport to the laboratory where the tails were stored at 4°C. After 2 days BMD at the proximal end of Cy9 was again measured using the SPA instrument as described above.

Measurements of specific gravity (SG) and ash density

The Cy9 vertebrae were carefully isolated from the resected tails by severing between adjoining vertebrae through the intervertebral spaces and then careful removal of all soft tissue. Maximum dorso-ventral tail bone thickness (TBT) at the proximal end of the dissected Cy9 vertebrae was measured with vernier calipers. Bone samples were stored in normal saline at 4°C pending further measurements. First, each defleshed Cy9 bone was weighed in air and in water to determine volume and SG. Then a 5 mm × 5 mm square dorso-ventral core of bone at the densitometer measurement site was cut from the tail bone using a fine-toothed band saw. Bone cores were washed and stored in normal saline. The remaining fragments of each Cy9 bone were quantitatively collected, incinerated at 550°C, and ash weights measured. Also the SG, volume and ash content of the cores were determined and ash density (g/cc) of the cores and the whole Cy9 bones were calculated.

Determination of TBT for calculating bone mineral density

In vivo measurement of tail bone BMD using SPA requires estimation of TBT, which is predicted from total tail thickness (TTT) (Murray 1989; Murray *et al*. 1994). In the present study measurements of actual TBT (TBT_{actual}) were made on the isolated defleshed Cy9 vertebrae. In addition TBT was predicted from the TTT measurements made with the SPA instrument. Predicted TBT ($TBT_{predict}$) was calculated from linear regressions relating TBT_{actual} to TTT where the latter was measured (i) *in vivo* and (ii) on resected tails. BMD was calculated using both TBT_{actual} and $TBT_{predict}$ values; the term BMD_{TBT_actual} denotes BMD calculated using TBT_{actual} whereas $BMD_{TBT\_predict}$ denotes BMD calculated using $TBT_{predict.}$

Statistical analyses

Statistical analysis was completed using GENSTAT release 16.1 (VSN International Ltd, Hemel Hemstead, UK).The laboratory determinations of SG by graviometry and of ash density in tail bone core samples were assumed to represent actual tail bone density (TBD) and actual BMD values, respectively. An assessment of the accuracy of SPA-calculated BMD was made

using ANOVA and also regression analysis relating SPA measures of BMD, made *in vivo* and on resected tails, to core ash density. Comparisons were also made between BMD measurements made *in vivo* and on resected tails. Analyses of variance of the factorial P- treatment × Animal age × Methods data required a multi-strata format because of the experimental design. The top stratum involved the 10 paddocks (replicated P-treatments P_{adeq} and P_{defic}), the second stratum was for the two animals (different ages) in each paddock, and the third stratum was for the different methods used to measure bone mineral in each animal (ash density of core samples; ash density of Cy9 tail bones; *in vivo* SPA measures BMD_{TBT_actual} and $BMD_{TBT\_predict}$; SPA measures of BMD_{TBT_actual} and $BMD_{TBT\_predict}$ made on resected tails).

Animal welfare and use of radioactive substances

Surgical and other experimental procedures were carried out according to the code of practice for the care and use of animals for scientific purposes and with the approval of the relevant Animal Ethics Committees operating at the time the experiments were conducted. Regulations regarding the use and storage of radioactive substances were followed.

Results

Hereafter the parameters TTT, TBT, SG, ash density and BMD refer to these measurements at the proximal end of Cy9 unless otherwise stated.

Specific gravity and ash density of tail bones

Both the SG and ash density of the tail bone cores (means 1.204 g/cc and 0.264 g/cc, respectively), were lower ($P < 0.001$) than in the whole Cy9 bones (means 1.240 g/cc and 0.305 g/cc, respectively). However, the values for the cores were closely correlated with those for whole Cy9 bones with correlation coefficients (r) of 0.95 and 0.92 for SG and ash density, respectively. SG and ash density were also highly correlated ($P < 0.001$) both within the bone core sample set ($r = 0.98$) and within the whole Cy9 bone sample set ($r = 0.99$). Both SG and ash density of whole Cy9 bones and of core samples were substantially higher ($P < 0.001$) in P_{adeq} steers than in P_{defic} steers with core ash density mean values of 0.339 and 0.191 g/cc, respectively. There was an age × treatment interaction effect ($P < 0.05$) such that, for the P_{adeq} steers, SG and ash density were higher in the older than in the younger steers, whereas no such effect of age was observed in the P_{defic} steers.

Total tail thickness and tail bone thickness

The linear regression relationships between TBT_{actual} measured in the laboratory on defleshed bone and TTT measured with the potentiometer differed between the TTT measurements made *in vivo* and those made on resected tails; the regressions differed in elevation ($P < 0.001$) but not in slope (Fig. 1). Measurements of TTT made *in vivo* (mean 2.865 cm, $n = 20$) were greater ($P < 0.001$) than measurements on resected tails (mean 2.742 cm); this indicated shrinkage in TTT (mean shrinkage of 4.25%) when tails were resected, presumably due primarily to exsanguination. The mean ratio of TBT_{actual} to TTT (TBT : TTT) was 0.602 for TTT measured *in vivo* and was lower ($P < 0.001$) than this ratio measured on resected tails (0.629). Despite the highly significant correlation between TBT_{actual} and TTT described above, measurements of TTT made either *in vivo* or on resected tails accounted for only ~60% of the variation in TBT_{actual}. This indicated that there was substantial error associated with the prediction of TBT_{actual} from TTT, with RSD values of 0.0864 cm and 0.0869 cm for measurements made *in vivo* and on resected tails, respectively. In the present study, because the slopes of the regressions in Fig. 1 did not differ ($P > 0.05$) from the mean TBT : TTT ratios, $TBT_{predict}$ was predicted from the relevant mean TBT : TTT ratio rather than from the relevant regression equation.

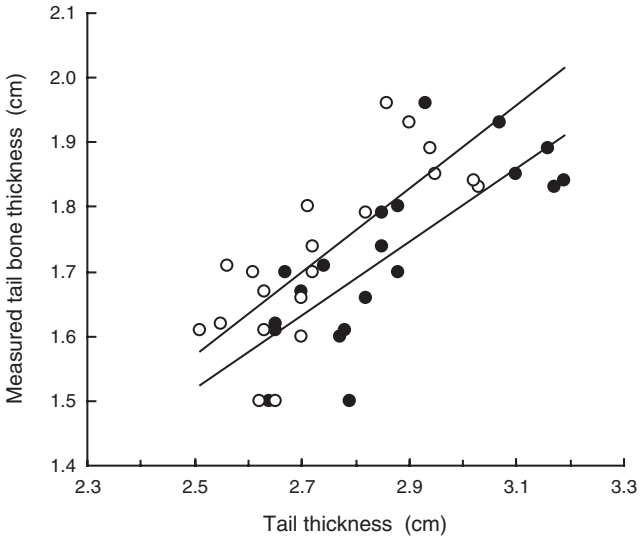

Fig. 1. The relationship between measured tail bone thickness (TBT) and total tail thickness (TTT) for TTT measured *in vivo* (●) and on resected tails (○). The regression equations were: TBT = 0.565 TTT + 0.106 ($n = 20$, $r = 0.78$, RSD = 0.086, $P < 0.001$) for TTT measured *in vivo*, TBT = 0.646 TTT – 0.0456 ($n = 20$, $r = 0.77$, RSD = 0.087, $P < 0.001$) for TTT measured on resected tails.

Relationships between measures of BMD and actual ash density of core samples

The critical comparison in this study for assessing the reliability of the SPA instrumentation for making on-farm measurements of tail bone BMD was the relationship between (i) *in vivo* measurements of BMD calculated using $TBT_{predict}$ and (ii) ash density of the bone cores measured in the laboratory (Fig. 2). There was a close correlation between these measures of mineral density ($r = 0.92$). Mean values for $BMD_{TBT\_predict}$ and core ash density were 0.2548 and 0.2643 g/cc, respectively, and the *in vivo* measurements of $BMD_{TBT\_predict}$ did not differ significantly from the ash density of core samples ($P > 0.05$). Measurements of BMD_{TBT_actual} and $BMD_{TBT\_predict}$ made *in vivo* did not differ significantly, and nor did the comparable measurements made on resected tails ($P > 0.05$). There were close linear regression relationships between all SPA measures of BMD (measurements calculated using TBT_{actual} as well as $TBT_{predict}$ and measurements on resected tails as well as measurements made *in vivo*) and the ash density of Cy9 core samples (Table 1). *In vivo* measurements of BMD were lower than those measured on resected tails ($P < 0.01$).

Effect of steer P status and age on BMD

Laboratory measurements of SG and ash density for whole Cy9 vertebrae and for bone cores, and all SPA measures of BMD, were higher ($P < 0.05$ or $P < 0.001$) for P_{adeq} steers than for P_{defic} steers (for example, Fig. 2). There was an interaction effect ($P < 0.05$) of steer age × P status on BMD; the older P_{adeq} steers had a higher BMD than the younger steers, but there was no such difference due to age in the P_{defic} steers.

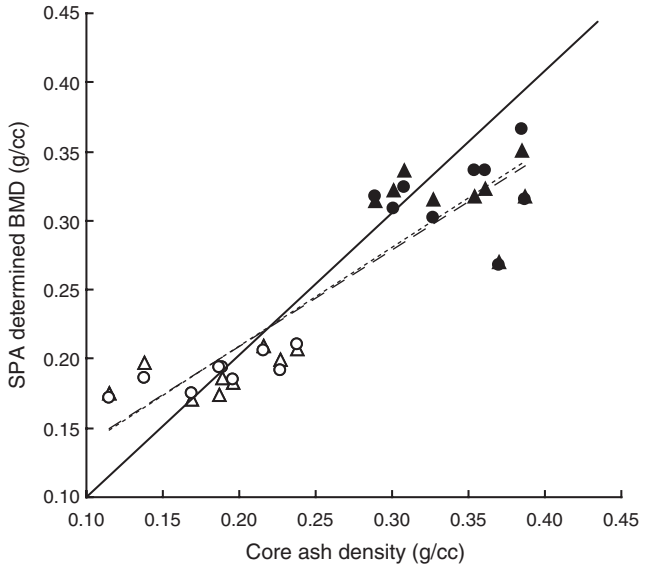

Fig. 2. The relationships between *in vivo* measurements of bone mineral density (BMD) at the proximal end of the ninth coccygeal vertebrae using single photon absorptiometry (SPA) and the ash density of bone core samples measured in the laboratory. ●, SPA BMD calculations based on predicted tail bone thickness, P_{adeq} steers; O, SPA BMD calculations based on predicted tail bone thickness, P_{defic} steers; ▲, SPA BMD calculations based on actual tail bone thickness, P_{adeq} steers; Δ, SPA BMD calculations based on actual tail bone thickness, P_{defic} steers. 1 : 1 line (—). Linear regression line for BMD based on predicted tail bone thickness (- - - -) was: BMD = 0.7144 core ash density + 0.066 ($n = 18$, $P < 0.001$, $r = 0.91$, RSD = 0.029). Linear regression line for BMD based on actual tail bone thickness (– – – –) was: BMD = 0.6976 core ash density + 0.069 ($n = 18$, $P < 0.001$, $r = 0.90$, RSD = 0.031).

Discussion

The ash density of the core samples determined by incineration was considered to be the most reliable measure of BMD at the target Cy9 measurement site and was thus used as the reference measurement to evaluate the SPA measurements. The potential of SPA for on-farm, crush-side estimations of tail bone BMD was therefore assessed primarily by comparing *in vivo* measurements of BMD based on predicted TBT ($BMD_{TBT\_predict}$) with the laboratory measurements of ash density of core samples. Although the correlation between SPA determined $BMD_{TBT\_predict}$ and core ash density was satisfactory ($r = 0.92$), the regression slope was less than unity ($P < 0.05$) and there was a tendency for the SPA measures to increasingly underestimate BMD as core ash density increased (Fig. 2). Differences between SPA determined $BMD_{TBT\_predict}$ and core ash density of individual steers varied between –0.102 and +0.056 g/cc with a mean difference of 0.029 g/cc and an overall bias of –0.009 g/cc. The lower SG and ash density of tail bone core samples than of whole Cy9 bones was presumably due to a lower proportion of compact bone in the core than in the entire Cy9 bone.

The relationships between BMD_{TBT_actual} and core ash density or between $BMD_{TBT\_predict}$ and core ash density were similar with the regressions differing neither in slope nor in vertical displacement. The absence of any improvement in the correlation coefficient associated with BMD_{TBT_actual} compared with $BMD_{TBT\_predict}$ was unexpected but was likely a consequence of the same set of animals being used to derive both the TBT : TTT ratio and the BMD measurements. It can be shown that use of a different TBT : TTT ratio to estimate TBT [for example, the ratio of 0.684 recommended by Murray (1989) and used by Coates and Murray (1994)] would lead to a displacement between the regression lines, but little if any effect on the regression slope or on the correlation coefficient. The observation that the correlation statistics between both BMD_{TBT_actual} and $BMD_{TBT\_predict}$ regressed on ash density was almost identical indicates that, for this dataset, factors other than errors in estimated TBT were primarily responsible for the differences between SPA determined BMD and the ash density of the core samples.

Table 1. Correlation coefficients and regression equations between parameters measured by γ-ray densitometry and ash density of bone cores from the proximal end of Cy9 tail bones measured by laboratory procedures

BMD_{TBT_actual} (resect) = BMD measured on resected tails and calculated using actual tail bone thickness; $BMD_{TBT\_predict}$ (resect) = BMD measured on resected tails and calculated using predicted tail bone thickness; BMD_{TBT_actual} (vivo) = BMD measured *in vivo* and calculated using actual tail bone thickness; $BMD_{TBT\_predict}$ (vivo) = BMD measured *in vivo* and calculated using predicted tail bone thickness. RSD, residual standard deviation

Regression	Parameters		Mean		Correlation	Regression	RSD
	Y	X	Y (g/cc)	X (g/cc)	coefficient (r)	equation; Y =	
1	BMD_{TBT_actual} (resect)	Ash density	0.2789	0.2643	0.925	0.7348x + 0.0846	0.0347
2	$BMD_{TBT\_predict}$ (resect)	Ash density	0.2798	0.2643	0.933	0.7664x + 0.0772	0.0328
3	BMD_{TBT_actual} (vivo)	Ash density	0.2538	0.2643	0.897	0.6976x + 0.0695	0.0313
4	$BMD_{TBT\_predict}$ (vivo)	Ash density	0.2548	0.2643	0.916	0.7144x + 0.0660	0.0294
5	$BMD_{TBT\_predict}$ (resect)	BMD_{TBT_actual} (resect)	0.2798	0.2789	0.988	1.0216x – 0.0051	0.0111
6	$BMD_{TBT\_predict}$ (vivo)	BMD_{TBT_actual} (vivo)	0.2548	0.2538	0.988	0.9952x + 0.0221	0.0111
7	BMD_{TBT_actual} (vivo)	BMD_{TBT_actual} (resect)	0.2538	0.2789	0.943	0.9233x – 0.0036	0.0236
8	$BMD_{TBT\_predict}$ (vivo)	$BMD_{TBT\_predict}$ (resect)	0.2548	0.2798	0.942	0.8986x + 0.0033	0.0240

Despite the similarity of the regression relationships of $BMD_{TBT\_predict}$ and BMD_{TBT_actual} on core ash density described above, there is, nevertheless, no doubt that errors in estimated TBT will lead to errors in SPA measured BMD of tail bone and that SPA measured BMD would be improved if the accuracy of estimated TBT could be improved. This study showed that TBT : TTT ratios differ between measurements made *in vivo* and measurements made on resected tails, likely due to shrinkage in TTT of resected tails as a result of exsanguination, and possibly also due to moisture loss during storage. Consequently TBT : TTT ratios derived from measurements made on exsanguinated tails are inappropriate for predicting TBT from TTT measured on live cattle.

The substantial between animal variation in the TBT : TTT ratio (see Fig. 1) is also a matter of concern. Murray *et al*. (1994) reported the TBT : TTT ratios derived from 225 abattoir tails to average 0.684, but in that dataset variation in TTT accounted for <40% of the variation in TBT. This mean ratio of 0.684 was substantially higher than the mean of 0.629 (range 0.566–0.685) measured on resected tails in the present study. An inspection of the data presented by Murray (1989) revealed a very wide range in TBT : TTT ratios of individual animals from 0.53 to 0.86 [fig. 1.3 in Murray (1989)], again indicating TTT to be a poor predictor of TBT. The disparity between the mean TBT : TTT ratio reported by Murray (1989) and those measured in the present study suggests the possibility of an operator effect on the defleshing of tail bones and the resulting measurement of actual TBT. The difficulty in obtaining accurate estimates of TBT, including the large between-animal variation in TBT : TTT ratios, indicates that there is a need for a more direct method of estimating or measuring TBT in the live animal. As suggested by Murray (1989), the error in estimating TBT could be markedly reduced if the densitometer were redesigned to provide a vertical measurement of the dorso-ventral TBT. However, even in its current form the SPA densitometer should still provide reliable measurements of changes with time and differences among groups of cattle.

A second approach to improve the accuracy of SPA measured BMD in tail bone would be to include a correction for the displacement of soft tissue in bone by bone mineral. Both soft tissue (collagen matrix, fat, other organic compounds and water) and bone mineral contribute to TBD. In the absence of evidence to the contrary, Murray (1989) and Murray *et al*. (1994) calculated TBD as equal to BMD + 1 (units in g/cc). This assumed that the contribution of soft tissue components to TBD is 1 g/cc and that no soft tissue is displaced by bone mineral. However, as Siemon *et al*. (1974) have pointed out, bone mineral must replace some soft tissue although these authors gave no indication of how much soft tissue is displaced by bone mineral. Nevertheless, it is logical that the contribution of soft tissue to TBD in g/cc = SG of bone – ash density in g/cc of bone. Therefore displacement of soft tissue by bone mineral (%) = [1 – (SG of bone – ash density of bone)]*100. Alternatively, the coefficient of displacement (COD) of soft tissue = 1 – (SG of bone – ash density of bone). Calculations using SG and ash density data of the core samples from the present study and rib bone data from other studies showed that displacement of soft tissue increases with increasing ash density and that, based on a fitted exponential regression, variation in measured ash density accounted for 83% of the variation in the COD of soft tissue (Fig. 3). The regression equation was:

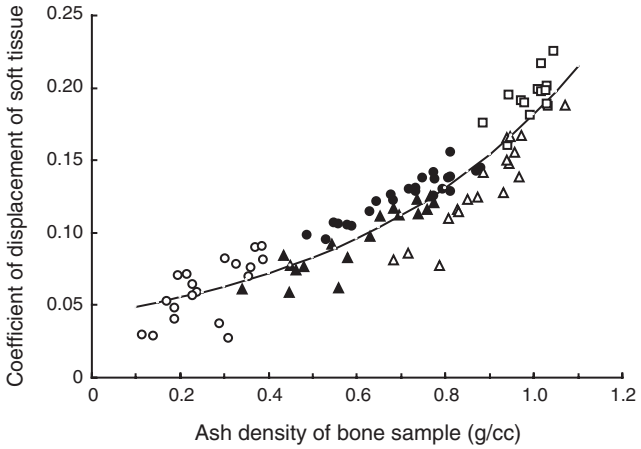

Fig. 3. The fitted exponential relationship between the coefficient of displacement (COD) of soft tissue by bone mineral and the ash density of the bone. ○, Cy9 core samples from the present study; ●, full core rib biopsy samples from steers in this study (unpublished); Δ, external compact bone from rib biopsy samples from steers in this study (unpublished); ▲, full core rib biopsy samples from mature cows (unpublished); □, calculated from data published by Holst *et al*. (2002). The relationship was: COD = 0.0185 + 0.0254*($6.43^{\text{Ash density in g/cc}}$) ($n = 114$, $P < 0.001$, $r = 0.93$, s.e. of observations = 0.0167).

$$\text{COD} = 0.0185 + 0.0254 * (6.43^{\text{Ash density in g/cc}}).$$

The method for calculating SPA measured BMD used by Murray (1989), Murray *et al*. (1994) and in the present study for calculating SPA measured BMD, but does not take account of displaced soft tissue in bone. As a consequence BMD was under-estimated, and the amount of under-estimation increased with increasing ash density. This is consistent with the data presented in Fig. 2 and the observed tendency for SPA measures of BMD (not adjusted for displacement of soft tissue) to increasingly underestimate BMD as core ash density increased. A modified formula that takes account of displaced soft tissue becomes:

$$\text{BMD g/cc} = [\text{Ln}(\text{Io}/\text{It}) - (\text{TTT} - (\text{TBT} * \text{COD})) * 0.198]/\text{TBT} * 0.404$$

where 0.198 and 0.404 are the mass attenuation coefficients for soft tissue and bone mineral, respectively. If BMD(1) and BMD (2) represent calculated BMD without allowance and with an allowance for displaced soft tissue, respectively, mathematical manipulation of the formula for calculating BMD shows that:

$$\text{BMD}(2) = \text{BMD}(1) + [(0.198 * \text{COD})/0.404] = \text{BMD}(1) + 0.49\text{COD}$$

It follows that a good approximation of BMD(2) can be made as follows:

$$\text{BMD}(2) = \text{BMD}(1) + \text{COD}/2.$$

The conversion of SPA determined BMD(1) to BMD(2) necessarily requires a reliable estimate of COD. Such an estimate can be derived from the data presented in Fig. 3, which shows the relationship between COD and bone ash density. As BMD(2) is the estimate of bone ash density that

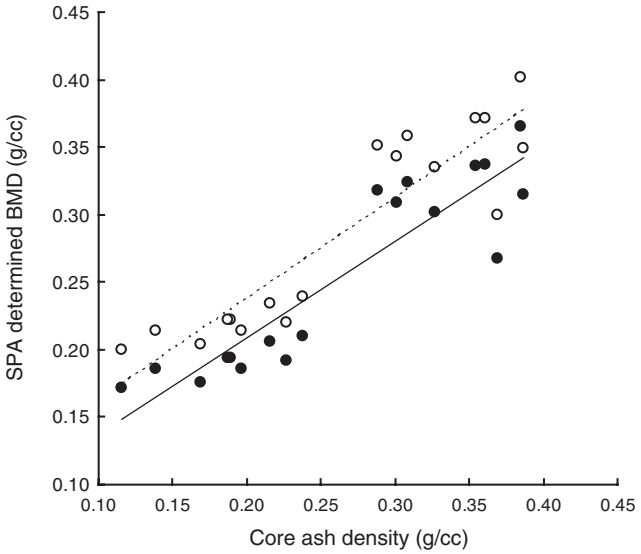

Fig. 4. The relationships between SPA determined BMD and core ash density at the proximal end of Cy9 tail bones. ●, BMD(1): calculated without making allowance for displaced soft tissue; O, BMD(2): calculated with an allowance for displaced soft tissue. (—) regression line for BMD(1): BMD(1) = 0.7144 ash density + 0.066 (n = 18, r = 0.91, P < 0.001, RSD = 0.038). (- - - -) regression line for BMD(2): BMD(2) = 0.7448 ash density + 0.0892 (n = 18, r = 0.91, P < 0.001, RSD = 0.038).

takes account of displaced soft tissue it follows that BMD(1) for the bone samples represented in Fig. 3 can be calculated as: BMD(1) = Ash density – COD/2. The exponential regression equation relating these estimates of BMD(1) to COD was:

$$\text{Predicted COD} = 0.0216 + 0.028[8.05^{\text{BMD(1)}}] \text{ with } R^2 = 0.842$$

This equation can then be used to estimate COD from SPA measurements of BMD(1). Accordingly, SPA measured BMD adjusted for displaced soft tissue [i.e. BMD(2)] then becomes SPA measured BMD(1) + (predicted COD/2). Using the above pathway, predicted BMD(2) values were derived from the *in vivo* SPA determined $BMD_{TBT\_predict}$ values so that comparisons could be made between the *in vivo* SPA determinations of $BMD_{TBT\_predict}$ with and without making allowance for displaced soft tissue (Fig. 4). When allowance was made for displaced soft tissue, calculated *in vivo* $BMD_{TBT\_predict}$ increased by an average of 0.031 g/cc. Because COD increases with increasing ash density the increase was greater for the P_{adeq} steers (0.0340 g/cc) than for the P_{defic} steers (0.0285 g/cc).

In conclusion the present study demonstrated that a purpose-built SPA instrument could be used on-farm to measure the mineral density of tail bone in cattle with acceptable accuracy. Making allowance for the displacement of soft tissue by bone mineral should improve the accuracy and is recommended. Errors in estimating TBT remain a problem with regard to accuracy of SPA measures of BMD made *in vivo*. However, it should be possible to modify the instrument to address this problem. Nevertheless, the SPA instrumentation provides a useful approach to obtain sequential non-invasive on-farm measurements of changes in tail bone BMD in cattle.

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