TAIL-BONE DENSITOMETRY AS A TECHNIQUE FOR MEASURING BONE MINERAL STATUS IN CATTLE

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SUMMARY

The paper describes the development and use of a bone densitometer (single photon absorptiometer) for the field measurement of bone mineral density in cattle. The densitometer consisted of a radioactive source of ^{241}Americium a sodium iodide detector plus photomultiplier tube, counter and ratemeter linked to a computer. The source and detector were mounted in a "U-shaped" frame which clamped over the Cy^{9} vertebra. A linear potentiometer measured tail thickness. Verification of the technique was carried out using postmortem material from abattoirs and spent animals for antimortem/postmortem data. Field data were obtained from a phosphorus depletion/repletion trial using mature cows.

Results obtained from the abattoir experiment suggested that densitometry gave a good indication of bone ash under controlled laboratory conditions. The field data indicated that densitometry, while capable of providing a measure of bone mineral status, may not be sensitive enough to replace current methods being used. Nevertheless the results do suggest that in more sensitive animals (young growing stock) the bone densitometer may be a useful diagnostic tool.

Keywords: mineral nutrition, densitometry, cattle.

INTRODUCTION

The widespread deficiency of phosphorus in the diets of cattle in tropical Australia makes the need for a rapid, field (crush-side) diagnostic test highly desirable. Present diagnostic tests are either considered unreliable (blood phosphorus (P) analysis, Cohen 1974), time consuming (bone biopsies, Little 1972) or require cumbersome equipment (neutron activation, Whineray *et al.* 1980). The close relationship between calcium (Ca) and P in bone mineral (Braithwaite 1976) would indicate that determination of total bone mineral, ie. bone density, would give a measure of bone phosphorus status. One non-invasive method of measuring bone mineral content *in vivo* is single photon absorptiometry or y-ray densitometry.

The absorption of y-rays, as they pass through bone, is proportional to the density (mineral content). This methodology is used routinely in human medicine for the diagnosis of osteoporosis (Cameron and Sorenson 1963) and as an aid to the determination of limb soundness in race horses (Jeffcott *et al.* 1986). The instrumentation used in these practices is quite cumbersome and not directly suitable for use with untrained cattle.

Siemon and Moodie (1974) and Siemon *et al.* (1974) used y-ray densitometry to investigate variability in density of the various bones of the bovine skeleton and concluded that the tail-bones were representative of the skeleton as a whole. This paper reports on initial work towards development of a small robust instrument to provide rapid, "crush-side", determination of the density, and hence mineral content, of the tail-bones of cattle.

EXPERIMENTAL

Establishment of the technique and formula

Mathematical formula Bones contain both soft tissue and mineral (hydroxyapatite) in varying proportions according to animal mineral (including P and Ca) status. Gamma-ray densitometry involves the determination of tissue density by measuring the attenuation of y-rays passing through a known thickness of tissue and calibration with substances of known density. The logarithmic decrease in y-ray counts through a tissue with increasing density and thickness (Cameron and Sorenson 1963) follows the formula:

$$I = I_o e^{-umpx}$$

where I = counts through the tissue, I, = counts through air, um = mass attenuation coefficient, p = density of tissue, and x = thickness of tissue.

Substituting for bone and soft tissue, this formula becomes:

$$\text{Bone Density},(p_B) = 1 + \frac{\ln \frac{I_O}{I_B} - um_{ST} p_{ST} x_{ST}}{um_B x_B}$$

where $p_{B,ST}$ = density (g/cm^3) of bone mineral, of soft tissue; $I_{,,B}$ = counts/second through air, through bone; $um_{ST,B}$ = attenuation coefficient for soft tissue, for bone; $x_{ST\,B}$ = thickness of soft tissue, of bone.

Equipment and abattoir material Initial measurements were made'at the Australian Institute of Marine Science using equipment based on single photon absorption of y-rays from ^{241}Americium.

Tail-bone (coccygeal (Cy) vertebrae) measurements were chosen because axial skeleton (trabecular bone) is more dynamic than cortical bone of limbs resulting in greater sensitivity to mineral status. The proximal end of Cy^9 was selected as Cy^9-17 have very similar specific gravity and ashed density; also Cy^9 is the largest coccygeal vertebra without major bony protruberences.

Tails were obtained at slaughter from 225 mature cattle from 8 properties for use in the feasibility study. These were frozen until densitometry measurements were made.

Determination of tissue attenuation coefficients Mass attenuation coefficients (counts/s.cm) were measured for water, 0.200; muscle, 0.201; tendon, 0.198; fat, 0.196; skin, 0.196; bone mineral, 0.404; and aluminium (Alcan 6063), 0.275; with regression coefficients greater than $r^2 = 0.9989$. Due to the very similar coefficients, all soft tissue, including demineralised bone matrix, were regarded as having the same attenuation coefficient of 0.198 counts/s.cm.

Relationship of tail thickness to bone thickness Bone density calculations involve estimating tail-bone and soft tissue thickness in live animals. Dissection of the 225 tails obtained from the abattoir found the relationship between tail-bone thickness and total tail thickness to be 0.684 ± 0.07 (mean ± sem) with a coefficient of variation of 0.1 ($P < 0.001$).

Inclusion of body condition score data from the abattoir cattle plus 39 animals from another experiment showed that the ratio of tail-bone thickness to total tail thickness was significantly ($P < 0.001$) related to condition score (estimated over a range of 1 to 10) by the regression:

Ratio tail-bone/total tail thickness = 0.78 - (0.013 x condition score)

Density of tail-bones from abattoir slaughtered cattle Following y-ray attenuation measurements through the Cy^9 bone of the abattoir specimens, this bone was dissected and a 5 mm disk cut from the proximal end. This disk was weighed in air and water to determine specific gravity, then ashed to determine ash density (ashed weight/original bone volume). There were significant correlations between y-ray absorption (bone densitometry) and ashed density (regression coefficient, 0.46; $r = 0.544$; $P < 0.01$), and between ashed density and specific gravity (regression coefficient, 0.99; $r = 0.97$; $P < 0.001$).

Repeatability of measurements and experimental error

Development of hardware and field technique The densitometer consists of a 3700 MBq (100 mCi) radioactive source of ^{241}Americium (which delivers 60 keV energy), a sodium iodide detector plus photomultiplier tube, counter and ratemeter (Canberra Packard, Chicago) linked via an IEEE interface card to a computer (Osborne, Australia). The detector output is recorded on the screen both numerically and graphically. The source and detector are mounted in a "U-shaped" frame which clamps onto the animal's tail. The source is collimated to direct a 3 mm y-ray beam through the tail to the detector. The high energy source was chosen to minimise time taken in reading and to reduce relative measurement error by increasing absolute counts.

A linear potentiometer is attached to measure tail thickness. As the tail is clamped and held for y-ray readings, the potentiometer measures the distance between the clamps as total tail thickness. The output is fed through an AD board and displayed on the computer screen. A moving stage has been devised to move the source and detector proximally and distally along the tail while maintaining the clamps in position. Slow movement along the tail while watching the graphical display of counts, enables precise location of the proximal part of Cy?

Crush-side method The animal is restrained by head-bail in a crush; no medication is required. The Cy^8-Cy^9 inter-vertebral space palpated; the instrument is clamped to the tail over this space. The proximal end of Cy^9 is located using the y-count rate meter to indicate the lowest count rate and hence thickest section of bone. An experienced operator can locate this spot quickly (2 minutes) and repeatedly. A computer program then directs the densitometer to record both 30 second count rate and tail thickness at this point.

The technique took an experienced operator with 1 assistant and reasonable crush facilities about 5 to 10 minutes/animal.

Temperature and time effects on machine performance The portable densitometer was tested for experimental error using the following steps:

The instrument was operated continuously for 6 hours, firstly under standardised laboratory conditions (18°C constant) and secondly under ambient temperature and humidity conditions outside in direct sunlight. Temperatures were recorded every 15 minutes from a thermometer placed on the upper surface

of the computer; these ranged from 20 to 44°C. Densitometer counts were taken every 3 minutes through air then through 10 mm standard aluminium (Alcan 6063; similar attenuation as an average tail). Apart from wide fluctuations during the first 60 minute "warm-up" period when recorded counts (I) decreased, the log ratio of counts through air to aluminium, $\ln(I_o/I)$, remained constant with a coefficient of variation of < 2%.

Repeatability and speed of measurement *(i) Inexperienced operator:* Repeatability of the densitometer and the ability of an inexperienced operator was tested with *42 Bos indicus* cross cattle on 3 times over 7 days. The cattle were cooperative while the instrument was in use, but readings became difficult when Buffalo flies caused irritation.

There was a significant day effect on tail density which was reduced if the first day's data was discarded. This was attributed to improvement of operator ability to consistently determine the point of maximum attenuation. Repeatability was highly significant ($P < 0.01$) for both bone density and for tail thickness data from the last 2 days.

Densitometer variation due to animals was significant at the 1% level, while variation between days was significant at the 5% level. Variation within animals was high due to errors in estimating the ratio of bone to soft tissue thickness and inaccurate location of Cy^9. As operator skill increased, variation in density decreased. Over the period, using 2 stockmen, speed increased from 3 to 8 animals/hour.

(ii) Experienced operator: A second study employing an experienced operator was made on 2 days over a week using 21 cows on which the position of the Cy^9 intervertebral space had been marked. Sufficient instrument "warm-up" time was allowed. Analysis of variance for tail-bone density showed neither between day ($P = 0.65$) nor within animal ($P = 0.06$) effects, and no interaction.

Comparison between densitometry and blood P

A group of 30 mature cows were studied over 140 days. Following initial densitometry and blood tests, 10 cows were slaughtered for measurement of tail/tail-bone thickness and bone density, and 20 cows had parotid salivary ducts catheterised and catheters exteriorised and these were placed on a barley straw diet in an attempt to create a P depletion regime. Difficulties with catheter maintenance and inability to obtain low P straw meant that the animals were in fact not on a depletion regime.

Blood P and bone density were monitored for 100 days (P intake = 10 g/day), after which 7 cows were slaughtered, 6 of the remaining cows were fed rice straw (P intake = 1 g/day), while 7 cows were fed Verano hay plus 60 g urea/day and a supplement of monosodium phosphate supplying 20 g P/day (P intake = 28 g/day). Blood P and bone density were monitored for a further 40 days before the remaining 13 cows were slaughtered for measurement of tail/tail-bone thickness and bone density.

Liveweight and condition score fell throughout the experiment except for the repleted group which gained in both following supplementation. Blood inorganic P levels fluctuated between 30 and 50 mg/L with a slight downwards trend throughout. Inorganic P in the supplemented group rose quickly to a mean of 90 mg/L ($P < 0.01$). Bone densitometry readings tended to rise throughout the trial from 1.40 g/cm^3 to 1.47 g/cm^3 as body condition fell in the unsupplemented animals; following supplementation, readings tended upwards to 1.52 g/cm^3. When densitometry readings were corrected for true tail-bone thickness measured at autopsy, the figures became 1.42, 1.49 and 1.55 g/cm^3 respectively.

Rib-bone cortical thickness (measured at autopsy on the 12th rib) and true tail-bone density both increased ($P < 0.05$) in response to P supplement (4.2 ± 0.01 v 3.9 ± 0.01 mm and 1.59 ± 0.03 v 1.51 ± 0.02 g/cm^3 respectively), but there was no reduction in either parameter due to attempted depletion. Phosphorus concentrations in the tail bone moved in parallel with those for rib bone. Although the differences between treatments were not great, the densitometer readings did reflect these differences. There was a good relationship of bone density to ash density, better than in earlier results.

DISCUSSION

Results obtained from the abattoir experiment suggest that densitometry gives a good indication of bone ash under controlled laboratory conditions. However, when an estimation of tail-bone thickness must be relied on, then the relationship is less reliable, particularly at the lower ash densities. Considering the regular composition of hydroxyapatite (bone mineral) and the constant ratio of Ca to P in bone ash, these results also gave an indication of bone P concentration.

Warm-up time required for the instrument (at least 60 minutes) is considerable and ambient temperatures can cause further instability. Nevertheless, with an appropriate warm-up period and use of a known density standard between animals, corrected readings can be quite repeatable.

Speed of operation is dependent on operator experience, increasing from 3 to 8 animals/hour. Further, as operator skill increased, variation in recorded tail-bone density decreased.

The fundamental requirement for the success of a diagnostic or survey tool is that of sensitivity to biological differences, ie. to the difference between P-adequate and subclinically P-deficient cattle. The data obtained in the depletion/repletion experiment indicate that densitometry, while capable of providing a measure of bone mineral status, may not be sensitive enough to replace current methods being used. This may in part be due to the age and history of the animals used. Such cattle probably had adequate stores of bone mineral which were resistant to depletion, and therefore would require a longer period to show signs of bone mineral disease. Nevertheless the results do suggest that in more sensitive animals (young growing stock) the bone densitometer may be a useful diagnostic tool. The opportunity to test the instrument on young animals known to have been subjected to different dietary P histories has been reported by Coates and Murray (1994).

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