10.1071/AN24213

Animal Production Science

Supplementary Material

Mobilisation and replenishment of phosphorus reserves in *Bos indicus* cows. 1. Mid-pregnant mature cows post-weaning

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Supplementary Appendix S1.

Description of the surgical procedures used to obtain bone biopsies from the rib cortical bone and tuber coxae trabecular bone

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Surgical techniques

For both sites, the skin was clipped and scrubbed with diluted chlorhexidine before being wiped with 4% chlorhexidine concentrate in methylated spirits. New, sterile scalpel blades and gloves were used for each animal and all instruments were cleaned and soaked in ethanol and 5% chlorhexidine for at least 20 minutes between animals. The skin, muscle and deeper tissue over the rib were infiltrated with 35–40 mL of lignocaine hydrochloride (20 mg lignocaine hydrochloride/mL; Troy laboratories; Glendenning, NSW, Australia). After 5 min an 8 cm longitudinal incision was made through the skin and muscle to the level of the bone. Muscle and periosteum were retracted using a periosteal elevator. The subcutaneous tissue and muscle were closed using absorbable suture (2/0 PDS). The skin incision was closed with a non-absorbable suture (size 0 or 1 Nylon). The surgical area was wiped clean using dry gauze swabs, and then sprayed with Chloromide antiseptic spray (Troy laboratories; Glendenning, NSW, Australia).

Sampling of rib bone

Rib bone biopsies were collected from the 12th rib at the site where the rib was intersected by a line from the tuber coxae to the point of the shoulder. A single biopsy was obtained from the central part of the outer cortex of the rib using a 16 mm bone trephine (Sontec Instruments Centennial, CO, USA) attached to a cordless drill. This trephine has a sharp central guide that was retracted once the cortical bone cut has been started. The surgeon could feel when the full outer cortex had been drilled and stopped before the inner cortex was impacted. The biopsy was easily removed.

In the laboratory a small hacksaw was used to divide the bone cores perpendicular to the orientation of the trabeculae such that samples were for the top and the bottom of the rib sample. These subsamples were (i) placed in saline and stored frozen for measurements of cortical bone thickness and specific gravity, or (ii) fixed with 10% normal buffered formalin for 4 to 12 weeks. The latter samples were decalcified in 10% EDTA (pH 7.0) for 12-18

weeks with solution changed every 2 weeks. Decalcification was considered as adequate when weekly measurements indicated that there was no further loss of weight of the sample. A small cut with a scalpel blade was also used to assess decalcification and both criteria were used to assess the bone as fully decalcified. The samples were then embedded in paraffin and multiple 5 µm thick sections transverse to the longitudinal direction of the bone axis were taken. The sections were stained with toluidine blue and Masson's trichrome, photographed twice (10 x objective, Olympus BX41 microscope (Olympus America Inc.; Melville, NY, USA) equipped with a digital camera Q-Imaging camera (Qimaging Corporation, Surrey, BC, Canada). One representative section from the thickest region of the rib for each animal was selected for histomorphometry measurements of bone porosity.

Sampling of *tuber coxae* bone

Bone biopsies were collected from the *tuber coxae* on the left and right side of each animal. A single biopsy 15-25 mm deep was obtained from the most central part of the tuber coxae of the ilium. A 16 mm bone hand-trephine (Sontec Instruments Centennial, CO, USA) was used to start the biopsy and then a 16 mm metal hole-saw (various brands, hardware stores) was used to obtain a deeper sample. One side of a long pair of scissors was used to loosen and separate the sample from the parent bone. This involved breaking the deep attachment of the bone core from the parent bone. The full core could then be pulled from the hole easily.

In the laboratory the bone cores were divided lengthwise using a scalpel blade and sub-samples were fixed with 10% normal buffered formalin (NBF) and 70% ethanol and placed on ice prior to transfer to the laboratory. The samples remained in fixative for 4 to 12 weeks. Bone samples fixed with 10% NBF were decalcified in 10% EDTA (pH 7.0) for 6-12 weeks with solution changed every 2 weeks. Adequate decalcification was determined when weekly weighing showed no further loss of weight of the sample. A small cut with a scalpel blade was also used to assess decalcification. Both criteria were used to determine full decalcification. After decalcification the samples were embedded in paraffin in a routine fashion and multiple 5 µm thick sections were taken parallel to the longitudinal direction of the bone axis. The sections were stained with toluidine blue and Masson's trichrome and photographed twice with a 10 x objective using a Olympus BX41 microscope (Olympus America Inc.; Melville, NY, USA) equipped with a digital camera Q-Imaging camera (Qimaging Corporation; Surrey, BC, Canada). Histomorphometry measurements were made in one representative section for each animal was selected for measures of trabecular bone

parameters. The pictures were taken at a standardised distance from the growth plate (300 μm). The images were then analysed using the software ImageJ (Schneider *et al.* 2012) and the plugin BoneJ (Doube *et al.* 2010) to obtain values for bone volume (BV.TV), trabecular separation (Tb.Sp) and trabecular thickness (Tb.Th).