DEFINITION OF AN OBJECTIVE CRITERION OF BODY PHOSPHORUS RESERVES IN CATTLE AND ITS EVALUATION IN VIVO

D. A. LITTLE[1]

CSIRO, Division of Tropical Crops and Pastures, St. Lucia, Q 4067 Australia.

Phosphorus concentration in total fresh ribs is a sensitive criterion of the body phosphorus reserves. It may be estimated from: (a) measurement, on a biopsy sample of the 12th rib, of thicknesses of compact bone and osteoid tissue on the endosteal surface, or (b) the ratio of cortical bone area: total area, calculated from midshaft dimensions of the metacarpal or metatarsal.

Key words: Cattle, phosphorus reserves, status, bone

Problems of P deficiency in cattle are widespread, but there is as yet no satisfactory method of objectively assessing the status of bovine P reserves. For dignostic purposes measurements of circulating P concentration are usually made, but it is now widely recognized that these are of very limited application (Gartner et al. 1980). Since the skeleton is the major body reserve of P (Duckworth and Hill 1953), the work described here was designed to develop an appropriate objective criterion of the status of the body P reserves based on skeletal measurements, and to demonstrate methods by which this might be evaluated in vivo. The experimental cattle were managed in different ways to produce different levels of body P storage. Animals were slaughtered and dissected to allow the measurement of total body P and its distribution among the respective tissue (muscles, viscera, blood, skin, sternum/costal cartilages, appendicular, axial and rib bones and "feet") (Little 1983).

Two groups of six Hereford cattle were slaughtered, at liveweights ranging from 170 to 280 kg, following dietary regimes high or low in P (designated HP and LP groups). In a third group of four Shorthorns, increased physiological stress with respect to P was produced by superimposing pregnancy and lactation upon a low P regime (designated low P lactation group, LPL, liveweight range 160–240 kg); two of these became osteophagic.

Radiological, chemical and physical measurements on the skeleton all indicated the development of osteomalacia in the P-stressed animals, increasing with the severity of P stress. For example, measurements made at the midshaft of the metatarsal showed significantly decreasing quantities of cortical bone, in that the mean ratios of medullary width:total width were 0.52, 0.64 and 0.74 for the HP, LP and LPL treatments, respectively ($P<0.05$), while the total widths were very similar.

Total body P (TBP) as a proportion of liveweight (LW) was influenced by nutritional effects, where the figures for the HP and LP groups were 0.64 and 0.58% ($P<0.01$), showing that TBP cannot be estimated from LW. The LPL group was intermediate, with TBP constituting 0.61% LW, which indicates this parameter to be an inappropriate index of the status of the body P reserves.

The skeleton was the only tissue in which increasing P stress produced significant differences in concentration of P in fresh tissue, and the rib fraction was the most sensitive to P stress. Significant differences occurred between all three groups in the proportion of TBP contained in ribs (10.8, 9.7 and 8.8% for HP, LP and LPL, $P<0.05$), but the rib fraction nevertheless constituted a constant 1.3% of fasted LW in all three groups; the loss of mineral (30.7, 26.5 and 24.4% ash) was largely compensated by an increase in fat content (9.9, 11.4 and 17.5%, respectively).

The concentration of P in the total fresh ribs, 5.41, 4.49 and 4.21%, respectively, for HP, LP and LPL provided a clear distinction, in that the lowest value from the HP treatment was 5.08%, and the highest of the P-stressed animals, 4.71%. It is therefore proposed that the concentration of P in total fresh ribs (RPC) be adopted as a criterion of the P reserves of cattle; a figure of 5% or more for this quantity appears to indicate adequate reserves.

Evaluation of RPC in vivo can be based on either: (a) Biopsy samples of the 12th rib (Little 1972); cores taken by trephine are sectioned along the midline, trabecular bone is removed from one-half and measurements of the thickness of compact bone (CBT) are made with a

[1]Present address (D.A.L.): CSIRO, PARD, P. O. Box 123, Bogor, Indonesia.

Can. J. Anim. Sci. 64 (Suppl.): 229–231 (Sept. 1984)

Vernier caliper along the line of section. The other half is fixed in ethanol and processed by the method of Tripp and Mackay (1972) which distinguishes osteoid tissue. The mean thickness of osteoid on the endosteal surface is calculated (a) by measurement with an eyepiece micrometer, and the proportion of the surface on which it occurs is estimated; or (b) calculation of the ratio cortical bone area:total area (CA:TA) based on transverse measurements of total and medullary diameter at the mid-shaft of the metacarpal or metatarsal, which can be made from contact radiographs as described in the human medical field by Barnett and Nordin (1960). The calculations assume cylindrical cross-section (Garn et al. 1967), and in the present work were based on measurements made on the actual bones following slaughter. These skeletal measurements are given in Table 1.

Table 1. Mean 12th rib compact bone thickness (CBT, mm) and osteoid thickness (OT, μm) and cortical bone area:total area ratios (CA:TA) from mediolateral dimensions of metatarsal (M/T) and metacarpal (M/C) bones of HP, LP and LPL groups of cattle

Parameter	HP	LP	LPL
CBT	3.28*a*	2.30*b*	1.42*c*
OT	0.14*a*	10.40*b*	6.21*b*
CA:TA (M/T)	0.74*a*	0.67*b*	0.53*c*
CA:TA (M/C)	0.68*a*	0.60*b*	0.49*c*

a–c Within rows, means with different letters differ significantly ($P < 0.05$).

Regression relationships were derived between RPC and the skeletal measurements described. These equations, given in Table 2, were tested by applying them to similar data derived from nine independent animals, and assessing the accuracy with which their RPC was estimated. The RPC of these independent animals ranged from 4.45 to 6.36% (cf. 3.81–5.80% for the HP, LP and LPL groups).

The relationship between RPC and individual CBT data showed that thicknesses > 3 mm are associated with adequacy, and those < 2 mm with deficiency. Some overlap between the HP and LP groups occurred, although the means of all three groups differed significantly from each other ($P<0.05$, Table 1). The combined index of osteoid thickness (OT) and CBT (Table 2) differentiated fully between the HP and P-stressed animals, since P stress provoked the occurrence of substantial but very variable quantities of endosteal osteoid, while the HP group had only trace amounts. This index therefore resolved the status of the P reserves of those animals whose CBT lay between 2 and 3 mm. The CA:TA ratios calculated from the medio-lateral dimensions of the metatarsals and metacarpals differed significantly between all three groups ($P<0.05$, Table 1). All of the regression equations given in Table 2 slightly underestimated RPC of the independent group, but only the mean based on CBT alone differed significantly from the actual figure. Although the accuracy of estimation based on the long bone dimensions was marginally superior (Table 2), slightly greater errors would be expected when the in vivo measurements are made from radiographs (Adams et al. 1969) than was the case here following direct measurements on the bones.

Table 2. Equations for estimation of total rib P concentration (RPC, Y) from various skeletal measurements (X), and mean errors of estimate of RPC of nine independent animals

Parameter (X)	$Y =$	r	Error of estimate
CBT	$3.40+0.577X$	0.85	−11.7*
Log (1 + CBT/OT)	$4.25+0.794X$	0.89	− 6.3
CA:TA M/T	$1.48+4.98X$	0.79	− 5.8
CA:TA M/C	$1.61+5.25X$	0.80	− 6.1

*Significantly lower than actual figure ($P < 0.05$).

Thus a basis is established for assessing the status of the P reserves of cattle in vivo; since the long bones may be easily obtained at slaughter, measurements taken on them could provide a useful retrospective assessment.

ADAMS, P., DAVIES, G. T. and SWEETNAM, P. M. 1969. Observer error and measurements of the metacarpal. Br. J. Radiol. **42**: 192–197.

BARNETT, E. and NORDIN, B. E. C. 1960. The radiological diagnosis of osteoporosis: a new approach. Clin. Radiol. **11**: 166–174.

DUCKWORTH, J. and HILL, R. 1953. The storage of elements in the skeleton. Nutr. Abstr. Rev. **23**: 1–17.

GARN, S. M., ROHMANN, C. G. and WAGNER, B. 1967. Bone loss as a general phenomenon in man. Fed. Proc. **26**: 1729–1736.

GARTNER, R. J. W., McLEAN, R. W., LITTLE, D. A. and WINKS, L. 1980. Mineral deficiencies limiting production of ruminants grazing tropical pastures in Australia. Trop. Grassl. **14**: 266–272.

LITTLE, D. A. 1972. Bone biopsy in cattle and sheep for studies of phosphorus status. Aust. Vet. J. **48**: 668–670.

LITTLE, D. A. 1983. Bovine body composition and phosphorus storage. Ph.D. thesis, University of Queensland.

TRIPP, E. J. and MacKAY, E. H. 1972. Silver staining of bone prior to decalcification for quantitative determination of osteoid in sections. Stain Technol. **47**: 129–136.