# FAILURE OF BONE PHOSPHORUS LEVELS TO INDICATE DIETARY INTAKE OF PHOSPHORUS BY SHEEP

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#### **ABSTRACT**

Belonje, P. C. & Van den Berg, A., 1983. Failure of bone phosphorus levels to indicate dietary intake of phosphorus by sheep. *Onderstepoort Journal of Veterinary Research*, 50 1-2 (1983)

Fifteen actively growing lambs were divided into 3 groups and fed diets similar in all respects except for their calcium (Ca) and phosphorus (P) levels. The diets (Group 1; 1,47% Ca, 0,36% P: Group 2; 0.85% Ca, 0,47% P: Group 3; 0,37% Ca, 0,64% P) were fed to the animals for 98 days, when rib biopsy specimens were removed and analysed for Ca and P (Group 1; 21,20% Ca, 10,49% P: Group 2; 19,54% Ca, 9,42% P: Group 3; 19,10% Ca, 9,24% P). Although there were no differences (P > 0,01) in the bone analyses between the groups, there was a tendency for bone calcium levels to follow dietary calcium levels. Bone phosphorus levels, again, followed bone calcium levels and were opposite to dietary phosphorus levels. This work re-emphasizes the dominance of calcium over phosphorus in bone formation. Implications of these findings are discussed in the light of the use of bone phosphorus analyses to estimate the phosphorus intake and status of grazing sheep.

### INTRODUCTION

As sheep are highly selective grazers (McDonald, 1968; Engels, 1972) hand-picked samples do not reflect forage intake. Although a primary deficiency of phosphorus has not been authenticated in sheep (McDonald, 1968), it may yet be found to occur. For this reason it is necessary to find a method to establish the phosphorus intake and status of free-ranging animals. Amongst other possibilities, it has been suggested that bone phosphorus may be used for this purpose (Cohen, 1973a, 1973b, Engels, 1981, Little, 1972). However, earlier work in this laboratory (Belonje, 1978) seemed to indicate that bone phosphorus levels followed more closely bone calcium and calcium intake levels than phosphorus intake levels.

To explore this suggestion further the following experiment was designed. Actively growing lambs were fed 3 diets similar in all respects except for their calcium and phosphorus content. The levels of these were set in such a fashion that they contained either high calcium, low phosphate or medium calcium medium phosphate or low calcium, high phosphate. After 98 days on these various diets, rib specimens were taken, and after analyses the rib calcium and phosphorus levels were compared with the dietary levels.

# MATERIALS AND METHODS

Animals

Fifteen healthy 6-month-old Merino lambs were selected. They were blocked by body mass into 3 equal groups and for the duration of the 98-day experimental period they were kept in pens on slatted floors in a shed with an open side allowing plenty of reflected sunlight to enter. On Day 0 they were treated with an anthelmintic†. This was followed by a further treatment‡ on Day 46. Feed

Three different balanced diets were formulated by a commercial undertaking. These diets were almost identical except that they differed in their calcium and phosphorus contents (Table 1).

The animals were adapted over 5 days to the new diets, after which the daily intake of each group was limited to the total amount consumed by the group eating the least the previous day. By this means the total intake of all 3 groups over the whole experimental period was about equal.

Received 27 August 1982-Editor

TABLE 1 The constituents and amounts (kg) used to compound the diets fed to the 3 groups of sheep

Constituent	Diet 1	Diet 2	Diet 3
Yellow maize	500	510	506
Straw	300	300	300
Sunflower oilcake meal		60	60
Limestone flour	60 30	12,5	1
Molasses	100	100	100
Salt	4	4	4
Urea	6	6	6
Monosodium phosphate		7,5	24

Specimens and data

Food samples: Grab samples of each of the diets were taken on a daily basis and pooled. Pool Sample 1 extended from Day 0-Day 48, Sample 2 from Day 49-Day 62, Sample 3 from Day 63-Day 76 and Sample 4 from Day 77-Day 98.

Body mass: The mass on the animals were determined on a weekly basis from Day 0-Day 91.

Rib specimens: On Day 98 rib biopsies were performed. Water and food was withheld for 16 h. The animals were anaesthetized with intravenous pentobarbitone\* and a portion of the distal part of the 7th rib was removed after reflecting and dissecting off the periosteum. Recovery in all cases was uneventful.

Processing of samples and analytical methods

Food and whole bone: These were processed as outlined before (Belonje, 1978) and analysed for phosphorus (Hanson, 1950). Calcium and magnesium levels were determined by atomic absorption spectrophotometry, using an air-acetylene flame and lanthanum as a releasing agent (Varian Techtron Manual, 1972).

## RESULTS AND DISCUSSION

Body mass

Data for body mass are set out in Table 2. It is gratifying that during the experimental period each group increased body mass by at least 50% and that the groups had similar body masses at the end. The obvious implication for this study is that there must have been bone growth and turnover during this time.

## Feed and bone

Data for feed and bone are set out in Table 3. The calcium and phosphorus levels and the calcium:phosphorus ratios in the 3 diets differed significantly from one another (P < 0.01). On the other hand there were no significant differences (P < 0.01) in the rib levels of calcium or phosphorus or the calcium:phosphorus ratio among the 3 groups.

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			D	ays			
	0	7	14	21	28	34	42
Group 1	27,1±4,9	28,6±4,7	28,3±4,4	31,1±5,1	30,4±5,7	32,1±5,4	33,2±5,6
Group 2	26,5±4,1	29,6±5,4	29,1±5,1	28,5±5,2	29,0±5,5	30,7±5,2	30,7±5,7
Group 3	26,7±2,0	28,3±3,0	29,3±3,8	29,6±1,8	29,7±1,7	31,3±1,8	32,9±1,4
			/ Da	ays			
	49	56	63	70	77	84	91
Group 1	35,6±6,0	36,4±5,9	37,4±6,1	38,6±6,2	40,9±6,2	41,4±5,7	42,2±6,4
Group 2	34,0±5,9	34,6±6,2	34,1±5,7	36,7±6,9	37,6±6,5	38,3±6,6	41,0±5,7
Group 3	35,4±1,9	34,6±1,8	35,4±1,6	36,6±1,4	38,4±1,8	39,2±1,8	40,4±2,0

TABLE 3 A comparison between the mean (±SD) levels of calcium and phosphorus and their ratio in the ribs of the sheep and in the feed the animals consumed

Determination	Specimen*	Group 1	Group 2	Group 3	Significance of differences among 3 groups	
					Not significant	P<0,01
Calcium %	Feed Bone	1,47±0,32 21,20±2,64	0,85±0,08 19,54±1,80	0,37±0,05 19,10±2,16	1v2:2v3:1v3	1v2:2v3:1v3
Phosphorus %	Feed Bone	0,36±0,01 10,49±0,73	0,47±0,01 9,42±0,26	0,64±0,03 9,24 ±0,56	1v2:2v3:1v3	1v2:2v3:1v3
Calcium: phosphorus ratio	Feed Bone	4,09±0,81 2,01±0,15	1,81±0,15 2,07±0,16	0,61±0,10 2,07±0,16	1v2:2v3:1v3	1v2:2v3:1v3

<sup>\*</sup> In each group there were 4 composite feed specimens and bone specimens from 5 animals

From the data it would appear that there was a trend for bone calcium to decrease as feed calcium decreased. But bone phosphorus also had a tendency to decrease in the face of rising feed phosphorus. This re-emphasizes previous work (Belonje, 1978) in which it was found that calcium is dominant in bone formation and phosphorus has a subservient role.

There are 3 main implications from this. Firstly, should animals be kept on a constant calcium intake and phosphorus be supplemented, then bone phosphorus analyses may possibly be used to monitor this change, as phosphorus does modify bone deposition slightly. Secondly, should the calcium intake in animals be on the decrease, particularly to levels below those required for balance, then bone analyses for phosphorus will also show a decrease and hence give a negative reflection of any supplementary dietary phosphorus. Thirdly, should animals be in a growing phase and be receiving increasing levels of calcium, then bone phosphorus is going to rise together with bone calcium and perhaps give a false impression that dietary phosphorus levels are actually rising at the same time.

These conclusions are reinforced by studying the calcium: phosphorus ratios (Table 3). Although the ratios in the 3 feeds showed a wide variation (0,61–4,09) there was, not unexpectedly, no change in the ratio in bone. Once again, then, as calcium is dominant, bone phosphorus levels follow closely those of calcium and therefore dietary calcium has a more profound effect on bone

phosphorus than does dietary phosphorus. The use of bone phosphorus analyses to assess phosphorus intake should therefore be viewed with caution.

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