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Supplementation of Phosphorus and Cholecalciferol and Effects on Absorption and Retention of Phosphorus in Sheep

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Abstract: It was evaluated effects of supplementation of P and cholecalciferol (CC) on P absorption and retention in sheep. Forty-two sheep were assigned randomly to 6 treatments, consisting of a basal diet supplemented with P and CC: 0 g P and CC (Basal), 0 g P + CC (Basal$^+$), 1 g P and no CC (P1), 1 g P + CC (P1$^+$), 2 g P and no CC (P2) and 2 g P + CC (P2$^+$). Total fecal and urinary collections were made for 10 day at the end of the experimental period and blood serum was collected. Rib bones were taken after sacrificing the sheep in the Basal$^+$ and P2$^+$ treatments. Samples were processed for P, Ca and Mg determination. Phosphorus and Ca body retention was calculated. Phosphorus retained in the body was greater (p = 0.01) in sheep supplemented with P and sheep of Basal$^+$ than in sheep of Basal. No differences (p >0.05) were found in the concentration of P, Ca, or Mg in bone. Supplementing either 1 or 2 g of P/d increases P absorption and retention, whereas supplementation of CC increases P absorption and retention only in sheep not supplemented with P.

Key words: Absorption, minerals, vitamin D, phosphorus, sheep

INTRODUCTION

Deficiency of phosphorus in ruminants has been reported over the world (Mejia *et al.*, 1999) mainly in ruminants grazing low-quality pastures. Absorption and retention of P and Ca was increased with supplementation of P (Ternouth and Coates, 1997) and vitamin D (Montgomery *et al.*, 2004). However, most of the studies were conducted in short periods and with periparturient cows and ewes. In addition, evaluation of Ca, rather than P, retention was the main purpose of those studies. It is necessary to carry out studies of P and cholecalciferol (CC) supplementation involving males in maintenance or production, grazing or being fed low-quality pastures and observe effects on P absorption and retention. For several decades, blood P analysis was the most common method of predicting P status of animals. However, in some cases, a lack of correlation between blood P content and P intake has lowered reliability of blood P content as an indicator of P intake (Erickson *et al.*, 2002). Based on the hypothesis that phosphorus, rather than cholecalciferol supplementation produces a higher body absorption and retention of phosphorus in sheep, the objective of this study was to measure effects of supplementation of cholecalciferol and P on body P absorption and retention.

MATERIALS AND METHODS

This study was carried out at the University of Nebraska-Lincoln, in the year 2003. A group of 42 8-mo-old sheep wethers (X = 41 kg) of mixed crossed breed (Suffolk, Dorset and Polypay) was fed a P-restricted diet (0.11% P; 0.34% Ca; 9.7% CP) for 48 day. After the P-restriction period, sheep were allocated in individual pens for 16 day for adaptation to the facilities and fed a basal diet (Table 1). Then, sheep were placed in metabolism crates and fed the same diet and amount of feed for 15 day to standardize the daily feed intake and fecal output.

After the pre-experimental period, sheep were assigned to a completely randomized design to one of 6 diet treatments: Basal (basal diet), Basal$^+$ (Basal supplemented with 2500 IU of CC/d) P1 (Basal supplemented with 1 g of P in the form of monosodium

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Table 1: Daily diet offered during the experimental period (Basal diet) and chemical composition of the feeds[1,2]

Ingredient	Amount (g d^{-1})	CP[3] (%)	P (%)	Ca (%)
Wheat straw[4]	984	6.56	0.21	0.41
Ground corn	89	11	0.33	0.03
Blood meal	24	90	0.04	0.03
Soybean meal	16	48.7	0.70	0.29
Trace mineral				
Premix[5]	0.2	-	-	-
Urea	5	281	-	-

[1]From lab analyses performed in the Department of Animal Science. UNL. [2]Expressed on dry matter basis, [3]Percentage of N×6,25, [4]Molasses was included at 13% of DM, [5]Contained 10% Mg, 6% Zn, 2% Mn, 4% Fe, 0.5% Cu, 0.3% I and 0.05% Co

phosphate), P1$^+$ (P1 supplemented with CC), P2 (Basal supplemented with 2 g of P), P2$^+$ (P2 supplemented with CC). Each animal was considered an experimental unit and each treatment consisted of 7 sheep.

Sheep were fitted with fecal-collection bags and total daily fecal and urinary collections were made for 10 day after feeding sheep the treatment diets for a period of 56 day. Feed samples were collected 2 day before and during the fecal collection. Feed and fecal samples were dried in a forced-air oven at 60°C for 3-4 day and ground (1 mm screen) through a Wiley mill. Approximately 1 g of sample was used to determine total DM (100°C, overnight), ash (550°C, 8 h), Ca, P and Mg content. Blood samples were collected at the beginning and the end of the experimental period. Approximately 15 mL of blood were drawn from the left jugular vein by using vacuum tubes for serum collection. Samples were taken 3 h after feeding at all times to avoid variation with time. In the laboratory, blood samples were centrifuged at 3700 × g for 10 min and serum was obtained. Bone samples were taken from sheep of Basal$^+$ and P2$^+$ treatments after slaughter at the end of the experiment. The 12th left rib in each sheep was taken and bone periosteum removed. Then, bone samples were weighed on fresh basis and specific gravity determined. Dry matter was determined at 100°C for 48 h and ash at 500°C for 8 h.

Phosphorus content was determined in ash solutions of feed, feces and bone and samples of urine and blood serum (deproteinized with a 5% trichloroacetic acid solution) (AOAC, 1996).

Calcium and Mg content was determined by atomic absorption spectrophotometry in feed, feces, urine, blood serum and bone (AOAC, 1996). Serum alkaline phosphatase and urinary creatinine were determined using a Beckman DV-650 spectrophotometer following the directions of SIGMA Diagnostics. In vivo dry matter digestibility, apparent absorption and true retention of P, Ca and Mg were calculated.

Statistical analysis was by analysis of variance using the General Linear Model Procedure of SAS (1997) and comparisons of means by tukey tests and correlation analyses among different variables were done.

RESULTS AND DISCUSSION

The amount of P excreted in feces was greater ($p<0.05$) in P2$^+$ and P2, in which sheep were supplemented with 2 g of P/d with and without supplementation of CC (Table 2) than in P1$^+$, P1, Basal$^+$ and Basal, in which no differences were observed. Apparent P absorption was greater ($p = 0.001$) in sheep from treatments P1, P1$^+$, P2 and P2$^+$ than sheep from Basal (Table 2). This is in agreement with Ternouth and Coates (1997), who found that amount of P absorbed increased in direct proportion to P intake. However, in our study, no differences ($p>0.05$) were found in the amount of P apparently absorbed by sheep in Basal$^+$, P1, P1$^+$ and P2 when expressed in mg d^{-1} kg^{-1} BW. But, expressed as a percentage of P intake, apparent P absorption in sheep from Basal was lower ($p<0.05$) than all the other treatments. Supplementation of CC had no effects on apparent P absorption in sheep supplemented with P but increased apparent P absorption in sheep not supplemented (Basal$^+$). The reduced amount of apparent P absorbed in Basal was due, in part, to the lower P intake and availability of organic than inorganic P sources, especially in cereal straws (Challa and Braithwaite, 1988a). Possibly, sheep not supplemented with P responded to supplementation of CC, enhancing P absorption because their intestinal ability to absorb P was not saturated (Braithwaite, 1979). No differences ($p>0.05$) in *in vivo* dry matter digestibility were found among treatments (Table 2). These findings agree with Milton and Ternouth (1985), who stated that diet dry matter digestibility remained unchanged when animals were fed varying amounts of P.

Amount of P excreted in urine was greater ($p = 0.01$) in sheep from the treatments supplemented with 1 and 2 g of P (P1, P1$^+$, P2 and P2$^+$), regardless of supplementation of CC than sheep not supplemented with P (Basal and Basal$^+$). Sheep not supplemented with P excreted insignificant amounts of P (Table 2). Amount of P excreted in urine was negligible in ruminants fed typical diets (Ternouth *et al*., 1996; Vitti *et al*., 2000; Rodehutscord *et al*., 2000). Daily amount of urinary P is not a good indicator of P intake when animals consume excessive amounts of P. Prediction of daily urine output and amount of P excreted in urine through analyses of urinary creatinine and creatinine: P ratio presented a large variability (Table 2).

Sheep from Basal were in negative balance and P retention was lower ($p<0.05$) than that from sheep receiving the rest of the treatments (Table 2). Supplementation of CC had no effect on P retention in sheep supplemented with P but increased the amount of P retained in sheep from Basal$^+$ compared to sheep fed

Table 2: Mean values for variables of phosphorus metabolism in sheep[1]

Item	Basal	Basal	P1	P1^{+}	P2	P2^{+}	SEM
P intake, g d^{-1}	2.1^{a}	2.2^{a}	3.1^{b}	3.2^{b}	4.1^{c}	4.1^{c}	0.1
Fecal P, mg d^{-1} kg^{-1} BW 61^{b}	49^{b}	58^{b}	61^{b}	82^{a}	84^{a}		5
P absorption^{2}, mg d^{-1} kg^{-1} BW	-0.6^{c}	13^{bc}	25^{ab}	28^{ab}	27^{ab}	31^{a}	4
P absorption^{2} %	-0.71^{b}	20.7^{a}	29.5^{a}	31.4^{a}	25.2^{a}	26.9^{a}	5.2
Urine P, mg d^{-1} kg^{-1} BW	0.8^{b}	1.5^{b}	9.9^{a}	9.9^{a}	12.9^{a}	10.2^{a}	2.6
TPE, mg d^{-1} kg^{-1} BW	61^{bc}	51^{c}	68^{b}	72^{b}	95^{a}	99^{a}	4
P retention, mg d^{-1} kg^{-1} BW	-1.3^{b}	11.6^{a}	18.7^{a}	17.8^{a}	14.4^{a}	20.5^{a}	3.9
P retention, %	-2^{b}	18^{a}	21^{a}	20^{a}	13^{a}	18^{a}	4
Blood serum P, mg L^{-1}	78	78	95	89	101	82	6
Serum alkaline phosphatase, U/L	267	254	212	254	200	202	24
In vivo DMD, %	57	59	60	57	57	60	1.5
Urinary P ratio^{3}	1.05	0.81	1.02	0.84	1.29	0.68	-

^{a,b,c}Different superscripts within rows mean significant differences ($p<0.05$), [1]Values are means of seven sheep per treatment, [2]Apparent absorption, [3]Predicted/actual values (using urinary creatinine: P ratio), *Treatments: Basal (control diet); Basal^{+} (Basal plus supplementation of cholecalciferol) {CC} equivalent to 2500 IU of vitamin D/d); P1 (Basal plus 1g P/d); P1+ (P1 plus CC); P2 (Basal plus 2 g P/d); P2+ (P2 plus CC), TPE = Total P excretion

Table 3: Mean values for variables of calcium and magnesium metabolism in sheep[1]

Item	Basal	Basal^{+}	P1	P1^{+}	P2	P2^{+}	SEM
Ca intake, mg d^{-1} kg^{-1}BW	93	98	92	96	86	93	4
Urine Ca, mg/d	14^{ab}	9^{b}	8^{b}	10^{b}	9^{b}	17^{a}	2
Ca retention, mg d^{-1} kg^{-1}BW	-39^{b}	-8^{a}	-8^{a}	-12^{a}	-16^{a}	-8^{a}	5
Ca retention, %	-42^{b}	-7^{a}	-9^{a}	-12^{a}	-20^{a}	-9^{a}	5.5
Blood Serum Ca, mg L^{-1}	104	104	98	99	91	104	4
Urine Mg, mg. d^{-1}. kg^{-1}BW	3.8^{a}	4.3^{a}	0.9^{b}	1.7^{b}	1.7^{b}	0.9	0.5
Mg retention, %	-15^{c}	-4^{bc}	21^{a}	11^{ab}	-0.5^{ab}	12^{ab}	-
Blood Serum Mg, ppm	22	22	21	22	21	23	0.8

^{a,b,c} Different superscripts within rows mean significant differences ($p<0.05$), [1]Values are means of seven sheep per treatment. *Treatments: Basal (control diet); Basal+ (Basal plus supplementation of cholecalciferol {CC} equivalent to 2500 IU of vitamin D /d); P1 (Basal plus 1g P/d); P1+ (P1 plus CC); P2 (Basal plus 2 g P/d); P2+ (P2 plus CC)

only the basal diet (Basal). Increase of P retention in sheep from Basal^{+} could be the result of stimulation of the renal enzyme 1 α-hydroxylase produced by the low absorption of P in the intestine (Horst, 1986). The lack of response to CC for P retention in sheep supplemented with P could be because the effects of supplementation of P and CC were not additive (Braithwaite, 1980). Amounts of P retained were not different ($p>0.05$) in sheep supplemented with 1 or 2 g of P/d and those sheep supplemented only CC were able to store similar amounts of P to those stored by sheep supplemented with 2 g of P/d. Challa and Braithwaite (1988b) found a limit in the amount of P retained when concentration of P in blood raises to certain level (> 50 mg L^{-1}) because the renal threshold for P is exceeded and P excretion in urine is substantially increased. However, large differences were reported between individual sheep in the way in which they partition the amounts of P lost via the urine and saliva, especially when the concentration of P in diets was different (Grace, 1981).

Calcium retention was in negative balance in all the treatments. However, the amount of Ca retained was more negative ($p = 0.009$) in sheep from Basal than the rest of the treatments (Table 3). The negative Ca retention was reduced substantially in sheep from treatments supplemented with 1 and 2 g P/d and in the treatment not supplemented with P but supplemented with CC (Basal^{+}). The average Ca intake was 93 mg d^{-1} kg^{-1} BW, which is below the amount suggested (250 mg d^{-1} kg^{-1} BW) by Hodge (1973) as adequate for young sheep and the requirements considered by the NRC (1985) of 165 mg d^{-1} kg^{-1} BW for lambs of 40 kg. Results of the present study are in agreement with Challa and Braithwaite (1988a), who reported that Ca retention in calves increased on high P diets even when Ca intake was adequate. Mg retention in sheep from Basal presented a negative balance and was lower ($p>0.05$) than sheep in the treatments supplemented with P. The fact of supplementing P reduced the negative Ca balance and also increased the body Mg retention (Table 3). No differences ($p>0.05$) were found in the concentration of P in blood serum among treatments, despite the differences in P intake (Table 2), samples were well above 45 mg L^{-1}, which is a concentration considered normal (McDowell, 1997). Ordinary concentration of P in blood was maintained by either feed or bone resorption. McMeniman and Little (1974) also reported no differences in blood P concentrations in sheep supplemented with P, P + molasses, or not supplemented. However, the degree of bone mineralization was higher in the supplemented groups than the control. Erickson *et al.* (2002) reported that plasma P data were quite variable in response to dietary P treatments in finishing feedlot calves fed diets containing 0.16, 0.22, 0.28, 0.34 and 0.40% P. On the contrary, Challa *et al.* (1989) reported high positive correlation between serum P concentration and the ssount of P absorbed in young calves. In the latter study, one important factor that is important to consider is the age of the animals, because very young ruminants may depend more on P contained in diet than in body P reserves compared with adult ruminants (Braithwaite, 1980). Blood P content is affected by several factors and the three principal hormones that regulate the metabolism of Ca (Parathyroid hormone, 1,25 $(OH)_2$ vitamin D_3 and calcitonin) have an important and very complex role in the regulation of the Ca and P concentration in blood (Lobaugh, 1995; Goltzman, 2005).

Although, a tendency to reduce the concentration of serum alkaline phosphatase in sheep of P2 and $P2^+$ was observed, values were not different ($p>0.05$) among treatments (Table 2). Cole (1992) also found no differences in serum alkaline phosphatase and inorganic P and Mg when using low and high P-diets in sheep when the concentration of the protein in diet was low.

Blood Ca and Mg concentration (Table 3) was not different ($p>0.05$) among treatments, it was observed that blood Ca level is maintained by the action of the regulatory hormones through feed and bone resorption.

No differences ($p>0.05$) were observed in the bone content of P, Ca and Mg between treatments $Basal^+$ and $P2^+$ when results were expressed in fresh and dry matter basis or mg mL^{-1} (Table 4). Apart from the lack of differences between treatments, level of bone mineralization of sheep in this study seemed not to be adequate because values of 127 and 121 mg of P/mL of fresh bone were found in sheep from treatments $Basal^+$ and $P2^+$, respectively. These amounts are below 140 mg mL^{-1}, which was suggested as evidence of satisfactory bone mineralization in sheep by McMeniman and Little (1974). It is possible that poor bone mineralization had occurred in sheep of all treatments, because mean values of Ca balance were negative in all treatments (Table 3) as was P in sheep on the basal diet. The negative Ca balance might be due, in part, to a Ca intake lower than requirements in both the depletion and the experimental period and a possible deficiency of protein intake, which could have restricted protein supply for bone matrix necessary for synthesis of collagen required in the mineralization process of bones (Garner *et al*., 1996).

Results of this study indicate that changes in P intake in short periods (less than 3 months) may not be reflected in significant changes in the concentration of P, Ca and Mg of bone samples. This finding agrees with Genuth (1993), who stated that, in the bone remodeling process, the formation phase lasts about 3 months. Erickson *et al.* (1999) also found no differences in bone ash content of steers fed diets containing P concentrations from 0.14-0.34% when fed for a 105 day period.

Amount of fecal P was highly correlated ($R = 0.70$, $p = 0.001$) to P intake (Table 5) and no correlations between urinary creatinine and urinary P were found. The lack of correlation, in part, was due to the large variation present in urinary P within animals, especially those supplemented with P. This technique could be useful in animals fed low-P diets, since excretion of P is less and the coefficient of variation is not as high. In our study Phosphorus retention was correlated positively (Table 5) to P intake, but this was not correlated to P content of blood serum ($p>0.05$). Challa *et al.* (1989) found a positive

Table 4: Mineral content and specific gravity of rib bone samples of sheep after slaughter

Item[1]	*Basal+	P2+	SEM
Bone ash content, %	46.7	46.6	1.2
Specific gravity	1.309	1.259	0.022
Bone P, %, FB[2]	9.7	9.6	0.4
Bone P, %, DM[3]	13.6	13.2	0.7
Bone P in ash, %	29.0	28.4	1.2
Bone P, mg/cc	127	121	6
Bone Ca, %, FB	11.2	11.5	0.3
Bone Ca, %, DM	15.6	15.9	0.5
Bone Ca in ash, %	33.4	34.1	0.5
Bone Ca, mg/cc	146	145	5
Ca:P ratio	1.16	1.21	0.04
Bone Mg, %, FB	0.339	0.342	0.01
Bone Mg, mg/cc	4.44	4.32	0.16

[1]No significant differences between treatments ($p>0.05$), [1]Values are means of seven sheep per treatment, [2]Fresh basis, [3]Dry matter basis, *Treatments: $Basal^+$ (control diet plus supplementation of cholecalciferol {CC} equivalent to 2500 IU of vitamin D /d); $P2^+$ (Control diet plus 2 g P/d plus CC)

Table 5: Phosphorus correlation among different variables

	F P[b]	U P[c]	F P C[d]	B P[e]	T P E[f]	AP A[g]	P R[h]
P intake[a]	0.70**	0.46**	0.69**	0.29NS	0.87**	0.59**	0.44**
F P		-0.07NS	0.77**	0.26NS	0.87**	0.06NS	-0.14NS
U P			0.14NS	0.26NS	0.87**	0.68**	0.36*
F P C				0.14NS	0.82**	0.03NS	-0.08NS
B P					0.32*	0.11NS	0.08NS
T P E						0.21NS	0.03NS
A P A							0.84**

[a]mg d^{-1} kg^{-1} BW, [b]Fecal P, mg d^{-1} kg^{-1} BW, [c]Urinary P, mg d^{-1} kg^{-1} BW, [d]Fecal P concentration, %, [e]Blood serum P, [f]Total P excretion mg d^{-1} kg^{-1} BW, [g]Apparent P absorption, mg d^{-1} kg^{-1} BW, [h]P retention, mg d^{-1} kg^{-1} BW

correlation among amount of P stored, blood P and P intake in growing calves fed diets supplying from 10-100 mg d^{-1} kg^{-1} BW.

CONCLUSION

Supplementation of phosphorus increases phosphorus absorption and retention in sheep at maintenance fed a low-phosphorus diet and the amount of phosphorus supplemented should be limited to the animal needs and supplementing cholecalciferol to sheep not supplemented with phosphorus enhances phosphorus absorption and retention, but does not have any additional effect in sheep supplemented with this mineral.

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