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Effects of Inclusion of Different Levels of Iron in Lamb Diets on Apparent Absorption and Retention of Phosphorus

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Abstract: An experiment was conducted to evaluate effects of high-iron (Fe) diets on apparent absorption and retention of P in lambs. Eighteen lambs approximately 7-mo old and averaging 33 kg were assigned randomly to one of three treatments, differing only in the concentration of Fe in diet; T_1 (100 ppm), T_2 (350 ppm) and T_3 (600 ppm). Diet of T_1 consisted of chopped switchgrass (*Panicum virgatum* L.) hay and diets of T_2 and T_3, the same feed plus supplementation of ferrous sulfate to make diets containing 350 and 600 ppm of Fe, respectively. Lambs were fed individually in the experimental period for 6 weeks, previous adaptation to the diet, placed in metabolism crates and fitted with fecal collection bags. The last 7 day of the experimental period, total fecal and urinary collections were made and representative samples were taken. Feed and fecal samples were processed for DM, P and Ca and urine for P and *in vivo* dry matter digestibility, apparent P and Ca absorption and net P and Ca body retention were calculated. Data were processed statistically by analyses of variance and orthogonal contrasts. Coefficients of apparent absorption of P and *in vivo* dry matter digestibility were not different ($p>0.05$) among treatments. Likewise, urinary P and net body retention of P and Ca were not different ($p>0.05$). Daily intakes of Ca and P were considered deficient for growing lambs and produced negative Ca balances and low P retention in lambs. Different than expected, diets containing 350 and 600 ppm of Fe had no negative effects on apparent absorption and retention of P and Ca in growing lambs consuming a low Ca and P diet.

Key words: Iron, lambs, phosphorus, sulfate, diets, absorption

INTRODUCTION

Phosphorus requirements of grazing ruminants usually are difficult to meet by forages alone; therefore, supplementation is required, especially in the dry season of each year. Furthermore, P availability can be adversely influenced by different factors of which excess of Fe is included. This element may be contained in high concentrations in some forages and soil, which may be consumed by ruminants. High level of Fe in diets has been suggested to interfere with P utilization by forming complexes, which are not absorbed. In addition, in spite of the low Fe requirements of ruminants, most of the commercial mineral mixtures contain considerable amounts of Fe. These mineral supplements may increase the Fe intake when they are supplemented to ruminants grazing high-Fe pastures and ultimately, interfere with P utilization. However, the concept of high-Fe diets has been widely used without defining a real safety range; furthermore, in most studies carried out in this specific area; extraordinary high concentrations of Fe have been used, even though, such concentrations are not common to find in practical situations. Studies using Fe concentrations, which are more frequently contained in forages, are required to find out whether or not P utilization is affected.

The objective of this study was to evaluate the effects of inclusion of different concentrations of iron in diet on apparent absorption and retention of phosphorus in lambs.

MATERIALS AND METHODS

This study was conducted in the facilities of the department of animal science of the university of Nebraska-Lincoln in the year 2003. Eighteen lambs

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approximately 7-mo old, averaging 33 kg and of mixed crossed breed (Suffolk, Dorset and Polypay) were assigned randomly to one of the following 3 diet treatments:

T_1 = A switchgrass (*Panicum virgatum* L.) hay diet containing 100 ppm of Fe (basal diet).
T_2 = Basal diet + supplementation of 1.13 g day^{-1} of ferrous sulfate to make diets containing 350 ppm Fe.
T_3 = Basal diet + supplementation of 2.27 g day^{-1} of ferrous sulfate to reach 600 ppm Fe in diet.

Lambs were allocated in individual pens with ambient temperature 20-30°C, adapted to the facilities and diet, weighed and administered a medication against internal parasites. After adaptation to the diet, lambs were fed individually 1.14 kg day^{-1} of switchgrass hay for 6 weeks and the amount of iron sulfate required per day to be added to the diet was mixed with 10 g of molasses and impregnated to a small amount of grass taken from the feeder of each lamb and fed immediately, taking care that each lamb ate completely the feed bolus. Lambs were fed the same amount of the experimental diet (1.135 kg day^{-1}) once a day 2 weeks before the beginning of the sample collection period and water was supplied at free access.

Seven days before the beginning of the sample collection period, lambs were moved from pens to the metabolism crates (fed individually and fitted with fecal collection bags) and daily feed intake, orts, urine and fecal outputs were recorded.

Feed samples were collected 2 days before and during the time in which fecal collection was made. Samples of approximately 400 g were taken in triplicate and oven-dried at 60°C for approximately 72 h. Once dried, samples were ground through a Wiley mill (1 mm screen, Arthur H. Thomas, Co, Philadelphia, PA) and approximately 1 g of sample was taken and processed for total dry matter (100°C, overnight) in a forced-air oven. Then, samples were processed for ash in a muffle furnace at 550°C for 8 h and prepared for mineral analyses. Total daily fecal collections were made for 7 days, weighed, sampled and composited to form a single sample per lamb. Then, samples were dried in a forced-air oven at 60°C for 3-4 days and ground through the Wiley mill (1 mm screen) and approximately 1 g of sample (in duplicate) was used to determine total dry matter (100°C, overnight) and processed for ash content (550°C for 8 h) and Ca and P analyses. Total daily urine collections in buckets placed under the crates were done for 7 days during the fecal collection and samples of about 200 mL were taken and processed for Ca and P determination.

Ash residues of approximately 0.5 g of feces and 1 g of feed were digested in porcelain crucibles using a 20% HCl solution and placed on a hot plate for approximately 30 min and filtered into 25 mL flasks using Whatman No 541 filter paper and phosphorus was determined by a Beckman DV 640 spectrophotometer, according to the technique of Fiske and Subbarow (1925).

Determination of Ca was done by atomic absorption spectrophotometry in feed, feces and urine samples according to Fick *et al.* (1979). Solutions of feed and feces and urine samples were diluted with a 1% lanthanum solution. Analysis of Fe in fecal samples was not considered because balance studies in Fe and some other trace minerals may have a low reliability because of the substantial contribution of the liver in the total excretion (Miller, 1975).

Analyses of variance were utilized to observe statistic differences among treatments using the General Linear model procedure of SAS. Orthogonal contrasts were used to compare different means. T_1 was contrasted to T_2 and T_3 and $T_{2\text{-}3}$. Dependent variables were: concentrations of fecal P and Ca, amount of P and Ca excreted in feces, apparent P absorption, urinary P, P retention, Ca retention and coefficient of dry matter digestibility. The independent variable was daily Fe intake.

RESULTS AND DISCUSSION

The daily amount of P consumed by lambs from all treatments (Table 1) was below the P recommendations (3.2 g day^{-1}) set by NRC (1985) for growing lambs. It is known that values greater than 500 ppm of Fe in sheep diets cause toxicity. However, addition of the tested levels of Fe in diets in the present study produced no negative effects on apparent P absorption. Neither coefficients of apparent P absorption (Table 2) nor coefficients of *in vivo* dry matter digestibility (Table 1) were different ($p>0.05$) among treatments. Likewise, the daily amount of P excreted in feces (mg kg^{-1} of body weight) was not different ($p>0.05$) among treatments (Table 2). Standish *et al.* (1971) also found no differences ($p>0.05$) in the coefficients of P absorption of steer diets supplemented with Fe and P to test levels of 100 and 1000 ppm of Fe and 0.23 and 0.46% of P. However, feed intake and average daily weight gains were substantially reduced in steers fed diets with 1000 ppm of Fe. In addition, the coefficient of dry matter digestibility was not affected by treatments, but apparent absorption of Ca and Mg was depressed by the high Fe diets and the high P diets resulted in decreased absorption of Ca and Mg. In this study, the tested Fe concentration was twice the

Table 1: Live body weight, feed and phosphorus intake and *in vivo* dry matter digestibility of lambs consuming diets with different levels of iron[1]

Item[2]	*T_1	T_2	T_3	SEM
Live body weight (kg)	34.100	36.400	33.700	1.000
Feed intake (kg day^{-1} DM)	0.808	0.918	0.914	0.034
P content of diet (DM%)	0.160	0.160	0.160	-
P intake (mg/kg/day BW)	39.000^{b}	41.000^{ab}	45.000^{a}	1.000
Ca content of diet (DM%)	0.460	0.460	0.460	-
Ca:P ratio	2.800	2.800	2.800	-
In vivo dry matter digestibility (%)	48.100	49.100	50.900	0.900

[1]Values are means of 6 lambs per treatment, [2]No significant differences among treatments (p>0.05), *ppm of Fe in diets: T_1, 100; T_2, 350; T_3, 600, [a,b]Different superscripts within rows mean significant differences (p<0.05)

Table 2: Excretion and net retention of phosphorus in lambs[1]

Item	*T_1	T_2	T_3	SEM
Fecal P (Content[2] %)	0.29	0.31	0.30	0.01
Fecal P (mg/kg/day)	37.00	41.00	42.00	2.00
Apparent P absorption (%)	3.86	0.21	6.43	-
Urinary P (mg/kg/day)	0.21	0.21	0.10	0.03
P retention (mg/kg/day)	1.70	0.00	2.80	2.40

[1]Values are means of 6 lambs in each treatment. [2]Dry matter basis, [a,b]Different superscripts within rows mean significant differences (p<0.05), *ppm of Fe in diets: T_1, 100; T_2, 350; T_3, 600

Table 3: Intake, excretion and net retention of calcium in lambs[1]

Item	*T_1	T_2	T_3	SEM
Ca intake (mg/kg/day)	109.000^{b}	116.000^{ab}	125.000^{a}	3.000
Fecal Ca content[2] (%)	0.984	0.906	0.898	0.030
Fecal Ca (mg/kg/day)	125.000	120.000	125.000	4.000
Ca retention (mg/kg/day)	-16.300	-4.200	0.140	5.400

[a,b]Different superscripts within rows mean significant differences (p<0.05); [1]Values are means of 6 lambs in each treatment; [2]Expressed in dry matter basis; *ppm of Fe in diets: T_1, 100; T_2, 350; T_3, 600

value considered toxic for sheep and up to 3 times the amount of P suggested by Erickson *et al.* (1999) for steers of similar body weight and diet.

Adverse effects of high Al diets on P utilization have been reported to be stronger than high Fe diets. Rosa *et al.* (1982) reported that sheep wethers consuming a low P and Ca diet (0.17 and 0.23%, respectively) reduced substantially feed intake when they were supplemented with 760 and 1450 mg of Fe and Al per kg of feed, respectively (745 vs 1326 g day^{-1} in the control group). Daily weight gain was also reduced from 182 g day^{-1} to 5 g. In addition, blood serum P was reduced substantially (63 vs 15 mg L^{-1}) in sheep fed the diet supplemented with Al + Fe but increased when Fe was added alone. In our study, P in blood plasma was not determined because of problems in handling of samples in the critical period.

Diets containing Fe concentrations of 350 and 600 ppm did not have any negative effect on body Ca retention (Table 3) and were not statistically different (p>0.05). Daily Ca intake (Table 3) was lower than the amount suggested by Hodge (1973) as adequate (250 mg/day/kg BW) for growing lambs. However, the mineral intake of lambs was supplied only by the forage consumed. In our study, using levels of minerals and other nutrients the most possibly close to those found in grazing systems was planned. The low Ca intake along with the low coefficient of dry matter digestibility should have influenced Ca body retention. Rajaratne *et al.* (1990) reported a strong negative Ca balance (-1.68 g day^{-1}) in lactating ewes fed diets low in Ca (70 mg/day/kg BW).

The low content of P in diets and the high demand of P by growing lambs, was reflected in insignificant amounts of P excreted in urine (Table 2). No differences (p>0.05) in the amount of P excreted in urine among treatments were found. Nevertheless, urinary P excretion in lambs of T_1 and T_2 was two-fold the amount excreted by lambs of T_3. Ternouth *et al.* (1996) mentioned that amount of P in urine is negligible in animals fed low P diets in which the renal threshold for P is not exceeded. In our study, the amount of P excreted in urine was insignificant because of the low P intakes of lambs from all treatments. Valdivia *et al.* (1982) found low P excretions in urine (7 and 15 mg day^{-1}) in sheep fed a low P diet (0.15%) with and without addition of 2000 mg of Al kg^{-1} of feed.

The amount of P retained in the body was very limited and no differences (p>0.05) were found among treatments (Table 2). Although, lambs were not in negative P balance, it is possible that with time, bone demineralization could have occurred if animals were fed the same diet, which was low in Ca and P content, for along time. Apparently, the Fe concentrations used in diets did not affect P retention. Hodge (1973) found no differences in average daily weight gains and serum Ca concentrations in growing lambs consuming diets supplying 250, 450 and 650 mg of Ca/day/kg BW. However, a Ca intake of 450 mg/day/kg was required before maximum bone mineralization was obtained. The authors suggested that the development of the skeleton was more affected at lower levels of Ca intake than feed intake, growth and blood serum Ca levels.

Valdivia *et al.* (1982) found negative P balances in all sheep fed diets containing 0.15% of P and 168 or 2168 ppm of Al. But the diet with a greater level of Al made more negative (p<0.05) the net retention of P (-404 vs - 48 mg day^{-1}sheep).

CONCLUSION

Diets containing Fe levels of 350 and 600 ppm do not have negative effects on apparent absorption and retention of phosphorus in lambs. Generally, it has been suggested that P absorption in ruminant diets containing high levels of Fe is decreased. But most of the studies have utilized Fe levels higher than 600 ppm. However, using diets below this concentration seems to have no problems on P utilization and amount of P excreted in urine of growing lambs fed a low P diet is negligible.

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