THE EFFECT OF PHYSICAL CHARACTERISTICS OF HAY DIETS ON PHOSPHORUS METABOLISM IN SHEEP

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Summary

The experiments were carried out to study the effect of physical forms of hay diet on phosphorus metabolism by offering sheep roughage diets containing a low phosphorus content. The sheep were fed coarse hay, short hay or finely ground pelleted hay. The physical forms of hay diet had little effect on the pathway of phosphorus excretion. In all groups, the most of phosphorus was excreted in feces and urinary phosphorus excretion was negligible. Duodenal fluid flow, rumen fluid outflow and estimated salivary flow appeared to be relatively high in sheep fed the coarse hay diet as compared to those in sheep fed the finely ground diet. The amount of phosphorus flow to the duodenum tended to be high in the coarse diet group as compared to those in other two groups. On the other hand, phosphorus concentrations in the duodenal fluid and the rumen fluid were lower in sheep fed the coarse hay diet than those in sheep fed the ground hay. Net intestinal phosphorus absorption tended to be higher in sheep fed the coarse hay than that in sheep fed the ground hay or pelleted hay. However, a negative phosphorus balance was observed in the coarse hay diet group although a positive phosphorus balance was found in other two groups. It was considered that salivary phosphorus secretion was greater than the amount of salivary phosphorus reabsorbed from the intestine, resulting in the negative of phosphorus balance in sheep fed the coarse hay.
(**Key Words**: P, Absorption, Duodenal Flow, Physical Form, Sheep)

Introduction

The relevance of dietary phosphorus deficiency in grazing ruminants is well documented as one of the mineral deficiencies, particularly in grazing area of tropical countries. The extended use of poor-quality roughages has increased the risk of phosphorus deficiency as those feedstuffs are characterized by low phosphorus contents.

Ruminants commonly excrete very little phosphorus in their urine (ARC, 1980). They do, however, secrete a large quantity of phosphorus into the digestive tract through their saliva (Clark et al., 1973; Tomas, 1973) and much of this salivary phosphorus lose in their feces. Therefore, the control of phosphorus homeostasis must be achieved in the gut either through the control of phosphorus absorption or control of saliva phosphorus secretion or a combination of the two.

The alternation of salivary phosphorus appears to play a major role in phosphorus homeostasis. Phosphorus in saliva is correlated with plasma phosphorus and the salivary phosphorus secretion appears to be dependent on salivary flow (Scott and Buchan, 1987). The physical nature of a diet seems to be an important factor influencing salivary secretion. The flow rate of saliva is higher in ruminants given poor quality roughages than that in ruminants given pelleted (Putnam et al., 1966) and finely ground diets (Wilson & Tribe 1963). Highly digestible concentrate diets are wellknown to be associated with a reduced rate of salivary secretion (Sato et al., 1976)

The present study is designed to examine the effect of physical form of diets on phosphorus metabolism. This was achieved by offering sheep poor quality roughage diets of either coarse or finely ground form. Changes in phosphorus and some other relevant parameters in rumen fluid, duodenal fluid and blood were observed.

Materials and Methods

Two experiments were carried out, the first

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Received August 1, 1991
Accepted April 15, 1992

studying the effects of diet on phosphorus balance, salivary phosphorus secretion and its absorption and the second studying salivary secretion rate and ruminal water balance.

Experiment 1

Three mature castrated male sheep of Suffolk breed averaging 50 kg body weight were used. Several months prior to the experiment, all animals were fitted with T-shape cannula in the rumen and duodenal. They were housed in metabolic cages to allow the separate collection of feces and urine. Orchard hay and barley were used as feeds. The hay was cut to provide three treatment differences in length, 6.0 and 1.3 cm, and the 1.3 cm hay was again passed to the hammer mill so as to produce a more finely ground material of about 0.2 cm in length. Barley and 0.2 cm hay were then pelleted prior to use. Details of composition and physical characteristics of diets are given in table 1 and 2, respectively. The experiment was planned to be carried out by a 3 × 3 latin square method.

TABLE 1. MINERAL COMPOSITION OF DIETS

		Analysis	
		Phosphorus	Calcium
		(g/kg)[1]	
Experiment			
1.	Hay	2.00	3.50
	Barley	2.80	0.60
2.	Hay	1.88	2.48
	Barley	2.56	0.60

[1] Air dry matter basis.

TABLE 2. THE DISTRIBUTION OF PARTICLE SIZE IN HAY

Hay	Length	%
6.0 cm.	> 6.0 cm.	33.7
	1.3-6.0 cm.	46.8
	1.3-0.2 cm.	17.8
	< 0.2 cm.	1.7
1.3 cm.	> 1.3 cm.	18.6
	1.3-0.2 cm.	78.1
	< 0.2 cm.	3.3
0.2 cm.[1]	< 0.2 cm.	100.0

[1] Prior to pelleting.

The experiment was conducted for 15 days. Sheep were fed 800 g/day of hay and 200 g/day of barley from an automatic feeding equipment set to deliver 1/8 th of the daily ration at 3 h. interval from A.M. 09:00 h. Water was available continuously and the water intake was measured. All sheep were allowed 7 days of a preliminary feeding before the start of each collection period. Details of the design of experiment and the sampling schedule were shown in figure 1.

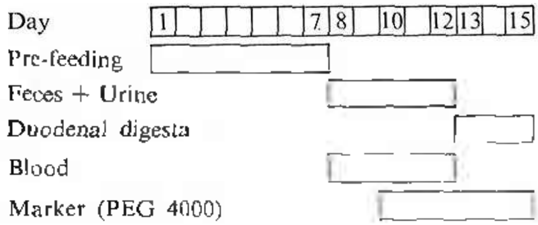

Figure 1. The plan of experiment 1 and sampling schedule.

Total urine and feces were collected once a every 5 days period. They were collected immediately before feeding at 09:00 h. Fecal air dry matter was determined each day and the remaining aliquot was later bulked and stored. Daily urine aliquot were also taken and bulked, and the acidity was adjusted with concentrated hydrocholic acid to approximately pH 4. Blood samples were taken from jugular vein on the third and fifth day of urine and fecal collection period. Digesta flow at the duodenum was measured by infusing 250 ml of polyethyleneglycol solution (PEG 4000, 18 g/d) as the liquid phase marker into the rumen on the third day of the feces and urine collection period with continuous infusion for further 6 day period. Digesta samples were collected during the last 3 days of each period. On each of these days, digesta samples were collected at 10:00, 12:00 and 17:00 h. The samples obtained in the 3 successive days from each cannula were pooled to give a comprise sample for each sheep. The subsamples were centrifuged at 18000 G for 30 minutes and the supernatant fraction was retained for marker and mineral analysis.

Experiment 2

Four ewes of Suffolk breed averaging 30 kg body weight were used. Two weeks prior to the experiment, all sheep were fitted with rumen cannula and were raised with timothy hay and barley in metabolic cages. Hay was cut to provide 2 treatment differences in length, 6.0 cm and 0.2 cm then the barley and 0.2 cm grounded timothy hay were pellected prior to use. Details of their mineral composition are shown in table 1.

The experiment was consisted of two 10 days period in which sheep were offered 600 g. of either hay and 200 g. of barley daily at 08:00 and 20:00 h Water was available consistently and the amount of intake was measured. All sheep were allowed about 7 days of preliminary feeding before the start of each collection period. Details of the design of experiment and the sampling schedule are shown in figure 2.

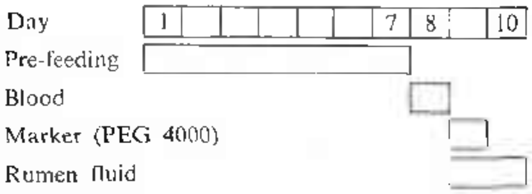

Figure 2. The plan of experiment 2 and sampling schedule.

Blood samples were collected from the jugular vein with heparinized centrifuge tubes twice a day at 10:00 and 16:00 h. on the first day of the sampling period. Plasma was stored at −20℃ prior to analysis. On the second day of each collection period, 100 ml of 20% of polyethyleneglycol (PEG 4000) was infused into the rumen at 09:00 h to determine the rumen volume and dilution rate. Samples of rumen fluid were taken immediately after the infusion of marker at 10:00, 12:00, 15:00 and 18:00 h. The sample was strained through the four layers of surgical gauze and then centrifuged at 18000 G for 30 min. The supernatant was stored at −20℃ prior to analysis.

Analytical Procedures

Grounded samples of feed, feces and dry whole duodenal digesta were wet-ashed with HNO_3 and 1:4 HNO_3: $HClO_4$, than made up with 1N HCl. Urine samples were also ashed by the same procedure. Inorganic phosphorus in samples of serum (or plasma in exp. 2), rumen and duodenal digesta supernatant was also determined by the method of Gomori (1942). The rumen volume, dilution rate and the amount of digesta and phosphorus flow from the abomasum to the small intestine were estimated by PEG as described by Malawer and Powel (1967).

Statistical analysis

Data in experiment 1 were analyzed by analysis of variance using Latin square design and significant differences between treatment were according to Statistical Analysis System (SAS) program package (Barr et al., 1985). Data in experiment 2 were analyzed by Student's t-test.

Results

As shown in table 3, the physical form of diet had little effect on the pathway of phosphorus excretion. Fecal phosphorus excretion was higher and urinary phosphorus excretion was negligible in sheep of all groups. Although statistically not significant, changing the physical form of diet had an obvious effect on phosphorus retention, which was negative in the 6.0 cm hay group and became increasingly positive in the 1.3 and pelleted 0.2 cm hay group. Apparent phosphorus absorption tended to be higher in animals fed 1.3 and pelleted 0.2 cm hay, compared to that in animals fed 6.0 cm hay.

Table 4. shows the effects of changing the physical form of hay diet on phosphorus flow to the duodenum, the amount of phosphorus secreted in saliva (duodenal P flow-P intake) and on the amount absorbed from the intestine (duodenal P flow-fecal P). Changing from 6.0 to 1.3 and pelleted 0.2 cm hay tended to decrease the amount of phosphorus secreted via saliva. This might play an important role on the reduction in both duodenal fluid and the amount of phosphorus flow to the duodenum in sheep fed short cut (1.3 cm) and finely ground pelleted hay (0.2 cm). Phosphorus concentrations in the duodenal fluid, however, were lower in sheep fed the coarse hay than those in sheep fed the short cut (1.3 cm) and the fine ground pelleted hay (0.2 cm). Apparent intestinal phosphorus absorption was higher in the coarse long hay group compared to that in the finely ground hay group.

As presented in table 5, the rumen volume, dilution rate and the estimated salivary flow

TABLE 3. THE EFFECTS OF PHYSICAL FORM OF HAY DIET ON PHOSPHORUS EXCRETION IN URINE AND FECES AND ON INORGANIC PHOSPHORUS LEVELS IN SERUM (EXP. 1)

	Treatment (cm)		
	6.0	1.3	0.2
		(g/d)	
P intake	2.13	2.13	2.13
Excretion			
Feces	2.25 ± 0.35[1]	1.89 ± 0.20	1.36 ± 0.10
Urine	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.00
Total	2.27 ± 0.35	1.92 ± 0.19	1.39 ± 0.14
P retention	− 0.14 ± 0.35	0.12 ± 0.19	0.74 ± 0.14
Serum Pi (mmol/l)	1.16 ± 0.10	1.49 ± 0.30	1.44 ± 0.09
		(%)	
Apparent			
P absorption	−5.79 ± 16.39	11.43 ± 9.22	20.66 ± 22.07

[1] Mean ± SE.

TABLE 4. THE EFFECTS OF PHYSICAL FORM OF HAY DIET ON PHOSPHORUS FLOW TO THE DUODENUM, SALIVARY PHOSPHORUS SECRETION AND NET INTESTINAL PHOSPHORUS ABSORPTION (EXP. 1)

	Treatment (cm)		
	6.0	1.3	0.2
P intake (g/d)	2.13	2.13	2.13
Duodenal fluid			
Flow (l/d)	23.30 ± 5.36[1]	11.60 ± 2.56	7.50 ± 3.62
P flow (g/d)	14.10 ± 4.29	7.00 ± 1.28	4.10 ± 0.04
P content (mmol/l)	16.61 ± 3.24	18.01 ± 1.51	18.46 ± 1.11
Salivary P flow (g/d)[2]	12.56 ± 4.29	5.81 ± 1.28	4.24 ± 0.04
Net intestinal P absorption (%)[3]	82.50	76.07	77.55

[1] Mean ± SE.
[2] Salivary P flow = duodenal P flow − P intake.
[3] Net intestinal P absorption = duodenal P flow − fecal P excretion.

tended to be decreased in the finely ground pelleted hay group compared to those in the coarse hay group. Rumen inorganic phosphorus contents were significantly higher in sheep fed with the finely ground hay than those in sheep fed the coarse long hay although there was no difference in phosphorus intake.

Discussion

According to Jacques et al. (1989), net trans-ruminal water flux is negligible and saliva flow can be estimated by the difference between rumen fluid outflow and water intake. Although a little amount of phosphorus is absorbed before the duodenum in sheep (Breves et al., 1988), it is assumed that the difference between duodenal phosphorus flow and phosphorus intake provides a reasonable estimated amount of phosphorus added to the digesta via the saliva (Scott and Buchan, 1987).

Physical forms of hay had little effect on the distribution of phosphorus excretion via urine and feces. This result might be due to the fact that hay used in the present study contained a relatively low amount of phosphorus and serum

TABLE 5. THE EFFECTS OF PHYSICAL FORM OF HAY DIET ON RUMEN FLUID PARAMETERS, THE AMOUNT OF SALIVARY FLOW AND INORGANIC PHOSPHORUS CONCENTRATION IN RUMEN FLUID AND PLASMA (EXP. 2)

	Treatment (cm)	
	6.0	0.2
P intake (g/d)	1.64	1.64
Rumen fluid		
Volume (l)	4.10 ± 0.19[1]	3.83 ± 0.16
Dilution rate (%/h)	8.65 ± 1.78	6.72 + 0.67
P content (mmol/l)	15.60 ± 0.31	18.39 ± 0.44**
P outflow (g/d)	4.30 ± 0.72	3.49 ± 0.36
Salivary flow (l/d)[2]	6.98 ± 1.92	4.74 ± 0.63
Plasma Pi (mmol/l)	1.02 ± 0.06	1.14 ± 0.16

[1] Mean ± SE.
** $p < 0.01$.
[2] Salivary flow = rumen fluid outflow − water intake.

inorganic phosphorus levels were lower than the renal threshold of phosphorus which was reported to be 2 mmol/l (Scott and McLean, 1981), and urinary phosphorus was very little amount in sheep of all groups.

Phosphorus concentrations in rumen fluid were significantly higher in sheep fed the finely ground hay than those in animal fed the coarse long hay. Most of phosphorus in the rumen seemed to be entered with saliva (Tomas et al., 1967). In the present study, grinding the diet led to a decrease in salivary flow and, hence, to a reduction in rumen volume, dilution rate and rumen fluid phosphorus outflow. It was reported by Cohen (1980) that there was an inverse relationship between salivary flow rates and phosphorus concentrations in saliva. As salivary flow increased, the PO_4 in saliva might be decreased in sheep fed the coarse long hay. Thus, phosphorus concentrations in saliva might be higher in animals given the finely ground hay than those in animals given the coarse hay. The increased phosphorus concentration in saliva was thought to induce the increase in a ruminal phosphorus concentration in sheep fed the finely ground hay.

Serum phosphorus concentrations appeared to be higher in sheep fed the finely ground hay than in sheep fed the coarse hay. The reduction of salivary phosphorus secretion might prevent the removal of phosphorus from the blood in animal fed the finely ground pelleted hay.

When the diet was changed from the finely ground hay to the coarse hay, the salivary phosphorus flow was increased. The increase in salivary phosphorus flow appeared to play an important role on the increase of the amount of phosphorus flow to the duodenum. Net intestinal phosphorus absorption tended to be higher in sheep fed the coarse hay group than those fed finely ground hay and pelleted hay group.

Phosphorus retention, however, became negative in the coarse hay group although positive in the finely ground hay group. The amount of phosphorus absorption in the gut was not enough for phosphorus retention in sheep fed the coarse hay. It was also considered that salivary phosphorus secretion was greater than the amount of salivary phosphorus reabsorbed from the intestine in sheep fed the coarse hay.

Conclusively, it may be described that coarse long hay of low phosphorus content is likely to induce phosphorus deficiency because of an increase in salivary phosphorus secretion although phosphorus absorbability in the digestive tract increases to some extent in sheep. On the contrary, when sheep are fed short cut or finely ground roughage diet containing relatively low phosphorus, phosphorus deficiency appears to be prevented through a reduction in salivary phosphorus secretion.

Acknowledgements

This work was supported by the General

Research Program B 63480084 from the Ministry of Education, Science and Culture, Japan.

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