In Situ Dry Matter, Nitrogen and Phosphorous Disappearance of Different Feeds for Ruminants

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ABSTRACT : Four feeds, three concentrates (rice bran, soybean meal and flaked corn) and one forage (corn silage) were incubated in four ruminally fistulated Holstein steers over three one week periods in a 3×4 incomplete latin square design where steers served as blocks and feeds as treatment. The objectives of the study were to investigate *in situ* DM, N and P degradability characteristics of feeds in order to assess availability of these nutrients by ruminants. In each period, all feeds were incubated in quadruplets (corn silage in triplicates) in the rumen of each steer in a reverse order for 3, 6, 9, 12, 18, 24 and 48 h. The DM 'a' fraction was higher and lower ($p<0.001$) in corn silage and rice bran respectively. Although corn silage contained the lowest ($p<0.01$) DM 'b' fraction, flaked corn contained the highest. Rate of DM degradation of flaked corn and corn silage were half ($p<0.05$) of the rate of DM degradation of either rice bran or soybean meal. Potential or effective DM degradability ($p<0.05$ to 0.001) at various passage rates were the lowest for rice bran and the highest for soybean meal. Corn silage N 'a' and 'b' was the highest and lowest, respectively ($p<0.01$). N 'c' of corn silage and rice bran was higher ($p<0.001$) than other feeds. Potential N degradability was the lowest in flaked corn ($p<0.05$). P 'a' was high ($p<0.01$) for corn silage and rice bran. P 'b' fraction was very high ($p<0.001$) in soybean meal but was absent in corn silage. Availability of DM ($p<0.01$ or 0.001), N ($p<0.001$) and P ($p<0.05$) differed between feeds at various passage rates except P availability at k=0.02 per h ($p>0.05$). The results demonstrate that the availability of DM, N and P by ruminants depends on feed as well as categories of animal.
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Key Words : Feeds, *In Situ*, Nitrogen, Phosphorous, Ruminants

INTRODUCTION

Studying nitrogen (N) and phosphorous (P) nutrition are becoming increasingly important in animal production systems due to their pressure on environment since their excretion leads to pollute the environment. In intensive ruminant production system high energy and protein feedstuffs are fed to meat and dairy animals for consistent production and microbial protein synthesis (Arieli et al., 1995). However, high energy and high protein feeds contain a high concentration of both N and P (AFRC, 1991), which may lead to higher excretion of these elements. Therefore, it is likely that both N and P excretion by ruminants would be much higher in Japan since concentrates consist of 50 to 70% of the total diet in intensive livestock production system. Although it is believed that P is largely degraded in the rumen by rumen microbes, there is limited evidence on the pattern of P degradation of different feeds in ruminants. Inherently, availability of P as well as N would be different for different classes of feed and animals. Therefore, it is necessary to determine availability of N and P for different classes of animals. P excretion depends on forage-concentrate ratio (Ishida et al., 1999), ratio of calcium and P (Martz et al., 1999) and levels of phosphorous (Ishida et al., 1999) in the diet. Availability of N and P by cattle may depend on the type of feed and degradability of N and P may affect their availability. In order to formulate a diet, which would excrete lower N and P, information on N and P composition and their degradation characteristics are important. In Japan, many dairy farmers use corn silage as forage whilst the farmers throughout Japan use concentrates such as rice bran, soybean meal and flaked corn.

The objectives of the study were therefore to determine *in situ* dry matter (DM), N and P degradability characteristics of some feeds those mostly used in Japan in order to assess the potential of N and P degradability and their availability in ruminants.

MATERIALS AND METHODS

Samples

Four feeds, one forage (corn silage) and three concentrate feeds (flaked corn, rice bran and soybean meal) those are widely used in preparation of dairy ration in Japan were used in this experiment. All concentrate feeds were ground through a 2.0 mm screen using a laboratory hammer mill. Corn silage was preserved at -18°C. Then the fresh corn silage was milled through a 4.0 mm sieve using an ultra centrifuge mill (Roetsch, Germany). Liquid nitrogen (ca. 20 ml) was added during grinding to facilitate grinding.

Animals and feeding

Four Holstein steers (3-4 years of age, 549.3 kg body weight (s.d. 48.4), each fitted with a rumen fistula were

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used. They were fed on 1.5 kg DM for 100 kg body weight. The diet consisted of round baled Italian ryegrass silage (7.42 kg DM, s.d. 0.65) and soybean meal (0.83 kg DM, s.d. 0.07); the ratio of silage to concentrate was 0.9:0.1 on DM basis. The crude protein (CP, % DM) content of Italian ryegrass silage, soybean meal and the whole diet was 7.9, 52.2 and 12.3, respectively. Feeds offered once a day at 09:00 h. Animals had free access of water and were offered a mineral-vitamin block *ad libitum* in the form of licks.

In situ trial

About 5 g of air dry samples of flaked corn, rice bran and soybean meal, and about 18 g (about 5 g on DM basis) of corn silage were weighed into polyester bags (10×20 cm; ANKOM, Tokyo, Japan) with a mean pore size (53 μm). Four feeds were incubated over three periods in four steers where each feed was incubated in a steer in a single period. For each feed, in each period at each time point, four bags (three in case of corn silage) were introduced serially into the rumen of each steer and incubated in a reverse order for 3, 6, 9, 12, 18, 24 and 48 h. Bags were first inserted in an especially designed polyester laundry net altogether of which were inserted into the rumen for incubation. One part of the net was tied with a strong polyester string that passed through a long (1.5 m) nylon cord to hold it to the outer fistula. The bottom of the net was tied with a weigh (100 g), and a side of the net was fitted with a chain to facilitate insertion of bags and removal at the end of incubation. Not more than 20 bags were incubated in a fishing net at a time. Before each incubation, samples were soaked in lukewarm water (39°C) for a minute. Disappearance at 0 h incubation (a) was estimated by washing in a domestic washing machine. After incubation, all bags were removed from the rumen at the same time and kept in ice water to stop further microbial degradation. Polyester bags along with laundry net were then washed roughly with tap water to remove outer particles entangled with them. Bags were then washed in a domestic washing machine. After washing, individual bags were washed with tap water to remove particles, which remain outside the bags and rubber bands were removed. Amount of residues in bags was measured after drying for 48 h at 60°C.

Kinetics of the degradation of DM, N, P and phytate P were determined as described by Ørskov and McDonald (1979). All data were fitted to the exponential equation as:

$$p=a+b\,(1-e^{-ct})$$

where p is the percentage of material in the bag disappeared at time t, a is the y-intercept denotes the amount of soluble material in the 0 h bags which has been subjected only to the washing procedure, fraction b is the amount of slowly degradable fraction and c represents the degradation rate of fraction b.

The effective DM, N and P degradability was calculated as described by Ørskov and McDonald (1979) as:

Effective degradability=a+(bc/c+k)

where a, b and c as described above and k represents the passage rate (0.02, 0.05 and 0.08 per hour) when $t_0 \leq 0$.

Chemical analysis

DM was determined after drying the samples at 100°C for 24 h. Ash was determined after ashing the samples at 600°C for 2 h. N of feeds was analyzed using Kjeldahl method (AOAC, 1984). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined by the method of Van Soest et al. (1991). P of feeds and residues was analyzed following Mizuno and Minami (1980).

Design and statistical analysis

The design was a 3×4 latin square where 4 feeds were incubated over 3 periods in 4 steers with 3 (corn silage) or 4 (all feeds except corn silage) replicates. The data were analyzed statistically using General Linear Model procedure of the Statistical Analysis System Institute (SAS, 1987).

RESULTS

Chemical composition

Table 1 shows chemical composition of feedstuffs. Concentrates contained high DM (89 to 91%), but corn silage contained 28% DM. The CP content of corn silage, flaked corn, rice bran and soybean meal was 8.3, 9.6, 21.3 and 53.9%, respectively. Rice bran contained a high phosphorous (2.36%) followed by soybean meal (0.58%), flaked corn (0.23%) and corn silage (0.14%).

DM, N and P degradability characteristics

Table 2 shows *in situ* DM, N and P degradability characteristics. Immediately soluble DM a (%) obtained after washing corresponded well with the fitted a (%) value in all cases. The DM of corn silage (i.e. DM 'a') was the highly ($p<0.001$) soluble where 48% of the total DM was soluble, but only about one-fifth (21%) of the total DM of rice bran was soluble. In contrast, concentrate feeds (rice bran, soybean meal and flaked corn) contained higher ($p<0.01$) b (%) than corn silage. The rate of degradation of rice bran and soybean meal was twice ($p<0.05$) than those of flaked corn and corn silage. Potential degradability (a+b) of soybean meal and flaked corn was higher ($p<0.05$) than

Table 1. Chemical composition of feeds (% DM)

Parameters	Rice bran	Soybean meal	Flaked corn	Corn silage
DM	89.4	91.0	89.6	90.8$^{\Psi}$
CP	21.3	53.9	9.6	8.3
Phosphorous	2.36	0.58	0.23	0.14

$^{\Psi}$ Contained 28% DM on fresh basis.

Table 2. *In situ* DM (%), N and P degradability (% DM or as stated) of feeds

Degradability	Feeds	Degradability constants							
		a	s.d.	b	s.d.	c, h^{-1}	s.d.	a + b	s.d.
DM	Rice bran	21.1	1.9	59.4	2.4	0.0759	0.008	80.7	1.9
	Soybean meal	31.4	3.4	70.1	6.7	0.0694	0.020	101.5	4.5
	Flaked corn	34.9	5.9	77.9	6.7	0.0289	0.009	112.8	11.9
	Corn silage	47.8	3.1	36.6	1.9	0.0317	0.006	84.5	3.9
	Significance	***		**		*		*	
Nitrogen	Rice bran	5.9	2.5	75.4	7.1	0.0708	0.009	80.6	3.5
	Soybean meal	9.1	4.5	96.0	6.9	0.0565	0.021	105.0	4.6
	Flaked corn	2.9	0.8	64.8	7.6	0.0416	0.008	67.8	7.0
	Corn silage	35.1	4.6	46.3	7.0	0.0766	0.019	81.4	4.0
	Significance	**		**		***		*	
Phosphorous	Rice bran	73.1	4.8	28.0	3.7	0.0538	0.016	101.0	1.3
	Soybean meal	12.0	5.3	89.5	8.0	0.0894	0.010	101.5	3.1
	Flaked corn	24.0	9.7	70.0	15.0	0.0922	0.049	93.8	5.7
	Corn silage	82.7	8.9	0.0	0.0	0.0	0.0	82.7	8.9
	Significance	**		***		*		*	

* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

rice bran and corn silage.

N solubility (a) of flaked corn was the low (6%; $p<0.01$). N 'a' of corn silage the high (35%; $p<0.01$). N 'b' fraction of soybean meal was 96% ($p<0.01$) in contrast to the corn silage, which was 46%. N 'c' of feeds did not differ ($p>0.05$). N 'a+b' was the lowest ($p<0.01$) for flaked corn.

Corn silage (83%) and rice bran (73%) contained high ($p<0.01$) amount of P 'a' but soybean meal contained only 12% P 'a'. In contrast, P 'b' fraction of soybean meal was very high (90%; $p<0.001$) while corn silage did not contain any P 'b' fraction. The P 'c' of rice bran was lower ($p<0.05$) than soybean meal and flaked corn. Potential P degradability of corn silage was 83%, but for concentrates it was more than 94%.

Effective degradability of DM, N and P

Table 3 shows effective degradability of DM, N and P at various passage rates. Effective DM degradability of soybean meal was always higher ($p<0.001$ or 0.01) and it was always lower for rice bran at all passage rates (passage rate, k per hour=0.02, 0.05 and 0.08). Effective N degradability of flaked corn at all passage rates was lower ($p<0.001$ or 0.01) than other feeds. On the other hand, effective P degradability of rice bran was higher ($p<0.05$) at passage rates 0.05 and 0.08 per h but similar for all feeds at passage rate 0.02 per h.

Relationships

Table 4 shows intra-relationship of the *in situ* DM, N and P degradability characteristics. Table 5 shows relationship between chemical composition and *in situ* degradability characteristics of DM, N and P. Table 5 also shows relationship between DM and N or P degradability characteristics as well as between N and P degradability characteristics.

DISCUSSION

Chemical composition

Corn silage, rice bran, flaked corn and soybean meal are widely used to formulate feeds for lactating dairy cows and beef cattle in Japan. These four feeds were therefore chosen to examine DM, N and P degradability pattern because excessive excretions of the latter two elements are considered as pollutant in the farm. The CP content of corn silage used in this study (8.3%) was lower than that reported by AFRC (1993). However, the CP content of rice bran in this study was higher (21.3%) than those reported (14.2 to 17.8%) by AFRC (1993). The P content of soybean meal used in the present study (0.58%) was lower than that reported (0.72%) by Ilyas et al. (1995). P content of corn silage used in this study (0.14%) was also slightly lower than that reported (0.19%) by Martz et al. (1999). Corn silage may supply 77 to 79% of total dietary P in non-lactating pregnant dairy cows (Martz et al., 1999). It was also reported that the true absorption of P was 80% for lactating cows (Martz et al., 1990) and 75% for sheep (Dayrell and Ivan, 1989).

In situ DM, N and P degradabilities of corn silage

Corn silage as a basal forage is widely used in the diet of lactating dairy cows in Japan because it provides a high content of rumen fermentable non-fibre carbohydrate (Mahanna, 1994; von Keyserlingk et al., 1996). The high content of DM 'a' (48%) of corn silage in the present study supports the stated facts because 'a' fraction is likely to be

Table 3. Effective DM, nitrogen and phosphorous degradability (% DM or as stated) of feeds

Degradability	Feeds	Rate of passage, h^{-1}					
		0.02	s.d.	0.05	s.d.	0.08	s.d.
DM	Rice bran	68.2	0.7	57.0	0.1	50.1	0.4
	Soybean meal	85.2	3.5	71.6	4.0	63.5	3.7
	Flaked corn	79.7	2.8	62.7	1.9	55.1	2.2
	Corn silage	70.1	2.0	61.9	1.9	58.1	2.1
	Significance	***		**		**	
Nitrogen	Rice bran	64.3	2.3	50.0	2.1	41.4	2.1
	Soybean meal	78.6	4.9	58.8	6.7	47.9	6.3
	Flaked corn	46.6	6.2	32.3	5.0	25.1	4.1
	Corn silage	71.7	4.2	63.1	3.7	57.8	3.1
	Significance	***		***		***	
Phosphorous	Rice bran	93.4	1.1	87.6	1.4	84.3	1.8
	Soybean meal	85.1	1.5	69.3	1.0	59.1	1.5
	Flaked corn	78.7	4.2	66.0	9.7	58.3	11.7
	Corn silage	82.7	8.9	82.7	8.9	82.7	8.9
	Significance	ns		*		*	

ns=not significant (p>0.05); * p<0.05; ** p<0.01; *** p<0.001.

Table 4. Intra-relationship (r) among *in situ* DM, nitrogen or phosphorous degradability characteristics of different feeds

	a	b	c	a + b	ED, 0.02	ED, 0.05	ED, 0.08
DM degradability:							
a	1						
b	-0.55	1					
c	-0.80	0.17	1				
a + b	0.08	0.79	-0.39	1			
ED, 0.02	-0.01	0.70	0.01	0.83	1		
ED, 0.05	0.20	0.33	0.08	0.54	0.90	1	
ED, 0.08	0.46	0.04	-0.07	0.38	0.74	0.95	1
N degradability:							
a	1						
b	-0.66	1					
c	0.70	-0.34	1				
a + b	0.07	0.70	0.20	1			
ED, 0.02	0.47	0.33	0.61	0.89	1		
ED, 0.05	0.72	0.01	0.79	0.69	0.94	1	
ED, 0.08	0.82	-0.16	0.84	0.56	0.87	0.99	1
P degradability:							
a	1						
b	-0.98	1					
c	-0.90	0.95	1				
a + b	-0.52	0.67	0.75	1			
ED, 0.02	0.43	-0.26	-0.14	0.53	1		
ED, 0.05	0.92	-0.83	-0.74	-0.16	0.75	1	
ED, 0.08	0.97	-0.92	-0.84	-0.33	0.61	0.98	1

attributed to the nonstructural carbohydrate content of corn silage. von Keyserlingk et al. (1996) reported that most non-structural carbohydrates of corn silages are water soluble and hence fermented during the ensiling process might lead to higher DM 'a' content. The DM 'b' content of corn silage in the present study was similar (36.6%) to that reported by other workers (38%; De Visser et al., 1993; von Keyserlingk et al., 1996). The rate of degradation of DM 'b' fraction of corn silage in the present study was slightly lower than those reported by von Keyserlingk et al., (1996).

Similar to DM 'a' and 'b', these fractions of N of corn silage followed the same trend. However, even though P 'a' of corn silage was the highest, P'b' fraction was completely absent. This suggests that the insoluble fraction of P is unlikely to be degraded by the rumen microbes may be due to its association with phytate. However there is no report to confirm this observation.

In situ DM, N and P degradabilities of flaked corn

Potential DM degradability of flaked corn used in the present study was higher, but effective DM degradability and DM 'c' were lower than those reported by Arieli et al. (1995). However, corn used by Arieli et al. (1995) was defined as control, extruded and expanded which were different from the flaked corn used in the present study. The low effective DM degradability despite high potential DM degradability of corn flakes may be attributed to the low rates of degradation.

In situ DM, N and P degradabilities of rice bran and soybean meal

The DM 'a' of rice bran was the least soluble of all feeds used in the study. The lower solubility associated with low DM availability of rice bran may be because it contains bypass protein, fat and starch (Elliot et al., 1978; Abidin and Kempton, 1981; Leng and Preston, 1985). The fact that some rice bran for feeding ruminants in Japan is prepared

Table 5. Relationship (r) between chemical composition and *in situ* DM, nitrogen and phosphorous degradability characteristics of feeds

Independent variables	Chemical composition			Degradability characteristics	
	DM%	N (%DM)	P (%DM)	DM (a+b)	N (a+b)
Dependent variables:					
DM degradability					
a	-0.84	-0.98	-0.67		
b	0.91	0.79	0.78		
c	0.54	-0.65	0.92		
a+b	0.47	0.12	0.92		
ED, 0.02	0.49	-0.29	0.24		
ED, 0.05	0.18	-0.58	-0.41		
ED, 0.08	-0.14	-0.71	-0.55		
N degradability					
a	-0.39	-0.32	-0.60	0.81	
b	0.36	0.92	0.67	0.64	
c	0.70	-0.13	0.50	0.25	
a+b	0.15	0.92	0.69	-0.11	
ED, 0.02	0.65	0.65	0.27	0.07	
ED, 0.05	0.76	0.36	-0.29	0.26	
ED, 0.08	0.62	0.20	-0.43	0.40	
P degradability					
A	-0.85	-0.41	0.35	0.20	0.62
B	0.04	0.33	-0.17	0.90	0.79
C	0.78	0.35	0.04	0.26	-0.87
a+b	-0.58	0.02	0.61	0.16	0.41
ED, 0.02	-0.49	0.05	0.94	-0.50	0.37
ED, 0.05	-0.55	-0.01	0.64	-0.70	0.48
ED, 0.08	-0.68	-0.07	0.51	-0.55	0.54

by heating. Presumably some of the protein and starch are denatured by heating and passed through the tract without being utilized either by rumen microbes or in the lower tract which may have been attributed to lower potential and effective DM degradability of rice bran in this study. Zhao et al. (1996) also reported lower DM degradability of heated rice bran than raw rice bran. Working with cereal grains, McAllister and Cheng (1996) reported that the heat treatment could have a profound effect on both the rate and extent of digestion. Several workers (Walhain et al., 1992; Malcom and Kiesling, 1993; Arieli et al., 1995) also reported the effect of thermal processing on various nutrient composition and digestion of cereal grains and legumes.

In contrast to the least DM 'a', P 'a' content of rice bran was the highest of all feeds used in this study although its P 'b' fraction was low compared to other concentrate feeds used. This result suggests that most of the P in rice bran is water-soluble and hence degraded in the rumen. This higher P degradability of rice bran occurred in the present study despite the fact, which is generally believed that rice bran may contain a large amount of phytate P. The rice bran used in this study was processed by heat. Konishi et al. (1999) using soybean meal reported that the heating reduces both CP and phytate P 'a' contents and makes a significant portion of both CP and P unavailable in the rumen. The N degradability pattern of soybean meal used in the study was similar to the unheated soybean meal used by Konishi et al. (1999). In case of rice bran although N solubility was low, P solubility was very high. Therefore, it remained to see the phytate P degradability pattern of rice bran. However, the low P 'b' fraction of rice bran which also had the lowest degradability compared to other feeds suggest that phytate P of rice bran likely to remain in P 'b' fraction. Interestingly, potential P degradability of rice bran was 100% and furthermore, effective P degradability of rice bran was the highest (8 and 15% higher than flaked corn and soybean meal at passage rate of 8% h^{-1}) of all feeds used in this study despite its lowest rate of degradation. Overall, P in rice bran is largely degradable in the rumen.

The present results suggest that 60-75% of the N and 16-42% of the P in concentrate feeds for dairy cows (i.e. k=0.08 per h) may pass through lower tracts to be digested and finally excreted. It is therefore important to find out N and P utilization mode in the lower tract using mobile nylon bag technique. It is interesting that only 16% of the P of rice bran likely to pass through the lower tract (k=0.08 per h). This low rate may mean that a significant portion of rice bran P consists of phytate P. This result therefore leads to suggest that most of the phytate P in rice bran likely to be degraded in the rumen. However, as stated earlier a significant portion of N and P likely to pass through the lower tract and hence utilization of N and P in the lower tract needs to be investigated to minimize their excretion.

Effective DM, N and P degradabilities of feed

Although effective DM and N degradabilities of soybean meal were the highest, effective P degradability of soybean meal was similar to all feeds used in this study. Moreover, in spite of the fact that the potential P degradability of soybean meal was 100%, effective P degradability (at the passage rate of 8% h^{-1}) of soybean meal was 41% lower than its potential P degradability. This result suggests that a large amount of P in soybean meal may escape rumen degradation especially in the high merit lactating dairy cows whose ruminal passage rate is higher. This seems logical because a large quantity of protein from soybean meal escapes rumen degradation (Obitsu et al., 1995) and may be utilized in the lower gut. Therefore, because of the association of P with protein (Fontaine et al., 1946; Saio et al., 1967). P could also be escaped with protein from rumen degradation; some portion of it may be utilized in the lower gut while the rest of it is likely to be excreted. Reddy et al. (1982) noted that more than 50% of P in soybean meal presents in the form of phytate P. Konishi et al. (1999) reported that 10-27% of the phytate P in untreated soybean meal and 25-55% of the phytate P of heated (153°C) soybean meal escapes from rumen degradation when ruminal outflow rate was 0.02-0.08 h^{-1} respectively.

The results suggest that the diet based on corn silage, rice bran and soybean meal in real feeding system may be used for high producing animals (k=0.05 to 0.08 h^{-1}), although it is better to limit the use of flaked corn owing to its low N degradability (tables 3 and 4), which may render high N excretion. Therefore, in a feeding system where corn silage fed *ad libitum* should be supplemented with a concentrate mixture ranking from soybean meal followed by rice bran and flaked corn for optimization of nutrient utilization and to minimize excretion of nutrients particularly N and P.

Correlations between degradability characteristics

Dry matter, N and P contents were negatively related to the respective *in situ* 'a' but positively to *in situ* 'b'. This suggests that the feed with high DM, N and P contents are likely to be less soluble in the rumen. However, the exception was the relationship between P content and P 'a' or 'b' which were positive and negative respectively. This suggests that the higher the P content in feed, the more P likely to be soluble. However, DM, N and P contents were positively related to their potential degradability despite their converse relationship with *in situ* 'a' and 'b'. The positive relationship between effective degradabilities (k=0.02 per h) of DM, N and P with the respective chemical components were high but decreased or became negative with the increase in passage rates suggests that the chemical composition may not explain their availability to different classes of animals.

DM degradability (a and b) were related to N a and b, and P 'b'. N degradability characteristics were related to the respective P degradability characteristics positively except for P 'c'. This suggests that the feed with high rate of degradation of N may have low rates of P degradation.

Finally, it is useful to select feeds for high genetic merit ruminants of which P of feeds degraded quickly in the rumen or utilized in the lower gut; or another option could be using feeds with low P content. More research is therefore needed using mobile nylon bags to study P utilization in the gut particularly in the high-producing animals where the P excretion is very high.

CONCLUSIONS

Availability of N and P of feedstuffs by the rumen microbes differ due to the difference in feedstuffs. A large fraction of these nutrients may pass through the lower tract utilization of which needs to be quantified for different categories of feeds and animals to minimize excretion of these nutrients because their excretion pollutes the environment.

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