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The effect of dietary energy level and rumen protozoa on urinary excretion of purine derivatives in goats

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Abstract The effect of dietary energy level and rumen protozoa on urinary PD excretion in goats was studied in two experiments. In experiment I, three treatment diets, low energy diet (LED), medium energy diet (MED), high energy diet (HED), with energy levels of 1.0, 1.2, 1.5 times the maintenance energy requirement respectively, were fed to three faunated goats in a 3 X 3 balanced Latin square design. The protein supply was 1.2 times the maintenance requirements. Experiment II was carried out in similar manner using the same goats after defaunation. Then, the animals were inoculated with protozoa (re-faunation) (Holotrichida and Oligotrichina, 1：9). After the 7th day of preliminary period, sampling was carried out for a period of 6 days with a diet supplying of 1.2 times the maintenance energy requirement. Total faeces and urine were collected daily before morning feeding for estimating nutrient digestibility and N balance, and urine samples were also used for determination of urinary purine derivatives (PD). The digestibilities of DM, OM, CP and NDF tended to increase ($P>0.05$) in defaunated animals than in faunated ones after feeding the diets with different energy level. Urinary nitrogen (N) excretion was significantly ($P<0.05$) increased with an increase of dietary energy, and was higher ($P<0.05$) in defaunated than in faunated group in three dietary treatments. Urinary PD excretion in both groups was significantly ($P<0.05$) lower in LED, however, urinary PD excretion in the defaunated group was significantly ($P<0.05$) higher than in the faunated group. Microbial N supply calculated was significantly ($P<0.05$) lower in LED feeding, and was significantly ($P<0.05$) higher in defaunated group, although the efficiency of microbial N supply in both groups was significantly ($P<0.05$) lower in HED feeding. From these results, it can be concluded that protozoa and dietary energy level obviously affects on urinary PD excretion and also N metabolism in goats.

Keywords: dietary energy levels, goat, protozoa, purine derivatives

INTRODUCTION

Allantoin (AN) is a final metabolic product of purine base catabolism in mammals except the Primates. In ruminants, purine bases absorbed in the lower digestive tract were excreted into urine as allantoin after catabolism via hypoxanthine, xanthine and uric acid (UA), as purine derivatives (PD), and sometimes, a part of these PD are excreted into urine as well as AN. In general, the dietary nucleic acids are degraded in the rumen, and therefore, the most of nucleic acids absorbed from the lower digestive tract are of mainly microbial origin synthesized in the rumen, *i. e.*, the nucleic acids flowing out of the rumen originated from microbes (McAllan 1982). Urinary PD are therefore, able to act as indicator for estimating microbial protein synthesized in the rumen, and also digested in the lower gut of ruminants (Fujihara *et al*. 1987；Chen *et al*. 1990). In the rumen microbes, bacteria and protozoa numbers are 10^9～10^{11} and 10^5～10^6 per ml of rumen fluid respectively, and they are most important protein source for ruminants. The

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amount of these microbes synthesized depends on fermentation and utilization of feed ingested in the rumen (Nakagawa *et al*. 1992; Takenaka *et al*. 1991), and estimating the amounts of synthesized microbes is important in assessing the nutritional status of ruminants. The bacteria and protozoon influence each other to produce microbial protein as one of important nutrition source through fermentation of fibrous feed, therefore, it is imperative to clear an effect of their relationship on protein nutrition in host animal. The contribution of protozoa to total protein supply for host animals may be so much as compared with that of bacteria, however, a proportionate contribution of protozoon to total N supply has to be established, if urinary PD excretion is used as an index for estimating microbial production in the rumen.

Then, to make a protozoa-free ruminant animal, there are some methods; rearing isolated from their dam just after birth, rumen washing with some reagents and/or water (Itabashi and Matsumoto 1993) and feeding a liquid diet (Fujihara *et al*. 2003a). Intragastric nutrition will be also valuable method to avoid an effect of protozoa on ruminal fermentation (Ørskov *et al*. 1979). About the effect of protozoa on fermentation of feed in the rumen, it has been reported that the number of bacteria decreases through an engulfment of protozoa (Eadie and Hobson 1962; Koenig *et al*. 2000) and the ammonia nitrogen in rumen fluid decreases, and volatile fatty acids level are also changed in defaunated animals (Nakagawa *et al*. 1992; Takenaka *et al*. 1991). However, there is limited information about an effect of protozoon on urinary PD excretion in ruminants (Matsumoto and Itabashi 1990; Fujihara *et al*. 2003 a,b).

Lindberg (1985) has reported that urinary PD excretion and microbial protein increased with an increase of dry matter intake (DMI) and digestible-organic matter intake (DOMI) in goats fed on roughage and/or mixed diets. Chen *et al*. (1992) has also reported that there was close relationship between urinary PD excretion and microbial protein supply and DMI and/or DOMI using 4 diets with different energy and protein levels in lambs. Sultan and Loerch (1992) and Merchen *et al*. (1987) have described that a difference in dietary energy level influences the nutrient digestibility and N metabolism in lambs. Furthermore, it has been reported that the amount of microbial protein synthesized will be limited by not only an amount of ammonia N, but also bioavailable energy obtained from feed in the rumen (Hogan and Weston 1970). Therefore, it is obvious that dietary energy level influences greatly on microbial protein synthesis in the rumen and also on N metabolism of animals.

In the present study, the effect of changes in dietary energy level on microbial protein production estimated using urinary PD excretion was investigated in faunated and defaunated goats fed on mixed diet of hay and concentrates. Part of this work has been briefly reported by Miyata *et al*. (2001).

MATERIALS AND METHODS

Experiment I

Animals and diets

Three Japanese Saanen goats (2 females and 1 castrated male: average body weight (BW), 38.2±1.6kg) were used, and they were fed on 3 diets mainly consisting of timothy hay (0.02DM/BW/d), rolled barley and corn starch at different energy level (low energy diet: LED, medium energy diet: MED, and high energy diet: HED; 1.0, 1.2 and 1.5 times above maintenance; 450kJ/$BW^{0.75}$/d (Fujihara *et al*., 1987), in a 3 X 3 Latin square design. The protein supply was 1.2 times the maintenance requirements (380mgN/$BW^{0.75}$/d) (Fujihara *et al*. 2005), and the dietary level was almost constant adjusted using soybean protein (see Table 1). Cornstarch was used to adjust dietary energy at a delicate level.

Experimental procedure

The experimental animals were kept in metabolism crates throughout the experiment. Six-day sampling periods were preceded by 7-day preliminary periods. Each lamb had access to fresh water and mineralized salt lick at all time. The daily ration was given in equal portions at 09.00h and 17.00h. During the 6-day sampling period total faeces and urine were collected daily just before the morning feed.

Experiment II

Animals and diets were similar to that in Experiment I. Prior to the experiment (digestion trial), animals were defaunated as same method described by Fujihara *et al*. (2003a). Defaunation of animals were accomplished by bottle-feeding milk replacer (MR) for 2-weeks, followed by feeding on a mixed diet (9:1 to 0:10 DM of MR and solid diet) for 10 days. Then, the animals were fed on a solid diet alone for 1 week.

After confirming they were protozoa-free in the rumen contents taken through a stomach tube, faeces and urine samples were collected just before the morning feed for consecutive 6 days. The experimental design was basically the same as Experiment I, but the date of completed defaunation was not always the same in all the animals, because someone still had protozoa in ruminal contents after bottle-feeding MR for 2-weeks, and then, the period of bottle-eeding MR was prolonged some days. The design (3 X 3 Latin square), therefore was a little anomalous.

After the completed the Experiment II, the animals were re-faunated as described by Fujihara *et al*. (2003a,b) to examine a changes in urinary PD excretion along with an increase of protozoa in the rumen. To examine whether a microbial condition in the rumen was stabilized or not, a 6-day digestion trial was performed at 8th day after inoculation using same experimental diet (MED).

Sampling and analytical procedure

Urine was collected with 100～150 ml of 1 M H_2SO_4 solution to adjust pH value below 3.0 to prevent disappearance of PD in urine (Fujihara *et al*. 1991), and stored at −20℃ until analysis.

Nitrogen (N) in feed, faeces and urine was analyzed using the Kjeldahl method, while DM, OM and neutral detergent fibre (NDF) in the feed and faeces were determined by AOAC method (Hoitz 1960) and Goering and Van Soest (1970), respectively. The PD in urine was analyzed by the methods of Young and Conway (1942) and Fujihara *et al*. (1987). Urinary creatinine was analyzed using a commercial kit (*Test* Wako, Wako Chemical Co., Osaka, Japan).

Statistical analysis

The statistical analysis In Experiment I was consisted of analysis of variance (Latin square) and a mean separation-using Tukey's multiple range test (Yoshida 1975). In Experiment II, the Latin square design was not completed, and so test for significance of only difference among dietary treatments was performed by an analysis of variance (one way) and a mean separation by Fisher' PLSD (Experiment II). The test for significance of difference between the two groups (Experiment I and II) was done using the *t*-test.

RESULTS

Diets, Feed intake and apparent digestibility

Chemical composition of the diets used in Experiments I and II were similar, although different batches of timothy hay were used (see Table 1).

As shown in Table 2, DMI, organic matter intake (OMI)

Table 1 Chemical composition and diet component of feed ingredients fed to experimental animals

	Timothy hay	Rolled barley	Soyabean protein	Corn starch
Experiment I				
Dry matter	898*	879	930	855
Organic matter	950**	977	956	998
Crude protein	84	133	845	−
NDF***	646	165	−	−
Experiment II				
Dry matter	909	882	942	855
Organic matter	950	977	956	998
Crude protein	71	120	897	−
NDF	650	165	−	−
Diet component, % DM				
Low energy diet (LED)	90.5	7.4	2.1	−
Medium energy diet (LED)	86.0	2.2	1.8	10.0
High energy diet (HED)	74.6	2.0	1.6	21.8

* g/kg

** g/kg dry matter.

*** Neutral detergent fibre.

Table 2 Feed intake of rumen faunated and defaunated goats fed with diet containing different energy level

Treatment		DMI*	OMI*	DOMI**	CPI*
LED	F	623.7± 5.2###	589.0± 3.0	338.7±15.3	64.0± 1.3
	D	570.3±36.7	544.7±34.9	365.9±34.6	63.1± 3.6
MED	F	726.3±49.1	687.3±40.1	410.5±15.6	64.6± 2.6
	D	679.7±46.7	652.2±44.6	446.2±48.4	67.3± 4.2
HED	F	826.3±50.9	787.7±42.9	495.5±27.1	64.3± 3.1
	D	815.3±60.2	786.7±57.8	537.1±60.6	68.8± 4.5

* g/day. ** g/DM/day. ### Mean±S.E. of 3 goats. F:faunated animals. D:defaunated animals.

Table 3 Comparison in apparent digestibility in faunated and defaunated goats (g/kg) fed with diets containing different energy level

Energy level		DM	OM	CP	NDF
LED	F	559±23#	575±23	644±44	489±34
	D	637±41	671±42	692±28	593±65
MED	F	585±23	599±23	588±39	487±27
	D	661±33	683±45	652±10	557±82
HED	F	612±05	616±18	502±47	465±15
	D	652±27	679±27	550±52	461±63
Comparison					
PI		652±09	633±09	667±20	531±11
MED1		585±23	599±23	588±39	487±27
MED2		661±33	683±45	652±10	557±82

Mean±S.E. of 3 goats. F:faunated animals. D:defaunated animals.
PI: after protozoa inoculation. MED1: Exp. 1, MED2: Exp. 2.

and DOMI increased apparently ($P>0.05$) with an increase of dietary energy level in both experiments. The intake of crude protein (CPI) was almost similar in all the diets and treatments.

As shown in Table 3, digestibilities of DM and organic matter (OM) tended to increase with an increase in dietary energy ($P>0.05$). However, slightly higher DM and OM digestibility values were observed in faunated and defaunated goats that were fed with MED and HED than those fed with LED. The crude protein (CP) digestibility tended to decrease with an increase in dietary energy in faunated goats; however, an opposite trend was observed in defaunated goats ($P>0.05$). The digestibility of NDF did not change with an increase in dietary energy level in faunated goats, however, it tended to decrease in defaunated goats with an increase of dietary energy level. Generally, the digestibilities of DM, OM and CP tended to be higher in defaunated animals (Expt. II) than in faunated ones (Expt. I), but not significantly ($P>0.05$). The result of digestion trial conducted 1 week after the protozoa inoculation was also shown, and the values in DM, OM, CP and NDF were almost similar to those after feeding MED to defaunated goats, although that tended to be higher than those after feeding MED to faunated goats.

Nitrogen balance

The Table 4 shows the effect of dietary energy level on the N balance of faunated and defaunated goats fed on hay and concentrate diet. Faecal N excretion was also increased with an increase of dietary energy in both groups of faunated and defaunated animals, but not significantly ($P>0.05$). Consequently, absorbed N resulted in decrease with an increase of dietary energy in both groups of faunated and defaunated animals. On the other hand, urinary N excretion was significantly ($P<0.05$) decreased with an increase of dietary energy in both faunated and defaunated goats, and it was significantly ($P<0.05$) smaller in the former than in the latter. Conse-

Table 4 Nitrogen balance（g/kg0.75/d）in faunated, defaunated and refaunated goats

Energy level		N intake	Faecal N	Urinary N	Absorbed N	Retained N
LED	F	0.79±0.01[1]	0.28±0.04	0.41±0.02[a]	0.51±0.03	0.10±0.07
	D	0.79±0.00	0.24±0.02	0.46±0.02[d]*	0.55±0.02	0.09±0.07
MED	F	0.79±0.02	0.32±0.04	0.29±0.01[b]	0.46±0.03	0.17±0.01
	D	0.80±0.00	0.28±0.01	0.39±0.02[e]*	0.52±0.01	0.13±0.07
HED	F	0.79±0.01	0.39±0.04	0.20±0.01[c]	0.40±0.03	0.20±0.02
	D	0.80±0.01	0.36±0.04	0.29±0.01[f]*	0.44±0.04	0.15±0.09
Comparison						
PI		0.80±0.00*	0.27±0.15	0.29±0.01	0.53±0.02	0.24±0.01
MED1		0.79±0.02	0.32±0.04	0.29±0.01	0.46±0.03	0.17±0.01
MED2		0.80±0.00	0.28±0.01	0.39±0.02	0.52±0.01	0.13±0.07

		Retained/intake	Absorbed/intake	Retained/absorbed
			（%）	
LED	F	12.5± 8.6[1]	64.4±4.41	17.9±11.9
	D	10.8± 9.1	69.2±2.85	14.7±13.1
MED	F	21.5± 1.5	58.6±2.27	37.0± 4.1
	D	16.1± 9.3	65.2±0.96	24.3±13.8
HED	F	24.8± 2.9	50.1±3.97	49.4± 2.3
	D	18.7±11.4	55.0±5.22	30.9±18.4
Comparison				
PI		30.1± 1.10	66.7±2.01	45.2± 1.0
MED1		21.5± 1.50	58.6±2.27	24.3±13.8
MED2		16.1± 9.30	65.2±0.96	24.3±13.8

1 Mean±S.E. of 3 goats. F:faunated animals D:defaunated animals. * :significantly difference between F and D（$P<0.05$）.

[a, b, c & d, e, f]: significantly difference among LED, MED & HED. PI:after protozoa inoculation. MED1:Exp.1 MED2:Exp. 2.

quently, the retained N tended to increase with an increase of dietary energy, and tended to decrease in defaunated goats than in faunated ones, but not significantly（$P>0.05$）.

In N balance of goats after inoculation, the faecal N excretion was lesser than those after feeding MED in both animal groups. Urinary N excretion of re-faunated goats was almost the same as that of faunated goats with MED in Experiment I. Consequently, absorbed N for re-faunated goats tended to increase as compared with those of defaunated and faunated goats. As a result, re-faunated goats tended to retain more N than the defaunated and faunated animals（$P>0.05$）.

Urinary PD excretion and microbial N supply

Table 5 shows the effect of dietary energy level on urinary PD excretion and microbial N supply. The AN excretion tended to increase with an increase of dietary energy level except faunated goats under HED feeding. After feedings MED and HED, the AN excretion was significantly（$P<0.05$）higher than that of LED feeding. The urinary excretion of uric acid（UA）included hypoxanthine and xanthine was significantly（$P<0.05$）lower for goats under HED than those under LED and MED, which was also lower than those under MED than LED fed goats（$P>0.05$）. On the ratios of AN and UA to total PD in both faunated and defaunated goats, the difference in UA excretion among 3 dietary treatments was not so broad, and consequently, the ratio of UA to total PD tended to decrease with an increase in ratio of AN to total PD.

The microbial N supply calculated was significantly（$P<0.05$）higher in goats under MED and HED than those under LED. However, the rate of microbial N supply（gN/kg DOMR）tended to decrease with an increase in dietary energy level, and in particular, the difference after feeding HED and

Table 5 Urinary excretion of PD and creatinine, and microbial N supply in faunated and defaunated goats

Energy level		Allantoin	Uric acid	Total PD	Microbial nitrogen supply		Creatinine	A/C
		(μmol/kg$^{0.75}$/d)			(gN/d)	(gN/kgRDOM)	(μmol/kg$^{0.75}$/d)	(μmol/μmol)
LED	F	506.2±16.4[#]	170.5± 7.6	676.7±17.1	7.5± 0.2	34.2	416.4± 9.8	1.2± 0.1
	D	639.7±30.4*	190.4± 8.1	816.9±23.5*	8.9± 0.3*	38.0	397.4±17.3	1.6± 0.1*
MED	F	619.3±15.7	163.2± 5.9	782.5±18.0	8.7± 0.2	32.8	408.4± 9.9	1.5± 0.1
	D	713.7±16.1*	174.8± 4.5	888.5±16.9*	10.3± 0.3*	35.6	422.5± 8.2	1.7± 0.1*
HED	F	611.6±22.7	147.5± 4.4	759.1±25.7	8.5± 0.3	26.9	354.5±11.0	1.7± 0.0
	D	780.9±36.4*	163.6± 3.2*	944.5±36.2*	11.1± 0.4*	32.5	426.4±11.9*	1.8± 0.1
Relative								
PI		619.1±13.6*[a]	203.3± 4.8[b]	822.4±15.4[a]	10.2± 0.3[b]	39.7		
MED1		619.3±15.7[a]	163.2± 5.9[a]	782.5±18.0[a]	8.7± 0.2[a]	32.8		
MED2		713.7±16.1[b]	174.8± 4.5[a]	888.5±16.9[b]	10.3± 0.3[b]	35.6		

[#]Mean±S.E. of 3 gaots. F:faunated animals. D:defaunated animals. A/C:allantoin/creatinine. PI:after protozoa inoculation. MED1:Exp. 1 MED2:Exp. 2.
*:significantly difference between F and D ($P<0.05$). [a,b]:significantly difference with the same line.

after feedings MED and LED was significant ($P<0.05$). On urinary PD excretion and microbial N supply in the rumen of defaunated and faunated goats, urinary AN excretion increased significantly ($P<0.05$) with an increase of dietary energy in defaunated goats. Uric acid excretion also tended to increase with increase of dietary energy in defaunated goat ($P>0.05$). The magnitude of difference was higher in defaunated goats under HED than the faunated ones. Consequently, urinary total PD excretion was significantly ($P<0.05$) higher in defaunated goats than in faunated ones.

Table 5 also shows urinary PD excretion and microbial N supply in re-faunated goats. Urinary AN excretion was almost similar excreted by faunated goats fed with MED, and significantly ($P<0.05$) lower than that of defaunated goats fed with MED. Urinary uric acid excreted by re-faunated goats was significantly ($P<0.05$) higher than that of faunated and defaunated ones. The urinary total PD excretion of re-faunated goats was significantly ($P<0.05$) lower than that of defaunated animals reflecting higher allantoin excretion. However, microbial N supply of re-faunated (gN/d) was almost the same as that of defaunated, and was significantly ($P<0.05$) higher than those of faunated goats. As a result, re-faunated goats exhibited higher rate of microbial N supply than faunated and defaunated goats under MED ($P<0.05$).

Urinary creatinine excretion and its ratio to AN

As shown in Table 5, urinary creatinine (CN) excretion was significantly ($P<0.05$) lower in faunated goats fed with HED than those fed with LED and MED. The CN excretion from defaunated goats was almost the same for all 3 diets. Then, the AN/CN tended to increase with an increase of dietary energy level, being significantly ($P<0.05$) higher in HED than that of MED and/or LED. The AN/AC tended to be higher in defaunated animals than in faunated one, and this would be due to an increase of urinary AN excretion in the former than in the latter.

DISCUSSION

About a contribution of protozoa for the continuing fermentation of feed in the rumen, an appraisal may be not always uneven, *i. e.*, defaunation increases significantly the flow of dietary and microbial protein to intestine (Veira *et al*. 1984; Broudiscou *et al*. 1994), and contrary, sometimes it causes a minor decrease in digestibility of the ration (Jouany and Ushida 1999), or it does not have any effects on intake and apparent digestibility (Hegarty *et al*. 2000; Fujihara *et al*. 2003b). In the current study, the digestibilities of DM, OM, CP and NDF tended to increase ($P>0.05$) in defaunated animals than in faunated ones, and these indicate a positive effect of protozoa in the fermentation of feed in the rumen as described above. In any case, these findings might suggest that the effect of protozoa on digestion will be not always similar after feeding a mixed diet consisted of hay and concentrates in ruminants.

On N balance result in this study, urinary N excretion was

significantly（$P<0.05$）increased with an increase of dietary energy, and was higher（$P<0.05$）in defaunated animals than that in faunated ones in each dietary energy level, although as a whole, the retained N tended to high in faunated animals than in defaunated animals, but not significantly（$P>0.05$）. This would result in an efficient utilization of dietary（absorbed）N with an increase of dietary energy in both of faunated and defaunated animals. However, the difference in retained N was not clear（$P>0.05$）with the changes in dietary energy level, and this would be due to small number of experimental animals used and/or relatively short term of experimental period in this study. In N balance of re-faunated goats, retained N was more than that of both faunated and defaunated ones, but not significantly（$P>0.05$）. These findings suggest that protozoa clearly have an effect on nitrogen utilization in ruminants.

The effect of dietary energy level on urinary PD excretion was clear, and in particular AN excretion after feeding LED was significantly（$P<0.05$）lower than that of MED and HED feeding in the present study. This probably be due to shortage of rumen digestible-organic matter（RDOM）in LED feeding as compared with those in MED and HED feedings, and this agrees with that reported by Offer *et al*.（1978）. On the other hand, urinary excretion of uric acid included hypoxanthine and xanthine tended to decrease with an increase of dietary energy, and then, it is presumed that an increase in dietary RDOM probably decreased the xanthine oxidase and/or uricase activity. As shown in Table 5, urinary excretion of uric acid included hypoxanthine and xanthine did not have big changes with an increase of dietary energy, then the ratio of uric acid to total PD tended to decrease with an increase of AN to total PD. This is in good agreement with result reported by Chen *et al*.（1992）. From this, it seems that the changes in total PD excretion is mainly due to the changes in AN excretion, and consequently, total PD excretion tended to increase with an increase in dietary energy level particular by MED and HED, which was significantly（$P<0.05$）higher than that observed from goats under LED. These findings support the results reported earlier（Chen *et al*. 1992；Fujihara *et al*. 2005）that urinary total PD excretion increased with an in crease in DMI and DOMI in lambs fed on hay and concentrate.

According to Chen and Gomes（1992）, microbial N supply can be calculated using excreted into urine, and they proposed the equations for cattle and sheep. Then, the equation for sheep was adopted in the current study, and the results showed an increasing of microbial N supply with an increase of dietary energy. This agrees with that reported by Fujihara *et al*.（2005）, who worked with lambs. However, it differ from the results obtained by Chen *et al*.（1992）using lambs, because in their study dietary protein supply was increased with an increase of DOMI. Leibholz（1972）also reported that the rate of microbial N supply in the rumen depend on the quality and quantity of dietary protein. The fact that in the current study the rate of microbial N supply in the rumen tended to decrease with an increase of DOMI would be due to uniform dietary protein regardless changing dietary energy level. In comparison with urinary PD and microbial N supply between faunated and defaunated animals, the decreased urinary PD in faunated goats would be due to the difference of retention time in the rumen between protozoa and bacteria, engulfment of protozoa, and utilization of bacterial protein by protozoa resulting some changes of microbial condition in the rumen. Urinary total PD excretion was greater in defaunated animals than in faunated animals, and therefore calculated microbial N supply（gN/d）was also significantly（$P<0.05$）higher in the former than in the latter. The rate of microbial N supply in the rumen was significantly（$P<0.05$）higher in defaunated than in faunated goats under MED and HED. From these findings, it can be suggested that the presence of protozoa in the rumen reduces urinary PD excretion resulting into under estimation of microbial protein if urinary PD is adopted for calculation.

Lindberg（1985）has formerly reported that urinary AN excretion was closely related to the urinary CN, and AN/CN could be useful for assuming in-directly microbial protein production in the rumen. Furthermore, the time course changes during 24 hours in AN/CN will be relatively small（Antoniewicz *et al*. 1981）, so that the microbial protein production in the rumen is thought to be assumed using a spot sample of urine（Lindberg 1985）. Antoniewicz *et al*.（1981）also reported that an increasing feeding level（metabolizable energy intake /metabolizable energy requirement）results into an increase in AN/CN particular by when feeding is lower than requirement（below 1.0）. These results suggest that AN/CN could be an index that shows whether an animal has consumed enough or not enough feed for their requirement. The results in the current study can be clearly explained by the suggestion of Lindberg（1985）, and also agrees with the findings by Antoniewicz *et al*.（1981）.

CONCLUSION

In the present study, the digestibility of crude protein tended to decrease, however urinary N excretion also tended to decrease with an increase of dietary energy level ($P<0.05$). The microbial synthesis in the rumen can be clearly presumed to increase along with an increase of dietary energy, though the rate of microbial N supply did no increase. From this, it is thought that microbial N supply would increase with an increase in dietary energy (DOMI) ; however, an increase in the rate of microbial N supply should be resulted from a simultaneous increase of dietary N as well as dietary energy. The microbial synthesis could be clearly assumed to decrease in faunated animals, and then, protozoon contribution to protein supply in the animal may be calculated too small if urinary PD is adopted for calculation. There was no clear evidence about the low correlation of urinary PD excretion and an increase of protozoa population in the rumen after inoculation, and they call further detail study.

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