Influence of Controlling Protozoa on the Degradation and Utilization of Dietary Fibre and Protein in the Rumen and Nitrogenous Flow Entering the Duodenum of Sheep

C. Y. Han, D. X. Lu*, M. Hu and Z. L. Tan
Inner Mongolian Academy of Animal Science, Huhhot, 010030, P. R. China

ABSTRACT : Nine two-year old sheep fitted with rumen and duodenum cannulas were used to study the effect of controlling protozoa flora on the degradation and utilization of dietary fibre and protein in the rumen and on nitrogenous flow to the duodenum. There were three groups in this experiment: defaunation (DF); partial defaunation (PDF); faunation (F) as control. Results showed that: 1,There were no differences between treatments in dietary DM degradation in the rumen, but defaunation and partial defaunation increased the quantity of nitrogenous material in the rumen and the flow of N to duodenum. 2, Partial defaunation and defaunation improved the degradabilities of dietary NDF, ADF and HC, but there were no differences between the defaunated and partially defaunated groups. 3, Partial defaunation decreased the degradability of dietary protein in the rumen. There was no difference between defaunated and faunated groups. 4, Defaunation and partial defaunation increased the quantity of total N (TN) and microbial N (MCN) in the rumen and the amounts entering the duodenum. The protozoa N (PN) flow in the faunated group was higher than that in the partially defaunated group, and the amino acid pattern in the digesta at the proximal duodenum in the defaunated group was closer to the ideal amino acid pattern. 5, There were differences in the mole percent of acetic, propionic, total-VFAs and the non-glucogenic to glucogenic VFAs ratio (NGR) value in the rumen fluids. The order was as follows: mole percent of acetate: F>PDF>DF; mole percent of propionate: DF>PDF>F; total-VFAs: PDF>F>DF; NGR: F>PDF>DF. *(Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 8 : 1241-1245)*

Key Words : Sheep, Fiber, Protein, Protozoa and Defaunation

INTRODUCTION

Protozoa have positive and negative effects on digestion in the rumen. Firstly protozoa may slow down the fast fermentation of dietary starch and soluble sugar and stabilize rumen pH when animals are fed diets rich in available starch, as well as decrease the redox potential of rumen digesta, which should indirectly stimulate the cellulolytic bacteria activity (Uside et al., 1991 and Mthieu et al., 1996). Protozoa may also supply some peptides that stimulate the growth of the rumen microbiota (Jouany and Ushida, 1998). Meanwhile, protozoa also have several negative effects. For example, the predation of bacteria increased the protein turnover in the rumen, and protozoa contribute to methane production (Jouany and Ushida, 1998). There have been many debates concerning the advantages and disadvantages for defaunation during the last two decades. In fact, the effect of defaunation depends on the balance between energy and protein needs for animals and nutrients supplied by diet. Defaunation is useful in case of diets low in protein but not limited in energy for animals with high protein requirements (Jouany and Ushida 1998; Smet et al., 1992). In practice, defaunation is difficult to maintain (Lu, 1998).

The control of rumen protozoa in terms of total concentrations or generic composition could be a way to improve animal production on low quality roughage diets. Lu (1993) suggested the use of partial defaunation in animals fed high fibre diets. The main principle of this proposal is: with low-quality roughage diets, animals are dosed with sufficient defaunating agent to decrease the population of protozoa by 30~50%, and to control the generic composition of protozoa, i.e. keeping the genera that could utilize more fibre and decreasing the genera that could utilize more starch and soluble sugar. The objectives of this trial were to validate this proposal.

MATERIALS AND METHODS

Nine two-year old sheep fitted with rumen and duodenum cannulas and weight 30±5 kg were used in a random design for this study. There were three groups: defaunation (DF); partial defaunation (PDF); faunation (F) as control. They were fed a ration comprising concentrate mixture and chopped hay in 31:69 ratio and based on 1.2 times maintenance (table 1). The concentrate mixture was fed in two equal portions at 07:00 h and 19:00 h and the hay as free choice all day long. They had free access to clean drinking water.

At the beginning of adaptation period, 1g sodium dodecylbenazene sulfonic acid as a defaunating agent was put into the rumen through rumen cannulas when the concentrate was provided. The amount of the

* Corresponding Author: D. X. Lu.
Received January 15, 1999; Accepted March 17, 1999

Table 1. The composition of experimental diet

Composition	Percentage (%)	ME (MJ/kg)	CP (%)	Ca (%)	P (%)
Concentrate Mixture	31	11.49	20.91	2.58	1.87
Hay	69	6.46	7.70	0.24	0.18
Total	100	8.02	11.19	0.97	0.69

* Concentrate mixture contains(%): ground shelled corn 66, soybean meal 10, wheat bran 18, bone meal 0.8, urea 3, mineral and vitamin premix 1 and salt 1.2.

Table 2. The population and generic compositions of protozoa in the rumen of the sheep in the treatment and control groups

Groups	Population ($\times 10^4$/ml)	Generic composition of protozoa(%)				
		Holotrichia	Oligotrich			
			Entodinium	Diplodinium	Ophryoscolex	Epidinium
Defaunation	0	0	0	0	0	0
Partial defaunation	1.11	3.85	78.41	14.41	0.92	2.41
Faunation	2.31	4.24	79.60	12.97	1.10	2.09

defaunating agent offered was increased by 0.5g every three days until the population of rumen protozoa in the sheep in the partially defaunated group was decreased by 30~50%, or the rumen protozoa in the sheep in the defaunated group disappeared. Rumen liquor was collected from each animal before feeding (0 h) and at 3, 6 and 9 hours after feeding and used to observe protozoa genera and population.

Total protozoa were counted and classified with a method described by Lu and Xie (1990), and Han and Chen (1988). The pH of rumen fluid was determined immediately after straining it through muslin cloth by a systronic pH meter (made in Shanghai, China). Rumen volatile fatty acids were determined by gas chromatography. The fractional outflow rate (Kd) of digesta in the rumen and the duodenum was estimated by using Cr_2O_3 as a marker. Fiber and protein degradation characteristics in the rumen were determined using the in sacco method. The microbial protein was determined by using RNA as a marker. The protozoan protein was determined by using AEP as marker (Julian and Czerkawski, 1974). The statistical analysis was done on Lotus 4.01 and SAS software.

RESULTS AND DISCUSSION

Effect on counts and classification of rumen protozoa

The population and generic compositions of protozoa in the rumen of the sheep in all groups are shown in table 2. The population of Holotrichs and Entadinia decreased, and that of other genera increased. As we know, Holotrich and Entadinia mainly ingest starch and soluble sugar to stabilize pH in the rumen, and other genera in Oligotrich could digest the cellulose in plants (Hungate, 1966).

Effect on degradation of dietary fiber

The effect of controlling protozoa on fibre degradation in the rumen is shown in table 3. The effective degradation (ED) of DM in hay was not significantly different between groups ($p>0.05$). The PED of DM in defaunated and partially defaunated groups was significantly higher than in the faunated groups ($p<0.05$). Because protozoa actively prey on bacteria, defaunation could increase the bacterial population. Moreover, SAFI and Kd were increased with increasing bacterial population. The results of partial defaunation indicated that the retained protozoa could ingest fewer bacteria in the rumen.

As reported by Romulo (1989), partial defaunation and defaunation significantly improved the ED of NDF, ADF and hemicellulose (HC), but there were no differences between the defaunated and partially defaunated groups ($p>0.05$) in this experiment. This result could indicate that the protozoa which can degrade NDF in the rumen were retained in the partially defaunated group, and the fungi and cellulolytic bacterial populations in both groups were increased.

Partial defaunation and defaunation may significantly improve the PED of DM and NDF, but no differences were observed. There were significant differences in PED of ADF and HC in the three groups, and the order was as follows: partial defaunation>defaunation>faunation. The reason may be that defaunation or partial defaunation changes the bacteria and protozoa population and generic composition.

Table 3. Effect of controlling protozoa flora on dietary fibre degradation in the rumen

Parameter	Nutrient	Groups		
		De-faunation	Partial defaunation	Faunation
ED (%)	DM	26.585[a]	26.625[a]	26.071[a]
	NDF	18.257[a]	19.625[a]	14.557[b]
	ADF	17.274[a]	17.208[a]	15.540[b]
	HC	19.224[a]	18.435[a]	13.782[c]
PED (%)	DM	49.570[a]	49.630[a]	47.810[b]
	NDF	44.703[a]	43.947[a]	39.803[b]
	ADF	42.050[b]	44.213[a]	39.647[c]
	HC	43.873[b]	50.443[a]	36.807[c]
Kd ($\%h^{-1}$)	DM	2.69[a]	2.49[b]	2.28[c]
	NDF	2.44[a]	2.51[a]	2.48[a]
	ADF	2.53[b]	2.74[a]	2.54[a]
	HC	2.68[b]	2.89[a]	2.59[c]
SAFI	DM	2.34[a]	2.18[b]	1.99[c]
	NDF	1.13[b]	1.57[a]	1.20[b]
	ADF	0.574[b]	0.674[a]	0.575[a]
	HC	0.364[b]	0.431[a]	0.221[c]
LT (h)	NDF	4.987[b]	5.969[a]	6.117[a]
	ADF	5.489[a]	4.929[b]	4.325[c]
	HC	6.703[b]	9.240[a]	6.531[b]
RF ($\%h^{-1}$)	NDF	21.566[a]	20.347[b]	21.187[a]
	ADF	21.804[a]	21.537[a]	21.237[a]
	HC	22.949[a]	22.601[a]	20.112[b]

* ED: effective degradability; PED: potentially effective degradation; Kd: rate of digestion; SAFI: surface area fermentability index; LT: lag time; RF: rumen fill.

Effect on rumen fermentation

The effect of controlling protozoa on total amount and molar proportion of VFAs in the rumen is shown in table 4. There was a significant decrease in total volatile fatty acid concentration in defaunated animals. Molar proportions of propionate increased and of acetate and butyrate decreased in defaunated animals, which is consistent with the observation of other workers (Whitelaw et al., 1984; Opnagia 1994). Molar proportions of propionate and acetate in the rumen of partially defaunated animals were inter- mediate between defaunated and faunated animals.

Table 4. Effect of controlling protozoa on VFAs and molar proportions of VFA in the rumen

	Groups		
	Defaunation	Partial defaunation	Faunation
Acetate(%)	47.03[c]	55.75[b]	64.54[a]
Propionate(%)	46.93[a]	31.80[b]	23.13[c]
Butyrate(%)	6.04[b]	13.45[a]	12.33[a]
Valerate(%)	-	-	-
Total VFA (mM)	62.15[c]	70.96[a]	67.85[b]
NGR	1.25[c]	2.59[b]	3.86[a]

$$* \text{ NRG} = \frac{\text{acetate (\%)}+2\times\text{butyrate (\%)}+\text{valerate (\%)}}{\text{Propionate (\%)}+\text{valerate (\%)}}$$

NGR is the ratio of non-glucogenic to glucogenic VFAs. When the NGR value is 2.25~3.00, the utilization of feed is the highest (Ørskov, 1975). Table 4 indicated that the value of NGR in partial defaunation group was in the range of 2.25~3.00, which proved that rumen protozoa in the partially defaunated animals fed on a roughage based diet could increase the feed utilization.

Effect on dietary protein degradability

Table 5 showed that partial defaunation could significantly decrease dietary protein degradability, and there was no difference between defaunated and faunated groups, but dietary protein degradability in the faunated groups was slightly higher than in the defaunated group, which is consistent with the result of the early researchers (Jouany and Thivend, 1983; Kayouli et al., 1986). The lower amount of dietary degraded protein in the rumen of sheep in partially defaunated group might be explained by the different protozoa genera and the concentration between partially defaunated and faunated groups (Jouany and Ushida, 1998).

Table 5. Effect of controlling protozoa on the effective degradability (ED%) of dietary protein and the flows of nitrogenous substances in the rumen and entering the duodenum

		Groups		
		Defaunation	Partial defaunation	Faunation
ED	(%)	46.187[a]	41.173[b]	46.583[a]
TN	R(g/d)	17.74[a]	17.96[a]	17.07[b]
	D(g/d)	15.02[a]	15.09[a]	14.43[b]
MCN	R(g/d)	5.77[a]	5.75[a]	5.02[b]
	D(g/d)	4.52[a]	4.66[a]	3.70[b]
PN	R(g/d)	-	1.66[b]	1.93[a]
	D(g/d)	-	0.94[b]	1.11[a]

* R: rumen; D: duodenum; TN: total N; MCN: microbial N; PN: protozoa N; ED: effective degradability.

The flow of TN and MCN in the rumen and the flow entering the duodenum in the defaunated and partially defaunated groups were significantly higher than in faunated group (table 5). There was no difference between the defaunated and partially defaunated groups. The result of defaunation agrees with other researchers (Meyer et al., 1986). The main reason is that the bacterial population in the defaunated and partially defaunated groups is increased, which increases microbial yield in the rumen and output of net protein into the duodenum.

Effect on the amino acid pattern

There were differences in the amounts of TAA,

Table 6. Effect of controlling protozoa on the amino acid amount and amino acid pattern in the digesta at the proximal duodenum of sheep

	Groups					
	Faunation		Partial defaunation		Defaunation	
	Amount (g/d)	Pattern (as Lys 100%)	Amount (g/d)	Pattern (as Lys 100%)	Amount (g/d)	Pattern (as Lys 100%)
Asp	7.008^{a}	184.8	7.853^{ab}	174.4	8.590^{a}	230.3
Thr	3.443^{a}	90.72	3.520^{a}	78.17	3.553^{a}	95.25
Ser	3.520^{a}	92.75	3.870^{a}	85.94	3.960^{a}	106.2
Glu	10.168^{b}	267.9	11.137^{ab}	247.36	12.860^{a}	344.8
Gly	4.104^{b}	109.1	4.473^{ab}	99.33	5.140^{a}	137.9
Ala	4.682^{b}	123.4	5.290^{ab}	117.5	5.940^{a}	159.2
Cys	1.387^{a}	36.54	1.430^{a}	31.76	1.515^{a}	40.62
Val	4.967^{a}	130.8	5.303^{a}	117.8	5.580^{a}	149.6
Met	1.885^{a}	49.67	1.823^{a}	40.48	1.950^{a}	52.28
Ile	4.165^{a}	109.7	4.450^{a}	98.82	4.520^{a}	121.2
Leu	7.682^{a}	202.4	8.497^{a}	188.7	9.270^{a}	248.5
Tyr	3.112^{a}	82.00	3.360^{a}	74.62	3.630^{a}	97.32
Phe	4.465^{a}	117.7	3.837^{a}	85.21	4.900^{a}	131.4
Lys	3.795^{a}	100.0	4.503^{a}	100.0	3.730^{a}	100.0
His	1.570^{a}	41.37	1.590^{a}	35.31	2.170^{a}	58.18
Arg	2.896^{b}	76.31	3.280^{ab}	72.84	3.890^{a}	104.3
Pro	3.575^{b}	94.20	3.207^{a}	71.22	3.460^{a}	107.8
EAA	34.868^{b}	-	36.863^{ab}	-	39.563^{a}	-
NEAA	37.592^{b}	-	40.620^{ab}	-	45.095^{a}	-
TAA	72.460^{b}	-	77.483^{ab}	-	84.685^{a}	-
EAA/NEAA	0.927^{a}		0.907^{a}		0.877^{a}	
EAA/TAA × 100%	48.12^{a}		47.58^{a}		46.73^{a}	

* EAA: essential amino acids; NEAA: nonessential amino acids; TAA: total amino acids.

EAA and NEAA in the digesta at the proximal duodenum of sheep in three groups (table 6), and the order was as follows: faunated<partially defaunated< defaunated ($p<0.05$). Data for all individual amino acids flows and the similar order of TAA, EAA and NEAA, especially for Arg, Glu and Ala in the digesta at the proximal duodenum of the animals in both defaunated groups indicated that increased amino acids flows mainly originated from rumen bacteria, because Arg, Glu and Ala were higher in rumen bacteria (Czerkawski, 1976).

The amino acid pattern in the digesta at the proximal duodenum of sheep in this experiment was compared with the ideal amino acid patterns proposed from other sources (Hongrong Wang, 1998; McCance and Widdowson, 1978). The comparison showed that the amino acid pattern in the digesta at the proximal duodenum of sheep in the partially defaunated group was the closest to the proposed ideal patterns. In view of certain essential amino acids (EAA) such as Lys, Glu, Leu and Ile are rich in the protein of rumen protozoa, although complete defaunation could improve the amount of microbial protein in the rumen and entering duodenum, the amino acid pattern was far from the ideal amino acid pattern.

CONCLUSIONS

In case of animals fed high fibre diets, partial defaunation not only decreased the negative effect of protozoa but also increased the positive effect of protozoa. The amino acid pattern in the digesta entering the duodenum of sheep in the partially defaunated group was the best in the three groups. Partial defaunation overcomes the difficulty of complete defaunation, because the defaunation agent could be easily added to the diet fed to partially defaunated animals.

REFERENCES

Czerkawski, J. W. 1976. Chemical composition of microbial matter in the rumen. J. Sci. Food Agric., 27:621-632.

Han Zhengkang and Jie Chen. 1988. The rumen digestion and metabolism in ruminants. Science Press, Beijing.

Hungate, R. E. 1966. The Rumen and its Microbes. Academic Press. New York.

Jouany, J. P. and J. Senaud. 1979. Role of rumen protozoa in the digestion of food cellulosic materials. Ann. Rech. Vet. 10:261-263.

Jouany, J. P. and K. Ushida. 1998. The role of protozoa in feed digestion. Proceedings Pre-conference Symposia, 8th

WCAP. pp. 552-569.

Jouany, J. P. and P. Thivend. 1983. Influence of protozoa on nitrogen digestion in the ruminant. Proc. IVth Symp. Protein Metabolism and Nutrition, Clermont-Ferrand, France, Vol. II. Les colloques de l'INRA, 16:287-290.

Julian, W. and J. W. Czerkawski. 1974. Methods for determining 2-6-diaminopimelic acid and AEP in gut content. J. Sci. Agric., 25: 45-55.

Kayouli, C. D., I. Demeyer and R. Dendooren. 1983-1984. Effect of defaunation on straw digestion in sacco and on particle retention in the rumen. Anim. Sci. Technol. 10: 165-172.

Kreuzer, H.. 1986. Methods and application of defaunation in the growing ruminant. J. Vet. Med., 33: 723-745.

Lu Dexun and Chongwen Xie. 1990. Recent methods and techniques for ruminant nutrition. Agriculture Press, Beijing.

Lu Dexun. 1993. Theory for nutritional manipulation and its application. Supplement: Inner Mongolian J. of Animal Sciences and Production, Huhhot.

Mathieu, F., J. P. Jouany, J. Senaud, J. Bohatier, G. Bertin and M. Mercier. 1996. The effect of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on fermentation in the rumen of defaunated and refaunated sheep; protozoal and probiotic interactions. Reprod. Nutr. Develop. 36:271-287.

Meyer, J. H. F., S. I. Van Der Walt and H. M. Schwartz. 1986. The influence of diet and protozoa numbers on the breakdown and synthesis of protein in the rumen of sheep. J. Anim. Sci. 62:509-520.

Opnagia and Riat Siiarma. 1994. Effect of defaunation of rumen function in buffaloes fed high concentrate diet. Indian J. Anim. Sci. 64: 1254-1258.

Ørskov, E. R. 1975. Manipulation of rumen fermentation for maximum food utilization. World Rev. Nutr. Diet., 22:152-182.

Romulo, B., S. H. Bird and R. A. Leng. 1989. Combined effect of defaunation and protein supplement on intake, digestibility, N retention and fungi counts in sheep fed straw-based diets. In: The Role of Protozoa and Fungi in Ruminant Digestion, J. V. Nolan, R. A. Leng and D. I. Demeyer Ed. Penambul Books, Armidale, Australia, pp. 285-288.

Smet, S., D. I. Demeyer and C. H. Van Nevel. 1992. Effect of defaunation and hay: concentrate ratio on fermentation, fibre digestion and passage in the rumen of sheep. Anim. Feed Sci. Technol. 37:333-344.

Ushida, K. 1986. Studies on protozoa contribution to the ruminant digesta. Ph.D. Dissertation. Tokyo University.

Ushida, K., J. P. Jouany and D. I. Demeyer. 1991. Effects of presence or absence of rumen protozoa on the efficiency of utilisation of concentrate and fibrous feeds. In: Physiological Aspects of Digestion and Metabolism in Ruminants, T. Tsuda, Y. Sasaki and R. Kawashima Ed. Academic Press, Tokyo. pp. 625-654.

Wang Hongrong. 1988. Limiting amino acids and ideal amino acid pattern at the proximal duodenum in growing sheep. Ph.D Thesis, Inner Mongolian Institute of Agriculture and Animal Husbandry.

Whitelaw, F. G., J. M. Eadie, L. A. Bruce and W. J. Shand. 1984. Methane production in faunated and ciliate-free cattle and its relationship with rumen volatile fatty acid proportion. Br. J. Nutr. 52:261-275.