

## Effects of Dietary Nitrogen Sources on Fiber Digestion and Ruminal Fluid Characteristics in Sheep Fed Wheat Straw

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**ABSTRACT :** Fifteen Inner Mongolian wethers with permanent ruminal and duodenal cannulas were used to study the effects of dietary rumen-undegradable protein (RUP) to rumen-degradable protein (RDP) ratios or protein sources on fiber digestion in the gastrointestinal tract and ruminal fluid characteristics. Fiber digestion and ruminal fermentation were not affected ( $p>0.05$ ) by dietary RUP to RDP ratios (from 1.54 to 0.72). Soybean meal supplementation improved ruminal digestion. Fish meal supplementation increased ( $p<0.05$ ) the ruminal degradability of fiber. The different RUP to RDP ratios (from 1.54 to 0.72) did not influence ( $p>0.05$ ) ruminal fluid pH, but there were differences ( $p<0.05$ ) in ruminal fluid  $\text{NH}_3\text{-N}$  concentration because of urea replacement. Soybean meal as a dietary protein source decreased ( $p<0.05$ ) ruminal fluid pH and increased ( $p<0.05$  or  $p<0.01$ )  $\text{NH}_3\text{-N}$ , acetate, propionate and butyrate concentrations in the rumen. Fish meal as a dietary protein source decreased ( $p<0.05$  or  $p<0.01$ ) ruminal  $\text{NH}_3\text{-N}$  and acetate concentrations and increased ( $p<0.05$ ) ruminal propionate concentration. It can be concluded that dietary protein sources have more significant effect on fiber digestion and ruminal fermentation than different dietary RUP to RDP ratios, when the dietary crude protein requirements of growing sheep are satisfied. (*Asian-Aust. J. Anim. Sci.* 2001. Vol 14, No. 10 : 1374-1382)

**Key Words :** Protein Source, Fiber Digestion, Wheat Straw, Sheep

### INTRODUCTION

In many developing countries, crop straw is one of the most important feed resources for ruminants. However, crop residues offered as the only feed, even supplemented with minerals, vitamins and non-protein nitrogen (NPN) sources, do not satisfy ruminant maintenance requirements because of their low digestibility and low voluntary intake resulting from their main nutritional limitations (i.e., low protein and low digestible energy contents). These problems have been traditionally solved by chemical or processing treatment of straw, or supplementation with energy or protein sources (McCullum and Galyean, 1985; Silva and Ørskov, 1988; Tan and Lu, 1998).

When higher production is required, it is necessary to provide supplements of energy and protein. Supplementation is especially important for animals fed low-quality roughage. A number of practical studies indicated that supplemental protein improved microbial growth and fiber digestion in the rumen when the supplemental nitrogen was added to straw-based diets for growing ruminants (Smith et al., 1985; Merry et al., 1990). In the

past decade, some studies discussed the effects of dietary protein degradability or protein sources on feed intake, rumen fermentation, and performance in ruminants (Newbold et al., 1987; Sloan and Rowlinson, 1988; Dawson et al., 1999). However, there were only limited data on the digestion of neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose (CEL) and hemicellulose (HC) in ruminants consuming diets having various rumen-undegradable protein (RUP) to rumen-degradable protein (RDP) ratios or protein sources. The objectives of the present study were to investigate the effects of various dietary RUP to RDP ratios and protein sources on the digestion of NDF, ADF, CEL and HC, as well as ruminal fluid characteristics for growing sheep fed a diet based on wheat straw.

### MATERIALS AND METHODS

#### Animals and feeds

Fifteen growing Inner Mongolian wethers with an initial live weight of  $28.9\pm 1.0$  kg fitted with permanent rumen and duodenal cannulas were penned individually, with free access to fresh water. The animals were randomly divided into five groups, 3 animals per group, on the basis of their body weight. Chemical compositions of their feedstuffs are in table 1. Each sheep was offered equal amounts (to 5% refusal) of chopped wheat straw (2 cm length) and concentrate at 07:00 and 19:00 daily to meet 1.1 times the maintenance requirements of Inner Mongolian sheep (SIMA, 1992). Before conducting the formal experiment, the rapidly degradable fraction (a), potentially degradable

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Received January 2, 2001; Accepted April 27, 2001

**Table 1.** Chemical composition of experimental feedstuffs (% DM)

Feedstuffs	DM	CP	FAT	NDF	ADF	ASH	NDFCP	CHO	NSC	SC
Wheat straw	92.0	4.2	1.3	82.1	57.7	8.5	1.3	86.0	5.1	80.8
Corn	86.3	8.7	3.2	13.1	5.5	1.7	1.4	86.4	74.7	11.7
Wheat bran	89.6	16.2	3.1	27.4	11.9	4.1	2.3	76.6	51.5	25.1
Rapeseed meal	94.1	27.2	8.5	39.4	33.5	16.8	9.2	44.0	13.8	30.2
Soybean meal	89.9	39.7	5.6	18.4	15.8	9.0	6.4	45.7	33.7	12.0
Corn starch	87.8	0.9	0.2	2.5	0.8	0.2	-	98.8	96.2	2.5
Fish meal	92.9	66.0	6.6	3.2	2.1	22.0	1.4	5.4	3.6	1.8
Blood meal	89.3	79.2	-	-	-	10.1	-	-	-	-
Urea	-	281.0	-	-	-	-	-	-	-	-

DM=dry matter, CP=crude protein, FAT= crude fat, NDF= neutral detergent fiber, ADF=acid detergent fiber, ASH=total crude ash, NDFCP=NDF bound crude protein, CHO=total carbohydrate, NSC=nonstructural carbohydrate, SC=structural carbohydrate.

**Table 2.** The potential dynamic crude protein degradability of main protein sources and urea

Feedstuffs	CP(%)	a(%)	b(%)	c(%/h)	k <sub>p</sub> (%/h)	PED (%)
Corn	9.2	36.7	45.2	7.2	4.8	63.8
Wheat bran	13.3	61.7	40.1	7.5	4.1	87.6
Soyabean meal	44.0	33.2	61.4	7.7	9.9	60.0
Blood meal	73.3	21.8	34.5	4.9	7.8	35.0
Fish meal	61.3	23.8	72.0	2.7	8.5	41.0
Urea	280	-	-	-	-	100.0
Rapeseed meal <sup>A</sup>	27.2	-	-	-	-	72.0

CP=crude protein, a= readily available fraction, b= degradable part of insoluble fraction, c=fraction degradation rate, k<sub>p</sub>= passage rate, PED=potentially effective degradability.

<sup>A</sup> The potential effective crude protein degradability of rapeseed meal was cited from Haenlein (1993).

fraction (b), fractional digestion rate (c), passage rate (k<sub>p</sub>) and potentially effective degradability (PED) (see table 2) of crude protein (CP) were measured according to the method described by Lu and Xie (1991) for main dietary nitrogen sources. The PED of five different concentrate supplements were calculated on the basis of these measured PED (see table 3). During the experimental period, the daily amounts of offered and refused wheat straw and concentrate were weighed to obtain actual intakes.

### Experimental procedures

The entire experiment lasted 40 days. The adaptation period was the first 15 days; five groups of sheep were offered equal amounts of wheat straw and concentrate. After the adaptation period, the rate of disappearance of wheat straw dry matter (DM), NDF, ADF, HC and CEL from nylon bags (70×100 mm, pore size: 45 µm) was determined as described by Ørskov and McDonald (1979). Wheat straw was ground to pass through a 1 mm screen using a mill (DF-2, Changsha Instrument Factory, China). Duplicate bags containing approximately 2.5 g of DM of wheat straw were incubated in the rumen of each sheep for periods of 0, 1, 2, 3, 6, 12, 24, 36, 48, 72 and 96 h. After withdrawal from the rumen, the bags were washed with cold water in a washing machine until the water ran clear. The length of the washing procedure was 30 min, consisting

of five rinsing cycles. A "0 h wash value" for calculation of the lag time of DM, NDF, ADF, HC and CEL degradation was determined twice in three bags. After washing, all samples were dried at 105°C for 24 h.

After a recovery period from Day 21 to Day 23, from Day 24 to Day 32, 40 g·day<sup>-1</sup> (4×10, i.e., dosed four times at 6h intervals each day) of chromium-mordanted wheat straw were administered via the rumen cannula at 06:00, 12:00, 18:00 and 24:00 respectively. The straw markers were prepared according to the method of Uden et al. (1980). From Day 29 to Day 32, ruminal digesta, duodenal digesta and rectal fecal samples were taken at 6h intervals. Equal amounts of samples within each segment were mixed, dried at 105 °C for 24 h, ground and analyzed for DM, OM, NDF, ADF, HC and CEL to determine their ruminal, duodenal and rectal flows as described by Lu and Xie (1991). Apparent digestibility of DM, OM, NDF, ADF, CEL and HC in the rumen, post-ruminal digestive tract and total gastrointestinal tract were then calculated.

Straw marker infusion was terminated at 06:00 h on Day 33 and from Day 33 to 35 ruminal digesta samples were taken from each sheep at 3 h intervals for 3 days to estimate the mean retention time (MRT) of wheat straw in the reticulo-rumen. The decline in the natural logarithm of Cr concentrations over time was used to estimate the fractional passage rate of ruminal digesta.

**Table 3.** The ingredients (%) and composition (%DM) of the five concentrates

Feedstuffs	C1	C2	C3	C4	C5
Corn	44.0	55.0	63.0	6.0	42.0
Wheat bran	8.0	3.0	1.0	8.0	0
Blood meal	46.0	38.0	30.0	0	0
Soybean meal	0	0	0	84.0	0
Fish meal	0	0	0	0	56.0
Urea	0	2.0	4.0	0	0
Bone meal	0.8	0.8	0.8	0.8	0.8
Salt	1.2	1.2	1.2	1.2	1.2
Total	100.0	100.0	100.0	100.0	100.0
Nutritional composition					
ME(Mcal/kg DM)	2.81	2.80	2.76	2.91	2.72
Dry matter (%)	88.1	88.0	86.9	88.8	90.2
Crude protein (%)	38.8	38.9	39.1	38.6	38.2
Rumen degradable protein (%)	15.3	18.9	22.7	23.5	16.2
RDP/CP (%)	39.4	48.6	58.1	60.8	42.4
RUP/CP (%)	60.6	51.4	41.9	39.2	57.6
RUP:RDP	1.54	1.06	0.72	0.65	1.31
Calcium (%)	0.29	0.28	0.28	0.46	2.78
Phosphorus (%)	0.35	0.31	0.30	0.63	1.68
Neutral detergent fibre (%)	7.9	8.0	8.5	17.8	7.3
Acid detergent fibre (%)	3.4	3.4	3.6	14.3	3.5
Total carbohydrate (%)	44.1	49.8	55.2	49.1	39.3
Non-structural carbohydrate (%)	37.0	42.6	47.6	36.8	33.4
Structural carbohydrate (%)	7.2	7.2	7.6	12.2	5.9

C1, C2, C3, C4 and C5 mean concentrate 1, 2, 3, 4 and 5 in the diets respectively. RDP=rumen degradable protein, RUP=rumen undegradable protein, CP=crude protein.

After recovery, Days 36 to Day 38, ruminal fluid was sampled on Days 39 to 40 via cannula according to the following timetable:

Day 39: 08:00, 09:00, 10:00, 11:00, 13:00, 16:00, 19:00, 23:00, 21:00, 22:00, 23:00.

Day 40: 01:00, 04:00, 07:00.

Ruminal fluid pH was measured immediately after sampling and fluid was then filtered through a metal mesh (1 mm pore size). Filtrate (20 ml) was diluted 1:1 with 0.2 M HCl and stored frozen until analysis of  $\text{NH}_3\text{-N}$ . A second 10 ml subsample was centrifuged at 25,000  $\times g$  for 20 min, mixed with 1 ml of a solution of formic acid and 25%  $\text{H}_3\text{PO}_4$  at a ratio of 1:3 (V/V) and kept frozen for volatile fatty acids (VFA) analysis as described by Erwin et al. (1961).

#### Chemical analyses

All samples were analysed for DM, OM and CP content by the Association of Official Analytical Chemist methods (AOAC, 1980), and for NDF, ADF, HC, CEL and NDF

bound CP (NDFCP) content by the procedure proposed by Van Soest et al. (1991) and Yang (1993). Ruminal fluid samples were analysed for  $\text{NH}_3\text{-N}$  according to the method of Feng and Gao (1993). Estimation of VFA contents was performed by gas-liquid chromatography in a GC-7A (Shimadzu Co. Kyoto, Japan). Measurement of chromium (Cr) was performed according to the procedure of Gao and Feng (1993).

#### Calculations

The flows of DM, OM, NDF, ADF, CEL and HC at duodenum and rectum were calculated using the single-marker method as described by Lu and Xie (1991). The disappearance of DM, NDF, ADF, HC and CEL from nylon bags was fitted to the equations of McDonald (1981) and Dhanoa (1988).

$$p = a' \quad \text{for } t \leq t_0 \quad (1)$$

$$p = a + b \times (1 - e^{-ct}) \quad \text{for } t > t_0 \quad (2)$$

where

P = some measured quantity such as percentage of dry matter or fiber loss,

$a'$  = soluble fraction or 0 h wash value

$a$  = readily available fraction fitted by the least squares method

$b$  = degradable part of insoluble fraction

$c$  = fractional degradation rate ( $\text{h}^{-1}$ )

The lag time (L) before the start of component b degradation was calculated as described by Khalili and Huhtanen (1991):

$$L = 1 / \{c \times (\ln(b/(a+b-a')))\} \quad (3)$$

The potentially effective degradability (PED) was calculated as

$$\text{PED} = a + b \times c / (c + k_p) \quad (4)$$

where  $k_p$  is the fractional passage rate of digesta from the rumen. The dietary structural carbohydrate (SC), non-structural carbohydrate (NSC) and carbohydrate (CHO) contents were calculated according to the following equations proposed by Van Soest et al. (1991).

$$\text{SC} = \text{NDF} - \text{NDFCP} \quad (5)$$

$$\text{NSC} = 100 - (\text{NDF} + \text{CP} + \text{FAT} + \text{ASH} - \text{NDFCP}) \quad (6)$$

$$\text{CHO} = 100 - \text{CP} - \text{FAT} - \text{ASH} \quad (7)$$

#### Statistical analyses

All degradation, apparent digestibility and ruminal fluid data were analyzed using ANOVA procedures of SAS (1985). The statistical models included group, and degradation, digestibility or ruminal fluid characteristics. The P-values that were  $< 0.05$  were considered significant.

## RESULTS AND DISCUSSION

#### Fiber digestion

The aims of the present experiment are made clear in table 3: effects of different dietary CP degradabilities (i.e.,

RUP:RDP) can be noted among the designed C1, C2 and C3. Further, we also investigated the effects of different protein sources by comparing C4 and C5. In other words, both protein sources and dietary RUP to RDP ratios were considered in this experiment so as to investigate their effects on fiber digestion and ruminal fluid characteristics.

The results for DM, OM, NDF, ADF, HC and CEL digestion in the different segments of the gastrointestinal tract are shown in table 4. No differences among five dietary treatments were found for ruminal DM, OM, NDF, ADF and CEL digestibilities, although ruminal DM, OM, NDF, and HC digestibilities were numerically higher in C4 and C5, and ruminal DM, OM, NDF, ADF and CEL digestibilities appeared to decline gradually among C1, C2 and C3 (except HC). These results suggested that supplementation with 2% and 4% urea might influence fiber digestion in the rumen of sheep fed blood meal; fiber digestion was probably more effective for diets containing soybean meal or fishmeal as the main protein source than diets with blood meal and urea as main protein sources. On the other hand, post-ruminal digestibilities of DM, OM, NDF, ADF, HC and CEL did not differ among the five dietary treatments except for post-ruminal DM digestibility of C2. In the C2 diet, post-ruminal digestibilities of DM, OM, NDF, ADF, HC and CEL seemed to be improved. Digestibilities in the total digestive tract did not differ among five treatments. Among three combinations of blood meal and urea (C1, C2, and C3), the total digestibilities of DM and OM were improved for 2% urea supplement diet; however, there was no apparent trend of improvement in total digestibilities of fiber. The total digestibilities of DM, OM, NDF, ADF, HC and CEL could be influenced by 4% urea supplementation in the diet, although the decreasing trends were not significant. When dietary RUP to RDP ratios were similar, such as C3 diet (RUP:RDP=0.72) and C4 diet (RUP:RDP=0.65), the former (blood meal and urea as protein sources) could restrain fiber digestion, the latter (soybean meal as protein source) might improve fiber digestion in the rumen and total digestive tract.

Petersen et al. (1985) reported that the supplementation of urea, soybean meal, or blood meal as a protein source in growing steer diets could significantly increase NDF digestibility and, especially, soybean meal supplementation could also significantly improve ADF digestibility. Stokes et al. (1988) found out that inclusion of increased concentrations of soybean meal in dairy diets might improve OM and NDF digestibility. Guthrie and Wagner (1988) reported that soybean supplementation in diets for growing beef cattle also increased DM, OM and ADF digestibility. Warly et al. (1992) reported that soybean meal supplementation could increase OM, NDF, ADF and CEL digestibility for growing sheep fed rice straw basal diets. In

the present experiment, trends towards higher NDF, ADF and HC digestibilities in the rumen and total digestive tract were noted when soybean meal was included as the main protein source compared to diets with blood meal and urea as main protein sources in the diets. On the other hand, it has also been reported that the urea supplementation of ruminant diets could improve fiber digestion. Punia et al. (1988) and Djajanegara and Doyle (1989) concluded that urea supplementation enhanced NDF digestibility in beef cattle fed wheat straw diets and sheep fed rice straw diets, respectively. However, the effects of urea on fiber digestion in this study were not similar to the previous reports. Inclusion of 2% urea (see C2) only tended to improve DM and OM digestibility compared to wheat straw diets including blood meal as the main protein source (see C1). No differences were noted in NDF, ADF and CEL digestibilities. On the contrary, the inclusion of 4% urea (see C3) could obviously reduce DM, OM, NDF, ADF, HC and CEL digestibilities. Ortigues et al. (1989) concluded that fishmeal supplementation in beef cattle diets could improve dietary fiber digestion, further enhancing the ability of digesting fiber in the hindgut. In this study, the diet including fishmeal were not digested better than those including soybean meal, nor was fiber digestion improved.

The digestibility results in table 4 showed that the large amounts of NDF, ADF and CEL could be digested in sheep rumen; post-ruminal digestibilities of NDF, ADF and CEL were lower than ruminal digestibilities. Although the ruminal and post-ruminal digestibilities of DM and OM also expressed the same trends, the post-ruminal digestibilities still were higher than those of NDF, ADF and CEL. Generally speaking, when dietary CP satisfies the requirements of ruminants, the main factors affecting fiber digestion in the digestive tract will be the protein source, and RUP or RDP concentration. According to the present study, fiber digestion tended to be improved with soybean meal or fishmeal as major CP sources. Urea as nitrogen sources increased the RDP concentration; however, both its ability to improve fiber digestibility and its supplemental amounts are limited.

#### **Wheat straw degradation *in situ***

The results for the ruminal degradation of DM, NDF, ADF, CEL and HC in wheat straw are shown in table 5. The  $k_p$  of particulate marker did not differ among diets with different RUP to RDP ratios or protein sources. The PED of DM in nylon bags was not influenced by diet (table 5), but fishmeal supplementation (see C5=1.31) tended to improve the ruminal degradability of DM. The rapidly degradable fraction (a) and degradation lag time (L) of DM were affected ( $p<0.05$  or  $p<0.01$ ) by dietary RUP to RDP ratios and protein sources. The inclusion of 4% urea, soybean meal and fishmeal (see C3=0.72, C4=0.65 and C5=1.31)

**Table 4.** Effect of dietary RUP to RDP ratios or protein sources on digestibilities of DM, OM, NDF, ADF, CEL and HC in different segments of the digestive tract of sheep\*

	Dietary RUP to RDP ratios or different protein sources					SEM
	C1=1.54	C2=1.06	C3=0.72	C4=0.65	C5=1.31	
Digestibility of DM (%)						
In the rumen	39.0 <sup>c</sup>	37.5 <sup>c</sup>	34.0 <sup>cd</sup>	41.5 <sup>bc</sup>	43.1 <sup>bc</sup>	1.58
Post-ruminal digestive tracts	19.3 <sup>e</sup>	23.3 <sup>dc</sup>	19.4 <sup>e</sup>	17.6 <sup>e</sup>	14.5 <sup>e</sup>	1.43
In the total digestive tract	58.2 <sup>a</sup>	60.8 <sup>a</sup>	53.4 <sup>ab</sup>	59.2 <sup>a</sup>	57.6 <sup>a</sup>	1.23
Digestibility of OM (%)						
In the rumen	48.3 <sup>cd</sup>	47.9 <sup>cd</sup>	43.2 <sup>d</sup>	51.0 <sup>cd</sup>	52.2 <sup>cde</sup>	1.57
Post-ruminal digestive tracts	14.6	17.8	14.4	12.6	10.8	1.18
In the total digestive tract	62.9 <sup>ab</sup>	65.7 <sup>a</sup>	57.5 <sup>abc</sup>	63.6 <sup>a</sup>	63.0 <sup>ab</sup>	1.35
Digestibility of NDF (%)						
In the rumen	44.9 <sup>ab</sup>	37.5 <sup>ab</sup>	36.1 <sup>b</sup>	50.4 <sup>ab</sup>	49.1 <sup>ab</sup>	2.93
Post-ruminal digestive tracts	4.7	10.0	4.0	2.5	0.9	1.53
In the total digestive tract	49.6 <sup>ab</sup>	47.5 <sup>ab</sup>	40.1 <sup>ab</sup>	52.9 <sup>a</sup>	50.0 <sup>ab</sup>	2.16
Digestibility of ADF (%)						
In the rumen	44.4	41.5	34.7	48.3	45.4	2.31
Post-ruminal digestive tracts	0.9	3.3	-0.5	1.4	-1.7	0.85
In the total digestive tract	45.3	44.7	34.2	49.7	43.8	2.55
Digestibility of HC (%)						
In the rumen	46.2 <sup>abc</sup>	29.2 <sup>dce</sup>	39.2 <sup>bcd</sup>	55.6 <sup>ab</sup>	57.4 <sup>ab</sup>	5.24
Post-ruminal digestive tracts	13.0 <sup>ef</sup>	24.3 <sup>edf</sup>	13.9 <sup>ef</sup>	5.2 <sup>f</sup>	6.6 <sup>f</sup>	3.38
In the total digestive tract	59.2 <sup>ab</sup>	53.5 <sup>ab</sup>	53.0 <sup>ab</sup>	60.7 <sup>a</sup>	64.0 <sup>a</sup>	2.12
Digestibility of CEL (%)						
In the rumen	48.0	45.7	40.2	47.8	48.8	1.57
Post-ruminal digestive tracts	1.6	2.9	-0.5	1.6	-1.1	0.74
In the total digestive tract	49.6	48.6	39.7	49.4	47.7	1.86

\* In the same items, the values with different superscripts in the same row or column are different ( $p < 0.05$ ). C1, C2, C3, C4 and C5 mean concentrate 1, 2, 3, 4 and 5 in the diets respectively. DM=dry matter, OM=organic matter, NDF=neutral detergent fiber, ADF=acid detergent fiber, HC=hemicellulose, CEL=cellulose, RDP=rumen degradable protein, RUP=rumen undegradable protein. SEM means standard error of mean.

not only decreased the rapidly degradable fraction of DM, but also increased the degradation lag time compared to diets C1 and C2. The slowly degradable fraction (b), fractional degradation rate (c) and potentially degradable fraction (a+b) were not affected by different RUP to RDP ratios or protein sources. The inclusion of 2% urea tended to improve the PED of DM among the former three different RUP to RDP ratio diets (see C1=1.54, C2=1.06, and C3=0.72).

Different RUP to RDP ratios or protein sources in the diets affected ( $p < 0.05$ ) the PED of NDF, ADF and CEL. Fishmeal supplementation of the diet especially increased

the PED of NDF, ADF and CEL (see table 5); however, there were no differences in the PED of NDF, ADF and CEL among the other four diets. Among other degradation parameters, a and L of NDF were affected ( $p < 0.01$  and  $p < 0.05$ ) by RUP to RDP ratios and protein sources. Inclusion of soybean meal or fishmeal increased ( $p < 0.01$  or  $p < 0.05$ , respectively) the a and L of NDF. Urea replacement had no effect on the a and L of NDF; similarly there were no differences for the b, c and a+b of NDF among different RUP to RDP ratios or protein sources. Furthermore, different RUP to RDP ratios or protein sources could influence ( $p < 0.05$ ) the c of ADF, but no effects were noted

**Table 5** Effect of different RUP to RDP ratios or protein sources on degradation parameters of DM, NDF, ADF, HC and CEL by the sheep<sup>1</sup>

Parameters	Dietary RUP to RDP ratios or different protein sources					Significance <sup>2</sup>	
	C1=1.54	C2=1.06	C3=0.72	C4=0.65	C5=1.31		
$k_p$ (%/h)	3.52	3.43	2.88	3.40	2.86	NS	
DM	a (%)	15.3 <sup>a</sup>	13.9 <sup>ab</sup>	12.9 <sup>b</sup>	12.6 <sup>b</sup>	12.3 <sup>b</sup>	*
	b (%)	31.7	35.3	38.2	36.2	39.1	NS
	c (%/h)	2.94	3.61	2.27	3.46	3.31	NS
	a+b (%)	47.0	49.2	51.1	48.8	51.4	NS
	L (h)	1.17 <sup>c</sup>	1.87 <sup>bc</sup>	4.00 <sup>a</sup>	3.17 <sup>ab</sup>	3.27 <sup>ab</sup>	**
	PED (%)	29.4	31.9	29.9	31.2	33.5	NS
NDF	a (%)	4.1 <sup>bc</sup>	2.8 <sup>c</sup>	2.9 <sup>c</sup>	6.0 <sup>ab</sup>	6.9 <sup>a</sup>	**
	b (%)	37.1	39.6	44.0	36.8	41.6	NS
	c (%/h)	2.55	3.61	2.11	2.74	3.39	NS
	a+b (%)	41.2	42.4	46.9	42.7	48.5	NS
	L (h)	-0.03 <sup>a</sup>	0.90 <sup>a</sup>	1.23 <sup>a</sup>	-1.70 <sup>b</sup>	-1.81 <sup>b</sup>	*
	PED (%)	19.4 <sup>b</sup>	23.3 <sup>ab</sup>	21.5 <sup>b</sup>	22.7 <sup>ab</sup>	29.6 <sup>a</sup>	*
ADF	a (%)	2.7	1.8	-1.1	4.4	4.6	NS
	b (%)	36.7	43.0	45.6	36.1	41.0	NS
	c (%/h)	2.60 <sup>ab</sup>	1.68 <sup>b</sup>	2.67 <sup>ab</sup>	2.68 <sup>ab</sup>	4.04 <sup>a</sup>	*
	a+b (%)	39.3	44.9	44.4	40.5	45.6	NS
	L (h)	7.55	10.90	9.04	4.89	2.47	NS
	PED (%)	17.7 <sup>b</sup>	16.0 <sup>b</sup>	20.8 <sup>b</sup>	20.4 <sup>b</sup>	28.9 <sup>a</sup>	*
HC	a (%)	7.2	7.9	7.4	3.1	4.8	NS
	b (%)	36.4 <sup>b</sup>	40.5 <sup>ab</sup>	44.6 <sup>ab</sup>	45.6 <sup>ab</sup>	48.1 <sup>a</sup>	*
	c (%/h)	3.57	2.80	2.78	3.56	3.18	NS
	a+b (%)	43.7 <sup>b</sup>	48.4 <sup>ab</sup>	52.1 <sup>a</sup>	48.7 <sup>ab</sup>	52.8 <sup>a</sup>	*
	L (h)	-5.18	-6.36	-5.55	-1.55	-3.39	NS
	PED (%)	25.4 <sup>a</sup>	26.3	29.5	26.3	30.4	NS
CEL	a (%)	3.1	-0.5	-2.0	1.8	2.0	NS
	b (%)	42.1	49.6	51.3	41.5	45.2	NS
	c (%/h)	2.37	2.39	2.48	3.08	4.34	NS
	a+b (%)	45.2	49.2	49.3	43.4	47.2	NS
	L (h)	6.49	8.89	10.14	6.62	4.10	NS
	PED (%)	20.0 <sup>b</sup>	20.1 <sup>b</sup>	21.4 <sup>b</sup>	21.3 <sup>b</sup>	29.2 <sup>a</sup>	*

<sup>1</sup> Means with different superscripts in the same line are significantly different ( $p < 0.05$ ).

<sup>2</sup> Statistical significance: NS, not significant; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ .

DM=dry matter, NDF=neutral detergent fiber, ADF=acid detergent fiber, HC=hemicellulose, CEL=cellulose,  $k_p$ =ruminal digesta passage rate, a=readily available fraction, b=degradable part of insoluble fraction, c=fractional degradation rate, L=lag time, a+b=potential degradable fraction, PED=potential effective degradability, RDP=rumen degradable protein, RUP=rumen undegradable protein.

C1, C2, C3, C4 and C5 mean concentrate 1, 2, 3, 4 and 5 in the diets respectively.

in the a, b, a+b and L of ADF. Also the a, b, c, a+b and L of CEL were not affected by RUP to RDP ratios or protein sources. In table 5, parameters describing the degradation of HC differed from those of DM, NDF, ADF and CEL. A possible reason was that the contents of HC were calculated from the differences of between NDF and ADF. According

to table 5, different RUP to RDP ratios (see C1, C2 and C3) did not affect the degradation parameters of DM, NDF, ADF, HC or CEL, but the inclusion of fishmeal influenced ( $p > 0.05$  or  $p > 0.01$ ) the PED of NDF, ADF and CEL.

Stokes et al. (1988) reported that increased concentrations of soybean meal in the diet could increase  $k_p$

from the rumen; however, similar results were not obtained in the present study. In this study, only the inclusion of 4% urea (C3) or fishmeal supplementation tended to decrease  $k_p$ . Silva and Ørskov (1988) reported that fishmeal supplementation could increase the degradability of barley straw, but soybean meal supplementation didn't influence the degradation of barley straw in sheep fed the barley straw basal diets. This conclusion was supported in the present experiment; fishmeal supplementation could also improve the degradation of wheat straw in the rumen.

### Ruminal fluid characteristics

The average ruminal pH and concentrations of  $\text{NH}_3\text{-N}$  and VFA are presented in table 6. Different RUP to RDP ratios or protein sources in the diet affected ( $p < 0.05$  or  $p < 0.01$ ) ruminal pH, and the concentrations of  $\text{NH}_3\text{-N}$ , acetate, propionate and butyrate. Inclusion of 2% urea or soybean meal (see C2 and C4 in table 6) decreased ruminal fluid pH ( $p < 0.05$  or  $p < 0.01$ ). When urea was included with blood meal in the diets, ruminal fluid  $\text{NH}_3\text{-N}$  concentration increased ( $p < 0.01$ ). The soybean meal diet had higher  $\text{NH}_3\text{-N}$  concentrations in the rumen than the blood meal and fishmeal diets. The lowest  $\text{NH}_3\text{-N}$  concentration was observed with the diet including fishmeal. Among the C1, C2 and C3 diets, 2% and 4% urea replacement did not influence the concentrations of acetate, propionate or butyrate in the rumen. Acetate, propionate and butyrate concentrations increased ( $p < 0.05$ ) in the rumen of sheep fed the diet that included soybean meal. Compared with C1, C2 and C3 diets, diet C5 decreased ( $p < 0.05$ ) the concentration of acetate and increased ( $p < 0.05$ ) the concentration of propionate in the rumen, but did not affect the concentration of butyrate. Inclusion of fishmeal decreased ( $p < 0.05$  or  $p < 0.01$ ) the concentrations of acetate and butyrate in the rumen compared to the diet that included soybean meal.

The  $\text{NH}_3\text{-N}$  concentration remained in the range (6.3 to 27.5 mg/100ml) which is suitable for the growth of ruminal microbes (table 6) according to the reports of Ortega et al. (1979) and Murphy and Kennelly (1987). As soybean meal concentrations increased in dairy cow diets,  $\text{NH}_3\text{-N}$  and

butyrate concentrations also increased, but ruminal fluid pH decreased and no effects were noted on the concentrations of acetate and propionate (Stokes et al., 1988). This is very similar to the results of the present study for pH, and  $\text{NH}_3\text{-N}$  and butyrate concentrations; however, the inclusion of soybean meal increased the concentrations of acetate and propionate in the rumen (see table 6). Silva and Ørskov (1988) reported that supplementation of sheep diets with soybean meal could increase the concentration of  $\text{NH}_3\text{-N}$  and decrease pH in the rumen. Warly et al. (1992) concluded that soybean meal supplementation could increase the concentrations of  $\text{NH}_3\text{-N}$  and propionate, and decrease pH, and the concentrations of acetate and butyrate in the rumen of sheep fed diets based on barley straw. These results are also consistent with those of the present study. Beever et al. (1990) also reported similar results to those of the present experiment, in that, when fishmeal was supplemented to diets for growing steers, the concentrations of  $\text{NH}_3\text{-N}$ , acetate and butyrate were decreased, but the concentration of propionate was significantly increased.

It can be concluded that, although urea can change the RUP to RDP ratio of the diet and increase the ruminal  $\text{NH}_3\text{-N}$  concentration, the pattern of ruminal fermentation (acetate:propionate:butyrate) did not differ among the diets with different RUP to RDP ratios (see C1, C2 and C3). However, by including soybean meal or fishmeal in the diets of ruminants, it is not only possible to change pH and  $\text{NH}_3\text{-N}$  concentration in the rumen, but also to change the pattern of ruminal fermentation.

### IMPLICATIONS

The present results confirm that various RUP to RDP ratios in the diet only slightly influence fiber digestion, ruminal fluid characteristics and fermentation pattern when crude protein concentrations in the diet satisfy the requirements of growing sheep. Although urea easily increased the RDP contents of diets, it did not influence fiber digestion or ruminal fluid characteristics. Inclusion of soybean meal decreased pH and increased the con-

**Table 6.** Effect of different dietary RUP to RDP ratios or protein sources on pH,  $\text{NH}_3\text{-N}$  (mg/100 ml) and VFA concentration (mmol/l) in the rumen of sheep

	Dietary RUP to RDP ratios or different protein sources					SEM
	C1=1.54	C2=1.06	C3=0.72	C4=0.65	C5=1.31	
pH	6.38 <sup>ab</sup>	6.32 <sup>b</sup>	6.46 <sup>a</sup>	6.22 <sup>c</sup>	6.39 <sup>ab</sup>	0.04
$\text{NH}_3\text{-N}$	20.2 <sup>c</sup>	32.3 <sup>a</sup>	30.1 <sup>a</sup>	24.9 <sup>b</sup>	16.4 <sup>d</sup>	2.97
Acetate	20.3 <sup>b</sup>	22.0 <sup>b</sup>	21.6 <sup>b</sup>	27.7 <sup>a</sup>	16.4 <sup>c</sup>	1.81
Propionate	9.3 <sup>b</sup>	9.1 <sup>b</sup>	9.6 <sup>b</sup>	12.2 <sup>a</sup>	13.6 <sup>a</sup>	0.92
Butyrate	2.7 <sup>c</sup>	3.8 <sup>b</sup>	3.4 <sup>bc</sup>	5.8 <sup>a</sup>	3.4 <sup>b</sup>	0.52

<sup>a,b,c</sup> means with different superscripts in the same row are significantly different ( $p < 0.05$ ). RDP= rumen degradable protein.

RUP=rumen undegradable protein. C1, C2, C3, C4 and C5 mean concentrate 1, 2, 3, 4 and 5 in the diets, respectively.

SEM means standard error of mean.

centrations of  $\text{NH}_3\text{-N}$ , acetate, propionate and butyrate in the rumen. Meanwhile the ruminal digestion of DM, NDF, ADF, HC and CEL tended to be improved with soybean meal; however, it did not improve the ruminal nylon bag degradability. Finally, the inclusion of fishmeal decreased  $\text{NH}_3\text{-N}$  and acetate concentrations, and increased propionate concentration in the rumen. Fishmeal supplementation also improved the ruminal digestibility of DM, OM, NDF and HC. There was significant improvement of ruminal degradabilities of NDF, ADF and CEL by fishmeal supplementation.

### ACKNOWLEDGMENT

The authors thank the Chinese Academy of Sciences for financial support and Dr. M. R. Murphy's valuable suggestion.

### REFERENCES

- AOAC, 1980. Official Methods of Analysis, 13th Ed. Association of Official Analytical Chemists, Washington, DC.
- Beever, D. E., M. Gill, J. M. Dawson and P. J. Buttery. 1990. The effect of fishmeal on the digestion of grass silage by growing cattle. *Br. J. Nutr.* 63:489-502.
- Dawson, L. E. R., A. F. Carson and D. J. Kilpatrick. 1999. The effect of the digestible undegradable protein concentration of concentrates and protein source offered to ewes in late pregnancy on colostrum production and lamb performance. *Anim. Feed Sci. Technol.* 82:21-36.
- Dhanao, M. S. 1988. On the analysis of dacron bag data for low degradability feeds. *Grass and Forage Sci.* 43:441-444.
- Djajanegaga, A. and P. T. Doyle. 1989. Urea supplementation compared with pretreatment: 2. Effects on ruminal and post-ruminal digestion in sheep fed a rice straw. *Anim. Feed Sci. Technol.* 27:31-47.
- Erwin, E. S., G. J. Marco and E. Emery. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *J. Dairy Sci.* 44:1768-1771.
- Feng, Z. C. and M. Gao. 1993. An improved method measuring ruminal  $\text{NH}_3\text{-N}$  concentration. *Inner Mongolian J. Anim. Sci.* 4:40-41.
- Gao, M. and Z. C. Feng. 1993. An improved method measuring Chromium. *Inner Mongolian J. Anim. Sci.* 3:42-43.
- Guthrie, M. J. and D. G. Wagner. 1988. Influence of protein or grain supplementation and increasing levels of soybean meal on intake, utilization and passage rate of prairie hay in beef steers and heifers. *J. Anim. Sci.* 66:1529-1537.
- Haenlein, G. F. W. 1993. Dietary Nutrient Allowances for Goats, Sheep: Ruminal undegradability of protein in selected feed. *Feedstuffs*, July 21, 1993.
- Khalili, H. and P. Huhtanen. 1991. Sucrose supplements in cattle given grass silage based diet. 2. Digestion of cell wall carbohydrates. *Anim. Feed Sci. Technol.* 33:263-273.
- Lu, D. X. and C. W. Xie. 1991. Modern Methods and Technology on Ruminant Nutrition Research. Agriculture Press, Beijing.
- McCullum, F. T. and M. L. Galyean. 1985. Influence of cottonseed meal supplementation on voluntary intake, rumen fermentation and rate of passage of prairie hay in beef steers. *J. Anim. Sci.* 60:570-577.
- McDonald, I. 1981. A revised model for the estimation of protein degradability in the rumen. *J. Agric. Sci.* 96:251-252.
- Merry, R. J., A. B. McAllan and R. H. Smith. 1990. *In vitro* continuous culture studies on the effect of nitrogen source on rumen microbial growth and fiber digestion. *Anim. Feed Sci. Technol.* 31:55-64.
- Murphy, J. J. and J. J. Kennelly. 1987. Effect of protein concentrate and protein source on the degradability of dry matter and protein in situ. *J. Dairy Sci.* 70:1841-1849.
- Newbold, J. R., P. C. Gansworthy, P. J. Buttery, D. J. A. Cole and W. Haresign. 1987. Protein nutrition of growing cattle: food intake and growth responses to rumen degradable protein and undegradable protein. *Anim. Prod.* 45:383-394.
- Ørskov, E. R. and I. McDonald. 1979. The estimation of crude protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.* 92:499-503.
- Ortega, M. E., M. D. Stern and L. D. Satter. 1979. The effect of rumen ammonia concentrate on dry matter disappearance in situ. *J. Dairy Sci.* 62(Suppl. 1):76(Abstr.).
- Ortigue, I., T. Smith, J. D. Oldham, A. B. McAllan and J. W. Siviter. 1989. Nutrient supply and growth of cattle offered straw-based diets. *Br. J. Nutr.* 62:601-619.
- Petersen, M. K., D. C. Clanton and R. Britton. 1985. Influence of protein degradability in range supplements on abomasal nitrogen flow, nitrogen balance and nutrient digestibility. *J. Anim. Sci.* 60:1324-1329.
- Punia, B. S., J. Leibholz and G. J. Faichney. 1988. Effects of level of intake and urea supplementation of alkali-treated straw on protozoal and bacterial nitrogen synthesis in the rumen. *Aust. J. Agric. Res.* 39:1181-1194.
- SAS. 1985. SAS User's Guide: Statistics (5th Ed.). SAS Inst. Inc., Cary, NC.
- Silva, A. T. and E. R. Ørskov. 1988. The effect of five different supplements on the degradation of straw in sheep given untreated barley straw. *Anim. Feed Sci. Technol.* 19:289-298.
- SIMA (Standards of Inner Mongolian Autonomy). 1992. The Local Standards of Inner Mongolian Autonomy (DB15/T30-92): Sheep Feeding Standard. Huhhot.
- Sloan, B. K. and P. Rowlinson. 1988. The influence of a formulated excess of rumen degradable protein or undegradable protein on milk production in dairy cow in early lactation. *Anim. Prod.* 46:13-22.
- Smith, T., J. W. Siviter and R. J. Merry. 1985. Further comparisons of energy and protein sources for growing cattle. *J. Agric. Sci.* 104:485-591.
- Stokes, S. R., A. L. Jones and K. M. Landis. 1988. Feed intake and digestion by beef cows fed prairie hay with different levels of soybean meal and receiving post-ruminal administration of antibiotics. *J. Anim. Sci.* 66:1778-1789.
- Tan, Z. L. and D. X. Lu. 1998. Effects of different dietary carbohydrate proportions on fiber digestion kinetics for growing sheep fed a wheat straw based diet. In Proceedings of the Third National Feed and Nutrition Symposium. the Chinese Animal Nutrition Association, Chengdu, pp. 146-155.
- Uden, P., P. E. Colucci and P. J. Van Soest. 1980. Investigation of



- chromium, cerium and cobalt as markers in digesta. Rate of passage studies. *J. Sci. Food Agric.* 31:625-632.
- Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Symposium: Carbohydrate Methodology, Metabolism and Nutritional Implications in Dairy Cattle. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.
- Warly, L., T. Matsui, T. Harumoto and T. Fujihara. 1992. Study on the utilization of rice straw by sheep. I. The effect of soybean meal supplementation on the voluntary intake of rice straw and ruminal fermentation. *Asian-Aus. J. Anim. Sci.* 5:687-693.
- Yang S. 1993. *The Measuring Technology of Feed Analysis and Feed Quality*. Beijing Agricultural University Press, Beijing.