

# Determining utilizable true protein digestibility of mixed rations for sheep using *in vitro* techniques

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## ABSTRACT

The objective of the experiment was to compare *in vitro* digestion techniques, i.e. 24, 48 and 72 h pepsin digestion, pepsin-small intestinal fluid (SIF) digestion and pepsin-pancreatin digestion, for the determination of *in vitro* digestibility of utilizable true protein (uTP) of mixed rations for sheep. Twelve typical mixed rations for sheep (CP content 7.64-13.02% DM) were formulated as feed samples. It was found that there was no significant difference in uTP digestibility of mixed rations between 24, 48 and 72 h pepsin digestion ( $P>0.05$ ), whereas uTP digestibility of pepsin-SIF treatment was higher than that of pepsin treatment ( $P<0.05$ ), and uTP digestibility of pepsin-pancreatin treatment was higher than that of all other treatments ( $P<0.05$ ). Further research is needed to study the reasons for the difference in *in vitro* uTP digestibility between pepsin digestion, pepsin-SIF digestion and pepsin-pancreatin digestion, and study the relationship between *in vitro* and *in vivo* uTP digestibility before a suitable *in vitro* technique for determining uTP digestibility is proposed.

KEY WORDS: utilizable true protein, digestibility, *in vitro*, sheep

## INTRODUCTION

In ruminants, the utilizable true protein (uTP) of feedstuffs flowing out of rumen to lower digestive tract mainly includes dietary undegradable true protein in rumen and rumen microbial true protein. The uTP of mixed rations for sheep could be estimated using *in vitro* incubation technique (Li and Zhao, 2007). The uTP digestibility in lower digestive tract is an important index for the evaluation of uTP nutritive value of feedstuffs. *In vitro* technique was widely used for the determination of dry matter digestibility of feedstuffs (Tilley and Terry, 1963). The aim of the study is to compare *in vitro* techniques with different enzymes and digestion time

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for the determination of uTP digestibility of mixed rations for sheep. A further aim is to propose an *in vitro* technique for determining uTP digestibility.

## MATERIAL AND METHODS

### *Feed samples*

Twelve typical rations for sheep (Li and Zhao, 2007) with graded levels of CP (7.64-13.02% DM) were formulated. The composition of the rations was the same as in Li and Zhao (2007).

### *Rumen fluid donors*

Three male adult sheep, weighing  $28.2 \pm 0.7$  kg, fitted with rumen cannulas, were used as the donor of rumen fluid. The animals were fed with 1200 g of total mixed ration. The ration contained 800 g hay and 400 g concentrate mixture. The concentrate mixture included, %: maize 60.3, soyabean meal 21, wheat bran 14, NaCl 1.7, dicalcium phosphate 2 and premix compound 1. The ration was given to the sheep in two equal meals at 8.00 and 18.00 h, respectively. Fresh water for the sheep was freely available.

### *Small intestinal fluid (SIF) donor*

One adult sheep weighing 26.0 kg, was slaughtered before morning feeding and all the digesta in the small intestine was collected. The digesta was strained through two layers of surgical gauze into centrifuge tubes. The intestinal fluid was centrifuged at 1000 g for 15 min at 4°C, and the supernatant was freeze-dried in a freeze-dryer (Freezone 6, Labconco, USA). The dried sample was milled through a 1 mm sieve, and stored at -50°C for later use.

### *Preparation of in vitro ruminal digesta*

Buffers and buffer-rumen fluid mixture were made up according to Zhao and Lebziem (2000). About 0.5 g of the mixed rations was weighed into 80 ml glass centrifuge tubes. Each feed sample had three duplicates. Approximately 120 ml rumen fluid was taken from each sheep three h after morning feeding. The rumen fluid from three sheep was well mixed and then strained through four layer of surgical gauze. The buffer-rumen fluid mixture was continuously gassed with CO<sub>2</sub> at 38°C. Fifty ml of buffer-rumen fluid mixture were transferred into each incubation tube. The samples were incubated at 38°C for 24 h. After incubation,

the incubation residues were dried at 65°C for 48 h, then milled through a 3 mm sieve and was kept at -20°C for later use.

### *Pepsin digestion*

The pepsin digestion procedure was according to Tilley and Terry (1963). Approximately 0.5 g dried *in vitro* ruminal digesta sample was incubated with 50 ml of 0.1 N HCl solution containing 20 g/l pepsin (Sinopharm Chemical Reagent Co., Ltd.; enzyme activity 1200 u/g) in 80 ml centrifuge tubes in three duplicates. The samples were incubated for 24, 48 or 72 h in a water bath at 38°C. After incubation, the incubation residues were centrifuged at 1800 g for 30 min and the supernatant was discarded. The residues were used for determination of TCA-insoluble N.

### *Pepsin-pancreatin digestion*

The procedures were according to Akeson and Stahmann (1964). Pancreatin solution was made up by dissolving 1.6 g pancreatin (Sigma, P7545-25G) in 400 ml phosphate buffer (52 ml 0.2M  $\text{KH}_2\text{PO}_4$ +348 ml 0.2M  $\text{Na}_2\text{HPO}_4$ ; pH 7.6). Approximately 0.5 g dried *in vitro* ruminal digesta sample was incubated with 50 ml of 0.1 N HCl solution containing 20 g/l pepsin in 80 ml centrifuge tubes in three duplicates. The samples were incubated in a water bath at 38°C for 3 h. At the end of incubation, the incubation residues were neutralized with 5 ml of 1N NaOH and centrifuged at 2400 g for 30 min. The supernatant was discarded and 10 ml freshly-made pancreatin solution was added into each tube containing incubation residues. The tubes were then incubated at 38°C in a water bath for 24 h. At the end of incubation, the samples were centrifuged at 2400 g for 15 min and the supernatant was discarded. The residues were used for the determination of TCA-insoluble N.

### *Pepsin-SIF digestion*

About 3.6 g freeze-dried SIF was dissolved in 400 ml phosphate buffer (52 ml 0.2 M  $\text{KH}_2\text{PO}_4$ +348 ml 0.2M  $\text{Na}_2\text{HPO}_4$ ; pH 7.6). The procedures were similar to those of pepsin-pancreatin digestion. In SIF digestion, the volume of SIF buffer used in each tube was 20 ml.

### *Determination and chemical analysis*

All samples were dried at 105°C for 6 h for dry matter (DM) determination. The true protein of the samples was determined using the TCA method (Licitra et al., 1996). The filter paper together with the residues was used for nitrogen determination using the Kjeldahl method.

### Calculation

The digestibility of true protein was calculated as following:

$$\text{uTP - digestibility} = (\text{TP}_1 - \text{TP}_2) / \text{TP}_1 \times 100$$

where: uTP-digestibility refers to utilizable true protein digestibility, %;  $\text{TP}_1$ , true protein content in *in vitro* digesta sample, g;  $\text{TP}_2$ , true protein content in residue after pepsin digestion, pepsin-SIF digestion or pepsin-pancreatin digestion, g.

### Statistical analysis

The EXCELL 2003 was used for one-way analysis of variance of the results between different treatments.

## RESULTS

The results indicated that although uTP digestibility of 48 h pepsin digestion was slightly higher than that of 24 and 72 h pepsin digestion, the difference in uTP digestibility between 24, 48 and 72 h pepsin digestion did not reach significant level ( $P > 0.05$ ).

Table 1. *In vitro* digestibility of utilizable true protein of mixed rations, %DM

Rations	Pepsin			SIF	Pancreatin
	24 h	48 h	72 h		
1	59.41 ± 0.36	59.39 ± 0.73	57.06 ± 0.69	62.14 ± 0.20	74.77 ± 0.13
2	66.42 ± 0.57	65.74 ± 0.77	64.51 ± 0.59	73.29 ± 0.27	75.09 ± 0.26
3	57.59 ± 0.41	59.42 ± 0.65	58.26 ± 0.47	66.99 ± 0.24	75.35 ± 0.26
4	69.82 ± 0.63	70.54 ± 0.07	69.35 ± 0.68	66.30 ± 0.44	76.79 ± 0.23
5	63.86 ± 0.41	63.84 ± 0.47	62.77 ± 0.48	71.45 ± 0.11	77.76 ± 0.15
6	59.26 ± 0.49	59.46 ± 0.51	59.19 ± 0.60	66.72 ± 0.62	72.47 ± 0.40
7	60.54 ± 0.38	61.66 ± 0.49	60.21 ± 0.15	72.89 ± 0.06	74.56 ± 0.00
8	61.93 ± 0.40	61.42 ± 0.77	59.00 ± 0.59	70.67 ± 0.20	75.34 ± 0.23
9	59.23 ± 0.35	59.07 ± 0.60	58.42 ± 0.47	74.53 ± 0.50	79.86 ± 0.11
10	59.87 ± 0.20	60.30 ± 0.41	58.98 ± 0.17	63.64 ± 0.34	81.01 ± 0.35
11	57.59 ± 0.70	59.04 ± 0.85	57.42 ± 0.35	58.61 ± 0.46	80.68 ± 0.35
12	55.60 ± 1.55	52.98 ± 1.01	53.02 ± 0.90	61.69 ± 0.03	76.81 ± 0.12
Mean	60.93 ± 0.81 <sup>a</sup>	61.07 ± 0.90 <sup>a</sup>	59.85 ± 0.88 <sup>a</sup>	67.41 ± 1.04 <sup>b</sup>	76.70 ± 0.53 <sup>c</sup>

values with different superscripts mean significant difference ( $P < 0.05$ )

uTP-digestibility of pepsin-SIF that digestion was significantly higher than of pepsin treatment ( $P < 0.05$ ). The uTP digestibility of pepsin-SIF digestion was about 7 percentage points higher than that of 24, 48 and 72 h pepsin digestion. Results also showed that uTP-digestibility of pepsin-pancreatin digestion was higher than that of pepsin digestion and that pepsin-SIF digestion ( $P < 0.05$ ).

## DISCUSSION

The results implied that the uTP of the samples could be completely digested within 24 h. Longer time digestion would not increase *in vitro* digestibility of uTP. The reasons for the difference between pepsin digestion and pepsin-SIF and pepsin-pancreatin digestion could be that the enzymes and the incubation time were different. In the future, it was necessary to study the reasons for the difference between different treatments and also study the relationship between *in vitro* uTP digestibility and *in vivo* digestibility, so that a suitable *in vitro* method for determining uTP digestibility could be proposed.

## CONCLUSIONS

No significant difference in *in vitro* digestibility of utilizable true protein (uTP) of mixed rations was found between 24, 48 and 72 h pepsin digestion. The *in vitro* uTP digestibility of pepsin-small intestinal fluid (SIF) and pepsin-pancreatin digestion was significantly higher than that of pepsin digestion. Further research is needed to study the reasons for the difference between pepsin digestion, pepsin-SIF digestion and pepsin-pancreatin digestion. It is also necessary to study the relationship between *in vitro* and *in vivo* uTP digestibility of feedstuffs before a suitable *in vitro* technique for determining of uTP digestibility is proposed.

## REFERENCES

- Akeson W.R., Stahmann M.A., 1964. A pepsin pancreatin digest index of protein quality evaluation. *J. Nutr.* 83, 257-261
- Li Y.X., Zhao G.Y., 2007. Prediction of utilizable true protein of mixed rations for sheep using an *in vitro* incubation technique. *Arch. Anim. Nutr.* 61, 203-213
- Licitra G., Hernandez T.M., Van Soest P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Tech.* 57, 347-358
- Tilley J.M.A., Terry R.A., 1963. A two-stage technique for the *in vitro* digestion of forage crops. *J. Brit. Grassl. Soc.* 18, 104-111
- Zhao G.Y., Lebzién P., 2000. Development of an *in vitro* incubation technique for the estimation of the utilizable crude protein (uCP) in feeds for cattle. *Arch. Anim. Nutr.* 53, 293-392