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Nutrient digestibility and ruminal fermentation of an unconventional feed estimated by the *in vivo* techniques

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Abstract

Chemical composition, feed intake and apparent digestibility of medicinal plant residue (MPR) used for ruminants were evaluated.MPR had higher crude protein (CP), crude fat (EE) and lower crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents than those of the roughage feed-peanut vines. Compared to the concentrated feeds, MPR showed a lower CF than that of other feeds except the maize, and NDF and ADF contents were also lower than those of other feeds except the soybean meal. Dry matter intake (DMI), dry matter (DM), organic matter (OM), CP, and EE digestibilities were significantly higher in sheep fed the MPR-based diet compared to the peanut vines-based diet group. However, the NDF and ADF digestibilities were lower in sheep that received MPR-based diet. The energy utilization in sheep fed MPR-based diet is more efficient than that of peanut vines-based diet group. Furthermore, we also found that MPR as a sheep diet showed no negative effect on the rumen fermentation profiles, and it could be expected to increase the microbial protein (MCP) production and reduce the acetate to propionate ratio (A/P). The results presented here clearly indicate the potential of MPR as an unconventional feedstuff for ruminants and could economize the ruminants production.

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Introduction

Nowadays, the critical limitation on profitable animal production in China is the inadequacy and current rising costs of quality conventional forage, because of ever growing human need for food, only limited cultivated land use for forage yield, further aggravated the situation (Liu and Wang, 2008). For sustainable intensification of ruminant industries, animal nutritionists have paid much more attention on the agro-industrial by-products, which are cheaper and available in large quantities, they are potential candidates to be used as unconventional feedstuff for ruminants. Recovering unconventional resources for use as animal feedstuff can reduced the cultivated land use, help food processors save money, while preventing pollution, it is an economical and environmental sound way (Khatunand Khan, 2015).

Chinese herbs as the traditional medicine have been widely used in East Asian countries, and has experienced a history of thousands of years (Liang *et al.,* 2013). The medicinal plant residue (MPR) is a major by-product from the pharmaceutical industry, and it has been well-known that MPR is of interest due to its high nutrient, low cost and availability in large quantities (Li *et al.,* 2010). It is considered tomight be the major source of nutrients for ruminant. Preliminary studies from our lab have revealed that MPR is good source of crude protein, has lower crude fiber, neutral detergent fiber, and acid detergent fiber contents compared with other common conventional roughage feeds, and it could be expected to increase the gas, microbial protein, and volatile fatty acids production, and reduce the acetate to propionate ratio using the *in vitro* ruminal fermentation system (Sheng *et al.,* 2016). As well known, the nutritive value of a ruminant feed is not only determined by the concentrations of its chemical components, but also their rate and extent of digestion (Hamid *et al.,* 2007).Previous studies have also shown that feed intake level is the most important factor affecting animal performance (Mertens, 1994), and voluntary intake of forage together with digestibility is a major determinant of forage quality (Andueza *et al.,* 2011).

However, to date, very little is known about the feed intake and apparent in vivo digestibility of the unconventional feedstuff-MPR.

The objective of this study was to evaluate the substitution of some common conventional feedstuffs such as peanut vines, maize, soybean meal and wheat bran by local unconventional material (MPR) in sheep feeding. The effect on apparent in vivo digestibility and ruminal fermentation were determined to evaluate the nutritional quality of the MPR.

Materials and methods

Animals, management and diets

Twelve sheep (40 ± 2 kg) were used in this experiment. Sheep were housed in an individual cage and had continuous access to fresh water and vitamin-mineral block. They were randomly divided into two groups to create the control and experimental groups. Before exposure to the experimental diets, sheep were given an adjustment period of 7 days. After7 days of adaptation to the environment, animals received a peanut vines-based diet (conventional diet: control group) and a MPR-based diet (unconventional diet: experimental group), and the ingredients, chemical compositions and nutritional levels of these two diets are shown in Table 1. These two diets were formulated according to the NRC guidelines, and were similar nutritionally. Diets were offered in 2 equal amounts twice daily (8:30 and 16:30).

Experimental procedure and sampling

Feed intake and apparent digestibility determination

The experiment conducted in a sequential 20-days period, with 14 days for adaptation to the diet and a 6 days sampling period. After a 14 days adaptation period to the diet, a digestion study of 6 days duration, involving quantitative collection of food offered, refusals and faeces was conducted to determine the apparent digestibility of these diets. The amount of food offered and refused, and the total amount of faeces excreted were measured for every 24 h (at 8:30 prior to feeding) during the6 days of digestion periods successively.

The food offered, refusals and faeces dried at 65°C in an oven to constant weight. Then the daily samples of food offered, refusals and faeces were mixed for the total collection period, and ground though a 1-mm screen (Wiley mill) for chemical analysis.

Ruminal fermentation

At the last day of the sampling period, the rumen fluid samples were collected immediately before the morning feeding (0 h) and then at 3 h and 6 h after feeding. At these times, a 200-mL sample of rumen contents was collected via the rumen fistula and strained through a four layer of cheesecloth.

The rumen fluid pH was immediately determined with a multi-parameter pH meter (Sartorius PB-10, Sartorius, Gottingen, Germany). A 5 ml of the rumen fluid was added to 5 ml of deproteinizing solution for VFA analyses (Agarwal *et al.*, 2009), and 2 ml was added to 2 ml of 0.5 M HCl for ammonia-N (NH_3-N) dertermination (Wheatherburn, 1967). Concentrations of the microbial protein (MCP) production were determined based on purines using the method of Zinn and Owens (1986), modified by Makkar and Becker (1997), and estimated from the ratio of purines to N of isolated bacteria with yeast RNA as standard.

Analytical methods

Samples of food offered, refusals and faeces were analyzed for dry matter (DM) by drying samples at 105 °C for 24 h (Horwitz, 2000). Crude protein (CP) was determined according to Kjeldahl method using 2400 Kjeltecanalyser unit (Foss tecator) and the crude fat (EE) content was determined by the ether extraction method according to AOAC (1990) (Horwitz, 2000). Crude fiber (CF), the neutral detergent fiber (NDF) and acid detergent fiber (ADF) was analysed according to Van Soest *et al.* (1991). The gross energy (GE) content of both feed and faecal samples was determined using a oxygen bomb calorimeter. Organic matter (OM) was determined as loss on ignition at 550°C for 6 hours. Chemical analyses were performed in triplicate.

DM intake (DMI) and apparent digestibility was calculated by using the following formula:

DMI (g/kg/d)=(DM in offered-DM in refusals)/live weight/day

% DM digestibility (DMD)=100×(% DM in offered-% DM in refusals-% DM in faeces)/ (% DM in offered-% DM in refusals)

% CP digestibility (CPD)=100×(% CP in DMI-% CP in faeces)/ % CP in DMI

The apparent digestibilities of the OM, EE, NDF, and ADF were calculated subsequently by using the approach for CP digestibility.

% efficiency of gross energy utilization (GEE)=100×(% GE in DMI-% GE in faeces)/ % GE in DMI

Results

Chemical composition

Chemical compositions of roughage feeds (peanut vines and MPR) and concentrated feeds (maize, soybean meal, and wheat bran) were shown in Table 1.There were observed variations in the chemical composition of the different feedstuffs, with DM ranging from 26.00 % to 91.30 %, CP from 10.17 % to 44.62 %, EE from 1.50 % to 7.26 %, CF from 1.20 % to 29.60 %, NDF from 13.14 % to 60.40 %, and ADF from 9.64 % to 49.65 %. Amongroughage feeds, MPR is high in CP(13.50 %) and EE (7.26 %), whereas peanut vines is rich in CF (29.60 %), NDF (60.40 %) and ADF (46.34 %)(Table 2), indicating thatthe MPR showed higher nutritive quality. Compared to the concentrated feeds, the MPR(13.50 %) showed a lower CP content than those of other feeds except the maize (10.17 %), whereas the EE (7.26 %) and CF (18.95 %) in MPR were higher than the others. For NDF and ADF, the MPR (33.03 % and 24.33 %) showed lower contents than those of other feeds except the soybean meal (13.14 % and 9.64 %)(Table 2).

Feed intake and apparent digestibility determination

Average values of dry matter intake (DMI) and the efficiency of gross energy utilization obtained in each group are shown in Table 3. For DMI values, experimental group showed significantly higher value than that of control group (41.03±0.75 g/kg/d vs 33.75±0.51g/kg/d) (Table 3).

And in the experimental group, the energy utilization in the rumen is more efficient than that of control group (72.68±1.08 % vs 69.26±3.89 %) (Table 3).

Using the total collection method, apparent digestibilities of DM, OM, CP, EE, NDF, and ADF of control group and experimental group were determined. As shown in Table 3, we found that DM, OM, CP, and EE digestibilities were better in the experimental group compared to the control group (75.56 % vs 49.93 %, 77.1 % vs 53.7 %, 67.62 % vs 58.96 %, and 86.30 % vs 66.15 %) (Table 3).

However, sheep in the experimental group showed a significant depression for the digestibility of NDF and ADF (7.51 % vs 75.83 % and 49.57 % vs 61.40 %) (Table 3).

Ruminal fermentation

pH, NH_3-N, and MCP

As shown in Figure 1, the ruminal pH in these two groups showed the similar variation tendency, they all decreased during the 3 hrs after the morning feeding, and then increased (Figure 1). The ruminal pH for experimental group was higher than those found with the control group but only at 0 and 3 h after the morning meal, and nearly similar pH at 6 h after feeding.

Table 1. Ingredients, compositions and nutritional levels of these two diets.

Control group				Experimental group			
Item	Content g/d	DM g/d	Percentage %	Item	Content g/d	DM g/d	Percentage %
Maize	440.4	385	19.90	MPR	6153.85	1600	92.72
Soybean meal	27.64	25	1.29	Soda	61.54	61.54	3.57
Wheat bran	11.18	10	0.52	Premix	64	64	3.71
Peanut vines	1577.22	1440	74.44				
Premix	74.4	74.4	3.85				
Total	2130.84	1934.4	100	Total	6279.39	1725.54	100
Nutritional level				Nutritional level			
Item	DE MJ/d	ME MJ/d	CP content g/d	Item	DE MJ/d	ME MJ/d	CP content g/d
Maize	5.47	4.49	39.15	MPR	20.16	16.53	208
Soybean meal	0.36	0.30	11.16				
Wheat bran	0.12	0.10	1.78				
Peanut vines	13.65	11.12	158.4				
Total	19.60	16.07	210.49	Total	20.16	16.53	208

Note: DE: digest energy; ME: metabolism energy; CP: crude protein; DM: dry matter; MPR: medicinal plant residue.

Table 2. Chemical composition of roughage and concentrated feeds.

Items	DM %	CP %	EE %	CF %	NDF %	ADF %
Peanut vines	91.30	11.00	1.50	29.60	60.40	46.34
MPR	26.00	13.50	7.26	18.95	33.03	24.33
Maize	87.42	10.17	3.43	1.20	37.39	49.65
Soybean meal	90.46	44.62	3.30	5.20	13.14	9.64
Wheat bran	89.44	17.74	3.16	6.80	46.73	45.26

Note: DM: dry matter; CP: crude protein; EE: crude fat; CF: crude fiber; NDF: neutral detergent fiber; ADF: acid detergent fiber; MPR: medicinal plant residue.

For NH_3-N, these two groups also showed a similar tendency, they all decreased at the first 3 hrs after feeding, and then increased.

At 0 and 6 h after feeding, the NH_3-N content in experimental group was significantly higher than that of the control group, however, at 3 h after feeding, a opposite phenomenon was observed (Figure 2).

Changes of the MCP contents were not obvious during the 6 hrs after the morning feeding. For the experimental group, there was a slightly increase during the first 3 hrs after feeding, and then decreased. But for the control group, the MCP content increased continuously during the 6 hrs after morning feeding.

And the MCP contents for experimental group were higher than those found with the control group at all times (Figure 3).

VFA production

As shown in Figure 4, the total VFA, acetate, propionate, and butyrate contents in both two groups showed inconspicuous changes at all times. The total VFA contents in both groups showed a similar tendency, they all increased during the first 3 hrs after feeding, and then decreased.

Table 3. Dry matter intake and apparent digestibility of sheep fed two different diets.

Items	DMI (g/kg/d)	DMD %	OMD %	CPD %	EED %	NDFD %	ADFD %	GEE %
Experimental group	41.03±0.75	75.56±0.45	77.1±1.1	67.62±4.36	86.30±4.62	7.51±0.09	49.57±2.01	72.68±1.08
Control group	33.75±0.51	49.93±2.91	53.7±2.4	58.96±4.92	66.15±4.63	75.83±1.93	61.40±2.39	69.26±3.89
P value	<0.05	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01	

Note: DMI: dry matter intake, DMD: dry matter digestibility, OMD: organic matter digestibility, CPD: crude protein digestibility, EED: crude fat digestibility, NDFD: neutral detergent fiber digestibility, ADFD: acid detergent fiber digestibility, GEE: efficiency of gross energy utilization.

The acetate contents in both two groups showed a little different tendency. There was no change in acetate content in the control group at 0 and 3 h after feeding, during the second 3 hrs after feeding, acetate content decreased.

For the experimental group, the acetate content decreased continuously during the 6 hrs after feeding. For propionate contents, they all increased during the 6 hrs after feeding in the both two groups.

The butyrate contents for the control group increased continuously during the 6 hrs after feeding, but for the experimental group, the butyrate content first increased and then decreased (Figure 4).

Furthermore, we also found an interesting phenomenon in this study, the total VFA, acetate, propionate, and butyrate contents for experimental group were lower than those of control group at all times.

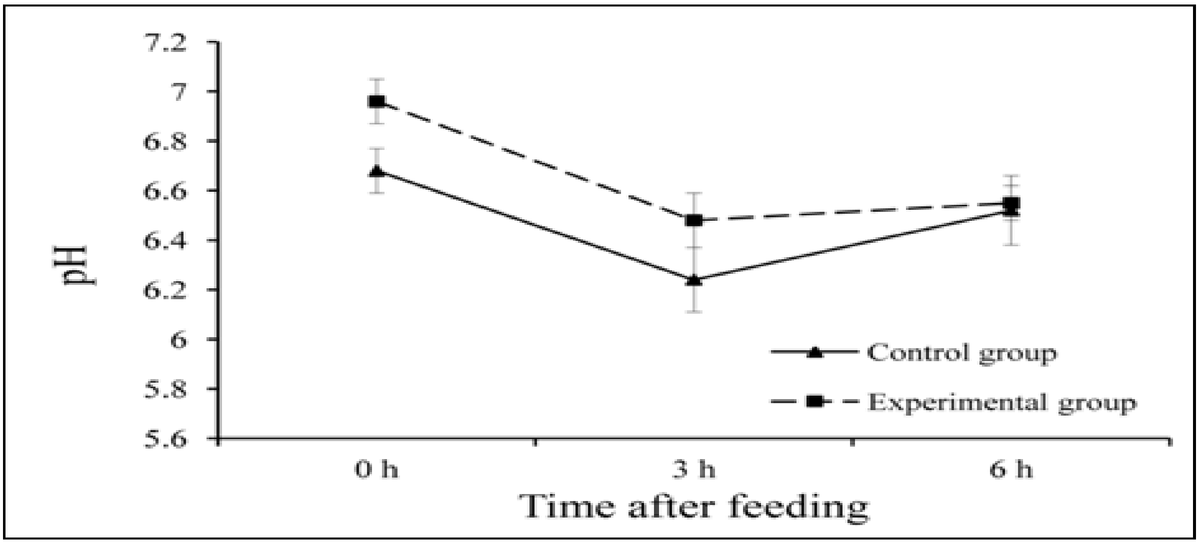

Fig. 1. Effect of MPR-based and peanut vines-based diets on ruminal Ph.

As shown in Figure 5, there was an decrease in acetate to propionate ratio (A:P) in the control and experimental groups, and at all times, the A:P ratio of experimental group was lower than of the control group (Figure 5).

Discussion

Forage quality

Crude protein and cell wall constituents (like crude fiber, neutral detergent fiber, and acid detergent fiber) are used to evaluate the nutritive quality of forages (Robinson and Mcqueen, 1997), the higher crude protein and lower cell wall contents indicate the higher nutritive quality of forages. From our results, we found that CP and EE contents in the MPR are higher than those of other roughage and some concentrated feeds, and the NDF content was lower than those of other feeds except the soybean meal, which indicate that the MPR supply more available energy for microbial growth and might be easily degraded.

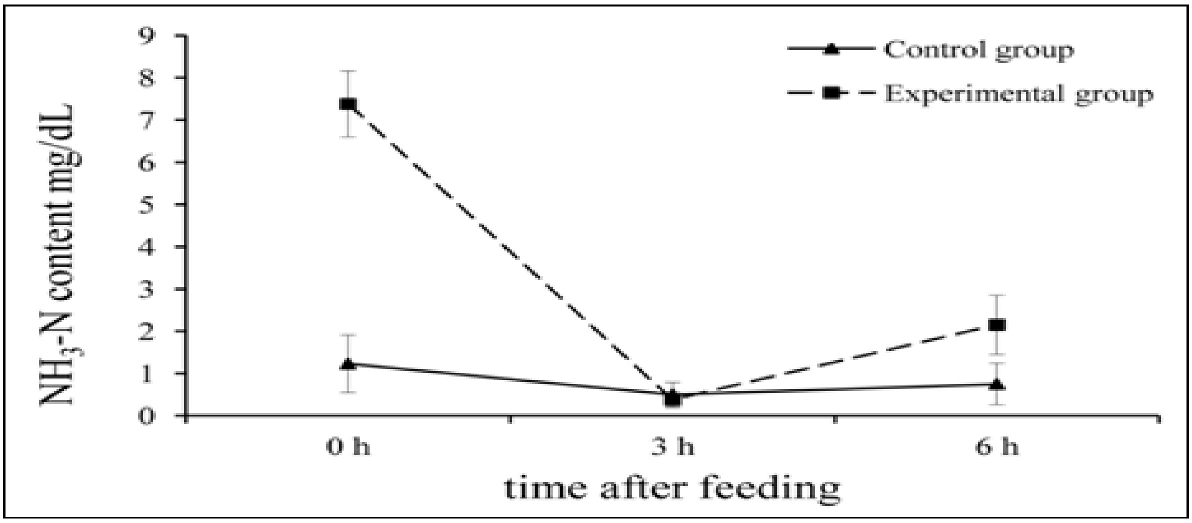

Fig. 2. Effect of MPR-based and peanut vines-based diets on ruminal NH_3-N.

Our previous in vitro ruminal fermentation results also showed that MPR was fermented faster than other roughage forages due to the presence of more soluble materials (data were not shown).

Apparent digestibility

Measurement of digestibility in sheep is the reference method for determining the nutritive value of forage in most of the feeding evaluation systems developed for ruminant nutrition (Van Soest, 1994). Aregheore *et al.* (1991) observed that the higher the cell wall constituents (crude fiber) of diets, the lower the DM digestibility values, besides, previous studies also demonstrated a highly negative effect of NDF (structural carbohydrate) on the digestibility of forages (Iantcheva *et al.,* 1999; Elghandour *et al.,* 2014). Our present study further confirmed those findings, the sheep fed completely on unconventional diet (MPR-based diet) showed a significant higher DM digestibility than the sheep which fed on conventional diet (peanut vines-based diet). However, the NDF and ADF digestibilities in the MPR-based diet group were lower than those of in the peanut vines-based diet group, this phenomenon could be due to two reasons, one is the rapid non-structural carbohydrate (starch and soluble sugars) degradation, diets with higher concentrations of non-structural carbohydrate and rapid degradation may depress fiber degradation as a result of lower ruminal passage rates and changes in ruminal microbial populations (McAllister *et al.,* 1990; Benchaar *et al.,*2007), and another reason is the shorter MPR retention time in the digestive system (Aregheore, 2000), retention time in the rumen is regulated by rumination that is required to comminute lignified fibrous (Van Soest, 1985), previous work also indicated that the feed retention time in rumen digesta affects its degradation efficiency (Goopy *et al.,* 2014).Our previous in vitro ruminal fermentation work also confirmed the above view, our results showed that the rate of MPR digestion was faster than those of some other roughage feeds, just like peanut vine, rice straw, etc.

The faster digestion rate of MPR could result in a shorter feed retention time in the rumen (Poppi *et al.,* 1981). Besides that, we also found that the efficiency of dietary gross energy utilization obtained in sheep fed peanut vines-based diet was lower than that of in sheep fed MPR-based diet, this could be due to fiber values present in the peanut vine diet (Aregheore, 2000).

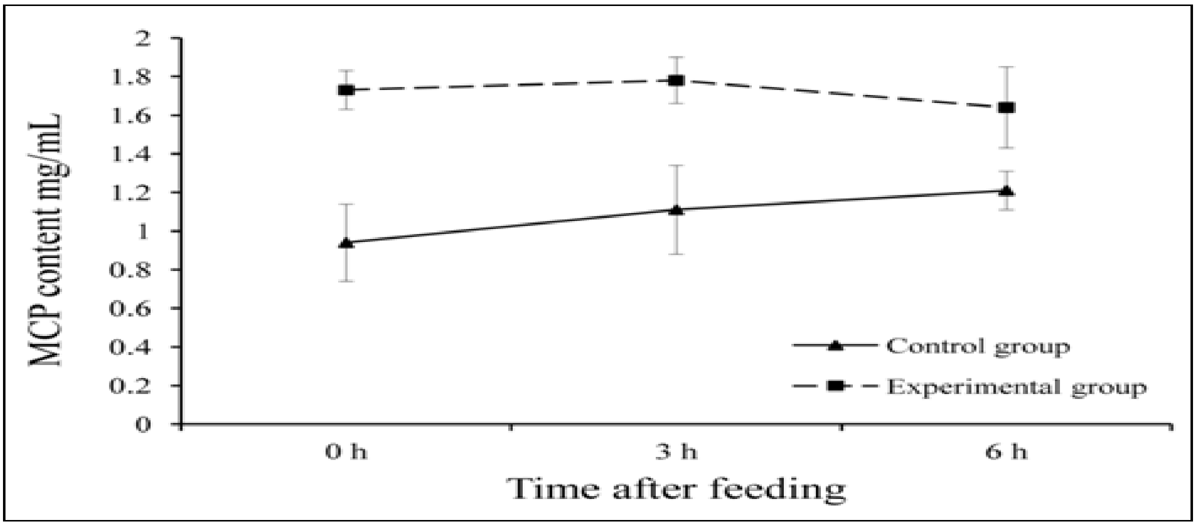

Fig. 3. Effect of MPR-based and peanut vines-based diets on ruminal MCP.

This is consistent with the findings reported by Donefer *et al.* (1963) and Olatunji *et al.* (1976), who also showed that increase in the fiber levels of diets decreased the efficiency of dietary gross energy utilization.

Furthermore, the nutritive value of MPR depends not only on their digestibility, but also on the amount of voluntary intake by an animal. Previous work with sheep showed that the higher feed intake was associated with the shorter feed retention time in the rumen (Laredo and Minson, 1973). In this trial similar trends were observed. This could be due to the faster digestion rate of MPR than that of peanut vines. Poppi *et al.* (1981) have indicated that the faster digestion rate could result in a shorter feed retention time in the rumen and a higher feed intake (Poppi *et al.,* 1981).

Ruminal fermentation

As we know, ruminal pH in digesta has been used as indicators of intestinal health, and it will drop below physiological levels when ruminants consume excessive amounts of rapidly fermentable (non-fiber) carbohydrates (Owens *et al.,* 1998; Krause and Oetzel, 2006). Previous studies in our group indicated that the MPR contains higher contents of rapidly fermentable materials than some other roughage feeds, such as peanut vines, rice straw, etc. However, our present study results showed that the MPR as a sheep diet showed no negative effect on their rumen pH, the pH in sheep fed the MPR-based diet was still in the normal range, and within a common shift during the digest process of a day. Previous studies suggested that shifts of 0.5-1.0 pH unit are common within a 24 h period (Nocek *et al.,* 2002).

Protein degradability is considered to be positively correlated with the ammonia-N, amino acids and peptides production in rumen (Taminga, 1983), and all of which could satisfy the microbial needs for N, maximize rate of fermentation in the rumen and finally enhance the synthesis of microbial protein in the rumen (Salman *et al.,* 2014).

A similar phenomenon was found in the present study, the increase in ruminal MCP concentration observed in experimental group is consistent with the higher ruminal ammonia-Ncontent and higher crude protein digestibility in this group. MCP is considered as an important protein resources for the ruminants, over half of the amino acids absorbed by ruminants, and often two-thirds to three-quarters, derive from microbial protein (Agricultural and Food Research Council, 1992).

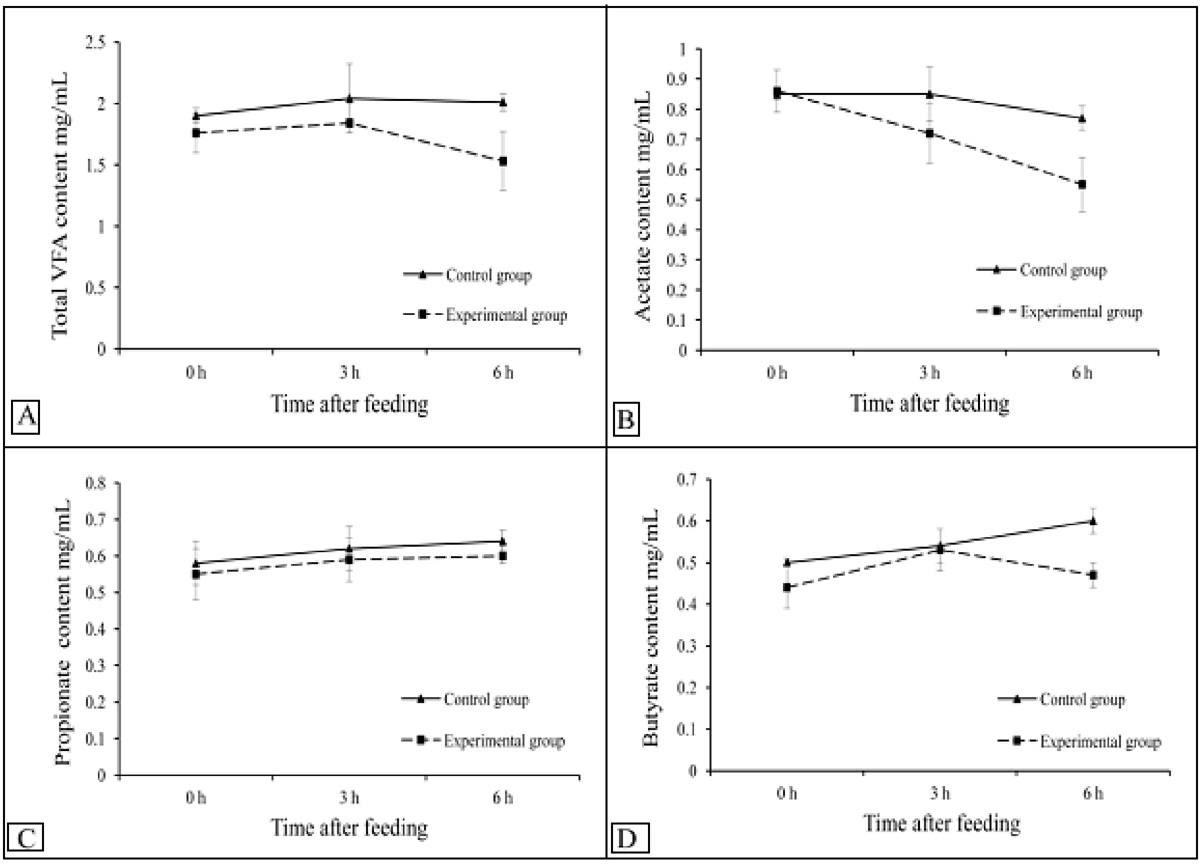

Fig. 4. Effect of MPR-based and peanut vines-based diets on ruminal VFA A: total VFA content, B: acetate content, C: propionate content, D: butyrate content.

Previous studies have reported that reduction in NDF digestibility have reported reduction in A:P ratio (Castillejos *et al.,* 2006), and starch and sugars fermentation by rumen microbes would produce more propionic acid (Dijkstra, 1994). In the present study, the A:P ratio of sheep fed the MPR-based diet was lower than that of sheep fed the peanut vines-based diet, a larger quantity of starch and soluble sugars were probably fermented in the rumen of sheep fed MPR-based diets, which would contribute to explaining the lower A:P observed in sheep fed MPR-based diets compared with those fed peanut vines-based diets (Benchaar *et al.,* 2007). Besides that, the A:P ratio was considered as a good indication of the energy and health of ruminants, because of the positive relationship between A:P ratio and methane production (Lana *et al.,* 1998; Russell, 1998), our present result could also indicated that the MPR-diet group could improve the feed efficiency compared with the peanut vines-based group. But this still need to be substantiated by methane detection.

Volatile fatty acids (VFA) are produced by fermentation of organic matter, they can have a major effect on production and product composition in ruminants (Dijkstra, 1994). However, in our present study, a interestingly phenomenon was observed, the total VFA, acetate, propionate, and butyrate contents for experimental group were lower than those of the control group, but the OM digestibility of the former group was significantly higher than that of the latter one, this could be due to the VFA absorption rates of the rumen. Previous study has also indicated that the proportions of VFA in rumen fluid do not accurately represent the proportions in which they are formed, the VFA absorption rates should be taken into account in estimating VFA production rates in the rumen (Dijkstra, 1994).

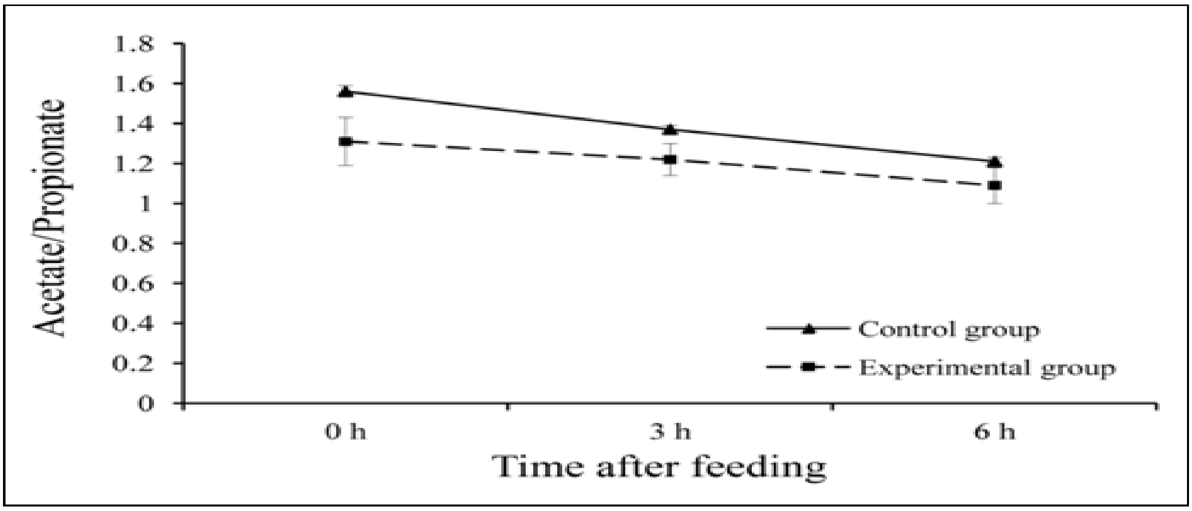

Fig. 5. Effect of MPR-based and peanut vines-based diets on acetate to propionate ratio.

Conclusion

MPR showed higher CP and lower CF, NDF and ADF contents than the common roughage feed-peanut vines. And sheep fed the MPR-based diet exhibited higher DMI, DM, OM, CP and EE digestibilities and efficiency of gross energy utilization than the control group.MCP content for sheep fed MPR-based diet was higher than that of the peanut vines-based diet group, and the A:P ratio for the former group was lower than that of the latter one. On the basis of chemical composition, apparent digestibility of nutrients and ruminal fermentation of peanut vines-based diets and MPR-based diets, the MPR proved to be excellent unconventional feedstuff for ruminants, equivalent to or better than some conventional feedstuff like peanut vines, it could be incorporated in feed mixture to replace conventional roughage sources in ruminant diets without major problem. However, further studies are still required and long-term animal feeding tests should be carried out to investigate the effect of using MPR as a ruminants feedstuff on their physiology and health status.

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