



Nutrition evaluation of an unconventional for ruminants estimated by the *in vitro* fermentation system

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Abstract

For sustainable intensification of ruminant industries, it would be wise to explore the potential of unconventional feedstuff resources. Chinese medicine residue (CMR) is of interest due to its high nutrient, low cost and large quantities, indicating its potential as an unconventional feedstuff for ruminants. In this study, *in vitro* ruminal fermentation technique was used to evaluate the potential ruminant feedstuff. The chemical analysis showed that CMR had higher concentrations of crude protein and lower concentrations of crude fiber, neutral detergent fiber and acid detergent fiber than those of some conventional feedstuff, such as peanut vines, chinese sedge and rice straw, indicating its high nutritional quality. Cumulative gas production was recorded at 6, 12, 18, 24 and 48 h of incubation periods and the pH, ammonia-N (NH₃-N), microbial protein production (MCP) and volatile fatty acid (VFA) were estimated at 48 h of incubation period. Our results showed that the fermentation gas volume was greatest in CMR group, followed by peanut vines and chinese sedge group, and the gas volume in rice straw group was only 53.60 % of that from CMR group at 24 h. For MCP and VFA, CMR group also showed the highest value (MCP: 1.34 mg/ml; VFA: 4.33 mg/ml) than other groups, whereas rice straw group showed the least value (MCP: 1.03 mg/ml; VFA: 3.09 mg/ml). Besides that, we also found that the acetate to propionate ratio (A/P) was significantly lower in CMR group (1.21) than others. Based on these results therefore, Chinese medicine residue could be a valuable alternative unconventional animal feed source in ruminant feeding and could economize the ruminants production.

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Introduction

In recent years, since the competition for grain between people and livestock, China ruminant industries are faced with the problems of meeting the nutritional requirement of the animals, lack of feed grain is one of the main choke points in livestock production (Liu and Wang, 2008). For sustainable intensification of ruminant industries, it is imperative to explore the potential of unconventional feedstuff resources. This is of economic and environmental benefits leading to a reduction in the cost of diets and consequently to a reduction in the production cost.

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). It has been well-known that Chinese medicine residues are of interest due to high nutrient, low cost and availability in large quantities (Li *et al.*, 2010). They are considered to be the major sources of nutrients for ruminant. However, very little is known about their nutrition evaluation.

The nutritive value of a ruminant feed is determined by the concentrations of its chemical components, as well as their rates and extent of digestion (Getachew *et al.*, 2004). Determining the digestibility of feeds *in vivo* is laborious, expensive, requires large quantities of feed, and is largely unsuitable for single feedstuff thereby making it unsuitable for routine feed evaluation (Coelho *et al.*, 1998). *In vitro* method of feed evaluation is less expensive, less time consuming and more rapid alternative (Getachew *et al.*, 2004; Akinfemi *et al.*, 2009). Usually, the use of *in vitro* fermentation methodology to estimate the ruminant feed is based on the gas production, and in combination with the feed's chemical composition (Menke and Steingass, 1988).

This present study was conducted to investigate Chinese medicine residue for assessment as potential sustainable feed for ruminants by comparing with some commonly used roughage forages using chemical analysis and *in vitro* rumen fermentation methods.

Materials and methods

Sample collection

Dried samples of commonly used forages (peanut vines, Chinese sedge, rice straw) were collected from the San Wang animal husbandry company, Le ping, Jiangxi, China. And the Chinese medicine residue was collected from a company in Nanchang, Jiangxi, China. All the samples were mill through a 1 mm screen and oven-treated at 65°C until a constant weight was obtained for dry matter determination.

Chemical analyses

Samples of peanut vines, Chinese sedge, rice straw and Chinese medicine residue were analysed for proximate analysis. Dry matter (DM) was determined at 105 °C for 24 h (Horwitz, 2000). Crude protein (CP) was determined according to Kjeldahl method using 2400 Kjeltex analyser 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 fibre (NDF) and acid detergent fibre (ADF) was analysed according to Van Soest *et al.* (1991). Chemical analyses were performed in triplicate.

In vitro incubation with sheep ruminal fluid

Five sheep, maintained on a basal diet, were used to provide rumen contents. After a adaptation period, approximately 500 ml of rumen contents was collected from each sheep using a rumen fluid sampling pump on the same day before the morning meal. The rumen liquid was immediately mixed with CO₂ to avoid O₂ contamination and transferred to the laboratory in a thermostatic box (39°C) under anaerobic conditions. The rumen liquid was then filtered through four layers of cheesecloth into a flask under a continuous flow of CO₂. An aliquot of the rumen liquid was buffered (1:3, v/v) by adding an artificial saliva solution (McDougall, 1948). Feeds (0.4 g of DM) were incubated in triplicate with 40 ml of inoculum. Gas production was measured over the range of 0-48 h of incubation at 6 h intervals. At the end of incubation period (48 h), pH, methane (CH₄), volatile fatty acid (VFA), ammonia-N (NH₃-N) and microbial protein production (MCP) contents were measured.

Effects on fermentation parameters

Fermented medium pH was determined with a multi-parameter pH meter (Sartorius PB-10, Sartorius, Gottingen, Germany). At the end of incubation (48 h), 1 ml of the fermented medium was collected in Eppendorf tubes containing 0.20 ml of 25 % metaphosphoric acid. The mixture was allowed to stand for 2 h at room temperature and centrifuged at 5,000×g for 10 min. The analysis of VFA was conducted according to the method described by Agarwal *et al.* (2009). The ammonia-N was analysed in the fermented medium as per the procedure described by Wheatherburn (1967). Concentrations of the MCP 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.

Statistical analysis

Data on chemical composition and fermentation parameters were analyzed using the one-way analysis of variance (ANOVA) procedure with SPSS 16.0.

Multiple comparison tests used Duncan's multiple-range test (Snedecor and Cochran, 1980).

The correlations between chemical constituents and fermentation products were established by calculating Pearson's correlation coefficient using SPSS 16.0.

Results

Chemical composition

Chemical compositions of peanut vines, Chinese sedge, rice straw and Chinese medicine residue were shown in Table 1. There were observed variations in the chemical composition of the different fermentation substrates, with DM ranging from 26.00 % to 91.77 %, CP from 6.20 % to 13.50 %, EE from 1.00 % to 7.26 %, CF from 18.95 % to 29.60 %, NDF from 33.03 % to 67.50 %, and ADF from 24.33 % to 46.34 %. Among them, the Chinese medicine residue showed the highest CP (13.50 %) and EE (7.26 %), the lowest CF (18.95 %), NDF (33.03 %) and ADF (24.33 %) (Table 1), which indicated its good feeding value for ruminants in terms of feed intake and digestibility and might be another good forage for ruminant.

Table 1. Chemical composition of fermentation substrates.

Items	Dry matter DM %	Crude protein CP %	Crude fat EE %	Crude fiber CF %	Neutral detergent fiber NDF %	Acid detergent fiber ADF %
Peanut vines	91.30	11.00	1.50	29.60	60.40	46.34
Chinese sedge	91.77	10.11	4.96	24.40	66.21	35.72
Rice straw	90.30	6.20	1.00	27.00	67.50	45.40
Chinese medicine residue	26.00	13.50	7.26	18.95	33.03	24.33

Effects on fermentation parameters

In vitro gas production

The fermentation gas accumulation curves displayed a nonlinear trend over the incubation period, the accumulated gas production reached asymptote around 24 h of incubation except rice straw group, gas production in rice straw group increased continuously over 48 h of incubation. Among these substrates, the fermentation gas accumulation rate and volume was greatest in Chinese medicine residue, followed by peanut vines and chinese sedge, the gas volume of rice straw was the least one, it was only 53.60 % of that from Chinese medicine residue at 24 h (Fig. 1).

pH, NH₃-N, and MCP

The pH varied in different groups, but was still in the normal range throughout the incubation, ranging from 6.44 to 6.57 (Table 2). Among these groups, fermenters with Chinese medicine residue as substrate had a significant lower pH value than the other substrates ($P < 0.01$). NH₃-N was significantly increased in Chinese sedge group (6.54 mg/dL, $P < 0.01$). No significant difference was observed among Chinese medicine residue (6.08 mg/dL), peanut vines (6.05 mg/dL) and rice straw (5.90 mg/dL) groups. Among all groups, MCP in Chinese medicine residue group was significantly higher than that in other groups (1.34 mg/ml), Rice straw group showed the least value (1.03 mg/ml) (Table 2).

Table 2. Effects of different substrate on pH, NH₃-N, and MCP concentration *in vitro* ruminal fermentation.

Items	pH	NH ₃ -N (mg/dl)	MCP (mg/ml)
Peanut vines	6.54a	6.05b	1.23ab
Chinese sedge	6.57a	6.54a	1.18ab
Rice straw	6.57a	5.90b	1.03b
Chinese medicine residue	6.44b	6.08b	1.34a
<i>P</i>	<0.01	<0.01	<0.01

In vitro VFA production

The effects of peanut vines, chinese sedge, rice straw, and Chinese medicine residue on concentration of VFA were shown in Table 3. Fermenters with chinese sedge and Chinese medicine

residue as substrate produced significantly higher concentrations of total VFA than others, with the value of 4.40 and 4.33 mg/ml. Fermenters with rice straw produced the least total VFA concentration (3.09 mg/ml).

Table 3. Effects of different substrate on volatile fatty acids concentration *in vitro* ruminal fermentation.

Items	Total VFA mg/ml	Acetate mg/ml	Propionate mg/ml	Butyrate mg/ml	Acetate / Propionate A/P
Peanut vines	3.26b	1.45b	1.08c	0.73c	1.34a
Chinese sedge	4.40a	1.96a	1.55b	0.89a	1.26b
Rice straw	3.09b	1.32c	1.00c	0.77b	1.32a
Chinese medicine residue	4.33a	1.92a	1.59a	0.83ab	1.21c
<i>P</i>	<0.01	<0.01	<0.01	<0.01	<0.01

Table 4. Correlation between chemical composition, gas production, ammonia-N, and microbial protein production.

	Gas production			NH ₃ -N	MCP
	6 h	24 h	48 h		
CP	0.969*	0.989**	0.902*	0.260	0.998**
EE	0.891	0.692	0.663	0.462	0.756
CF	-0.769	-0.534	-0.620	-0.204	-0.590
NDF	-0.887	-0.841	-0.955*	0.172	-0.839
ADF	-0.874	-0.670	-0.701	-0.307	-0.726
NH ₃ -N	-	-	-0.159	-	0.204
MCP	-	-	0.926*	0.204	-

Note: CP: crude protein, EE: crude fat, CF: crude fiber, NDF: neutral detergent fibre, ADF: acid detergent fibre, MCP: microbial protein production, * $P < 0.05$, ** $P < 0.01$.

The concentration of acetate in these different fermenters ranged from 1.32 to 1.96 mg/ml, among them, chinese sedge (1.96 mg/ml) and Chinese medicine residue (1.92 mg/ml) groups showed the higher concentrations, rice straw group showed the least value (1.32 mg/ml).

Propionate concentrations were higher in Chinese sedge and Chinese medicine residue groups, with concentrations reaching as high as 1.55 and 1.59 mg/ml, respectively. The rice straw group was the least one (1.00 mg/ml).

The concentration of butyrate produced in the different groups also showed a similar trend to the former acids, Chinese sedge (0.89 mg/ml) and Chinese medicine residue (0.83 mg/ml) groups produced the higher concentrations than other groups, the peanut group (0.73 mg/ml) showed the least one (Table 3).

The acetate to propionate ratio (A/P) was significantly lower in Chinese medicine residue group (1.21) than others. The peanut and rice straw groups showed the higher ratio (1.34 and 1.32, respectively) (Table 3).

Relationship between chemical constituents and fermentation products

The CP was positively correlated with 6 h ($P<0.05$), 24 h ($P<0.01$), 48 h ($P<0.05$) gas production, and MCP ($P<0.01$). NDF was negatively correlated ($P<0.05$) with 48 h gas production, however the MCP showed a significant positive correlation with 48 h gas production ($P<0.05$) (Table 4).

Positive correlations ($P<0.05$) were observed between EE level and total VFA, acetate, and propionate production. However there was a negative association between EE and A/P ratio ($P<0.05$). Besides that, we also found the A/P ratio was positively affected by CF and ADF ($P<0.01$). And propionate showed negative correlation with ADF ($P<0.05$) (Table 5).

Table 5. Correlation between chemical composition, volatile fatty acid, ammonia-N, and microbial protein production for feeds incubated in buffered rumen fluid for 48 h.

	VFA				A/P
	Total	Acetate	Propionate	Butyrate	
CP	0.653	0.684	0.699	0.243	-0.635
EE	0.934*	0.928*	0.963*	0.731	-0.978*
CF	-0.792	-0.766	-0.836	-0.646	0.981**
NDF	-0.493	-0.492	-0.573	-0.130	0.741
ADF	-0.863	-0.849	-0.905*	-0.658	0.988**
Gas 48 h	0.412	0.430	0.489	-0.020	-0.585
NH ₃ -N	0.746	0.760	0.682	0.821	-0.384
MCP	0.622	0.653	0.673	0.203	-0.629

Note: CP: crude protein, EE: crude fat, CF: crude fiber, NDF: neutral detergent fibre, ADF: acid detergent fibre, MCP: microbial protein production, VFA: volatile fatty acid, * $P<0.05$, ** $P<0.01$.

Discussion

Forage quality

Crude protein and crude fiber are used to evaluate the nutritive quality of forages (Robinson and Mcqueen, 1997), the higher crude protein and lower crude fiber contents indicate the higher nutritive quality of forage. Besides, previous studies also demonstrated a highly negative effect of neutral detergent fibre (NDF) on the digestibility of forages (Iantcheva *et al.*, 1999; Elghandour *et al.*, 2014).

This study has provided interesting information on the nutritive value of Chinese medicine residue for use as a ruminant feed.

From our results, we found that the Chinese medicine residue might be easily degraded and was considered to be of good to high quality based on high CP concentration and low CF, NDF and ADF concentrations, indicating it may be as a potential feed for ruminants in China in the future.

Effects on *in vitro* fermentation parameters

To our knowledge, *in vitro* gas production is used to measure fermentation properties of ruminant feedstuffs (Rymer *et al.*, 2005; Posada *et al.*, 2012).

Gases can be regarded as an indicator of soluble and insoluble carbohydrates degradation, and are important end products of microbial fermentation (Wolin *et al.*, 1979; Menke and Steingass, 1988). Some previous studies also showed that gas arises directly from microbial degradation of feeds (Getachew *et al.*, 2004). By using *in vitro* fermentation technique, we found the gas production was greatest in Chinese medicine residue group, and least in rice straw group. This demonstrates that this

substrate may be more easily degraded by microorganisms than other common feedstuffs. Besides that, we also found the gas production increased with the decrease of NDF content of these forages. The decrease of NDF led to the increase of soluble carbohydrate fractions in forages, which could be easily utilized by microorganisms, this may be caused the increase of *in vitro* gas production in this study (Chumpawadee *et al.*, 2007). Our result is also in agreement with studies on the fermentation of tropical trees and several ruminant feeds, a negative relationship between NDF level and gas production was also reported (Nsahlai *et al.*, 1994; Larbi *et al.*, 1998; Getachew *et al.*, 2004).

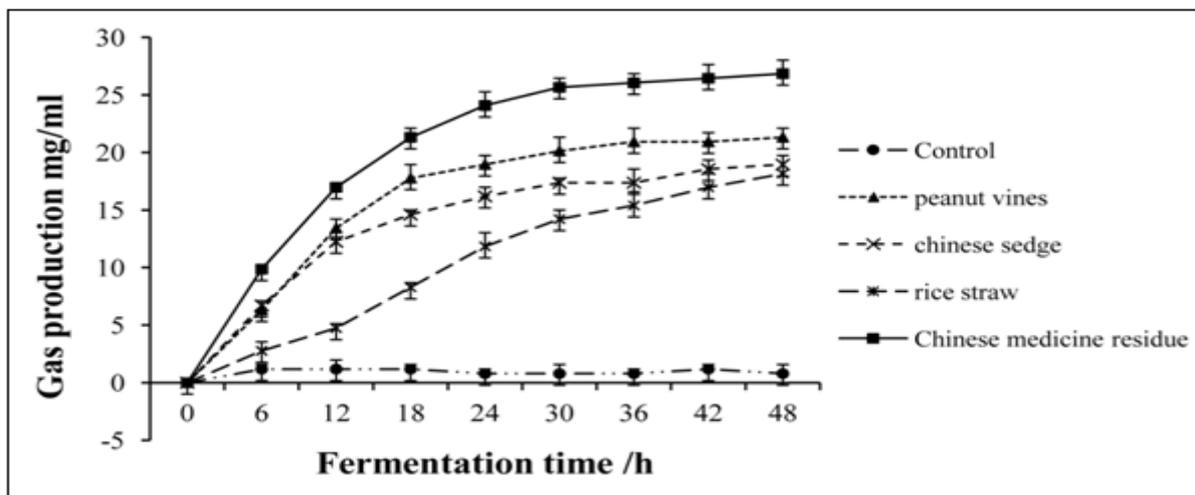


Fig. 1. Effects of different substrate on gas production *in vitro* ruminal fermentation.

pH in digesta has been used as indicators of intestinal health. Previous study showed that maintenance of rumen pH and prevention of rumen acidosis is important for efficient productivity, rumen function, and animal health (Owens *et al.*, 1998). In the present study, we found that the pH in the Chinese medicine residue group was still in the normal range throughout the whole incubation, suggesting the Chinese medicine residue as a fermentation substrate showed no negative effect on the rumen pH.

Nowadays, ruminant nutritional research has been focused on decreasing ammoniogenesis (Patra and Yu, 2014). Excessive amino acid deamination in the rumen are nutritionally

wasteful processes because more ammonia is produced than rumen microorganisms can utilize (Firkins *et al.*, 2007). In the present study, the Chinese medicine residue group produced a relatively lower ammonia-N, suggesting that it might decrease deamination of dietary amino acids (Wang *et al.*, 2008) and improve utilization of dietary protein and reduced excretion of excess N as urea to the environment (Mueller-Harvey, 2006). This still need to be substantiated by investigating the effects on amino-deaminating and protein-degrading rumen bacteria and analyzing the proportion of branched-chain VFA and valeric acid (Parta and Yu, 2014).

Volatile fatty acids (VFA) is considered to be the main source of energy for maintenance, growth, and lipogenesis for ruminants (Russell *et al.*, 1992; Bergman, 1990). It represent the rumen fermentation pattern and efficiency of nutrient digestion (France and Dijkstra, 2005). Higher VFA concentrations reflect higher amounts of fermented substrate, higher quantities of microbial activity and higher energy intake, increase in propionate could increase the efficiency of acetate utilization (Kinley and Fredeen, 2015). As summarized by Bergman, acetate was the major end product of microbial fermentation in the rumen and it contributed about 40 % of a ruminant's daily energy requirements (Bergman, 1990). The present results showed that the effect of Chinese medicine residue on rumen fermentation is considered positive when there is an increased total volatile fatty acid (TVFA), acetate, propionate and butyrate production and decreased A/P ratio (Castillejos *et al.*, 2008), which are good indications of the energy and health of animals.

Relationship between chemical constituents and fermentation products

Gas volume is a good parameter from which to predict substrate digestibility, fermentation end product and microbial protein synthesis by rumen microbes in the *in vitro* system (Sommart *et al.*, 2000; Sallam, 2005). In the present study, we found that the gas production is positively affected by the CP level in feedstuffs, which is consistent with the finding of Larbi *et al.* (1998) (Larbi *et al.*, 1998). The high correlation between CP and gas production might support high CP degradability. On the basis of our results, it is not surprised that the CP and gas production both showed a significant positive correlation with MCP in this study. Beyond that, Krishnamoorthy *et al.* (1991) also showed the gas production is positively related to microbial protein synthesis (Krishnamoorthy *et al.*, 1991). Furthermore, we also found that the gas production at 48 h is negatively affected by NDF level, which is also consistent with some other studies (Nsahlai *et al.*, 1994; Getachew *et al.*, 2004).

A high NDF level of feedstuffs directly decreased its digestibility (Elghandour *et al.*, 2014), then resulting in the decrease of gases, the important end products of microbial fermentation (Wolin, 1979; Chumpawadee *et al.*, 2007).

Volatile fatty acid represent the efficiency of nutrient digestion (France and Dijkstra, 2005). Higher VFA concentrations reflect higher quantities of microbial activity. In our study, a significantly positive correlation was observed between EE level and total VFA production. However, EE showed a negative effect on the A/P ratio. Previous studies showed that increased EE content in the feedstuff could inhibit the growth of protozoa and further increase the MCP, VFA and propionate production (Johnson and Johnson, 1995; Jouany, 1996), and the efficiency of microbial fermentation was also increased. Beyond that, we also found that the A/P ratio was positively correlated with CF, considering that lower A/P ratio and lower CF content in feedstuffs indicate the higher nutritive quality of them, our result further indicate the Chinese medicine residue was considered to be of good to high quality and might be another good unconventional feedstuff resource for ruminants in China.

Conclusion

On the basis of chemical analysis of these feedstuffs, we found that the Chinese medicine residue showed a higher crude protein and crude fat contents, and a lower crude fiber, neutral detergent fibre and acid detergent fibre contents, indicating the Chinese medicine residue was nutritionally better and more suitable as forage for ruminants. Results of *in vitro* gas production and rumen fermentation profiles from this study also indicated that the Chinese medicine residue could be expected to increase the gas, microbial protein and volatile fatty acids production, and reduce the acetate to propionate ratio, which are good indications of the energy and health of animals.

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