Effect of traditional Chinese medicine compounds on rumen fermentation, methanogenesis and microbial flora in vitro

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Abstract

This study was conducted to investigate the effects of traditional Chinese medicine compounds (TCMC) on rumen fermentation, methane emission and populations of ruminal microbes using an in vitro gas production technique. Cablin patchouli herb (CPH), Atractylodes rhizome (AR), Amur Cork-tree (AC) and Cypsum were mixed with the weight ratios of 1:1:1:0.5 and 1:1:1:1 to make up TCMC1 and TCMC2, respectively. Both TCMC were added at level of 25 g/kg of substrate dry matter. In vitro gas production was recorded and methane concentration was determined at 12 and 24 h of incubation. After 24 h, the incubation was terminated and the inoculants were measured for pH, ammonia nitrogen, volatile fatty acids (VFA). Total deoxyribonucleic acid of ruminal microbes was extracted from the inocula, and populations were determined by a real-time quantitative polymerase chain reaction. Populations of total rumen methanogens, protozoa, total fungi, \textit{Ruminococcus albus}, \textit{Fibrobacter succinogenes} and \textit{Ruminococcus flavefaciens} were expressed as a proportion of total rumen bacterial 16S ribosomal deoxyribonucleic acid. Compared with the control, the 2 TCMC decreased (\(P<0.05\)) total VFA concentration, acetate molar proportion, acetate to propionate ratio, gas and methane productions at 12 and 24 h, hydrogen (H) produced and consumed, and methanogens and total fungi populations, while the 2 TCMC increased (\(P<0.05\)) propionate molar proportion. Traditional Chinese medicine compound 1 also decreased (\(P<0.05\)) \textit{R. flavefaciens} population. From the present study, it is inferred that there is an effect of the TCMC in suppressing methanogenesis, probably mediated via indirect mode by channeling H\textsubscript{2} utilized for methanogenesis to synthesis of propionate and direct action against the rumen microbes involved in methane formation. In addition, the relative methane reduction potential (RMRP) of TCMC2 was superior to that of TCMC1.

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1. Introduction

In major developing countries, a large proportion of roughages is widely utilized in the diets of ruminants. Modulating rumen microbial ecosystem to improve digestibility of fibrous feeds and mitigate enteric methane emissions from ruminants are some of the most important fields for animal nutritionists. Methane produced during anaerobic fermentation in the rumen results in a 2% to 12% of gross energy lost from the animal, and contributes to emissions of greenhouse gases into the atmosphere which may lead to a damaging impact on the environment. The methane abatement strategies involve utilization of feed additives (chemicals, organic acids, and probiotics) and oils supplementation in diets, alteration of...
feeding practices, and complementation of low-quality or high-fiber diets with deficient nutrients (Gerber et al., 2013). However, the utilization of above-mentioned strategies by livestock producers has been limited for some reasons, such as decrease in fiber utilization efficiency, short-term duration of effectiveness, controversial research results between in vitro and in vivo, potential risk to animal health, and high cost of feed or labor (Kumar et al., 2014).

Up to date, traditional Chinese medicine (TCM) has still been remaining the most thrilling vitality with philosophical, experiential and experimental bases. In China, TCM has been warmly used to prevent and treat human and animal diseases, and also taken as health care products for human and green feed additives for animal, because TCM is easy and cheap to get, and is effective with fewer side effects. TCM is a kind of natural drugs, and keeps its natural structure and biological activity, and owns double features of nutrient and drug which endues it with the function of regulating physiological status of the body from a holistic perspective (Liu et al., 2011; Wang and Wang, 2016). There is a great variety of TCM in China, and every TCM has complicatedly various components which generate diversely biological functions, let alone TCM compound (TCMC) which is comprised of more than one TCM. The overwhelming majority of TCM is botanical medicines called "Chinese herbal medicine (CHMs)" in China. According to the theories of modern pharmacology and nutriology, TCM contains a variety of biologically active substances, such as antibacterial materials, alkaloids, polysaccharides, glycosides, essential oils, tannins and organic acids, as well as a certain amount of amino acids, minerals, vitamins, pigments and unknown growth-regulatory factors (Lu, 2011). Therefore, the possible mechanisms of TCM would associated with exerting nutrition supplements, ameliorating nonspecific immunity, inhibiting or killing bacteria, producing hormone-like or vitamin-like effects, resisting stress and oxidation, protecting feed from oxidation and mildew, and so on (Lu, 2011; Liu et al., 2011).

Wang et al. (2010) and Wang et al. (2011) found that the 2 TCMC composed of Cablin patchouli herb (CPH), Atractylodes rhizome (AR), Amur Cork-tree (AC) and Cypsum with the weight ratios of 1:1:1:0.5 and 1:1:1:1 could serve beef cattle via alleviating stress from the intense heat of summer and improving fattening performance. Wang et al. (2012), Wang et al. (2012a, 2012b) and Wang et al. (2013) also observed those above-mentioned phenomena when the TCMC as feed additives were supplemented to the diets composed of 50% of dried rice straw and 50% of mixed concentrate in goats. In addition, they also reported that the TCMC had some positive actions on the ruminal concentrations and proportions of volatile fatty acids (VFA), the activity of cellulolytic enzymes, and the ruminal degradability and total apparent digestibility of dietary nutrients, which inferred that the TCMC had the potential of modulating rumen fermentation as well as microbial ecosystem, and further acted on ruminal methanogenesis. Thus, the aim of the present study was to evaluate the effects of the TCMC on rumen fermentation, methanogenesis and microbial flora in vitro.

2. Materials and methods

The experimental procedures were approved by and conformed to the requirements of the Animal Care and Use Committee of Southwest University located in Chongqing City, southwestern China.

2.1. Experimental additives

Four types of TCM were used in this study. The dried aerial part of CPH is commonly known as *Pogostemon cablin* in Latin and guàng huò xiāng in Chinese pinyin. The dried rhizome of AR is commonly known as *Atractylodes lancea* in Latin and nán cang zhú in Chinese pinyin. The dried bark of AC is commonly known as *Phellodendron chinensis* in Latin and chuán huáng bò in Chinese pinyin. Cypsum, a natural mineral medicine with hydro calcium sulfate fibribrom crystallized polymeric, is commonly known as *Cypsum Fibrosum* in Latin and shèng shí gào in Chinese pinyin. All of them were usually and easily available on the TCM market in China and were purchased from Rongchang County Hospital of TCM located in Chongqing City, southwestern China. All medicines were naturally and gradually air-dried in the shade in summer and finely ground to powders through a screen of 1 mm. Cablin patchouli herb, AR, AC and Cypsum were mixed according to the weight ratios of 1:1:1:0.5 and 1:1:1:1 to make up TCMC1 and TCMC2, respectively. The difference between TCMC1 and TCMC2 was the dosage of Cypsum, and TCMC2 had 2-fold dosage of Cypsum compared with TCMC1. Then the 2 TCMC were preserved in tightly closed plastic jars and stored in a dry, dark and cool place. After mixing, a sample of each TCMC was taken for the analyses of gross energy (GE), dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), amylase-treated ash-free neutral detergent fiber (aNDFom), ash-free acid detergent fiber (ADFom), calcium (Ca) and phosphorous (P). On the basis of DM, TCMC1 and TCMC2 contained 16.30 and 14.24 MJ/kg of GE, 757 and 663 g/kg of OM, 61 and 68 g/kg of CP, 436 and 362 g/kg of aNDFom, 283 and 202 g/kg of ADFom, 38 and 24 g/kg of EE, 59 and 83 g/kg of Ca, 10 and 15 g/kg of P, respectively.

2.2. In vitro fermentations

An in vitro gas production (GP) test was conducted with the semi-automated Reading Pressure Technology (RPT; Mauricio et al., 1999). Traditional Chinese medicine compound 1 and TCMC2 were added at the level of 25 g/kg of DM of the substrate, which was the same as the supplementation level in the diets as described in the previous studies of Wang et al. (2010, 2012) when the 2 TCMC were fed to beef cattle and goats. Meanwhile, a control was set up with the substitution of the substrate for the TCMC in an equivalent amount. The substrate had the same ingredients as the basal diet used in the study of Wang et al. (2012), which consisted of 500 g of rice straw, 260 g of corn, 60 g of wheat bran, 120 g of soybean meal, 35 g of rapeseed meal, 10 g of calcium carbonate, 5 g of sodium chloride and 10 g of vitamin and trace mineral premix per kg of DM. According to the measured chemical composition, the substrate per kg could provide 19.92 MJ GE, 1864 kcal of GE, 757 and 663 g/kg of OM, 61 and 68 g/kg of CP, 436 and 362 g/kg of aNDFom, 283 and 202 g/kg of ADFom, 38 and 24 g/kg of EE, 59 and 83 g/kg of Ca, 10 and 15 g/kg of P, respectively. The incubation procedures were carried out in 180-ml serum bottles. Every bottle contained 750 mg of the substrate (Theodorou et al., 1994). Subsequently, the designated amounts of the TCMC or the equivalent substrate were added into the bottles as the TCMC treatments or the control, respectively. Before weighing, both the TCMC and the substrate were finely milled using a 1-mm screen and dried at 65 °C for 4 h in an oven. After that, 90 mL of artificial saliva prepared by the method of Menke and Steingass (1988) was poured into the bottles using a syringe. Finally, the bottles with the substrate, the TCMC and the buffer medium were placed in an incubator at 39 °C overnight after sealing with butyl rubber stoppers and aluminum caps. At the same time, 4 bottles containing incubation medium without any substrate and TCMC were incubated as the blanks to correct the GP resulting from the activity of the rumen fluid. Furthermore, the in vitro GP test with the same treatment sequence was performed twice to provide replication, and each treatment of 2 separate runs had 4 repeats.

Three healthy wethers of Dazú black goat (25.2 ± 1.2 kg), fitted with permanent rumen fistula, were used as donors of rumen fluid. They were fed twice daily at 07:00 and 19:00 on 600 g/d of a mixed diet in which ingredients and chemical compositions were identical with the above-mentioned substrate, and had free access to water. In
the morning of the second day, mixed rumen contents were obtained before the morning feeding from the 3 wethers in equal proportion, and transported to the laboratory quickly then filtered through 4 layers of cheesecloth into a flask under CO2 in the water bath at 39 °C until used. Ten milliliter of filtered rumen fluid was injected through the stopper using a syringe into the incubation bottles. Shortly afterwards, the bottles were shaken to mix the contents completely and put into the incubator at 39 °C. According to methods described by Zhang et al. (2008), the gas pressure was recorded at 12 and 24 h of incubation using a pressure transducer to calculate total GP (mL/g DM incubated) and then 20 μL of gas was drawn out by a needle through the stopper to determine methane concentration by a gas chromatography (GC) to estimate methane production (mmol/g DM incubated). After termination of incubation at 24 h, the incubation fluids were sampled. A portion of samples was stored at −20 °C for later analysis of end-products, and another was stored at −80 °C immediately for the later analysis of microbe communities by real-time polymerase chain reaction (PCR).

2.3. Chemical analytical procedures

Gross energy was determined by an isoperibol bomb calorimeter (Model number 1281, Parr Instrument Co., Moline, IL) with benzoic acid used as a standard. Dry matter was determined by loss of weight after drying a 2-g aliquot of each sample for 24 h at 105 °C, and OM was calculated as weight loss upon ignition at 600 °C for 18 h in a muffle furnace (AOAC, 2005). Crude protein was measured by multiplying nitrogen (N) obtained from a Leco model FP-2000 N analyser (Leco Corp., St. Joseph, MI) according to the Dumas Combustion Method using ethylenediamine tetra-acetate (EDTA) as a standard with a factor of 6.25 (AOAC, 2005). Ether extract was quantified using diethyl ether as an extraction fluid in a Soxhlet apparatus (AOAC, 2005). Amylase-treated ash-free neutral detergent fiber and ADfom were analyzed with a fiber analyzer (FIW6E, VELP, Italy) using reagents described by van Soest et al. (1991). Sodium sulfate and heat-stable z-amylase were used in the nDNFom determination. Calcium and P were determined by inductively coupled plasma atomic emission spectroscopy after dry ashing at 550 °C to prepare the homogenized samples (AOAC, 2005). All assays were conducted in triplicate.

The methane concentration in the headspace gas at 12 and 24 h of incubation were determined by GC (GC-2010, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a capillary column (HP-INNOWAX, 19091N-133) of 30 m × 0.25 mm × 0.5 μm in size (Hu et al., 2005). Fermentation parameters of the incubation fluids at the end of 24 h, such as ammonia N and VFA, were determined using methods described by van Soest et al. (1991). Sodium sulfitre and heat-stable z-amylase were used in the nDNFom determination. Calcium and P were determined by inductively coupled plasma atomic emission spectroscopy after dry ashing at 550 °C to prepare the homogenized samples (AOAC, 2005). All assays were conducted in triplicate.

The concentration of ammonia N was measured by colorimetry with a 721 spectrophotometer (Shanghai, China). The VFA concentration was analyzed by GC (GC-2010, Shimadzu, Kyoto, Japan).

2.4. Analysis of rumen microbial population

The genomic deoxyribonucleic acid (DNA) of rumen microbes from the incubation fluids was extracted by the bead beating method with a mini-bead beater (Biospec Products, Bartlesville, OK, USA), as described by Zoetendal et al. (1998). Quantitative PCR was conducted with a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using the SYBR Premix Ex Taq II Perfect Real Time (Takara Bio, Dalian, China). The PCR mixture incorporated 2 μL template DNA, 0.2 mmol dNTP, 0.3 μmol Primers, 1.5 mmol MgCl2, and 1.25 U Taq in a total 20-μL volume. The real-time PCR assays for microorganisms were completed as follows: one cycle at 95 °C for 10 s for initial denaturation, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing at 60 °C for 34 s. Melting curve analysis was performed after amplification to verify the specificity of the real-time PCR. The amplification efficiencies for each primer pair were investigated by examining the dilution series of the total rumen microbial DNA template on the same plate in triplicate. The primers of total bacteria, total fungi, Fibrobacter succinogenes and Ruminococcus flavefaciens were cited from Denman and McSweeney (2006), the primers of methanogens and protozoa were cited from Denman et al. (2007), and the primer Ruminococcus albus of was cited from Koike and Kobayashi (2001).

2.5. Data calculation and statistical analysis

According to Demeyer (1991), during 24 h of incubation, hydrogen (H) produced (mmol/g DM incubated) was estimated as 

\[ (2A + P + 4B) \]

and H consumed (mmol/g DM incubated) was estimated as 

\[ (4M + 2P + 2B) \]

thus H recovery (%) was calculated as 

\[ \frac{H\text{ consumed}}{H\text{ produced}} \times 100 \]

where acetate (A), propionate (P), butyrate (B) and methane (M) were expressed as net molar production (mmol). Moreover, H consumed via CH4/VFA was evaluated as 

\[ \frac{4A}{2P + 2B} \]

The reduction of methane production by TCMC was usually followed by a reduction of VFA production. The reductions of methane and VFA were expressed as a proportion of total production in controls, and were calculated as 

\[ (1 - \text{CH}_4/\text{VFA production in treatment incubations}/\text{CH}_4/\text{VFA production in control}) \]

Therefore, the relative methane reduction potential (RMRP) of a treatment versus control was estimated as a ratio of reduction in methane production to reduction in VFA production, which was used for the selection of optimal combination, as described by Lin et al. (2012).

Quantification for methanogen, protozoa, fungi, R. albus, F. succinogenes and R. flavefaciens were expressed as a proportion relative to total rumen bacterial 16S ribosomal DNA (rDNA) according to the following equation: relative quantification = \[ \frac{ct}{ct} \]

where ct represents threshold cycle.

Data were analyzed with an one-way ANOVA analysis of variance using the PROC GLM procedure of SAS (SAS Institute, 2005). Multiple comparisons of means among treatments were conducted by the Duncan's multiple range tests. Degree of significance was defined as follows: P > 0.05, not significant and P ≤ 0.05, significant.

3. Results

3.1. Effects of the TCMC on in vitro rumen fermentation parameters

No differences (P > 0.05) were observed in pH value, ammonia N concentration, and butyrate molar proportion among different

| Item                     | Control | TCMC1 | TCMC2 | SEM | P-value |
|--------------------------|---------|-------|-------|-----|---------|
| pH                       | 6.71    | 6.69  | 6.70  | 0.000 | 0.056   |
| Ammonia nitrogen, mg/dL  | 19.96   | 19.70 | 20.19 | 0.272 | 0.055   |
| Total VFA, mmol/L        | 5.36    | 4.39  | 4.73  | 0.113 | 0.000   |
| VFA, mol/100 mol         |         |       |       |       |         |
| Acetate                  | 81.06   | 79.64 | 78.89 | 0.305 | 0.007   |
| Propionate               | 15.21   | 17.35 | 17.29 | 0.242 | 0.000   |
| Butyrate                 | 3.72    | 2.99  | 3.80  | 0.168 | 0.116   |
| Acetate to propionate ratio | 5.32  | 4.58  | 4.57  | 0.082 | 0.000   |

SEM = standard error of the mean; VFA = volatile fatty acids. TCMC1 is comprised of Cablin patchouli herb (CPH), Atractylodes rhizome (AR), Amur Cork-tree (AC) and Cypsum with the weight ratio of 1:1:1:0.5. TCMC2 is comprised of CPH, AR, AC and Cypsum with the weight ratio of 1:1:1:1.

Within a raw, means with different superscripts differ at P ≤ 0.05.
treatments (Table 1). The addition of TCMC1 and TCMC2 decreased (P ≤ 0.05) total VFA concentration, acetate molar proportion, and acetate to propionate ratio, but increased (P ≤ 0.05) propionate molar proportion. In addition, total VFA concentration of TCMC2 treatment was higher (P ≤ 0.05) than that of TCMC1 treatment.

3.2. Effects of the TCMC on in vitro GP, methane production and H balance

The addition of TCMC1 and TCMC2 reduced (P ≤ 0.05) GP and methane production at 12 and 24 h of incubation, and H produced and consumed, but unaffected (P > 0.05) H recovery (Table 2). Hydrogen produced of TCMC2 treatment was higher (P ≤ 0.05) than that of TCMC1 treatment. With the addition of TCMC2, H consumed via CH4 to via VFA was lowered (P ≤ 0.05). In addition, the RMRP of TCMC2 was higher (P = 0.002) than that of TCMC1 (Fig. 1).

3.3. Effects of the TCMC on in vitro rumen microbe population

Both TCMC1 and TCMC2 did not mediate (P > 0.05) the evolution of protozoa, R. albus, and F. succinogenes, but inhibited (P < 0.05) the growth of methanogens and total fungi (Table 3). Traditional Chinese medicine compound 2 treatment had less quantity of methanogens, relative to total bacterial 16S rDNA, than TCMC1 treatment. The proliferation of R. flavefaciens was suppressed (P ≤ 0.05) with the addition of TCMC1, but was unaffected (P > 0.05) with the addition of TCMC2.

4. Discussion

In the present study, the 2 TCMC were composed of CPH, AR, AC and Cypsum. Cablin Patchouli Herb, AR and AC fall into the category of natural plant products, which produce an enormous variety of secondary metabolites to provide protection against microbial and insect attack (Hart et al., 2008). Nowadays there is an increasing interest in the use of plants containing phytochemicals and their extracts to manipulate the function of gastrointestinal tract in both ruminant and non-ruminant livestock (Greathed, 2003; Wang and Wang, 2016). Generally, these bioactive substances can be classified into terpenes or isoprenoids, phenols and alkaldoids, and 4 major classes with current potential use in ruminant nutrition are saponins, tannins, organosulphur compounds and essential oils (Bodas et al., 2012). Indeed, except for some nutrient substances, the 3 raw plant medicinal drugs used in this study contain a certain quantity of one or more classes of bioactive compounds listed above (Wang and Wang, 2016). It has been confirmed that CPH contains essential oils, tannins and amaroids, AR contains various alkaloids like berberine, N free crystalline substances, epressed oils, mucoid substances, steroids and closely related sterols, as well as AC contains essential oils, carotene or carotenoid and hiamine (Wang and Wang, 2016). Although the definitive categories and actual concentrations of these secondary compounds contained with the 3 raw plant materials above were not determined in the present study, the concentration of each kind of secondary compound might be relatively low. Therefore, a considerable amount of each TCMC (25 mg TCMC per g of total DM incubated) was added into the substrate. Moreover, because more than two-thirds of the 2 TCMC might be composed of OM, which can be degraded and fermented to some extent, an extra substrate (18.75 mg) was added into the control cultures so that the amount of total DM incubated was the same in all cases.

It has been well known that the mitigation of enteric methane in ruminants has significant economical and environmental benefits, which attracts the scientific community to explore various ways to manipulate rumen microbial population to change fermentation pattern. Whilst numerous chemical additives and antibiotics have been studied and utilized for this purpose, the use of ‘natural products’ to modify rumen fermentation is attracting the closest attention with a concept of ‘clean, green and ethical’ animal production being promoted (Durmic and Blache, 2012). Based on this background, bioactive plant metabolites become an important contemporary research field for the replacement of chemical feed additives because some of these metabolites show potential to alter rumen fermentation and

Table 2

| Item                  | Control | TCMC1  | TCMC2  | SEM   | P-value |
|-----------------------|---------|--------|--------|-------|---------|
| Gas production, ml/g  |         |        |        |       |         |
| 12 h                  | 148.4a  | 112.14b| 117.38b| 3.565 | 0.000   |
| 24 h                  | 251.14a | 203.13b| 206.15b| 5.152 | 0.000   |
| Methane production, mmol/g | 0.84a    | 0.46b  | 0.52b  | 0.033 | 0.000   |
| 24 h                  | 1.70a   | 1.30b  | 1.28b  | 0.056 | 0.000   |
| Hydrogen balance      |         |        |        |       |         |
| Produced, mmol/g      | 10.70a  | 8.28b  | 9.01b  | 0.242 | 0.000   |
| Consumed, mmol/g     | 9.29a   | 6.99b  | 7.15b  | 0.271 | 0.000   |
| Recovery, %           | 86.73   | 84.22  | 79.77  | 1.816 | 0.303   |
| Consumed via methane  | 3.40a   | 2.90b  | 2.63b  | 0.121 | 0.022   |
| via VFA               |         |        |        |       |         |

SEM = standard error of the mean; VFA = volatile fatty acids.

TCMC1 is comprised of Cablin patchouli herb (CPH), Atractylodes rhizome (AR), Amur Cork-tree (AC) and Cypsum with the weight ratio of 1:1:1:1.05. TCMC2 is comprised of CPH, AR, AC and Cypsum with the weight ratio of 1:1:1:1.05.

Table 3

| Item                  | Control | TCMC1  | TCMC2  | SEM   | P-value |
|-----------------------|---------|--------|--------|-------|---------|
| Methanogens           | 191.52a | 80.98b | 53.19b | 10.101| 0.000   |
| Protozoa              | 204.58  | 202.89 | 193.54 | 2.234 | 0.091   |
| Total fungi           | 44.57a  | 24.31b | 24.99b | 1.626 | 0.000   |
| Ruminococcus albus    | 309.08  | 297.91 | 293.79 | 3.208 | 0.131   |
| Fibrobacter succinogenes | 20.66  | 20.65  | 20.67  | 0.576 | 1.000   |
| Ruminococcus flavefaciens | 379.31a | 192.23b | 384.21b | 15.512| 0.000   |

SEM = standard error of the mean.

TCMC1 is comprised of Cablin patchouli herb (CPH), Atractylodes rhizome (AR), Amur Cork-tree (AC) and Cypsum with the weight ratio of 1:1:1:1.05. TCMC2 is comprised of CPH, AR, AC and Cypsum with the weight ratio of 1:1:1:1.05.

a, b, cWithin a raw, means with different superscripts differ at P ≤ 0.05.
decrease methanogenic methane production (Patra, 2012). The anti-
methanogenic activity of saponins, tannins and essential oils 
extracted from a diverse array of plant materials has been 
extensively demonstrated in many in vitro and in vivo studies 
with variable efficacy depending on their chemical nature and 
ruminial concentration though their modes of action are so 
tremendously different and have not been completely elucidated 
(Benchaar and Greathead, 2011; Goel and Makkar, 2012). In fact, 
there is still a long way to go before the phytochemical fractions 
involved in reduction of methane production are extracted and 
isolated, or artificially synthesized (once their structure are 
identified) and then used as feed additives. However, directly 
feeding the plants containing these bioactive compounds could 
be an alternative approach to motivate similar changes in rumen 
fermentation, just as the results demonstrated by the 2 TCMC in 
the present study. Furthermore, Wang et al. (2010) suggested that 
the 2 TCMC could give rise to more efficient animal production.

Gas production during incubation in vitro was well correlated 
with OM digestibility of fermented feedstuffs (Menke et al., 1979). 
Higher GP meant more violent fermentation in the rumen for 
feedstuffs. The VFA were usually regarded as one of rumen 
fermentation indexes, and typified rumen fermentation pattern 
and nutrient digestion efficiency (Pitt et al., 1996). In the present 
study, the addition of TCMC1 and TCMC2 decreased GP at 12 
and 24 h of incubation, indicating that the 2 TCMC could inhibit in vitro 
fermentation of substrate, then decrease degradability of OM of 
substrate, finally induce a reduction in total VFA production. The 
inference above could be verified by the detection results of total 
VFA concentration in the incubation fluids, which further implied 
that H produced might be declined by the 2 TCMC because of the 
decrease of total fermentable OM (Jordan et al., 2006). The VFA 
profile in the rumen was mainly affected by the compositions of 
diet fed to ruminants (Pitt et al., 1996). In the present study, acetate 
molar proportion of every group was relatively high since 50% of 
substrate was dried rice straw which can be categorized into the 
roughage with low quality. However, the addition of TCMC1 and 
TCMC2 decreased acetate molar proportion, but increased propio-
nate molar proportion, which suggested that the 2 TCMC could 
hibit acetate fermentation with the promotion of propionate 
fermentation, consequently induce a decrement in the ratio of ac-
etate to propionate as well as a specific shift in the fermentation 
pattern without any drastic effects on ruminal pH. Higher propio-
nate yield in the rumen always means better performance for ru-
migators. Thus, the improvement in the efficiency of ruminal 
fermentation by the 2 TCMC should be one of reasons in the study 
of Wang et al. (2010) who declared an amelioration in the 
fermentation by the 2 TCMC should be one of reasons in the study 
minants. Thus, the improvement in the ef

5. Conclusions

Addition of the 2 TCMC abated methane release along with a 
shift of fermentation pattern towards a reduced acetate to prop-
nionate ratio, while TCMC2 had a greater advantage over TCMC1. 
Methanogens and total fungi populations, relative to total bacte-
rial 16S rDNA, were decreased by the 2 TCMC, with the prolifer-
ation of R. flavefaciens being depressed only by TCMC1. Further 
research is required to elucidate the actions on fibrolytic micro-
organisms surviving in the rumen, to evaluate the persistence of 
in vivo antimethanogenic effects, to specify the chemical nature of 
active compounds being responsible for such effects, and to testify 
the usefulness and applicability under diverse practical conditions.

Conflicts of interest

None.

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