



## Effects of Momordica charantia polysaccharide on in vitro ruminal fermentation and cellulolytic bacteria

Jinhe Kang, Bo Zeng, Shaoxun Tang, Min Wang, Xuefeng Han, Chuanshe Zhou, Qiongxin Yan, Jinfu Liu & Zhiliang Tan

To cite this article: Jinhe Kang, Bo Zeng, Shaoxun Tang, Min Wang, Xuefeng Han, Chuanshe Zhou, Qiongxin Yan, Jinfu Liu & Zhiliang Tan (2017) Effects of Momordica charantia polysaccharide on in vitro ruminal fermentation and cellulolytic bacteria, Italian Journal of Animal Science, 16:2, 226-233, DOI: [10.1080/1828051X.2017.1289825](https://doi.org/10.1080/1828051X.2017.1289825)

To link to this article: <https://doi.org/10.1080/1828051X.2017.1289825>



© 2017 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



Published online: 21 Feb 2017.



Submit your article to this journal [↗](#)



Article views: 266



View related articles [↗](#)



View Crossmark data [↗](#)

## Effects of *Momordica charantia* polysaccharide on *in vitro* ruminal fermentation and cellulolytic bacteria

Jinhe Kang<sup>a</sup>, Bo Zeng<sup>a</sup>, Shaoxun Tang<sup>a</sup>, Min Wang<sup>a</sup>, Xuefeng Han<sup>a</sup>, Chuanshe Zhou<sup>a</sup>, Qiongxin Yan<sup>a</sup>, Jinfu Liu<sup>b</sup> and Zhiliang Tan<sup>a</sup>

<sup>a</sup>Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha, Hunan, China; <sup>b</sup>Department of Food Science, Tianjin Agricultural University, Tianjin, China

### ABSTRACT

Four levels of *Momordica charantia* polysaccharide (MCP) supplements (0, 0.1, 0.3, 0.6 mg/ml) were designed to investigate the effects of MCP on ruminal fermentation and cellulolytic bacteria *in vitro*. The pH, ammonia-N (NH<sub>3</sub>-N) and volatile fatty acids (VFA) were measured at 6, 24, 48 h, whilst the cellulolytic bacteria population was determined at 6 and 24 h. The 0.6 mg/ml MCP inclusion decreased the theoretical maximum of gas production and the half-life. The NH<sub>3</sub>-N concentration was decreased by MCP at all doses at 24 h. The MCP inclusion increased the concentration of total VFA at 24 and 48 h and the acetate to propionate ratio, the molar proportion of isovalerate at 6 h, while decreased that of isobutyrate at 24 h and that of isovalerate, valerate at 24 and 48 h, respectively. The relative abundances of *Ruminococcus albus* and *Ruminococcus flavefaciens* were decreased at 6 h, while that of *Butyrivibrio fibrisolvens* was increased at all times of incubation and that of *Fibrobacter succinogenes* reached the greatest value at 0.6 mg/ml MCP supplementation at 24 h. This study demonstrated that the MCP had the ability to enhance the total VFA production, modulate the rumen fermentation pathway and influence the number of cellulolytic bacteria population.

### ARTICLE HISTORY

Received 25 July 2016  
Revised 6 November 2016  
Accepted 15 December 2016

### KEYWORDS

Plant components; *in vitro* fermentation; cellulolytic bacteria; roughage

### Introduction

Polysaccharides are the most commonly studied phytochemicals as feed additives. Recent studies have demonstrated that polysaccharides have the potential to modulate rumen fermentation, such as decreasing the acetate to propionate (A/P) ratio in ruminal fluid and changing the individual volatile fatty acid production (Poulsen et al. 2012; Zhong et al. 2012). Meanwhile, polysaccharides have been shown to modulate the ruminal microbial communities (Poulsen et al. 2012). However, the reported effects of polysaccharides on gastrointestinal fermentation have been inconsistent, depending on different types of polysaccharides and microbial inocula (Guo et al. 2003; Marzorati et al. 2013). *Momordica charantia* (MC), known as bitter melon, is cultivated throughout the world for use as vegetable as well as medicine, and *Momordica charantia* polysaccharide (MCP) is one of the important bioactive components in MC (Guan 2012). Previous studies showed that MCP significantly

influenced the *in vitro* fermentation of caecal digesta from pigs (Bo et al. 2010). However, there is little information on the activity of MCP in manipulating rumen fermentation. Our hypothesis is that MCP will affect *in vitro* rumen fermentation.

Maize stover is an important roughage in the diets of ruminants in China. The objective of this study was to investigate the effect of different doses of MCP on *in vitro* rumen fermentation characteristics and rumen cellulolytic bacteria, when maize stover was used as fermented substrate. Moreover, the correlations between rumen cellulolytic bacteria, gas production (GP) parameters and VFA profiles when maize stover was used as fermented substrates were studied.

### Materials and methods

This experiment was approved by the Animal Care Committee, Institute of Subtropical Agriculture (ISA), the Chinese Academy of Sciences, Changsha, China.

**CONTACT** Dr. Jinfu Liu ✉ [nxyljf@163.com](mailto:nxyljf@163.com) Department of Food Science, Tianjin Agricultural University, Tianjin, 300384, China; Prof. Zhiliang Tan ✉ [zltan@isa.ac.cn](mailto:zltan@isa.ac.cn) Key Laboratory for Agro-Ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha, Hunan, China

© 2017 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

## Reagents

The MCP was supplied by department of Food Science and Bioengineering, Tianjin Agricultural University, Tianjin, China. The preparation of MCP was as follows: Dried materials of *Momordica charantia* were powdered and sieved (60 mesh). *Momordica charantia* powder (200 g) was incubated with 4000 ml 0.5% pectinase at 50 °C for 2 h, followed by heating at 70 °C for 1 h, and repeated two times. The solution was concentrated by ultrafiltration on a polysulfone hollow fibre membrane (6000 molecular mass cut-off) in the pressure of 0.05 Mpa, and repeated three times. The leaching liquid was discarded, and the concentrated fluid was dried under the pressure of 0.07 Mpa, giving the crude polysaccharide powder. Sevage method (Navarini et al. 1999) was used to remove the proteins in the extract. After 24 h of dialysis, the extract was precipitated with 80% alcohol solution, and further purified on a Sephadex G-75 column, pre-equilibrated and eluted with distilled water. Fractions (5 ml each) were collected at a flow rate of 1 ml/min. The eluted fraction was collected and concentrated by dryness under vacuum. The total carbohydrate content of MCP was determined by anthrone-sulfuric acid colorimetric assay (Laurentin & Edwards 2003) using glucose as standard sample. The reducing sugar content was determined by DNS (3, 5-dinitrosalicylic acid) method (Wang et al. 2013b) using glucose as standard sample. The purity of MCP was 87.5%. The other components of the MCP were mainly reducing sugar (1%), H<sub>2</sub>O (3%) and protein (6%). The MCP was dissolved in deionized water as a stock solution. The stock was diluted to the required concentration immediately before use. All the other reagents were of analytical reagent quality.

## Experimental design

The experiment was conducted to measure *in vitro* gas production (GP) characteristics of maize stover. Inclusive levels of MCP were 0, 0.1, 0.3, 0.6 mg/ml *in vitro* incubation fluid, respectively. The MCP was mixed with the substrate before the commencement of the experiment.

## *In vitro* fermentation and chemical analyses

Three castrated Xiangdong black goats (a local breed in southern China, mean live weight 25 ± 2 kg) fitted with the permanent rumen cannulas were individually housed in stainless metabolic cages (100 cm × 75 cm × 80 cm) with free access to fresh water. Each goat was fed 140 g of concentrate and 210 g of maize stover per meal at 0800 and 1900 h

daily. The mixed diet contained 10% CP, 52.5% NDF, 31.5% ADF. The maize stover contained 91.6% OM, 9.0% CP, 71.4% NDF, 44.8% ADF. The equal volume of rumen fluid was collected from each goat before morning feeding, and immediately transported to laboratory. Ruminal contents were strained through four layers of cheesecloth under a continuous CO<sub>2</sub> stream. Two-hundred ± 1 mg of oven-dried maize stover was weighed into a 100 ml serum bottle, which was followed by 10 ml of rumen fluid and 20 ml of McDougall's buffer (Cone & Becker 2012) at 39 °C under the anaerobic conditions. All bottles were connected with pressure sensors (CYG130-12, Sqsensor, Kunshan, China; pressure range from 0 to +100 kPa; accuracy of ±0.1% of measured value) (Wang et al. 2016). The pressures in the bottles were recorded at 0, 1, 2, 3, 6, 12, 24, 36 and 48 h during the process of *in vitro* fermentation.

Triplicate bottles were used for each sampling time point. Bottles were removed from fermentation at 6, 24 and 48 h of incubation, and fluid samples from the bottles were immediately stored at -80 °C for later analyses. The pH value of *in vitro* fermentation liquid was determined using a pH metre (Model PHS-3C, Shanghai precision & scientific instrument Co., LTD, China). The NH<sub>3</sub>-N content was determined as described by Weatherburn (1967) using a spectrophotometer (8500II, Thermo Electron Corporation, Waltham, MA). VFA contents were calculated as described by Tang et al. (2013). Briefly, two millilitres of incubation solution was centrifuged at 10,000 × g and 4 °C for 15 min, then 1.5 ml of supernatant solution was taken and 0.15 ml metaphosphoric acid was added and homogenised. The mixed solution was centrifuged at 10000 × g and 4 °C for 15 min again, and the supernatant solution was used to determine VFA content with a gas chromatograph (HP5890, Agilent 5890; Agilent Technologies Co. Ltd, Santa Clara, CA). A DBFFAP column (30m in length with a 0.25 mm i.d.) was used for the separation. The attenuation was set at a nitrogen diffuent ratio of 1:50, hydrogen flow was 30 ml/min, airflow was 365 ml/min, injector temperature was 250 °C, column temperature was 150 °C and detector temperature was 220 °C. The N<sub>2</sub> was used as carrier gas at a flow rate of 0.8 ml/min. The quantitative and qualitative evaluations of individual peaks were made using the external standard method prepared by mixing individual VFA (Supelco) and analysed with the Millennium 2001 software (version 2.15, Waters Corporation, Manchester, England). Total molar proportion was calculated by taking the sum of individual VFA as 100%. To determine the relative abundance of selected microbes to total bacterial

16S rDNA, the fluid from each bottle was sampled under oxygen-free CO<sub>2</sub> and immediately stored at -80 °C.

The *in vitro* fermentation was separately run three times on different days of collecting mixed rumen fluids, so that each treatment was conducted in triplicate. Bottles containing maize stover substrate without MCP were included as controls. Bottles containing buffered ruminal fluid alone or buffered ruminal fluid plus MCP without substrates were also prepared and used to correct for GP and fermentation residues resulting from the inoculum or MCP itself.

### Real-time quantitative PCR analysis

Total DNA was extracted from each sample as previously described by Zeng et al. (2012). The primers used for the real-time PCR were as shown in Table 1. The oligonucleotides were synthesised by Sangon Biotech Co., Ltd. (Shanghai, China). Real-time PCR was performed using the Power SYBR Green PCR Master Mix kit (Applied Biosystems, Foster City, CA) in accordance with the manufacturer's instructions. Then, real-time PCR was carried out in a ABI-7900 HT Fast Real Time PCR system (Applied Biosystems, Foster City, CA) according to the following protocol: 2 min at 50 °C; 10 min at 95 °C; and 40 cycles of 15 s at 95 °C and 60 s at 60 °C. Specificity of amplified products was confirmed by melting temperatures and dissociation curves after each amplification according to the following protocol: 15 s at 95 °C; 15 s at 60 °C and 15 s at 95 °C. Dilutions of samples were used to check the PCR amplification efficiencies for each primer pair. Each DNA extract from each MCP level of inclusion *in vitro* incubation were run in triplicate, and a no-template (sterile distilled water) negative control was loaded on each plate run to screen for possible contamination and dimer formation. The individual relative abundance of rumen cellulolytic bacteria was expressed as a proportion of total rumen bacterial 16S rDNA according to the following equation: relative

quantification of target =  $2^{-(Ct_{\text{target}} - Ct_{\text{total bacteria}})}$ , where Ct represents threshold cycle.

### Calculation and statistical analysis

Before the MCP experiment was conducted, the correlation between the pressure in bottle and gas volume was measured at 39 °C, and then a regression equation was established:

$y = (x - 0.816) / 0.805$  ( $n = 20$ ,  $R^2 = .999$ ,  $p < .001$ ), where,  $y$  represents gas volume (ml),  $x$  is the pressure in bottle (kPa), 0.816 and 0.805 are constants. A logistic-exponential model with the initial value being zero ( $LE_0$ ) was employed to fit the kinetics of GP (Wang et al. 2011) using NLREG (Version 5.0) software and expressed as:

$$V = \frac{V_F(1 - \exp(-kt))}{1 + \exp(b - kt)}$$

where  $V$  is the cumulative GP (ml) at time point  $t$ ,  $V_F$  is the theoretical maximum of GP (ml),  $k$  is fractional rate of gas production at particular time (1/h),  $b$  is a shape parameter.

The half-life ( $t_{0.5}$ ,  $h$ ; represents the time at which half of the final gas production) and initial fractional rate of degradation ( $FRD_0$ ,/h) was calculated according to the equation provided by Wang et al. (2013a), and expressed as:

$$t_{0.5} = \frac{\ln(2 + \exp(b))}{k}$$

$$FRD_0 = \frac{k}{1 + \exp(b)}$$

SAS 8.02 software was used for all statistical analysis including fermentation factors, cellulolytic bacteria population and the correlations between rumen cellulolytic bacteria population and fermentation factors. *In vitro* GP parameters and measures of pH value, NH<sub>3</sub>-N, VFA production and cellulolytic bacteria population were subjected to the GLM procedure of the SAS 8.02 programme. In the model, MCP was the only fixed effect considered. MCP treatment effects on dependent variables are expressed as least-square-means

**Table 1.** Primers for real-time quantitative PCR analysis.

Microbial species	Primer sequence (5'-3')	Product size, bp	Reference
Total bacteria	FP: CGGCAACGAGCGCAACCC RP: CCATTGTAGCACGTGTGTAGCC	130	(Zeng et al. 2012)
<i>Ruminococcus flavefaciens</i>	FP: CGAACGGAGATAATTTGAGTTTACTTAGG RP: CGGTCTGTATGTTATGAGGTATTACC	132	(Zeng et al. 2012)
<i>Fibrobacter succinogenes</i>	FP: GTTCGGAATTACTGGGCGTAA A RP: CGCCTGCCCTGAACTATC	121	(Zeng et al. 2012)
<i>Ruminococcus albus</i>	FP: CCCTAAAAGCAGTCTTAGTTCC RP: CCTCCTTGCGGTTAGAACA	176	(Zeng et al. 2012)
<i>Butyrivibrio fibrisolvens</i>	FP: GAGGAAGTAAAAGTCGTAACAAGGTTTC RP: CAAATTCACAAAGGGTAGGATGATT	160	(Zeng et al. 2012)

(LSM). Tukey's test was used for pair-wise comparisons of individual treatments from significant GLM models, and comparisons among treatments containing increasing levels of MCP were made using orthogonal polynomial contrasts. The IML procedure of SAS was used to correct the contrast coefficients of orthogonal polynomial. Pearson correlation analysis was used to test the correlations between rumen cellulolytic bacteria population and fermentation factors. The  $p < .05$  was considered statistically significant.

## Results

### Kinetics of *in vitro* gas production

Effects of MCP on *in vitro* GP parameters of maize stover are presented in Table 2. The results showed that inclusion of MCP at increasing amounts significantly affected values of  $V_F$ ,  $k$ ,  $FRD_0$  (linear and quadratic,  $p < .01$ ). The value of  $V_F$  was increased by 4.94% and 6.35%, respectively, for 0.1 and 0.3 mg/ml MCP inclusion compared with the control, although it was not significant. The highest dose of MCP (0.6 mg/ml) decreased ( $p < .05$ ) the  $V_F$  value, and increased ( $p < .05$ ) the  $k$  value, while no differences ( $p > .05$ ) were observed in the 0.1, 0.3 mg/ml MCP-treated groups compared with the control. The value of  $FRD_0$  reached the greatest value at 0.3 mg/ml MCP supplementation, while decreased to the least value at 0.6 mg/ml MCP supplementation ( $p < .05$ ). The value of  $t_{0.5}$  was decreased (quadratic,  $p < .01$ ) in response to MCP supplementation and no differences ( $p > .05$ ) were observed in the 0.1, 0.3, 0.6 mg/ml MCP-treated groups compared with the control.

### pH and $NH_3$ -N

Effects of MCP inclusion on pH and  $NH_3$ -N concentration at different times of *in vitro* incubation are given in Table 3. The pH values were increased (linear,  $p < .001$ ) by the inclusion of MCP at all times of incubation, ranged within 6.60 to 6.78. The  $NH_3$ -N concentration was decreased (quadratic,  $p < .01$ ) by the inclusion of MCP at 24 h. The MCP supplementation

resulted in a comparable concentration of  $NH_3$ -N with the control at 6 h and 48 h of *in vitro* incubation.

### VFA

Effects of MCP inclusion on VFA production at different times of *in vitro* incubation are shown in Table 3. At 6 h of *in vitro* incubation, the inclusion of MCP at 0.6 mg/ml had a greater molar proportion of acetate ( $p < .05$ ) compared to the control, but no significant changes were observed among the other three groups. The MCP inclusion increased the molar proportion of isovalerate (linear,  $p < .05$ ) and the A/P ratio (linear,  $p < .01$ ), but decreased the molar proportion of propionate (linear,  $p < .001$ ; quadratic,  $p < .05$ ). The molar proportion of isobutyrate, butyrate, valerate and the concentration of total VFA were not affected by MCP at all doses.

The MCP inclusion increased the molar proportion of propionate (linear,  $p < .01$ ) and the concentration of total VFA (quadratic and cubic,  $p < .001$ ), but decreased the molar proportion of isobutyrate (quadratic and cubic,  $p < .01$ ), isovalerate (quadratic,  $p < .001$ ; cubic,  $p < .05$ ), valerate (linear,  $p < .01$ ; quadratic and cubic,  $p < .05$ ) at 24 h. The molar proportion of acetate, butyrate and the A/P ratio were not affected by MCP for all doses at 24 h.

After 48 h of incubation, the MCP inclusion increased the concentration of total VFA (linear and quadratic,  $p < .01$ ), but decreased the molar proportion of isovalerate (linear and cubic,  $p < .05$ ; quadratic,  $p < .01$ ) and valerate (linear,  $p < .05$ ; quadratic,  $p < .01$ ). The molar proportion of acetate, propionate, isobutyrate, butyrate and the A/P ratio were not affected by MCS at all doses.

### Cellulolytic bacteria population

Effects of MCP inclusion on the relative abundance of cellulolytic bacteria at different times of *in vitro* incubation are showed in Table 4. At 6 h of *in vitro* incubation, the relative abundance of *B. fibrisolvans* (quadratic,  $p < .05$ ; cubic,  $p < .001$ ) was increased and

**Table 2.** Effect of *Momordica charantia* polysaccharide (MCP) inclusion on *in vitro* gas production parameters.

Item	MCP (mg/ml)				SEM	MCP effect		
	0	0.1	0.3	0.6		Linear	Quadratic	Cubic
$V_F$ , ml	40.93 <sup>a</sup>	42.95 <sup>a</sup>	43.53 <sup>a</sup>	36.45 <sup>b</sup>	0.85	**	**	ns
$k$	0.08 <sup>b</sup>	0.08 <sup>b</sup>	0.06 <sup>b</sup>	0.15 <sup>a</sup>	0.16	***	**	*
$FRD_0$ , /h	0.03 <sup>b</sup>	0.03 <sup>b</sup>	0.04 <sup>a</sup>	0.02 <sup>c</sup>	0.002	**	***	ns
$t_{0.5}$ , h	17.84 <sup>a</sup>	14.90 <sup>b</sup>	14.18 <sup>b</sup>	15.68 <sup>b</sup>	0.65	ns	**	ns

$V_F$ : the theoretical maximum of gas production (ml);  $k$ : the fractional rate of gas production at time (1/h);  $t_{0.5}$ : the time at which half of the final gas production (h);  $FRD_0$ : the initial fractional rate of degradation (/h).

<sup>a-c</sup>Means within a row with different superscripts differ ( $p < .05$ ). \* $p < .05$ ; \*\*\* $p < .001$ ; ns: not significant.

**Table 3.** Effects of *Momordica charantia* polysaccharide (MCP) inclusion on *in vitro* fermentation characteristics.

Item	Incubation time, h	MCP, mg/ml				SEM	MCP effect		
		0	0.1	0.3	0.6		Linear	Quadratic	Cubic
pH	6	6.72 <sup>c</sup>	6.77 <sup>b</sup>	6.77 <sup>b</sup>	6.78 <sup>a</sup>	0.003	***	***	***
	24	6.64 <sup>b</sup>	6.69 <sup>a</sup>	6.69 <sup>a</sup>	6.70 <sup>a</sup>	0.006	***	*	***
	48	6.60 <sup>b</sup>	6.63 <sup>a</sup>	6.65 <sup>a</sup>	6.65 <sup>a</sup>	0.010	**	ns	ns
NH <sub>3</sub> -N, mM	6	33.95	29.29	31.99	30.79	1.997	ns	ns	ns
	24	44.53 <sup>a</sup>	34.91 <sup>b</sup>	33.65 <sup>b</sup>	36.71 <sup>b</sup>	2.007	ns	**	ns
	48	46.13	40.48	46.43	45.49	3.736	ns	ns	ns
Total VFA, mM	6	16.90	18.80	16.57	13.44	1.852	ns	ns	ns
	24	19.43 <sup>b</sup>	35.30 <sup>a</sup>	34.90 <sup>a</sup>	23.47 <sup>b</sup>	1.479	ns	***	***
	48	35.04 <sup>b</sup>	45.92 <sup>a</sup>	52.02 <sup>a</sup>	50.77 <sup>a</sup>	2.816	**	**	ns
Acetate, molar % of total VFA	6	38.77 <sup>b</sup>	42.82 <sup>ab</sup>	43.97 <sup>ab</sup>	44.73 <sup>a</sup>	1.823	ns	ns	ns
	24	40.28	50.47	47.08	43.90	3.314	ns	ns	ns
	48	46.27	50.79	52.11	48.21	2.105	ns	ns	ns
Propionate, molar % of total VFA	6	31.31 <sup>a</sup>	26.83 <sup>b</sup>	25.78 <sup>b</sup>	25.10 <sup>b</sup>	0.883	***	*	ns
	24	21.98 <sup>b</sup>	25.80 <sup>a</sup>	25.20 <sup>a</sup>	26.92 <sup>a</sup>	0.879	**	ns	ns
	48	23.25 <sup>ab</sup>	24.23 <sup>ab</sup>	22.85 <sup>b</sup>	25.43 <sup>a</sup>	0.753	ns	ns	ns
Isobutyrate, molar % of total VFA	6	3.84	4.05	3.95	4.70	0.331	ns	ns	ns
	24	4.53 <sup>a</sup>	2.71 <sup>c</sup>	3.57 <sup>b</sup>	4.18 <sup>ab</sup>	0.247	ns	**	**
	48	3.07	3.09	3.47	3.57	0.303	ns	ns	ns
Butyrate, molar % of total VFA	6	19.89	20.27	19.66	17.19	1.091	ns	ns	ns
	24	18.92	18.27	18.53	18.23	1.271	ns	ns	ns
	48	17.44	15.19	15.27	15.66	0.826	ns	ns	ns
Isovalerate, molar % of total VFA	6	4.56 <sup>b</sup>	4.67 <sup>b</sup>	4.57 <sup>b</sup>	6.54 <sup>a</sup>	0.553	*	ns	ns
	24	8.20 <sup>a</sup>	4.91 <sup>b</sup>	4.69 <sup>b</sup>	6.03 <sup>b</sup>	0.394	ns	***	*
	48	7.41 <sup>a</sup>	4.97 <sup>b</sup>	4.73 <sup>b</sup>	5.33 <sup>b</sup>	0.416	*	**	*
Valerate, molar % of total VFA	6	1.64	1.37	2.07	1.75	0.239	ns	ns	ns
	24	4.80 <sup>a</sup>	1.67 <sup>b</sup>	1.84 <sup>b</sup>	1.53 <sup>b</sup>	0.520	**	*	*
	48	2.55 <sup>a</sup>	1.74 <sup>b</sup>	1.56 <sup>b</sup>	1.79 <sup>b</sup>	0.165	*	**	ns
A/P	6	1.25 <sup>b</sup>	1.60 <sup>a</sup>	1.72 <sup>a</sup>	1.81 <sup>a</sup>	0.108	**	ns	ns
	24	1.83	2.14	1.73	1.58	0.196	ns	ns	ns
	48	2.00	2.11	2.32	1.93	0.156	ns	ns	ns

<sup>a-c</sup>Means within a row with different superscripts differ ( $p < .05$ ).

\* $p < .05$ ; \*\* $p < .01$ ; \*\*\* $p < .001$ ; ns: not significant.

**Table 4.** Effects of *Momordica charantia* polysaccharide (MCP) on *in vitro* cellulolytic bacteria (as a fraction of total bacterial 16S rDNA).

Item	Incubation time, h	MCP, mg/ml				SEM	MCP effect		
		0	0.1	0.3	0.6		Linear	Quadratic	Cubic
<i>Fibrobacter succinogenes</i>	6	0.003	0.002	0.003	0.002	0.001	ns	ns	ns
	24	0.007 <sup>b</sup>	0.005 <sup>c</sup>	0.007 <sup>b</sup>	0.010 <sup>a</sup>	0.000	***	**	*
<i>Ruminococcus albus</i>	6	0.004 <sup>a</sup>	0.004 <sup>a</sup>	0.003 <sup>ab</sup>	0.002 <sup>b</sup>	0.000	**	ns	ns
	24	0.012	0.010	0.008	0.012	0.002	ns	ns	ns
<i>Butyrivibrio fibrisolvens</i> ( $10^{-3}$ )	6	0.002 <sup>c</sup>	0.016 <sup>a</sup>	0.008 <sup>b</sup>	0.007 <sup>b,c</sup>	0.002	ns	*	***
	24	0.092 <sup>c</sup>	0.317 <sup>b,c</sup>	0.528 <sup>ab</sup>	0.829 <sup>a</sup>	0.115	**	ns	ns
<i>Ruminococcus flavefaciens</i> ( $10^{-3}$ )	6	0.031 <sup>a</sup>	0.006 <sup>b</sup>	0.006 <sup>b</sup>	0.006 <sup>b</sup>	0.002	***	***	***
	24	0.030 <sup>a</sup>	0.003 <sup>b</sup>	0.005 <sup>b</sup>	0.010 <sup>b</sup>	0.002	**	***	**

<sup>a-c</sup>Means within a row with different superscripts differ ( $p < .05$ ).

\* $p < .05$ ; \*\* $p < .01$ ; \*\*\* $p < .001$ ; ns: not significant.

the relative abundance of *Ruminococcus albus* (linear,  $p < .01$ ) and *R. flavefaciens* (linear, quadratic and cubic,  $p < .001$ ) were decreased with the increment of MCP doses. The relative abundances of *F. succinogenes* were comparable among four groups.

After 24 h of incubation, the relative abundance of *B. fibrisolvens* (linear,  $p < .01$ ) was increased, while that of *R. flavefaciens* was decreased (linear and cubic,  $p < .01$ ; quadratic,  $p < .001$ ) with the increase of MCP doses. There was a U-shaped curvilinear relationship between the relative abundance of *F. succinogenes* and MCP, with the least value being for the level of 0.1 mg/ml MCP and the greatest value being for the

level of 0.6 mg/ml MCP. The MCP inclusion did not affect the relative abundance of *R. albus* in incubation fluid.

### Correlation analysis of ruminal microbial population, GP parameters and VFA

*Ruminococcus albus* displayed a significant negative correlation with  $FRD_0$  ( $R = -.963$ ,  $p < .05$ ) (Table 5). *Ruminococcus flavefaciens* had a significant positive correlation with  $t_{0.5}$  ( $R = .965$ ,  $p < .05$ ). As well, *B. fibrisolvens* ( $R = .982$ ,  $p < .05$ ) displayed significant positive correlation with the molar proportion of propionate.

**Table 5.** Correlations between rumen cellulolytic bacteria population and gas production.

Item	V <sub>F</sub>	FRD <sub>0</sub>	t <sub>0.5</sub>	Acetate, % of tVFA	Propionate, % of tVFA	Isobutyrate, % of tVFA	Butyrate, % of tVFA	Isovalerate, % of tVFA	Valerate, % of tVFA	tVFA
<i>F. succinogenes</i>	-0.911	-0.740	0.231	-0.671	0.555	0.605	0.880	0.191	-0.028	-0.500
<i>R. albus</i>	-0.829	-0.963*	0.791	-0.855	-0.051	0.883	0.821	0.686	0.440	-0.847
<i>B. fibrisolvens</i>	-0.583	-0.285	-0.530	0.079	0.982*	-0.157	0.257	-0.583	-0.739	0.283
<i>R. flavefaciens</i>	-0.241	-0.440	0.965*	-0.867	-0.687	0.894	0.656	0.996**	0.962*	-0.943

\* $p < .05$ ; \*\* $p < .01$

*Ruminococcus flavefaciens* had a significant positive correlation with the molar proportion of isovalerate ( $R = .996$ ,  $p < .05$ ) and valerate ( $R = .962$ ,  $p < .05$ ). *Fibrobacter succinogenes* showed no significant correlation with molar proportion of any individual VFA, or the concentration of tVFA (Table 5).

## Discussion

Results in the current study showed that a high level of MCP inclusion (0.6 mg/ml) significantly decreased the V<sub>F</sub>. This finding was consistent with previous *in vitro* experiments which reported that the polysaccharide from *Saponaria officinalis* decreased the total gas production using dairy cow rumen fluid as inoculum (Cieslak et al. 2014). A decrease in FRD<sub>0</sub>, V<sub>F</sub>, t<sub>0.5</sub> value with 0.6 mg/ml MCP addition in the current study indicated that the *in vitro* fermentation of maize stover could be inhibited quickly by a high dose of MCP. An increase in FRD<sub>0</sub> value, and a decrease in t<sub>0.5</sub> value with 0.1, 0.3 mg/ml MCP addition in the current study indicated that the *in vitro* fermentation of maize stover could be promoted by low doses of MCP.

VFA production in the rumen depends highly on the degree and rate of fermentation (Dijkstra et al. 2005; Cone & Becker 2012). Cieslak et al. (2014) found that *Saponaria officinalis* polysaccharide did not change the concentration of the total VFA *in vitro* fermentation using rumen fluid from dairy cows. In present study, it could be clearly noted that the MCP significantly increased the total VFA concentration and pH (> 6.3). It seemed that MCP had the function of maintaining healthy rumen environment. Previous *in vivo* measurements have also shown that *Astragalus* polysaccharide supplementation stabilised ruminal pH in lambs (Zhong et al. 2012) and chitosan, a kind of polysaccharide biopolymer, stabilised ruminal pH in Nellore steers (Araújo et al. 2015).

NH<sub>3</sub>-N is a main source of nitrogen in the synthesis of ruminal bacteria, and the ruminal NH<sub>3</sub>-N concentration is a crude predictor of the efficiency of dietary N conversion to microbial N (Salter et al. 1979). In present study, ruminal NH<sub>3</sub>-N concentration was decreased in response to MCP inclusion at 24 h of

incubation, and it was consistent with earlier *in vitro* fermentation report (Cieslak et al. 2014) that *Saponaria officinalis* polysaccharide decreased NH<sub>3</sub> contents using the rumen fluid of dairy cows as inoculum. Therefore, the results of current study indicated that MCP inclusion might increase the efficiency of dietary N conversion to microbial N at 24 h of *in vitro* fermentation. Furthermore, the current study showed that MCP inclusion decreased the molar proportion of isobutyrate and isovalerate at 24 h of incubation, suggesting that deamination activity *in vitro* incubation fluid may have been inhibited by MCP inclusion, because branch-chain VFAs are derived from deamination of amino acids in the rumen (Hino & Russell 1985). Increment of total VFA concentration and reduction of NH<sub>3</sub>-N at low doses of MCP at 24 h of incubation synergistically reflected the enhancement of the activities of ruminal microbes, because the growth of microbes required NH<sub>3</sub>-N as nitrogen sources. Certainly, the total bacteria population will need to be determined to confirm this hypothesis in future.

In present study, it could be clearly noted that the MCP inclusion significantly increased the molar proportion of acetate and decreased the proportion of propionate. Therefore, the A/P ratio was increased at 6 h of incubation. This result indicated that the MCP changed the fermentation pathway at 6 h of incubation. Liu et al. (2015) reported that addition of pectin, a kind of polysaccharide, increased acetate production *in vitro* fermentation using rumen fluid at 24 h of incubation. Cieslak et al. (2014) found that *Saponaria officinalis* polysaccharide decreased the molar proportion of acetate and increased that of propionate *in vitro* fermentation using rumen fluid from dairy cows. In present study, the A/P ratio appeared to be lower than we expected, but it was consistent with earlier *in vitro* fermentation reports (Tang et al. 2013; Wu et al. 2013) that the A/P ratios were below 2.0 *in vitro* fermentation.

Acetate formation is due mainly to structural carbohydrate fermentation by cellulolytic bacteria, whereas the fermentation of non-structural carbohydrate is degraded by amylolytic bacteria, leading to a relatively higher production of propionate (Wanapat et al. 2013). In present study, MCP addition increased the

population of *B. fibrisolvans* and *F. succinogenes*, but decreased population of *R. flavefaciens* and *R. albus*. The result indicated that cellulolytic bacteria population responded differently to MCP inclusion. Zhao et al. (2015) have reported that pectin increased the copy numbers of *R. albus* and *R. flavefaciens in vitro* using goat rumen fluid as inoculum. Joshua & Daniel (1993) have reported that ruminal bacterial strains (*Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *Ruminococcus albus* and *Butyrivibrio fibrisolvans*) solubilised cotton stalks cell walls polysaccharides differently and the utilisation of solubilised cellulose, xylan, hemicellulose sugars and total carbohydrates by these ruminal bacterial strains was also varied. Hall and Weimer (2016) have reported that the growth rate of ruminal bacteria on glucose, fructose, maltose and sucrose varied. The possible reason for the different responses of rumen bacteria to the polysaccharide may be the difference of bacteria in the degradation and utilisation of polysaccharides (Gradel & Dehority 1972).

Simultaneously, the examined relationships between rumen cellulolytic bacteria population and the GP parameters, VFA concentrations indicated that the change of the abundances of *R. albus*, *B. fibrisolvans* and *R. flavefaciens* in rumen liquor may affect the ruminal fermentation. Certainly, the effectiveness of MCP for the improvement of *in vitro* ruminal fermentation of roughages needed to be further validated *in vivo*.

## Conclusions

The current study indicated that 0.1 and 0.3 mg/ml MCP addition promoted the *in vitro* fermentation of maize stover, with increased the total VFA production, changed the rumen fermentation pathway and increased the population of *B. fibrisolvans* and *F. succinogenes*. Studies are needed to further verify the effect of MCP *in vivo*, expanding possibly ultimate use of MCP supplementation for various rations in animal production.

## Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

## Funding

The authors wish to acknowledge the financial support received from the National Natural Science Foundation of China (Grant No. 31201826, 31320103917) and Tianjin

Science and Technology Support Plan Project (13ZCZDNC01800).

## References

- Araújo APC, Venturelli BC, Santos MCB, Gardinal R, Cônsolo NRB, Calomeni GD, Freitas JE, Barletta RV, Gandra JR, Paiva PG, et al. 2015. Chitosan affects total nutrient digestion and ruminal fermentation in Nelore steers. *Anim Feed Sci Tech.* 206:114–118.
- Bo MJ, Yang F, Kong XF, Zhang YZ, Tan ZL, Yin YL. 2010. Comparative study on *in vitro* fermentation characteristics of three fractions of *Momordica charantia* polysaccharides. *Nat Prod Res Dev.* 22:625–629.
- Cieslak A, Zmora P, Stochmal A, Pecio L, Oleszek W, Perskamczyc E, Szczechowiak J, Nowak A, Szumacher-strabel M. 2014. Rumen antimethanogenic effect of *Saponaria officinalis* L. phytochemicals *in vitro*. *J Agr Sci.* 152:981–993.
- Cone JW, Becker PM. 2012. Fermentation kinetics and production of volatile fatty acids and microbial protein by starchy feedstuffs. *Anim Feed Sci Tech.* 172:34–41.
- Dijkstra J, Kebreab E, Bannink A, France J, Lopez S. 2005. Application of the gas production technique to feed evaluation systems for ruminants. *Anim Feed Sci Tech.* 123:561–578.
- Gradel CM, Dehority B. 1972. Fermentation of isolated pectin and pectin from intact forages by pure cultures of rumen bacteria. *Appl Microbiol.* 23:332–340.
- Guan L. 2012. Synthesis and anti-tumour activities of sulphated polysaccharide obtained from *Momordica charantia*. *Nat Prod Res.* 26:1303–1309.
- Guo FC, Williams BA, Kwakkel RP, Verstegen MW. 2003. *In vitro* fermentation characteristics of two mushroom species, an herb, and their polysaccharide fractions, using chicken cecal contents as inoculum. *Poult Sci.* 82:1608–1615.
- Hall MB, Weimer PJ. 2016. Divergent utilization patterns of grass fructan, inulin, and other nonfiber carbohydrates by ruminal microbes. *J Dairy Sci.* 99:245–257.
- Hino T, Russell JB. 1985. Effect of reducing-equivalent disposal and NADH/NAD on deamination of amino acids by intact rumen microorganisms and their cell extracts. *Appl Environ Microb.* 50:1368–1374.
- Joshua M, Daniel BG. 1993. Untreated and delignified cotton stalks as model substrates for degradation and utilization of cell-wall monosaccharide components by defined ruminal cellulolytic bacteria. *Bioresour Technol.* 43:241–247.
- Laurentin A, Edwards CA. 2003. A microtiter modification of the anthrone-sulfuric acid colorimetric assay for glucose-based carbohydrates. *Anal Biochem.* 315:143–145.
- Liu J, Pu YY, Xie Q, Wang JK, Liu JX. 2015. Pectin induces an *in vitro* rumen microbial population shift attributed to the pectinolytic *treponema* group. *Curr Microbiol.* 70:67–74.
- Marzorati M, Maignien L, Verhelst A, Luta G, Sinnott R, Kerckhof FM, Boon N, Van de Wiele T, Possemiers S. 2013. Barcoded pyrosequencing analysis of the microbial community in a simulator of the human gastrointestinal tract showed a colon region-specific microbiota modulation for



- two plant-derived polysaccharide blends. *Anton Van Leeuw.* 103:409–420.
- Navarini L, Gilli R, Gombac V, Abatangelo A, Bosco M, Toffanin R. 1999. Polysaccharides from hot water extracts of roasted *Coffea arabica* beans: Isolation and characterization. *Carbohydr Polym.* 40:71–81.
- Poulsen M, Jensen BB, Engberg RM. 2012. The effect of pectin, corn and wheat starch, inulin and pH on *in vitro* production of methane, short chain fatty acids and on the microbial community composition in rumen fluid. *Anaerobe.* 18:83–90.
- Salter DN, Daneshvar K, Smith RH. 1979. Origin of nitrogen incorporated into compounds in the rumen bacteria of steers given protein-containing and urea-containing diets. *Br J Nutr.* 41:197–209.
- Tang SX, Zou Y, Wang M, Salem AZM, Odongo NE, Zhou CS, Han XF, Tan ZL, Zhang M, Fu YF, et al. 2013. Effects of exogenous cellulase source on *in vitro* fermentation characteristics and methane production of crop straws and grasses. *Anim Feed Sci Tech.* 13:489–505.
- Wanapat M, Gunun P, Anantasook N, Kang S. 2013. Changes of rumen pH, fermentation and microbial population as influenced by different ratios of roughage (rice straw) to concentrate in dairy steers. *J Agr Sci-Cambridge.* 152:675–685.
- Wang M, Sun XZ, Tang SX, Tan ZL, Pacheco D. 2013a. Deriving fractional rate of degradation of logistic-exponential (LE) model to evaluate early *in vitro* fermentation. *Anim Feed Sci Tech.* 7:920–929.
- Wang M, Tang SX, Tan ZL. 2011. Modeling *in vitro* gas production kinetics: Derivation of Logistic-Exponential (LE) equations and comparison of models. *Anim Feed Sci Tech.* 165:137–150.
- Wang M, Wang R, Tang SX, Tan ZL, Zhou CS, Han XF, Kang JH. 2016. Comparisons of manual and automated incubation systems: effects of venting procedures on *in vitro* ruminal fermentation. *Livest Sci.* 184:41–45.
- Wang Y, Yuan B, Ji YC, Li H. 2013b. Hydrolysis of hemicellulose to produce fermentable monosaccharides by plasma acid. *Carbohydr Polym.* 97:518–522.
- Weatherburn MW. 1967. Phenol-hypochlorite reaction for determination of ammonia. *Anal Biochem.* 39:971–974.
- Wu DQ, Tang SX, He ZX, Odongo dwin NE, Tan ZL, Han XF, Zhou CS, Kang JH, Wang M. 2013. Oleic and linoleic acids alter fermentation characteristics, methane and fatty acid isomers production during *in vitro* incubation with mixed ruminal microbes. *J Food Agric Environ.* 11:464–469.
- Zeng B, Tan ZL, Zeng JY, Tang SX, Tan CY, Zhou CS, Han XF, Zhong RZ. 2012. Effects of dietary non-ionic surfactant and forage to concentrate ratio on bacterial population and fatty acid composition of rumen bacteria and plasma of goats. *Anim Feed Sci Tech.* 173:167–176.
- Zhao XH, Gong JM, Zhou S, Fu CB, Liu CJ, Xu LJ, Pan K, Qu MR. 2015. Effects of degradable protein and non-fibre carbohydrates on microbial growth and fermentation in the rumen simulating fermenter (rusitec). *Ital J Anim Sci.* 14:220–225.
- Zhong R, Yu M, Liu H, Sun H, Cao Y, Zhou D. 2012. Effects of dietary Astragalus polysaccharide and Astragalusmembranaceus root supplementation on growth performance, rumen fermentation, immune responses, and antioxidant status of lambs. *Anim Feed Sci Tech.* 174:60–67.