Journal of Animal and Veterinary Advances 11 (1): 36-42, 2012
ISSN: 1680-5593

Effects of Adding Mannan-Oligosaccharide to Different Concentrate-to-Roughage Diets on Ruminal Fermentation *in vitro*

[1, 2]Zheng Chen, [1]Hao Zhengli, [1, 2]Li Fadi, [1]Guo Yanli and [3]Jin Yanmei
[1]Faculty of Animal Science and Technology,
Gansu Agricultural University, Lanzhou, Gansu, P.R. China
[2]Gansu Feed Engineer Research Center, Lanzhou, Gansu, P.R. China
[3]College of Marine Studies, Shandong University at Weihai, Weihai, Shandong, P.R. China

Abstract: This study was conducted to investigate the effects of adding Mannan-Oligosaccharide (MOS) to different concentrate-to-roughage diets on ruminal fermentation *in vitro*. A 4×6 two factorial experimental design (the concentrate-to-roughage ratio, 20:80, 30:70, 40:60, 50:50 and the MOS level, 0, 0.4, 0.8, 1.2, 1.6 and 2.0%) was carried out. The gas production technique was adopted and used the 100 mL cultivated bottles, 50 mL graduated syringes and thermostatic water bath pans as *in vitro* cultivated device. The *in vitro* incubation continued 24 h and collected samples at 3, 6, 9, 12 and 24 h. Each treatment executed five replicates at each fermentation time. The results showed the gas and methane production were not impacted by concentrate-to-roughage and MOS and the combination of two factors did not influence all items significantly ($p>0.05$). The *In vitro* Dry Matter Digestibility (IVDMD) was impacted by concentrate-to-roughage ratio (20:80<40:60, 9 and 12 h, $p<0.05$, 24 h, $p<0.01$; 20:80<50:50, 3, 12 and 24 h, $p<0.01$). The *In vitro* Crude Protein Digestibility (IVCPD) was affected by both concentrate-to-roughage ratio (20:80>40:60, 3 h, $p<0.05$; 20:80>50:50, 12 h, $p<0.01$; 30:70>40:60, 3 h, $p<0.01$, 6 h, $p<0.05$; 30:70>50:50, 3 and 12 h, $p<0.01$; 6 and 9 h, $p<0.05$) and MOS (0<1.6%, 24 h, $p<0.01$; 0<2.0%, 24 h, $p<0.05$). The pH also influenced by concentrate-to-roughage ratio (50:50<20:80, 3, 6, 9 and 12 h, $p<0.01$; 50:50<30:70, 3, 6 and 12 h, $p<0.01$; 50:50<40:60, 12 h, $p<0.05$; 20:80>40:60, 6 and 12 h, $p<0.01$; 3 and 9 h, $p<0.05$) and MOS (0>1.6%, 6 and 12 h, $p<0.01$; 0>2.0%, 6, 12 and 24 h, $p<0.05$; 0.4>2.0%, 6 h, $p<0.05$). The NH_3-N content was affected by concentrate-to-roughage ratio (50:50<20:80, 3 and 9 h, $p<0.05$; 50:50<30:70, 12 h, $p<0.01$) and MOS (2.0>0%, 24 h, $p<0.01$). The concentrate-to-roughage impacted the ratio of acetic acid to propionic acid (20:80>50:50, $p<0.05$), acetic acid to TVFAs (20:80>40:60 and 20:80>50:50, $p<0.01$) and butyric acid to TVFAs (20:80<40:60 and 20:80<50:50, $p<0.05$). We could find the IVCPD, pH, NH_3-N content increased with the reducing of concentrate approximately while opposite to the IVDMD and the IVCPD and NH_3-N content increased with the rising of MOS roughly while opposite to pH.

Key words: Mannan-oligosaccharide, concentrate-to-roughage, ruminal fermentation, *in vitro*, China

INTRODUCTION

Mannan-Oligosaccharides (MOS) is another yeast cell wall derived feed ingredient working locally in the gut. MOS are known to improve digestion and gut health in animals by binding to and blocking glycoprotein receptors on pathogens (Newman and Newman, 2001; Fernandez *et al*., 2002; Refstie *et al*., 2010). Additionally, it improved digestive morphology and modulation of gut microbial populations (Torrecillas *et al*., 2007; Dimitroglou *et al*., 2009; Daniels *et al*., 2010). There were many studies about animals growth using MOS such as increasing growth on broilers turkeys (Hooge, 2004 a, b), enhancing rate of daily weight gain and feed conversion on weaning pigs (Rosenboom *et al*., 2001; Davis *et al*., 2002), improving faecal consistency on calves (Heinrichs *et al*., 2003) and raising concentrate intake in early life on prewean dairy calves (Morrison *et al*., 2010). The incorporation of concentrates into ruminant diets was intended to increase dietary energy, proteins, minerals and vitamins and to optimize the efficiency of feed utilization (Cantalapiedra-Hijar *et al*., 2009). However, ruminal fermentation varied obviously by concentrate-to-roughage ratio. Depending on ruminal pH decreased sharply with the rising of concentrate ratio, the gas and methane production, N metabolism, VFAs yield and proportion, digestibility of DM, OM, NDF and ADF, chymus flowing speed were impacted extensively.

Corresponding Author: Li Fadi, Faculty of Animal Science and Technology, Gansu Feed Engineer Research Center, Gansu Agricultural University, Gansu, 730070 Lanzhou, P.R. China

The *in vitro* gas production technique has gained general recognition over the last decade being used increasingly for feed evaluation, to investigate mechanisms of microbial fermentation and for studying the mode of action of anti-nutritive factors, additives and feed supplements. The technique is based on the assumption that the gas produced in batch cultures is just the consequence of the fermentation of a given amount of substrate (Lopez *et al*., 2007). One of the advantages of the *in vitro* gas production technique is that it can be automated to obtain a large number of data points allowing more accurate parameter estimation than the gravimetric *in vitro* techniques or *in situ* methods (Huhtanen *et al*., 2008). There were many studies focus on the effects of MOS on nonruminants, a few studies researched ruminants directly and most of them paied attention to small ruminants not alone the influences of MOS on ruminal fermentation *in vitro*. Therefore, this experiment was conducted to investigate the efficiency of adding MOS to different concentrate-to-roughage diets on ruminal fermentation *in vitro*.

MATERIALS AND METHODS

Experimental design: A 4×6 two factorial experimental design was carried out. The two factors were the ratio of concentrate-to-roughage (20:80, 30:70, 40:60 and 50:50) and the level of adding MOS (BIO-MOS®, Alltech; 0, 0.4, 0.8, 1.2, 1.6 and 2.0%). Five replicates in each level per each point and fermentation times were 3, 6, 9, 12 and 24 h.

Diets: The diets were formulated to meet or exceed the recommendations for all nutrients of ram hogg of China Agricultural Industry Standard (NY/T816-2004), regardless of each treatment (Table 1).

Table1: The composition and nutritional level of the experiment diets (air dry basis)

	C:R[a] treatment			
Items	20:80	30:70	40:60	50:50
Ingredients (%)				
Corn	12.490	19.290	29.290	37.990
Soybean meal	7.000	9.000	9.000	9.000
Cotton seed meal	1.500	1.500	1.500	2.500
Alfalfa	37.800	29.000	26.000	19.000
Tall oat grass	40.000	40.000	33.000	30.300
Salt	0.700	0.700	0.700	0.700
Additive premix[b]	0.510	0.510	0.510	0.510
Total	100.000	100.000	100.000	100.000
Nutrient				
DM (%)	90.860	90.620	90.270	89.970
DE (MJ kg^{-1})	9.120	9.690	10.320	10.930
CP (%)	10.460	10.910	11.030	11.160
E/P (MJ g^{-1})[c]	0.087	0.089	0.094	0.098
Ca (%)	0.670	0.570	0.530	0.440
P (%)	0.210	0.220	0.230	0.240

[a]Concentrate-to-roughage ratio; [b]Additive premix includes: mineral elements (mg kg^{-1}): S, 200; Fe, 25; Zn, 40; Cu, 8; I, 0.3; Mn, 40; Se, 0.2; Co, 0.1; vitamin (IU kg^{-1}): VA, 940; VE, 20; [c]Energy-to-protein ratio

***In vitro* operation:** Six crossbred wethers fitted with permanent rumen fistula were chosen as the source of rumen liquid. A 100 mL cultivated bottles, 50 mL graduated syringes and thermostatic water bath pans were used as *in vitro* cultivated installation. The cultivated bottles were plugged with airtight rubber plugs and connected with syringes by needles. The syringes should be clean and smeared liquid paraffin on pistons before using avoiding air leak and reducing resistance. The ruminal liquid were collected from six crossbred wethers before morning meal and filtered with four layers gauzes and then stored in the thermos which was full with CO_2 beforehand and brought to lab as soon as possible. Afterward, 20 mL ruminal liquid was transfered to each cultivated bottle which was added 1 g substrate (different diets), full with CO_2, added 40 mL buffer and thermostatic at 39°C beforehand. Then the bottles and syringes were connected with needles. The blank treatment should be carried out at the same time.

Laboratory analysis: The gas production at different periods was read on graduated syringes directly and calibrated by blank treatment. The methane and VFA content of cultivated liquid was analyzed by gas chromatography (Agilent 6890N) and the calculation of the methane content was calibrated by blank treatment and the headspace volume of the bottle (Lopez *et al*., 2007). The pH of cultivated liquid was measured by Sartorius PB-10 acidity meter. The NH_3-N content was determined by colorimetric method. The DM (method 934.01) and CP (method 984.13) contents were determined according to the AOAC (1999).

Statistical analysis: The data were analyzed by Univariate procedure of General linear model (SPSS 16.0). Tukey post hoc tests were used to determine any significant differences between groups and factors. Because of no significant differences observed from each combination ($p>0.3$), these data were not listed in tables for clearness.

RESULTS

The gas and methane production: The methane production was affected by MOS at 9 h significantly ($p<0.01$) and there was no influence on the gas and methane production by both concentrate-to-roughage and MOS at other time ($p>0.05$; Table 2 and 3). The methane production in 1.2% MOS was higher than 0 and 0.8% MOS significantly ($p<0.05$).

The IVDMD and IVCPD: The IVDMD at 9, 12 and 24 h was impacted significantly by concentrate-to-roughage ($p<0.01$; Table 4). The IVDMD in 50:50

Table 2: The gas production in different periods (mL g^{-1})

	Cultivated period (h)				
Items	3	6	9	12	24
C:R*					
20:80	33.550	44.990	69.160	76.910	91.970
30:70	37.670	49.620	60.790	72.640	100.390
40:60	39.480	55.240	67.050	79.040	110.490
50:50	37.530	47.200	59.380	70.940	95.760
MOS (%)					
0	37.860	50.110	55.480	62.080	90.940
0.4	33.490	45.340	54.580	71.220	85.560
0.8	38.120	45.060	62.270	73.180	95.590
1.2	38.460	47.130	72.700	88.890	114.560
1.6	37.490	51.920	66.850	78.120	105.280
2.0	38.290	55.040	68.780	75.450	99.560
SEM**	1.460	1.900	2.580	3.270	4.540
p-value					
C:R	0.366	0.340	0.501	0.845	0.561
MOS	0.741	0.678	0.379	0.522	0.403
C:R×MOS	0.673	0.998	0.997	0.950	0.862

$p<0.05$; $p<0.01$; *Concentrate-to-roughage ratio; **SEM: SE Mean

Table 3: The methane production in different periods (mL g^{-1})

	Cultivated period (h)				
Items	3	6	9	12	24
C:R*					
20:80	2.830	5.600	7.780	9.730	12.080
30:70	3.310	4.840	8.760	9.480	12.340
40:60	3.530	5.100	7.850	10.170	11.920
50:50	3.060	5.600	7.410	7.550	9.450
MOS (%)					
0	3.200	4.680	6.510^{b}	7.650	11.070
0.4	2.730	5.170	7.020^{ab}	9.340	10.690
0.8	3.160	5.320	6.490^{b}	8.460	11.060
1.2	3.450	5.610	9.970^{a}	10.550	13.270
1.6	3.050	5.140	8.600^{ab}	10.780	11.600
2.0	3.540	5.760	9.170^{ab}	10.510	11.520
SEM**	0.140	0.200	0.320	0.450	0.640
p-value					
C:R	0.328	0.548	0.575	0.702	0.780
MOS	0.730	0.761	0.003	0.194	0.965
C:R×MOS	0.929	0.983	0.525	0.849	0.944

$^{a-c}p<0.05$; $p<0.01$; *Concentrate-to-roughage ratio; **SEM: SE Mean

Table 4: The IVDMD in different periods (%)

	Cultivated period (h)				
Items	3	6	9	12	24
C:R*					
20:80	41.580	43.170	45.450^{Bc}	48.810^{Bb}	55.460^{B}
30:70	44.970	46.320	48.030^{ABbc}	50.870^{AB}	58.970^{AB}
40:60	42.120	45.980	49.050^{ABab}	53.470^{ABa}	60.960^{A}
50:50	44.780	47.400	51.980^{Aa}	54.940^{A}	62.710^{A}
MOS (%)					
0	43.250	46.050	47.280	51.980	59.200
0.4	43.880	45.470	48.990	51.890	59.640
0.8	42.470	43.960	48.430	52.780	60.100
1.2	42.880	45.510	48.820	51.720	58.680
1.6	43.310	46.770	49.150	52.360	60.140
2.0	43.490	46.900	49.210	50.760	59.400
SEM**	0.620	0.730	0.490	0.550	0.600
p-value					
C:R	0.127	0.311	0.000	0.001	0.000
MOS	0.982	0.905	0.799	0.990	0.980
C:R×MOS	0.640	0.999	0.999	0.999	0.998

$^{a-c}p<0.05$; $^{A-C}p<0.01$; *Concentrate-to-roughage ratio; **SEM: SE Mean

Table 5: The IVCPD in different periods (%)

	Cultivated period (h)				
Items	3	6	9	12	24
C:R*					
20:80	20.580^{ABa}	25.740^{ab}	28.490^{ab}	32.000^{A}	41.560
30:70	24.010^{A}	27.230^{a}	30.270^{a}	33.430^{A}	42.840
40:60	14.020^{Bb}	18.690^{b}	22.350^{ab}	27.460^{AB}	38.440
50:50	15.540^{B}	18.520^{b}	21.770^{b}	24.200^{B}	36.580
MOS (%)					
0	17.100	21.310	23.900	27.520	33.160^{Bb}
0.4	18.100	23.000	21.410	26.280	37.610^{AB}
0.8	19.940	23.790	26.210	28.870	39.810^{AB}
1.2	15.600	19.010	26.120	28.590	40.130^{AB}
1.6	21.720	24.020	25.530	31.540	45.130^{A}
2.0	20.560	25.360	29.090	32.400	43.280^{ABa}
SEM**	0.850	1.080	1.040	0.890	0.930
p-value					
C:R	0.000	0.002	0.022	0.010	0.059
MOS	0.089	0.358	0.627	0.465	0.004
C:R×MOS	0.412	0.869	0.672	0.795	0.859

$^{a-c}p<0.05$; $^{A-C}p<0.01$; *Concentrate-to-roughage ratio; **SEM: SE mean

concentrate-to-roughage was higher than 20:80 concentrate-to-roughage significantly (9, 12 and 24 h, $p<0.01$) and the IVDMD in 40:60 concentrate-to-roughage was higher than 20:80 concentrate-to-roughage significantly (9 and 12 h, $p<0.05$; 24 h, $p<0.01$).

From the Table 4, researchers could observe the IVDMD rose accompanied with the increase of concentrate-to-roughage ratio approximately. The IVCPD was affected by both concentrate-to-roughage (3, 6, 9 and 12 h, $p<0.05$) and MOS significantly (24 h, $p<0.05$; Table 5). The IVCPD in 30:70 concentrate-to-roughage was higher than 50:50 concentrate-to-roughage (3 and 12 h, $p<0.01$; 6 and 9 h, $p<0.05$) and 40:60 concentrate-to-roughage significantly (3 h, $p<0.01$; 6 h, $p<0.05$). The IVCPD in 20:80 concentrate-to-roughage was higher than 40:60 concentrate-to-roughage (3 h, $p<0.05$) and 50:50 concentrate-to-roughage significantly (12 h, $p<0.01$). The IVCPD in 1.6 and 2.0% MOS was higher than 0% MOS significantly (24 h, $p<0.01$, $p<0.05$, respectively). They also roughly concluded the IVCPD rose with the decrease of concentrate-to-roughage or increase of MOS.

The pH, NH_3-N content and VFAs of cultivation liquid: Except 24 h, the pH of cultivation liquid *in vitro* was impacted by concentrate-to-roughage significantly ($p<0.01$; Table 6) and was significantly affected by MOS at 12 h ($p<0.01$), 6 h and 24 h ($p<0.05$). The pH in 20:80 and 30:70 concentrate-to-roughage was higher than 50:50 concentrate-to-roughage significantly (3, 6 and 12 h, $p<0.01$) and the pH in 20:80 concentrate-to-roughage was higher than 50:50 concentrate-to-roughage significantly (9 h, $p<0.01$).

The pH in 20:80 concentrate-to-roughage was higher than 40:60 concentrate-to-roughage significantly (6 and 12 h, $p<0.01$; 3 and 9 h, $p<0.05$). The pH in 40:60

Table 6: The pH of cultivation liquid *in vitro* at different periods

	Cultivated period (h)				
Items	3	6	9	12	24
C:R*					
20:80	6.650^{Aa}	6.590^{A}	6.650^{Aa}	6.530^{A}	6.450
30:70	6.600^{A}	6.550^{AB}	6.529^{AB}	6.480^{AB}	6.430
40:60	6.580^{ABb}	6.480^{BC}	6.460^{ABb}	6.420^{BCa}	6.350
50:50	6.510^{B}	6.420^{C}	6.410^{B}	6.360^{Cb}	6.400
MOS (%)					
0	6.600	6.550^{a}	6.540	6.510^{A}	6.590^{a}
0.4	6.610	6.550^{ab}	6.510	6.470^{AB}	6.430^{ab}
0.8	6.590	6.520^{abc}	6.640	6.460^{AB}	6.390^{ab}
1.2	6.580	6.490^{abc}	6.480	6.440^{AB}	6.350^{ab}
1.6	6.560	6.480^{bc}	6.450	6.410^{B}	6.360^{ab}
2.0	6.560	6.460^{c}	6.430	6.400^{B}	6.320^{b}
SEM**	0.010	0.010	0.030	0.010	0.030
p-value					
C:R	0.000	0.000	0.005	0.000	0.591
MOS	0.535	0.023	0.190	0.002	0.036
C:R×MOS	0.994	1.000	0.401	1.000	0.375

$^{a-c}$p<0.05; $^{A-C}$p<0.01; *Concentrate-to-roughage ratio; **SEM: SE Mean

Table 7: The NH_3-N content of cultivation liquid *in vitro* at different periods (mg mL^{-1})

	Cultivated period (h)				
Items	3	6	9	12	24
C:R*					
20:80	0.530^{a}	0.490	0.520^{a}	0.530^{ABa}	0.760
30:70	0.480^{ab}	0.470	0.480^{ab}	0.550^{A}	0.750
40:60	0.490^{ab}	0.440	0.470^{ab}	0.500^{AB}	0.680
50:50	0.440^{b}	0.420	0.450^{b}	0.450^{Bb}	0.670
MOS (%)					
0	0.470	0.450	0.460	0.490	0.620^{B}
0.4	0.460	0.460	0.450	0.500	0.680^{AB}
0.8	0.480	0.450	0.470	0.520	0.700^{AB}
1.2	0.510	0.480	0.500	0.520	0.730^{AB}
1.6	0.470	0.420	0.490	0.500	0.730^{AB}
2.0	0.520	0.460	0.500	0.520	0.820^{A}
SEM**	0.010	0.010	0.010	0.010	0.020
p-value					
C:R	0.028	0.054	0.041	0.001	0.119
MOS	0.507	0.656	0.473	0.838	0.016
C:R×MOS	0.771	0.978	0.859	0.553	0.982

$^{a-c}$p<0.05; $^{A-C}$p<0.01; *Concentrate-to-roughage ratio; **SEM: SE Mean

concentrate-to-roughage was higher than 50:50 concentrate-to-roughage significantly (12 h, p<0.05). The pH in 0% MOS was higher than 1.6 and 2.0% MOS significantly (6 and 12 h, p<0.01, p<0.05, respectively). The pH in 0% MOS was higher than 2.0% MOS significantly (24 h, p<0.05). The pH in 0.4% MOS was higher than 2.0% MOS significantly (6 h, p<0.05).

The NH_3-N content of cultivation liquid *in vitro* was significantly affected by concentrate-to-roughage at 3 h, 9 h (p<0.05) and 12 h (p<0.01) and by MOS at 24 h (p<0.05; Table 7). The NH_3-N content in 20:80 concentrate-to-roughage was higher than 50:50 concentrate-to-roughage significantly at 3 h and 9 h (p<0.05). The NH_3-N content in 30:70 concentrate-to-roughage was higher than 50:50 concentrate-to-roughage

Table 8: The TVFAs content and the ratio of each acid to TVFAs of cultivation liquid *in vitro*

			The ratio of every acid to TVFAs (%)			
Items	TVFAs (mmol L^{-1})	A:P*	Acetic acid	Propionic acid	Butyric acid	others
C:R**						
20:80	33.32	3.930^{a}	68.45^{A}	18.360	10.210^{b}	2.980
30:70	34.43	3.870^{ab}	67.61^{AB}	18.480	10.970^{ab}	2.940
40:60	33.80	3.640^{ab}	66.39^{B}	19.140	11.460^{a}	3.000
50:50	33.47	3.610^{b}	66.22^{B}	19.360	11.500^{a}	2.920
MOS (%)						
0	32.11	4.000	68.30	17.900	10.920	2.880
0.4	32.02	3.810	67.41	18.680	10.860	3.050
0.8	34.04	3.710	67.10	19.110	10.870	2.930
1.2	33.94	3.740	66.91	18.930	11.100	3.060
1.6	34.59	3.620	66.75	19.160	11.150	2.940
2.0	35.84	3.700	66.54	19.220	11.330	2.910
SEM***	0.670	0.040	0.210	0.160	0.150	0.030
p-value						
C:R	0.944	0.010	0.000	0.079	0.009	0.831
MOS	0.571	0.160	0.180	0.166	0.940	0.502
C:R×MOS	0.998	0.756	0.994	0.879	0.999	0.990

$^{a-c}$p<0.05; $^{A-C}$p<0.01; *Acetic acid? Propionic acid; **Concentrate-to-roughage ratio; ***SEM: SE Mean

significantly at 12 h (p<0.01). The NH_3-N content in 2.0% MOS was higher than 0% MOS significantly at 24 h (p<0.01).

The ratio of acetic acid to propionic acid the ratio of acetic acid and butyric acid to TVFAs of cultivation liquid *in vitro* were affected by concentrate-to-roughage significantly (p<0.01; Table 8). The ratio of acetic acid to propionic acid in 20:80 concentrate-to-roughage was higher than 50:50 concentrate-to-roughage significantly (p<0.05). The ratio of acetic acid to TVFAs in 20:80 concentrate-to-roughage was higher than 40:60 and 50:50 concentrate-to-roughage significantly (p<0.01) while the ratio of butyric acid to TVFAs was lower than the latter significantly (p<0.05). In addition, we could find the pH, NH_3-N content, ratio of acetic acid to TVFAs and acetic acid:propionic acid of cultivation liquid reduced and the ratio of propionic acid and butyric acid to TVFAs increased with the ascending of concentrate and the pH reduced and NH_3-N content increased with the rising of MOS in this experiment.

DISCUSSION

Effects on the gas and methane production when adding MOS to different concentrate-to-roughage ratio diets: Tang *et al.* (2008) reported that adding yeast culture (0, 2.5, 5.0 and 7.5 g kg^{-1}) on four cereal straws (rice straw, wheat straw, maize stover and maize stover silage) could increase the cumulative gas production, theoretical maximum of gas production and rate of gas production *in vitro*. Goiri *et al.* (2010) declared the methane in adding chitosan substrate (50% alfalfa hay and 50% concentrate,

starch or cellulose) was lower than control substrate significantly by gas production experiment *in vitro*. But in this experiment, MOS did not significantly influence the gas and methane production *in vitro*.

In general, the gas production is increased accompanying with the increase of concentrate in diets. But some reports showed diverse conclusion on gas production by different concentrate-to-roughage ratio. Samanta *et al.* (2000) indicated when substrates were a grass mixture (*Cenchrus ciliaris* and *Heteropogon contortus*) only (T_0) and 50:50 (T_1), 60:40 (T_2) and 70:30 (T_3) grass:concentrate ratios the highest gas production was in T_3 (110.62+ or -4.52 mL) followed by T_2 (110.05+ or -5.62 mL), T_1 (97.37+ or 7.66 mL) and T_0 (75.16+ or -5.42 mL) *in vitro* fermentation. In the experiment of Mohini *et al.* (2007) however, 18 Lactating cattle were divided in 3 groups and animals of group 1 were fed green fodder (maize) and concentrate mixture according to their requirements while group 2 and 3 requirement of roughage was divided in fresh and dry portion in the ratio of 50:50 and 40:60, respectively. Results showed total methane produced was 218.15+ or -11.03, 233.50+ or -15.24 and -252.82+ or -10.82 g day^{-1} in group 1-3, respectively and the difference among the groups was not significant.

There were a few relative studies and theories about experiments with adding MOS *in vitro* and no regular changes of the gas and methane production between groups were observed from this experiment so, it needed further research to investigate the reasons.

Effects on the IVDMD and IVCPD when adding MOS to different concentrate-to-roughage ratio diets: The IVDMD and IVCPD were the important parameters about descriptive diets digestibility *in vitro* and they can be used to forecast the digestibility of the diets in rumen. Hinman *et al.* (1998) reported there were no significant effects on the DM, CP, NDF, ADF and GE apparent digestibilities of steers when adding yeast culture to diet which used in a barley and potato finishing diet (85 g $steer^{-1}$ daily for the 1st 28 days and then 28 g $steer^{-1}$ daily for the remaining 85 days). The experiment was conducted to investigate the effects on the DM, CP, OM and GE apparent digestibilities of adding yeast culture to higher fat and lower fat diets of horse and the results implied no significant effects on these apparent digestibilities (Markey and Kline, 2006).

After all, there were some differences between *in vitro* and *in vivo* experiments. Tang *et al.* (2008) showed that adding yeast culture (0, 2.5, 5.0 and 7.5 g kg^{-1}) on four cereal straws (rice straw, wheat straw, maize stover and maize stover silage) could increase the IVDMD and IVOMD. Opposite to the above *in vitro* experiment, MOS only influenced the IVCPD at 24 h but concentrate-to-roughage affected both the IVDMD and IVCPD in this experiment. Variety of concentrate-to-roughage ratio leaded to different consists in diets although under the similar nutritional level and these changes might denote different utilization of nutrients by sheep. As a result, the IVDMD and IVCPD were altered by concentrate-to-roughage *in vitro*.

It conformed to the results which the IVDMD in AC diet (30% alfalfa hay and 70% concentrate) and GC diet (30% grass hay and 70% concentrate) was higher than AF diet (70% alfalfa hay and 30% concentrate) and GF diet (70% grass hay and 30% concentrate) significantly and the IVCPD in GC diet higher than GF diet while no difference between AC and AF diet (Cantalapiedra-Hijar *et al.*, 2009). From the present standpoint of nutrition, MOS could tune up microorganism system in the lower bowel of nonruminents but there was not clear theory about MOS impacting ruminal microbe.

Hence, MOS might affect digestion and utilization of nutrients in gastrointestine via a finely tuned microbe action and biochemical process. However, MOS did not influence DM digestibility but improve N metabolism in this experiment, it might be for further research.

The crude fiber would decrease with the climbing of concentrate to diet and resulted in the increase of IVDMD. Meanwhile, the CP level would grow slightly, nevertheless, the digestive speed of CP was slower than other nutrients such as starch, sugar and so on. So, the phenomenon was observed the IVCPD reduced with the rise of concentrate in this experiment and according with Orskov and McDonald (1979) who thought different diets might leaded to differences of microflora and pH in rumen. Moreover, the reason of the IVCPD augmented with ascending of MOS might be that chemical probiotic could improve the multiplication and activity of ruminal microbe.

Effects on the pH, NH_3-N content and VFAs of cultivation liquid when adding MOS to different concentrate-to-roughage ratio diets: The pH, N-NH_3 content and VFAs of ruminal liquid were important parameters reflecting ruminal environment. The normal range of pH and N-NH_3 content were fluctuating 5.0-7.5 and 10-50 mg/100 mL, respectively. In general, pH achieved the minimum level after feeding 2~6 h and N-NH_3 content reached peak after feeding 1.0~1.5 h. In rumen, the ratio of acetic acid, propionic acid and butyric acid to TVFAs were 50~65, 18~25 and 12~20%, respectively.

The content and ratio of individual acid was impacted by a lot of ingredients, especially feeds and feeding condition (Zhengkang and Jie, 1988). Rotger *et al.* (2005) showed compared with 12:88 forage-to-concentrate diet, the molar proportion of acetate tended to increase and

ammonia N concentration and branched-chain VFA tended to decrease in 30:70 forage-to-concentrate diet on Holstein heifers and no differences in pH, total VFAs, the molar proportions of propionate, buryrate and valerate between diets. Ramos *et al.* (2009) reported the similar results, Merino sheep received 30:70 forage-to-concentrate diet (DM basis) had lesser ruminal pH values and higher N-NH_3 content compared sheep received 70:30 forage-to-concentrate diet (DM basis).

Cantalapiedra-Hijar *et al.* (2009) indicated however, ruminal NH_3-N concentration increased with increasing concentrate in goats fed diets based on grass hay but not when diets included alfalfa hay. The forage:concentrate affected ruminal pH significantly but not total VFA concentration. In this experiment, the pH and NH_3-N content of cultivation liquid reduced with the ascending of concentrate *in vitro*.

Although, there was likely that ruminal pH was more dependent on the absolute quantities of food ingested than on concentrate:forage ration (Sauvant *et al*., 1999; Commun *et al*., 2009) *in vitro* compulsory condition might remove the effects of quantities of food in this experiment. For NH_3-N, the cotton meal was used in diets and its degradation was lower than other protein feeds moreover, IVCPD reduced with the increase of concentrate and mycoprotein synthesis improved because of much concentrate offering energy and other sources contributed to this outcome. In common knowledge, ruminal acetic acid decreased and propionic acid rose with concentrate ascending and the same result was gained in this experiment so, leaded obvious variation on acetic acid:propionic acid.

Goiri *et al.* (2010) demonstrated butyrate content in adding chitosan 50% alfalfa hay and 50% concentrate substrate was lower than control substrate by gas production experiment *in vitro* and no differences on pH, total VFA, NH_3-N content, propionate:acetate and acetate, propionate, valerate content between two treatments. Malcolm and Kiesling (1990) also considered, except adding 1.6% yeast culture reduced butyrate proportion significantly in control diet, adding 1.6% yeast culture to control diet or control plus 10% whole cottonseed did not influence pH, NH_3-N content and other individual VFA on steers.

Lattimer *et al.* (2007) reported whereas adding yeast culture could not heighten acetate acid content on high-fiber diet but high-concentrate diet using horse feces for *in vitro* fermentation while adding yeast culture did not impact pH, NH_3-N content and propionate acid content on both high-concentrate and high-fiber diet. In this experiment, the pH of cultivation liquid reduced and NH_3-N content increased with the rising of MOS *in vitro*, it might be chemical probiotic could improve the multiplication and activity of ruminal microbe.

CONCLUSION

The IVDMD, IVCPD, pH, NH_3-N content, ratio of acetic acid to propionic acid, ratio of acetic acid and butyric acid to TVFAs were affected by concentrate-to-roughage significantly; while the IVCPD, pH and NH_3-N content were affected by MOS significantly. The gas and methane production were not impacted by two factors and the combination of two factors did not influence all items significantly. The IVCPD, pH, NH_3-N content increased with the reducing of concentrate approximately while opposite to the IVDMD and the IVCPD and NH_3-N content increased with the rising of MOS roughly, while opposite to the pH.

ACKNOWLEDGEMENTS

The researchers wish to acknowledge supported by the earmarked fund for Modern Agro-industry Technology Research System. And also sincerely thank Liu Ting, Ren Chunyan, Wen Xiuxiu, Wang Zhipeng and Wei Zhanhu for their selfless help.

REFERENCES

AOAC, 1999. Official Methods of Analysis of the Association of Official Agricultural Chemists. 16th Edn., AOAC International, Gaithersburg, MD, USA.

Cantalapiedra-Hijar, G., D.R. Yanez-Ruiz, A.I. Martin-Garcia and E. Molina-Alcaide, 2009. Effects of forage:concentrate ratio and forage type on apparent digestibility, ruminal fermentation and microbial growth in goats. J. Anim. Sci., 87: 622-631.

Commun, L., M.M. Mialon, C. Martin, R. Baumont and I. Veissier, 2009. Risk of subacute ruminal acidosis in sheep with separate access to forage and concentrate. J. Anim. Sci., 87: 3372-3379.

Daniels, C.L., D.L. Merrifield, D.P. Boothroyd, S.J. Davies, J.R. Factor and K.E. Arnold, 2010. Effect of dietary *Bacillus* spp and mannan oligosaccharides (MOS) on European lobster (*Homarus gammarus* L.) larvae growth performance, gut morphology and gut microbiota. Aquaculture, 304: 49-57.

Davis, M.E., C.V. Maxwell, D.C. Brown, B.Z. de Rodas and Z.B. Johnson *et al*., 2002. Effect of dietary mannan oligosaccharides and (or) pharmacological additions of copper sulfate on growth performance and immunocompetence of weanling and growing/? finishing pigs. J. Anim. Sci., 80: 2887-289.

Dimitroglou, A., D.L. Merrifield, R. Moate, S.J. Davies, P. Spring, J. Sweetman and G. Bradley, 2009. Dietary mannan oligosaccharide supplementation modulates intestinal microbial ecology and improves gut morphology of rainbow trout, Oncorhynchus mykiss (Walbaum). J. Anim. Sci., 87: 3226-3234.

Fernandez, F., M. Hinton and B. van Gils, 2002. Dietary mannan-oligosaccharides and their effect on chicken caecal micro?ora in relation to *Salmonella enteritidis* colonization. Avian Pathol., 31: 49-58.

Goiri, I., L. M. Oregui and A. Garcia-Rodriguez, 2010. Use of chitosans to modulate ruminal fermentation of a 50:50 forage-to-concentrate diet in sheep. J. Anim. Sci., 88: 749-755.

Heinrichs, A.J., C.M. Jones and B.S. Heinrichs, 2003. Effects of mannan oligosaccharide or antibiotics in neonatal diets on health and growth of dairy calves. J. Dairy Sci., 86: 4064-4069.

Hinman, D.D., S.J. Sorensen and P.A. Momont, 1998. Effect of yeast culture on steer performance, apparent diet digestibility and carcass measurements when used in a barley and potato finishing diet. Prof. Anim. Sci., 14: 173-177.

Hooge, D.M., 2004a. Meta-analysis of broiler pen trials evaluating dietary mannan oligosaccharide 1993-2003. Int. J. Poul. Sci., 3: 163-174.

Hooge, D.M., 2004b. Turkey pen trials with dietary mannan oligosaccharide: Meta-analysis, 1993-2003. Int. J. Poult. Sci., 3: 179-188.

Huhtanen, P., A. Seppala, M. Ots, S. Ahvenjarvi and M. Rinne, 2008. *In vitro* gas production profiles to estimate extent and effective first-order rate of neutral detergent fiber digestion in the rumen. J. Anim. Sci., 86: 651-659.

Lattimer, J.M., S.R. Cooper, D.W. Freeman and D.L. Lalman, 2007. Effect of yeast culture on *in vitro* fermentation of a high-concentrate or high-fiber diet using equine fecal inoculum in a Daisy II incubator. J. Animal Sci., 85: 2484-2491.

Lopez, S., M.S. Dhanoa, J. Dijkstra, A. Bannink, E. Kebreab and J. France, 2007. Some methodological and analytical considerations regarding application of the gas production technique. Anim. Feed Sci. Technol., 135: 139-156.

Malcolm, K.J. and H.E. Kiesling, 1990. Effects of whole cottonseed and live yeast culture on ruminal fermentation and fluid passage rate in steers. J. Anim. Sci., 68: 1965-1970.

Markey, A.D. and K.H. Kline, 2006. Effects of dietary fat and yeast culture supplementation on total tract digestibility by horses. Prof. Anim. Sci., 22: 261-266.

Mohini, M., V. Mani and G.P. Singh, 2007. Effect of different ratios of green and dry roughage on milk production and methane emission in cattle. Indian J. Anim. Sci., 77: 79-82.

Morrison, S.J., S. Dawson and A.F. Carson, 2010. The effects of mannan oligosaccharide and Streptococcus faecium addition to milk replacer on calf health and performance. Livest. Sci., 131: 292-296.

Newman, K.E. and M.C. Newman, 2001. Evaluation of mannan oligosaccharide on the micro?ora and immunoglobulin status of sows and piglet performance. J. Anim. Sci., 79: 189-189.

Orskov, E.R. and I. McDonald, 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci., 92: 499-503.

Refstie, S., G. Baeverfjord, R.R. Seim and O. Elvebo, 2010. Effects of dietary yeast cell wall β-glucans and MOS on performance, gut health and salmon lice resistance in Atlantic salmon (*Salmo salar*) fed sunflower and soybean meal. Aquaculture, 305: 109-116.

Rosenboom, D.W., D.T. Shaw, J.E. Pettigrew and A. Conolly, 2001. Comparative effect of mannanoligosaccharide and an antibiotic in nurcery dietson performance of pigs reared on three farms. J. Anim. Sci., 79: 102-102.

Rotger, A., A. Ferret, S. Calsamiglia and X. Manteca, 2005. Changes in ruminal fermentation and protein degradation in growing holstein heifers from 80 to 250 kg fed high-concentrate diets with different forage-to-concentrate ratios. J. Anim. Sci., 83: 1616-1624.

Samanta, A.K., K.K. Singh and M.M. Das, 2000. Effect of roughage and concentrate ratio on rumen metabolites and dry matter digestibility. Indian Vet. J., 85: 795-796.

Sauvant. D., F. Meschy and D. Mertens, 1999. Components of ruminal acidosis and acidogenic effects of diets. Prod. Anim., 12: 49-60.

Tang, S.X., G.O. Tayo, Z.L. Tan, Z.H. Sun and L.X. Shen *et al*., 2008. Effects of yeast culture and fibrolytic enzyme supplementation on *in vitro* fermentation characteristics of low-quality cereal straws. J. Anim. Sci., 86: 1164-1172.

Torrecillas, S., A. Makol, M.J. Caballero, D. Montero and L. Robaina *et al*., 2007. Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. Fish Shellfish Immunol., 23: 969-981.

Zhengkang, H. and C. Jie, 1988. Digestibility and Metabolism of Rumen. Scientific Press, Beijing, China.