# Effects of adding mannan oligosaccharides on digestibility and metabolism of nutrients, ruminal fermentation parameters, immunity, and antioxidant capacity of sheep<sup>1</sup>

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**ABSTRACT:** The purpose of this study was to investigate the effects of adding mannan-oligosaccharides (MOS) on the following parameters in sheep: digestibility and retention rate of nutrients, ruminal fermentation, immunity, and antioxidant capacity. Twelve healthy crossbred wethers (Suffolk  $\sigma \times Small \ tail \ Han-yang \ Q)$  with external ruminal fistula and similar body weights  $(28.04 \pm 2.07 \text{ kg})$ were fed individually four treatments, three repeats of each treatment. The wethers diets were supplemental MOS at 0%, 1.2%, 1.6%, and 2.0% kg<sup>-1</sup> of basal diet (as fed basis). The experiment lasted 17 d, including 10 d of acclimation and 7 d of formal experimentation. The results showed that MOS did not influence the apparent digestibility and retention rate of nutrients, ruminal fermentation, and immunity or concentration of serum nitric oxide and activity of serum nitric oxide synthase  $(P \ge 0.07)$ . However, the apparent digestibility of neutral detergent fiber and acid detergent fiber at MOS supplementation rates of 1.6% and 2.0%both tended to be greater than the control group  $(P \le 0.103)$ . There was also moderate evidence that MOS might increase the nitrogen retention rate (P = 0.082). MOS increased the antioxidant ability of sheep ( $P \le 0.018$ ), especially at a dose of 1.6%: an increase in activity of total superoxide dismutase (P = 0.007), glutathione peroxidase (P = 0.018) and total antioxidant capacity (P < 0.001), and a decrease in concentration of malondialdehyde (P < 0.001) were found. The results indicated that in sheep MOS improved fiber digestion, N retention and some antioxidant abilities, but these effects may be too small to improve health and performance.

Key words: antioxidant, digestibility, immunity, mannan-oligosaccharides, ruminal fermentation, sheep

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doi: 10.1093/jas/skx040

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# **INTRODUCTION**

Functional oligosaccharides are widely used in animal feed because of the benefits to animal growth and immunity (Duan et al., 2016). Mannanoligosaccharide (MOS) has been identified as a typical functional oligosaccharide (Patel and Goyal, 2011) and has been the subject of several studies on animals including pigs (Giannenas et al., 2016; Valpotic et al., 2016), laying hens (Bozkurt et al., 2016; Ghasemian and Jahanian, 2016), broiler chickens (Attia et al., 2014), rabbits (Abdel-Hamid

<sup>&</sup>lt;sup>1</sup>This work was supported by grants from the National Natural Science Foundation of China (grant number 31560646), the China Agriculture Research System (grant number CARS38), and the Special Fund for Agro-scientific Research in the Public Interest (grant number 201503134). We thank all the people who participated in this experiment. We also thank Mr. Zhiyuan Ma from Institute of Subtropical Agriculture, the Chinese Academy of Science for his comments on this paper, and the native English speaking scientists of Elixigen Company (Huntington Beach, California) for editing this manuscript.

and Farahat, 2016), and aquaculture (Akter et al., 2016; Torrecillas et al., 2016). Most previous studies concentrated on monogastric animals, because it is believed that oligosaccharides are degraded by ruminal microbes and therefore bring no additional benefit to ruminant diets. However, a small number of studies show a benefit of oligosaccharides to ruminants. MOS was found to limit body condition scoring (BCS) loss following parturition in spring-calving beef cows (Linneen et al., 2014), and increased concentrate intake in very young pre-wean dairy calves without affecting their performance (Morrison et al., 2010). Similarly, another study found that supplementary MOS given to cows during the dry period enhanced not only their immune response to rotavirus but also the subsequent transfer of rotavirus antibodies to their calves (Franklin et al., 2005). However, very few studies on the effect of MOS in sheep were found (Demirel et al., 2007). Therefore, the objective of the current study was to investigate the effects of adding different doses of MOS to sheep diets on the digestibility and metabolism of nutrients, ruminal parameters, immunity and antioxidant capacity.

# MATERIALS AND METHODS

# Ethical Statement

This experiment was conducted according to the guidelines established by the Biological Studies Animal Care and Use Committee of Gansu Province, China (2005–12).

# Experimental Design, Animals, and Housing

The experimental group consisted of 12 healthy crossbred wethers (*Suffolk*  $\circ$  × *Small tail Han-yang*  $\bigcirc$ ) fitted with an external ruminal fistula and with a similar body weight (28.04 ± 2.07 kg). They were fed individually four treatments, three repeats of each treatment. The wethers diets were supplemental MOS (Bio-Mos, Alltech, Nicholasville, KY) at 0%, 1.2%, 1.6%, and 2.0% kg<sup>-1</sup> of basal diet (as fed basis). The test period included a 10-d acclimation and a 7-d formal experiment. All sheep were housed individually in an environmentally-controlled room. Each pen was equipped with a feeder, providing feed at 0.8 kg·sheep<sup>-1</sup>·d<sup>-1</sup> (1.77 times maintenance level), and a drinker providing ad libitum access to water.

# **Experimental Diets**

The basal diet was formulated to meet or exceed the recommendations for all nutrients of ram sheep

under China Agricultural Industry Standard (NY/ T816-2004, Table 1).

# Samples Collecting and Analysis

During the formal experimental period, 10% of total diet, 10% of total feces output, and 5% of total urine output was sampled for sequential 6 d and stored at -20 °C. For nitrogen analysis, samples of 3% of total feces output were taken daily, stored in wide-mouth bottles with 20 mL 10% sulfuric acid and pooled for 6 d. At the end of the data collection period, diets and fecal samples were thawed and pooled by sheep and then dried at 65 °C for 72 h in a forced-air oven for partial dry matter (DM) determination. Dried pooled samples of diets and feces were ground through a 1-mm screen in a Wiley Mill (Ogaw Seiki Co., Ltd., Tokyo, Japan) and examined for the following using Association of Official Analytical Chemists methods (AOAC, 2002): analytical DM (method 930.15), ash (method 942.05), Ca (method 978.02), total P (method 946.06); neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Goering and Soest (1970). The nitrogen content of diets, feces with 10% sulfuric acid and urine samples were determined by the Kjeldahl method (AOAC, 2002, method 990.03).

On the seventh day, the rumen fluid and blood samples before feeding and at 1 h, 3 h, 5 h, and 7 h

Table1.Compositionandnutritionallevel of the experiment diets (air dry basis,concentrate:roughage = 40:60)

Feedstuff		%
Corn		29.29
Soybean meal		9.00
Cotton seed meal		1.50
Alfalfa hay		26.00
Tall oat grass		33.00
Salt		0.70
Additive premix <sup>1</sup>		0.51
Nutritional level	DM (%)	90.27
	DE (digest-	10.32
	ible energy)	
	$(MJ \cdot kg^{-1})$	
	CP (%)	11.03
	DE/CP (energy	0.094
	: protein	
	ratio) ( $MJ \cdot g^{-1}$ )	
	NDF (%)	29.57
	ADF (%)	16.61
	Ca (%)	0.53
	P (%)	0.23

<sup>1</sup>Additive premix includes: mineral elements (mg/kg): S, 200; Fe, 25; Zn, 40; Cu, 8; I, 0.3; Mn, 40; Se, 0.2; Co, 0.1; vitamins (IU/kg):vitamin A, 940; vitamin E, 20.

RESULTS

# Nutrient Apparent Digestibility and Retention Rate

The nutrient apparent digestibility and retention rate are shown in Tables 2 and 3. There were no differences between the groups fed supplementary MOS in either nutrient apparent digestibility or retention rate (P > 0.07). However, a tendency of MOS to increase the NDF and ADF apparent digestibility and N retention rate was observed ( $P \le 0.103$ ).

# **Ruminal Fermentation Parameters**

The ruminal fermentation parameters are shown in Tables 4 and 5. Apart from the before feeding butyrate: total volatile fatty acid (TVFA) ratio, no differences were found ( $P \ge 0.073$ ). The before feeding butyrate:TVFA ratio in sheep given the 1.2% MOS diet was less than the other groups (P = 0.001). Supplementary MOS led to a slight decrease in ammonia nitrogen concentration 1 h to 5 h after feeding and a slight increase in protein nitrogen concentration 5 h to 7 h after feeding ( $P \ge 0.203$ ).

#### Immune and Antioxidant Parameters

The immune and antioxidant parameters of sheep serum are shown in Table 6. There was no effect of MOS on immunity parameters or on serum NO, TNOS, iNOS, or cNOS concentration ( $P \ge 0.112$ ). However, there was an increase in antioxidant parameters: the values of T-SOD and T-AOC in sheep given the 1.6% and 2.0% MOS diet were greater than that of the control group (P = 0.007, P < 0.001); similarly, the value of GSH-Px in sheep given the 1.6% MOS diet was greater than that of the control group (P = 0.018). However, the value for MDA was less than in the control group (P < 0.001).

# DISCUSSION

#### Nutrient Apparent Digestibility and Retention Rate

In the current study, the only parameters increased by MOS were apparent digestibility of NDF and ADF, and N retention rate. The reason for these could be that MOS promotes cellulolytic bacteria, causing a greater degradation of fiber in the rumen; and promotes ruminal microbes to synthesize more degradable microbial protein

after feeding were collected. Approximately 50 mL of rumen fluid was taken from three places in the rumen, strained through four layers of cheesecloth and preserved in individual plastic tubes for each sheep. Immediately after collection, samples were evaluated with a pH meter (PB-10; Sartorius Co. Limited, Göttingen, Germany) before storage at -20 °C. Volatile fatty acids (VFA) were measured by Agilent 6890N gas chromatography (Agilent Co. Limited, Santa Clara, CA, USA) with a 30-m (0.32-mm i.d.) fused silica column (HP-19091N-213; Agilent Co. Limited). The total nitrogen content was determined by the Kjeldahl method (AOAC, 2002, method 990.03). The ammonia nitrogen content was measured using a spectrophotometer (SP-723; Spectrum Instruments, Ltd., Shanghai, China) according to the Berthelot reaction (phenol-hypochlorite) described by Broderick and Kang (1980). A 5 mL blood sample was collected from the cervical vein into a nonheparinized vacuum tube, incubated at 37 °C for 4 h, stored at 4 °C overnight and then centrifuged  $(3,000 \times g)$  for 10 min at 4 °C to obtain the serum. The serum activity or concentration of the following were measured by colorimetric kits (Nanjing Jiancheng, Ltd., Nanjing, China) using a spectrophotometer (SP-723; Spectrum Instruments, Ltd): total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), malondialdehyde (MDA), total nitric oxide synthase (TNOS), inducible nitric oxide synthase (iNOS), constitutive nitric oxide synthase (cNOS) and nitric oxide (NO). The serum concentration of the following were determined using an ELISA kit (Assay Design Co. Limited, Michigan, USA) by a microplate reader (iMARK; BIO-RAD, Ltd., Hercules, CA, USA): immunoglobulin A (IgA), immunoglobulin G (IgG), interleukin 2 (IL-2) and complement 3 (C3).

# Statistical Analysis

The data were analyzed by one-way ANOVA (SPSS 19.0, IBM Co. Limited, Chicago, USA) using the following model:

$$X_{ij} = m + \alpha_i + e_{ij}$$

Where  $X_{ij}$  is the observation of the dependent variable (i = 1 to 4, j = 1 to 3),  $\mu$  is the population mean,  $\alpha_i$  is the random effect of treatment, and  $e_{ij}$  is the random error associated with the observation.

Significance was declared at  $P \le 0.05$ , and tendency at  $0.05 < P \le 0.10$ , using Tukey's multiple comparison test.

		MO				
Item	0%	1.2%	1.6%	2.0%	$SEM^2$	P value
DM						
Intake	685.48	716.92	699.80	650.59	13.28	0.376
DM in feces	235.52	222.46	213.90	201.28	7.67	0.507
Digested DM	449.95	494.46	485.90	449.31	11.04	0.364
Apparent digestibility	65.55	68.98	69.40	69.23	0.95	0.474
OM						
Intake	639.22	668.54	652.57	606.68	12.38	0.376
OM in feces	210.85	191.60	183.34	172.47	7.35	0.334
Digested OM	428.37	476.94	469.24	434.21	10.66	0.289
Apparent digestibility	66.92	71.34	71.87	71.76	1.01	0.259
NDF						
Intake	224.62	234.92	229.31	213.19	4.35	0.376
NDF in feces	128.38	114.68	104.03	96.58	5.23	0.142
Digested NDF	96.24	120.24	125.29	116.60	4.76	0.129
Apparent digestibility	42.67	51.19	54.59	55.12	2.09	0.103
ADF						
Intake	126.17	131.96	128.81	119.75	2.44	0.376
ADF in feces	74.49	68.42	60.74	55.29	3.07	0.105
Digested ADF	51.68	63.54	68.07	64.46	2.56	0.097
Apparent digestibility	40.83	48.16	52.80	54.26	2.10	0.070

<sup>1</sup>Sheep were fed 0%, 1.2%, 1.6%, and 2.0% mannan-oligosaccharides (MOS) (n = 3 per treatment). Mean results of digestion are shown for the six-day collection phase of the study for each treatment.

 $^{2}$ SEM = standard error of the mean.

	$MOS^1$					
Item	0%	1.2%	1.6%	2.0%	SEM <sup>2</sup>	P value
N						
Intake	15.11	15.80	15.42	14.34	0.29	0.376
N in feces	5.07	5.00	4.60	4.47	0.13	0.326
N in urine	6.76	7.07	6.68	6.41	0.19	0.724
Digested N	10.03	10.80	10.82	9.87	0.24	0.395
Apparent digestibility	66.41	68.31	70.15	68.78	0.70	0.334
Retained N	3.27	3.72	4.14	3.46	0.14	0.105
Retention rate	21.62	23.56	26.84	24.20	0.76	0.082
Ca						
Intake	4.71	4.93	4.81	4.47	0.09	0.376
Ca in feces	3.68	3.86	3.67	3.44	0.07	0.207
Digested Ca	1.03	1.07	1.14	1.03	0.03	0.724
Apparent digestibility	21.84	21.69	23.62	22.96	0.49	0.499
Р						
Intake	1.60	1.67	1.63	1.51	0.03	0.376
P in feces	1.07	1.11	1.06	1.00	0.03	0.583
Digested P	0.53	0.56	0.57	0.52	0.01	0.329
Apparent digestibility	32.94	33.32	34.70	34.41	0.72	0.842

Table 3. Apparent digestibility of N, Ca, and P and retention rate of N of sheep  $(g \cdot d^{-1}, \%)$ 

<sup>1</sup>Sheep were fed 0%, 1.2%, 1.6%, and 2.0% mannan-oligosaccharides (MOS) (n = 3 per treatment). Mean results of digestion and retention are shown for the six-day collection phase of the study for each treatment.

 $^{2}$ SEM = standard error of the mean.

to increase N digestion, although the effect was slight. These findings are consistent with various other reports which found that MOS could increase the digestibility and retention rate of various nutrients in monogastric animals, such as pigs and piglets (Nochta et al., 2010; Conejos et al., 2012; Zhao et al., 2012; Giannenas et al., 2016), broiler chickens (Corrigan et al., 2015),

**Table 4.** Parameters related to nitrogen metabolism in rumen fluid (mg $\cdot$ 100 mL<sup>-1</sup>)

Item		MO				
	0%	1.2%	1.6%	2.0%	$SEM^2$	P value
Total nitrogen						
Before feeding	157.60	124.80	152.00	164.80	12.19	0.733
After 1 h	160.80	165.87	201.60	177.07	13.43	0.778
After 3 h	183.20	130.13	181.87	146.40	12.48	0.354
After 5 h	145.60	153.07	149.87	160.80	12.78	0.990
After 7 h	133.60	173.33	188.00	170.40	13.12	0.646
Ammonia nitrogen						
Before feeding	71.24	81.43	71.72	68.42	3.97	0.794
After 1 h	179.97	97.87	105.00	95.44	15.02	0.203
After 3 h	131.78	89.35	91.56	89.81	8.45	0.305
After 5 h	136.26	96.96	50.86	53.75	17.97	0.385
After 7 h	72.18	70.32	74.35	90.67	5.95	0.716
Protein nitrogen						
Before feeding	81.69	68.87	77.19	93.48	13.58	0.960
After 1 h	66.93	65.64	94.95	111.52	11.22	0.446
After 3 h	48.76	38.19	89.25	54.44	11.03	0.360
After 5 h	43.89	54.48	115.32	105.58	14.88	0.237
After 7 h	58.78	100.08	111.25	78.49	13.96	0.679

Sheep were fed 0%, 1.2%, 1.6%, and 2.0% mannan-oligosaccharides (MOS) (n = 3 per treatment). Mean results of nitrogen metabolism of rumen fluid are shown for the five time point collection at seventh day of the study for each treatment.

<sup>2</sup>SEM: standard error of the mean.

# and aquaculture (Anguiano et al., 2013; Safari et al., 2014).

# **Ruminal Fermentation Parameters**

There are fewer studies in ruminants, and the results are less conclusive. For example, MOS did not affect apparent nutrient digestibility in steers (Jin et al., 2014), and yeast culture did not alter apparent nutrient digestibility in dairy heifers (Lascano and Heinrichs, 2009), dairy cows (Moallem et al., 2009), steers (Hinman et al., 1998), or lambs (Adams et al., 1981). In addition, Holstein cows given diet containing 56 g·d<sup>-1</sup> of yeast culture showed only a small numerical increase in NDF digestibility (White et al., 2008).

Other experiments had different conclusions. For example, Holstein cross calves fed MOS at 4 g·d<sup>-1</sup> had greater use of DM, total digestible nutrients (TDN) and crude protein (CP) (Ghosh and Mehla, 2012). Yeast culture supplementation increased true organic matter (OM) digestibility of steers early in the grazing season (Olson et al., 1994b), and also increased in situ NDF and CP degradation in beef cows in June (Olson et al., 1994a). An earlier study concluded that yeast culture improved N, Zn, and Fe metabolism in lambs (Cole et al., 1992). A more recent study found that NDF decreased the apparent digestibility in sheep fed 136 mg·kg<sup>-1</sup> of chitosan (Goiri et al., 2010). These differences may be attributed to different species, additives, diets or feeding strategies.

In ruminants, nitrogen metabolism parameters are used to predict ruminal health and assess whether nitrogen supplementation is appropriate to the need. In this study, MOS decreased ammonia nitrogen concentration and increased protein nitrogen concentration of rumen fluid to a small degree. A less ammonia nitrogen concentration is commonly considered a sign of flourishing ruminal microorganisms. Therefore, MOS could provide some benefits to ruminal microorganisms by enhancing their ability to synthesize microbial protein, even though this is less important than in monogastric animals.

Some similar results were found from previous studies in ruminants. Yeast culture was able to decrease ammonia nitrogen concentration in dairy cows (Moallem et al., 2009), similar to results found in lambs (Adams et al., 1981) and steers (Malcolm and Kiesling, 1990; Olson et al., 1994b). One study found that galacto-oligosaccharide (GOS) could decrease ruminal ammonia nitrogen concentration in sheep, although there was no influence of yeast culture (Mwenya et al., 2004). A later study also reported that GOS marginally decreased the ruminal ammonia nitrogen concentration in sheep (Pen et al., 2007). A study into chitosans found that they decreased ammonia nitrogen concentration of

Table 5. Parameters related to pH and volatile fatty acid in rumen fluid (mM, %)

Item		M	$OS^1$		SEM <sup>2</sup>	
	0%	1.2%	1.6%	2.0%		P value
pН						
Before feeding	7.17	7.10	7.05	7.37	0.08	0.653
After 1 h	6.40	6.54	6.49	6.68	0.12	0.933
After 3 h	6.46	6.22	6.81	6.60	0.13	0.443
After 5 h	6.55	5.76	6.40	6.11	0.22	0.648
After 7 h	6.94	6.80	6.94	6.83	0.16	0.992
Total volatile fatty acid						
Before feeding	57.48	55.58	61.20	48.67	5.29	0.916
After 1 h	95.25	67.23	90.03	69.44	9.50	0.730
After 3 h	93.69	66.28	75.26	65.94	8.36	0.747
After 5 h	69.48	55.10	110.02	87.33	14.69	0.660
After 7 h	54.87	70.45	77.61	65.07	8.74	0.907
Acetate:TVFA						
Before feeding	69.83	68.84	69.51	70.94	0.66	0.810
After 1 h	67.02	67.47	69.38	67.83	0.82	0.822
After 3 h	67.56	68.61	67.74	66.96	0.74	0.924
After 5 h	66.94	70.86	68.55	66.08	1.50	0.742
After 7 h	68.70	64.50	69.83	69.82	1.28	0.396
Propionate:TVFA						
Before feeding	13.34	13.23	9.55	10.61	0.82	0.242
After 1 h	21.08	17.30	16.85	18.29	1.14	0.703
After 3 h	18.59	17.01	16.95	20.54	0.94	0.601
After 5 h	18.06	14.81	15.70	22.34	1.58	0.395
After 7 h	13.48	20.72	14.13	13.86	1.52	0.240
Butyrate:TVFA						
Before feeding	12.21ª	10.43 <sup>b</sup>	12.89ª	12.42 <sup>a</sup>	0.35	0.001
After 1 h	9.58	10.82	10.31	9.91	0.48	0.878
After 3 h	10.02	10.87	11.54	9.45	0.35	0.156
After 5 h	11.14	11.61	12.04	8.56	0.54	0.073
After 7 h	12.82	10.72	10.37	12.13	0.41	0.113
Acetate:Propionate						
Before feeding	5.24	5.47	7.39	6.69	0.42	0.202
After 1 h	3.22	4.07	4.14	4.02	0.28	0.760
After 3 h	3.67	4.06	4.12	3.38	0.22	0.716
After 5 h	3.87	5.15	4.39	3.14	0.49	0.578
After 7 h	5.11	3.28	5.41	5.40	0.53	0.420

<sup>1</sup>Sheep were fed 0%, 1.2%, 1.6%, and 2.0% mannan-oligosaccharides (MOS) (n = 3 per treatment). Mean results of pH and volatile fatty acid of rumen fluid are shown for the five time point collection at seventh day of the study for each treatment.

 $^{2}SEM$  = standard error of the mean.

<sup>a,b</sup>Means within rows with different superscript letters differ (P < 0.05).

sheep rumen fluid (Goiri et al., 2010). These results, including those from the current study, indicate that MOS has the potential to affect ruminal fermentation, depending on the species, diet, and the quality of MOS.

In the current study, there was little evidence of supplementary MOS effect on ruminal pH or TVFA concentration. This could be because the effect of MOS on ruminal microorganisms was too small to change ruminal fermentation, although there was a small effect on butyrate concentration. This seems to be consistent with previous studies where yeast culture supplementation did not affect the major fermentation acids or pH in vitro (Kung et al., 1997) or in steers (Malcolm and Kiesling, 1990). Similarly, a more recent study found that GOS had no impact on propionate or acetate molar proportions in sheep (Pen et al., 2007). In contrast, a study found that TVFA, propionate, acetate and isoacid concentration increased when yeast culture was added to dairy heifer diets (Lascano and Heinrichs, 2009). This finding corroborates an earlier paper

**Table 6.** The immune and antioxidant parameters of sheep serum

		$MOS^2$				
Item <sup>1</sup>	0%	1.2%	1.6%	2.0%	SEM <sup>3</sup>	P value
IgA (µg·mL <sup>−1</sup> )	467.66	496.79	558.88	512.88	13.55	0.112
IgG (µg·mL⁻¹)	12,496.47	12,853.87	14,181.94	13,302.01	334.96	0.321
C3 (ng·mL <sup>-1</sup> )	944.67	958.84	1050.25	1044.26	30.30	0.481
IL-2 (ng·L <sup>-1</sup> )	16,217.48	16,899.82	17,563.05	17,749.03	571.28	0.783
GSH-Px (U·mL <sup>−1</sup> )	151.41 <sup>b</sup>	183.55 <sup>a,b</sup>	259.14ª	189.14 <sup>a,b</sup>	11.10	0.018
T-SOD $(U \cdot mL^{-1})$	35.62 <sup>b</sup>	39.49 <sup>a,b</sup>	41.27ª	41.02 <sup>a</sup>	0.71	0.007
T-AOC $(U \cdot mL^{-1})$	3.37°	4.81 <sup>b,c</sup>	9.03ª	6.48 <sup>a,b</sup>	0.60	< 0.001
MDA (nmol·mL <sup>-1</sup> )	45.08ª	36.25 <sup>a,b</sup>	24.68 <sup>b</sup>	27.98ь	2.32	< 0.001
NO (µM)	2.38	2.09	2.43	3.37	0.20	0.202
TNOS (U·mL <sup>-1</sup> )	33.38	32.59	30.93	29.88	0.88	0.250
iNOS (U·mL <sup>-1</sup> )	22.18	23.50	21.51	19.90	1.00	0.426
$cNOS (U \cdot mL^{-1})$	11.20	9.09	9.41	9.98	0.48	0.543

 $^{1}$ IgA = immunoglobulin A; IgG = immunoglobulin G; IL-2 = interleukin 2; C3 = complement 3; GSH-Px = glutathione peroxidase; T-SOD = total superoxide dismutase; T-AOC = total antioxidant capacity; MDA = malondialdehyde; NO = nitric oxide; TNOS = total nitric oxide synthase; iNOS = inducible nitric oxide synthase; cNOS = constitutive nitric oxide synthase.

<sup>2</sup>Sheep were fed 0%, 1.2%, 1.6%, and 2.0% mannan-oligosaccharides (MOS) (n = 3 per treatment). Mean results of immune and antioxidant parameters are shown for collection at seventh day of the study for each treatment.

<sup>3</sup>SEM: standard error of the mean.

<sup>a,b,c</sup>Means within rows with different superscript letters differ (P < 0.05).

which suggested that adding yeast culture to a high-concentrate diet increased TVFA production, butyrate and valerate concentrations, but decreased acetate concentration in vitro (Carro et al., 1992).

# Immune and Antioxidant Parameters

In the current research, MOS influenced neither the immune factors nor the serum NO concentration or activity of nitric oxide synthase. However, MOS supplementation did increase antioxidant capacity, especially at the 1.6% dose. This suggests that MOS could increase antioxidase activity, which would decrease oxygen radicals and MDA concentration. One previous study found no effect on IgG level and antibody response in lambs (Demirel et al., 2007). Another previous study found no effect on passive immunity characteristics transferred from beef cows to their calves (Linneen et al., 2014). In contrast, another study concluded that MOS supplementation in dairy cows during their dry period enhanced their immunity response to rotavirus, and tended to enhance the subsequent transfer of rotavirus antibodies to their calves (Franklin et al., 2005).

#### **CONCLUSION**

Additional MOS did not impact the apparent digestibility and retention rate of the vast majority of nutrients, nor did it affect ruminal fermentation, immunity, or serum NO concentration or activity of nitric oxide synthase in sheep. However, MOS did appear to increase apparent digestibility of NDF and ADF, and there was moderate evidence that it also increased the rate of N retention. In addition, MOS increased the antioxidant ability of sheep. The results suggest that MOS improved fiber digestion, N retention and improved antioxidant capacity; however these effects were too small to lead to substantial changes in sheep.

*Conflict of interest statement*. The authors declare no conflicts of interest.

# LITERATURE CITED

- Abdel-Hamid, T. M., and M. H. Farahat. 2016. Effect of dietary mannan-oligosaccharides on some blood biochemical, haematological parameters and carcass traits in purebred New Zealand White and crossbred rabbits. Anim. Prod. Sci. 56:2133–2139. doi:10.1071/AN15032
- Adams, D. C., M. L. Galyean, H. E. Kiesling, Joe D. Wallace, and M. D. Finkner. 1981. Influence of viable yeast culture, sodium bicarbonate and monensin on liquid dilution rate, rumen fermentation and feedlot performance of growing steers and digestibility in lambs. J. Anim. Sci. 53:780–789.
- Akter, M. N., A. Sutriana, A. D. Talpur, and R. Hashim. 2016. Dietary supplementation with mannan oligosaccharide influences growth, digestive enzymes, gut morphology, and microbiota in juvenile striped catfish, Pangasianodon hypophthalmus. Aquacult. Int. 24:127–144. doi:10.1007/s10499-015-9913-8
- Anguiano, M., C. Pohlenz, A. Buentello, and D. M. Gatlin. 2013. The effects of prebiotics on the digestive enzymes and gut histomorphology of red drum (Sciaenops ocellatus) and hybrid striped bass (Morone chrysops 3 M. saxatilis). Brit. J. Nutr. 109:623–629. doi:10.1017/ S0007114512001754

- AOAC. 2002. Official methods of analysis of AOAC international, 17th ed. In: AOAC (ed.). Gaithersburg (MD), Arlington, VA.
- Attia, Y. A., A. E. AbdAl-Hamid, M. S. Ibrahim, M. A. Al-Harthi, F. Bovera, A. Sh. Elnaggar. 2014. Productive performance, biochemical and hematological traits of broiler chickens supplemented with propolis, bee pollen, and mannan oligosaccharides continuously or intermittently. Livest. Sci. 164:87–95. doi:10.1016/j. livsci.2014.03.005
- Bozkurt, M., E. Bintas, S. Kirkan, H. Aksit, K. Kucukyilmaz, G. Erbas, M. Cabuk, D. Aksit, U. Parin, G. Ege, et al. 2016. Comparative evaluation of dietary supplementation with mannan oligosaccharide and oregano essential oil in forced molted and fully fed laying hens between 82 and 106 weeks of age. Poultry Sci. 95:2576–2591. doi:10.3382/ ps/pew140
- Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63:64–75. doi:10.3168/jds. S0022-0302(80)82888-8
- Carro, M. D., P. Lebzien, and K. Rohr. 1992. Influence of yeast culture on the *in vitro* fermentation (Rusitec) of diets containing variable portions of concentrates. Anim. Feed. Sci. Tech. 37:209–220.
- Cole, N. A., C. W. Purdy, and D. P. Hutcheson. 1992. Influence of yeast culture on feeder calves and lambs. J. Anim. Sci. 70:1682–1690.
- Conejos, J. R. V., S. P. Acda, S. S. Capitan, E. M. Agbisit, and F. E. Merca. 2012. Mannan oligosaccharides from yeast (*Saccharomyces cerevisiae*) cell wall improves nutrient digestibility and intestinal morphology of growing pigs [*Sus domesticus* (Erxleben)]. Philipp. Agric. Sci. 95:305–311.
- Corrigan, A., M. de Leeuw, S. Penaud-Frezet, D. Dimova, and R. A. Murphy. 2015. Phylogenetic and functional alterations in bacterial community compositions in broiler ceca as a result of mannan oligosaccharide supplementation. Appl. Environ. Microb. 81:3460–3470. doi:10.1128/ Aem.04194-14
- Demirel, G., N. Turan, A. Tanor, N. Kocabagli, M. Alp, M. Hasoksuz, H. Yilmaz. 2007. Effects of dietary mannanoligosaccharide on performance, some blood parameters, IgG levels and antibody response of lambs to parenterally administered *E. coli* O157:H7. Arch. Anim. Nutr. 61: 126–134. doi:10.1080/17450390701222964
- Duan, X. D., D. W. Chen, P. Zheng, G. Tian, J. P. Wang, X. B. Mao, J. Hu, J. He, B. Li, Z. Q. Huang, et al. 2016. Effects of dietary mannan oligosaccharide supplementation on performance and immune response of sows and their offspring. Anim. Feed. Sci. Tech. 218:17–25. doi:10.1016/j. anifeedsci.2016.05.002
- Franklin, S. T., M. C. Newman, K. E. Newman, and K. I. Meek. 2005. Immune parameters of dry cows fed mannan oligosaccharide and subsequent transfer of immunity to calves. J. Dairy Sci. 88:766–775. doi:10.3168/jds. S0022-0302(05)72740–5
- Ghasemian, M., and R. Jahanian. 2016. Dietary mannan-oligosaccharides supplementation could affect performance, immunocompetence, serum lipid metabolites, intestinal bacterial populations, and ileal nutrient digestibility in aged laying hens. Anim. Feed. Sci. Tech. 213:81–89. doi:10.1016/j.anifeedsci.2015.12.012

- Ghosh, S., and R. K. Mehla. 2012. Influence of dietary supplementation of prebiotics (mannanoligosaccharide) on the performance of crossbred calves. Trop. Anim. Health Prod. 44:617–622. doi:10.1007/s11250-011-9944-8
- Giannenas, I., D. Doukas, A. Karamoutsios, A. Tzora, E. Bonos, I. Skoufos, A. Tsinas, E. Christaki, D. Tontis, P. Florou-Paneri. 2016. Effects of *Enterococcus* faecium, mannan oligosaccharide, benzoic acid and their mixture on growth performance, intestinal microbiota, intestinal morphology and blood lymphocyte subpopulations of fattening pigs. Anim. Feed. Sci. Tech. 220:159–167. doi:10.1016/j.anifeedsci.2016.08.003
- Goering, H. R., and P. J. V. Soest. 1970. Forage fiber analysis. Agricultural handbook No. 379. Washington (DC): Department of Agriculture.
- 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. doi:10.2527/jas.2009–2377
- 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. Scientist 14:173–177.
- Jin, L., G. Z. Dong, C. L. Lei, J. Zhou, and S. Zhang. 2014. Effects of dietary supplementation of glutamine and mannan oligosaccharides on plasma endotoxin and acute phase protein concentrations and nutrient digestibility in finishing steers. J. Appl. Anim. Res. 42:160–165. doi:10.10 80/09712119.2013.823863
- Kung, L., Jr., E. M. Kreck, R. S. Tung, A. O. Hession, A. C. Sheperd, M. A. Cohen, H. E. Swain, J. A. Z. Leedle. 1997. Effects of a live yeast culture and enzymes on *in vitro* ruminal fermentation and milk production of dairy cows. J. Dairy Sci. 80:2045–2051.
- Lascano, G. J., and A. J. Heinrichs. 2009. Rumen fermentation pattern of dairy heifers fed restricted amounts of low, medium, and high concentrate diets without and with yeast culture. Livest. Sci. 124:48–57. doi:10.1016/j.livsci.2008.12.007
- Linneen, S. K., G. L. Mourer, J. D. Sparks, J. S. Jennings, C. L. Goad, D. L. Lalman. 2014. Effects of mannan oligosaccharide on beef-cow performance and passive immunity transfer to calves. Prof. Anim. Scientist 30:311–317. doi:10.15232/S1080-7446(15)30122–4
- 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.
- Moallem, U., H. Lehrer, L. Livshitz, M. Zachut, and S. Yakoby. 2009. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. J Dairy Sci 92:343–351. doi:10.3168/ jds.2007-0839
- 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. doi:10.1016/j.livsci.2010.04.002
- Mwenya, B., B. Santoso, C. Sar, Y. Gamo, T. Kobayashi, I. Arai, J. Takahashi. 2004. Effects of including β-1,4 galacto-oligosaccharides, lactic acid bacteria or yeast culture on methanogenesis as well as energy and nitrogen metabolism in sheep. Anim. Feed. Sci. Tech. 115:313–326. doi:10.1016/j.anifeedsci.2004.03.007

- Nochta, I. V. H., J. Tossenberger, and L. Babinszky. 2010. Effect of different levels of mannan-oligosaccharide supplementation on the apparent ileal digestibility of nutrients, N-balance and growth performance of weaned piglets. J. Anim. Physiol. an N. 94:747–756. doi:10.1111/j.1439-0396.2009.00957.x
- Olson, K. C., J. S. Caton, D. R. Kirby, and P. L. Norton. 1994a. Influence of yeast #ulture supplementation and advancing season on steers grazing mixed-grass prairie in the northern great plains: I. Dietary composition, intake, and in situ nutrient disappearance. J. Anim. Sci. 72:2149–2157.
- Olson, K. C., J. S. Caton, D. R. Kirby, and P. L. Norton. 1994b. Influence of yeast culture supplementation and advancing season on steers grazing mixed-grass prairie in the northern great plains: II. Ruminal fermentation, site of digestion, and microbial efficiency. J. Anim. Sci. 72:2158–2170.
- Patel, S., and A.Goyal. 2011. Functional oligosaccharides: production, properties and applications. World J. Microb. Biot. 27:1119–1128. doi:10.1007/s11274-010-0558-5
- Pen, B., K. Takaura, S. Yamaguchi, R. Asa, and J. Takahashi. 2007. Effects of yucca schidigera and Quillaja saponaria with or without β1–4 galacto-oligosaccharides on ruminal fermentation, methane production and nitrogen utilization in sheep. Anim. Feed. Sci. Tech. 138:75–88. doi:10.1016/j.anifeedsci.2006.11.018
- Safari, O., D. Shahsavani, M. Paolucci, and M. M. S. Atash. 2014. Single or combined effects of fructo- and mannan oligosaccharide supplements on the growth

performance, nutrient digestibility, immune responses and stress resistance of juvenile narrow clawed crayfish, *Astacus leptodactylus leptodactylus* Eschscholtz, 1823. Aquacult. 432:192–203. doi:10.1016/j. aquaculture.2014.05.012

- Torrecillas, S., M. J. Caballero, D. Montero, J. Sweetman, and M. Izquierdo. 2016. Combined effects of dietary mannan oligosaccharides and total fish oil substitution by soybean oil on European sea bass (*Dicentrarchus labrax*) juvenile diets. Aquacult. Nutr. 22:1079–1090. doi:10.1111/ anu.12322
- Valpotic, H., M. Samardzija, S. Terziic, S. Vince, M. Speranda, G. Lackovic, B. Habrun, N. Mas, D. Duricic, P. Kocila, et al. 2016. Effect of mannan oligosaccharide supplementation on blood and intestinal immune cells, bacteria numbers and performance in weaned pigs. Acta. Vet. Brno 85:267–276. doi:10.2754/avb201685030267
- White, R. A., J. H. Harrison PAS, I. Yoon PAS, W. K. Sanchez, and N. Nicholson. 2008. Effect of yeast culture on efficiency of nutrient utilization for milk production and impact on fiber digestibility and fecal particle size. Prof. Anim. Scientist 24:114–119. doi:10.15232/ S1080-7446(15)30825-1
- Zhao, P. Y., J. H. Jung, and I. H. Kim. 2012. Effect of mannan oligosaccharides and fructan on growth performance, nutrient digestibility, blood profile, and diarrhea score in weanling pigs. J. Anim. Sci. 90:833–839. doi:10.2527/ jas.2011–3921