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EFFECTS OF MANNAN-OLIGOSACCHARIDES ON GROWTH PERFORMANCE, SERUM BIOCHEMICAL INDICES, IMMUNE FUNCTION, AND INTESTINAL MORPHOLOGY IN FATTENING TAN SHEEP

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ABSTRACT

This study examined the effects of dietary Mannan-Oligosaccharides (MOS) on growth performance, serum biochemical indices, immune function, and intestinal morphology in fattening Tan sheep. Twenty-eight four-month-old male Tan sheep were assigned to four groups and received a basal diet supplemented with 0%, 1%, 2%, or 3% MOS for 56 days following a 14-day adaptation period.Results indicated that MOS supplementation significantly increased average daily gain (ADG) in a dose-dependent manner ($P \leq 0.05$). Serum biochemical analyses showed a linear and quadratic reduction in triglyceride (TG) levels, and a linear and quadratic increase in alkaline phosphatase (ALP) levels ($P \leq 0.05$). Total cholesterol (TC) levels decreased linearly, whereas urea nitrogen (UN) levels increased linearly with higher MOS levels ($P \leq 0.05$). Mannan-Oligosaccharides also enhanced total antioxidant capacity (T-AOC) and catalase (CAT) activity, with both parameters increasing linearly ($P \leq 0.05$). Immune function was significantly improved, demonstrated by elevated levels of immunoglobulin A (IgA), interleukin-6 (IL-6), IL-10, and interferon-gamma (IFN-γ) ($P \leq 0.05$). Additionally, 2% and 3% MOS groups showed increased immunoglobulin G (IgG) and tumor necrosis factor-alpha (TNF-α) levels ($P \leq 0.05$). Linear increases were noted in serum IgG, IgA, IgM, IL-6, and TNF-α levels, while IL-10 and IFN-γ levels exhibited both linear and quadratic increases ($P \leq 0.05$).Intestinal morphology analysis revealed significant improvements in jejunal villus height, crypt depth reduction, and the villus-to-crypt ratio, with these parameters showing both linear and quadratic changes in response to increasing MOS levels ($P \leq 0.05$). In conclusion, 2% MOS supplementation is recommended for enhanced growth performance, metabolic health, immune function, and intestinal integrity in fattening Tan sheep.

Keywords: Tan sheep; growth performance; antioxidant; immune function; intestinal morphology

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INTRODUCTION

Antibiotics have traditionally been pivotal in animal disease prevention, treatment, and production, but their overuse has led to rising concerns about food safety and environmental contamination. In response to China's ban on antibiotics in animal feed, the search for sustainable, eco-friendly alternatives has intensified. Mannan-oligosaccharides (MOS), a non-antibiotic, anti-pathogenic compound derived from yeast cell cultures, are recognized for their environmentally friendly and safe properties, making them promising substitutes for antibiotics. Studies have shown positive impact of MOS supplementation on gut health, including the promotion of beneficial bacteria, the inhibition of harmful ones, the enhancement of intestinal function, and the increased nutrient digestibility, all contributing to animal growth and health. For instance, (Halas and Nochta 2012) found that adding MOS to post-weaning pig diets preserved intestinal integrity, enhanced digestive function, and bolstered immunity through improved antigen presentation. Halas and Nochta (2012) showed that the addition of MOS to the ration enhances the improvement of rumen epithelial health in crossbred lambs by decreasing the thickness of the cuticle and facilitates rumen pH stabilization. (Yang *et al.* 2021) documented that neonatal lambs supplemented with MOS exhibited improved antioxidant capacity, reduced inflammation, and increased IgA secretion and lactobacilli colonization in the ileum. While there has been considerable depth in MOS research in monogastric animals, studies in ruminants, particularly in Tan sheep, appear relatively lacking. Therefore, this study aimed to investigate the specific effects of MOS on growth performance, meat quality, serum biochemical parameters, immune function, and intestinal morphology in Tan sheep.

MATERIALS AND METHODS

Experimental Design and Husbandry Management: All animal procedures performed in this study were approved by the Ethics Committee of the Institute of Animal Science, Ningxia Academy of Agriculture and Forestry (approval number: AEWC-GAU-2022-003). A controlled experimental design was employed for this study. Twenty-eight healthy, 3-month-old male Tan sheep with similar body weights (28.31±0.98kg) were selected and randomly divided into four groups, each consisting of seven individuals，each group was fed in a single pen. A 14-day acclimation period was followed by a 56-day experimental period. At the beginning of the experiment, all sheep were fed the same basic diet. During the experimental period, the basic feed was supplemented with 0%, 1%, 2%, and 3% MOS, respectively, we integrated MOS into the pre-mixed concentrate through a stepwise homogenization process, preparing a one-week supply of the concentrate in advance. Thereafter, the concentrate was daily blended with roughage to create the TMR diet, which was subsequently fed to the respective groups of sheep. The MOS used in the experiment was provided by a biotechnology company in Shandong and was derived from food-grade brewer's yeast cell walls, with an effective content of ≥99%. The trial was conducted at the Tan Sheep Breeding Farm of the Binhai Tan Sheep Breeding Co., Ltd. in Yan chi County, Ningxia. The sheep enclosure features slatted flooring, undergoes weekly cleansing throughout the trial period, and is routinely subjected to disinfection protocols, Feeding took place at 08:00 and 16:00 daily with a total mixed ration, and feed quantity was adjusted based on the leftover amount to maintain it at 10% of the feeding amount, with unrestricted access to water. The feed formula and nutritional level were designed according to the NRC (2007), targeting a body weight of 30 kg and a daily weight gain of 100 g (

Table **1**).

Sample Collection and Index Measurement

Growth Performance Indices: The sheep were weighed on an empty stomach before the morning feed on the first day and the last day of the experimental period to determine initial and final body weights. Before the daily feeding, we collected and weighed the leftover feed to calculate the Dry Matter Intake (DMI), Average Daily Gain (ADG), and Feed to Gain Ratio (F/G).

Slaughter Performance and Meat Quality Indices: On day 56 of the trial period, six sheep from each group that were quarantined, qualified, and had similar body conditions were selected for slaughter after fasting and weighing. Post-slaughter, the carcass weight was recorded, and the backfat thickness and pH of the longissimus dorsi muscle were measured. Additionally, approximately 100 g of the longissimus dorsi muscle was sampled for the assessment of other meat quality indicators. These specific indicators included meat color, shear force, cooking loss rate, and water-holding capacity (Ge *et al.* 2021)

Serum Biochemical, Antioxidant, and Immune Indices: On the last day of the test period (Day 56), blood was collected from all sheep (aged 5 months) via jugular venipuncture into 10 mL tubes, centrifuged at 3,600 rpm for 5 minutes to isolate serum, which was stored at -20°C.Using kits from Shanghai Enzyme-linked Biotechnology Co., Ltd., we measured serum indices including total protein (TP), albumin (ALB), globulin (GLB), glucose (GLU), total cholesterol (TC), triglycerides (TG), urea nitrogen (UN), alkaline phosphatase (ALP), total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), catalase (CAT), malondialdehyde (MDA), total antioxidant capacity (T-AOC), and cytokines: immunoglobulin A (IgA), IgG, IgM, interleukin-1β (IL-1β), IL-2, IL-6, IL-10, IL-12, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ).

Intestinal Tissue Structure: Intestinal Tissue Structure: Following the feeding trial, sheep were weighed, and six healthy, similarly weighted animals were selected for slaughter. Duodenum and jejunum tissues were excised, rinsed in PBS, and fixed in 4% paraformaldehyde. After H&E staining, intestinal villus height, crypt depth, and muscle layer thickness were measured with Case Viewer software, and the villus height to crypt depth ratio (V/C) was calculated (Li *et al.* 2016).

Villous Height to Crypt Depth Ratio (V/C) = Villous Height (VH) / Crypt Depth (CD)

Statistical Analysis: A one-way ANOVA was performed to assess the overall effects of the different treatments. Additionally, contrast analysis was employed to evaluate both linear and quadratic trends in the dose-response relationship. The results are expressed as arithmetic mean and SEM, and $P \leq 0.05$ was considered to be significant. The analysis was conducted using SPSS 25.0 software (IBM Corp., Armonk, NY, USA).

RESULTS

The Effect of Mannan-Oligosaccharides on the Growth Indicators of Fattening Tan Sheep: As shown in

Table **2**, the addition of 1%, 2%, and 3% MOS to the diet significantly increased the ADG of Tan sheep compared to the control group (*P*≤0.05). Moreover, ADG increased linearly with the MOS levels (*P*≤0.05). However, different proportions of MOS in the diet had no significant effect on other growth performance indicators of Tan sheep.

Effect of Mannan-Oligosaccharides on Slaughter Performance and Meat Quality of Fattening Tan Sheep: As shown in

Table **3**, with increasing proportions of MOS in the diet, the water loss rate of the longissimus dorsi muscle decreased linearly compared to the control group($P \leq 0.05$). However, the addition of 1%, 2%, and 3% MOS in the diet had no significant impact on the slaughter performance and meat quality indicators of fattening Tan sheep ($P > 0.05$).

Effect of Mannan-oligosaccharides on Serum Biochemical Indicators of Fattening Tan Sheep: As shown in

Table **4**, the addition of 1%, 2%, and 3% MOS to the diet significantly decreased serum TG levels and increased ALP levels in fattening Tan sheep compared to the control group ($P \leq 0.05$). Serum TG levels demonstrated both linear and quadratic decreases with increasing MOS levels, while ALP levels showed both linear and quadratic increases. TC levels exhibited a linear decrease, and UN levels showed a linear increase with higher MOS levels in the diet ($P \leq 0.05$).

Effect of Mannan-oligosaccharides on Antioxidant Capacity of Fattening Tan Sheep: As shown in Table 5, the addition of 1%, 2%, and 3% MOS to the diet significantly increased serum T-AOC and CAT levels in fattening Tan sheep compared to the control group ($P \leq 0.05$). Furthermore, T-AOC and CAT levels increased linearly with higher MOS levels in the diet ($P \leq 0.05$).

Effect of Mannan-oligosaccharides on Immune Function of Fattening Tan Sheep: As shown in

Table **6**, the addition of 1%, 2%, and 3% MOS to the diet significantly increased serum IgA, IL-6, IL-10, and IFN-γ levels in fattening Tan sheep compared to the control group (*P*≤0.05). Adding 2% and 3% MOS significantly elevated serum IgG and TNF-α levels (*P*≤0.05). Serum IgG, IgA, IgM, IL-6, and TNF-α levels increased linearly with higher MOS levels in the diet (*P*≤0.05), while serum IL-10 and IFN-γ levels exhibited both linear and quadratic increases (*P*≤0.05).

Effect of Mannan-oligosaccharides on Intestinal Morphology of Fattening Tan Sheep: As shown in Table 7, the addition of 1%, 2%, and 3% MOS to the diet significantly increased jejunal villus height, decreased crypt depth, and increased the villus-to-crypt ratio in fattening Tan sheep compared to the control group (*P*≤0.05). With higher MOS levels in the diet, jejunal villus height and the villus-to-crypt ratio showed both linear and quadratic increases, while crypt depth exhibited both linear and quadratic decreases (*P*≤0.05).

Table 1. Composition and nutrient levels of basal diets (dry matter basis).

Item	Content %
Ingredient composition (% DM)	
Maize silage	15.00
Alfalfa hay	30.00
Corn grain	29.00
Bean pulp	15.00
Wheat bran	6.00
Premix[1]	5.00
Nutrient composition[2]	
Metabolic energy (MJ/kg DM)	9.40
Crude protein (% DM)	12.39
Neutral detergent fiber (% DM)	32.75
Acid detergent fiber (% DM)	21.35
Starch (% DM)	22.64
Calcium (% DM)	0.52
Phosphorus (% DM)	0.36

[1]The premix (per kg) contained: vitamin A 224,000 IU, vitamin D_3 100,000 IU, vitamin E 700 mg, Zn 1900 mg, Cu 370 mg, Fe 1500 mg, Mn 1 200 mg, I 35 mg, Se 4.50 mg, lysine 1.5%.
[2] Metabolic energy was a calculated value, while the others were measured values.

Table 2 The Influence of Mannan-Oligosaccharide Supplementation on Growth Indices in Fattening Tan Sheep.

Items*	Control group	Mannan-Oligosaccharide added levels (%)			SEM	*P*-value		
		1	2	3		ANOVA	Linear	Quadratic
IBW/(kg)	28.56	28.67	27.90	28.10	0.20	0.498	0.252	0.913
FBW/(kg)	33.18	34.24	34.34	34.27	0.23	0.225	0.103	0.212
ADG/ (g/d)	99.36[b]	104.48[ba]	104.87[a]	105.40[a]	0.75	0.006	0.002	0.068
DMI/(kg/d)	0.87	0.88	0.87	0.89	0.12	0.979	0.787	0.920

[ab]Values in a row with different superscripts differ significantly (*P*≤0.05).
[*]**IBW**: Initial body weight; **FBW**: Final body weight; **ADG**: Average daily gain; **DMI**: Dry matter intake

Table 3 The Influence of Mannan-Oligosaccharide Supplementation on Slaughtering Performance and Meat Quality in Fattening Tan Sheep.

Items		Control group	Mannan-Oligosaccharide added levels (%)			SEM	*P*-value		
			1	2	3		ANOVA	Linear	Quadratic
Carcass weight/(kg)		16.18	16.71	16.88	16.88	0.14	0.226	0.068	0.333
Slaughter rate/ (%)		48.75	48.79	49.20	49.28	0.27	0.869	0.436	0.966
Back fat thickness/ (mm)		10.91	11.12	12.01	12.24	0.45	0.692	0.256	0.992
Drip loss/ (%)		3.69	3.61	3.70	3.84	0.04	0.231	0.132	0.168
Shear force/ (N)		65.44	65.65	65.15	65.16	0.72	0.995	0.851	0.950
Cooking loss/ (%)		62.39	63.53	64.29	64.47	0.44	0.333	0.084	0.587
Water loss rate/ (%)		34.12	32.51	32.86	31.51	0.38	0.100	0.027	0.857
Cooked meat rate/ (%)		55.39	55.53	55.26	54.89	0.22	0.796	0.410	0.594
$PH_{45\ min}$		6.42	6.46	6.42	6.51	0.02	0.402	0.243	0.581
$PH_{24\ h}$		5.82	5.79	5.77	5.74	0.16	0.358	0.082	0.898
Meat color	**L^***	34.46	33.62	33.53	33.54	0.40	0.835	0.460	0.618
	a^*	21.55	21.97	21.89	21.55	0.15	0.695	0.939	0.246
	b^*	8.19	8.05	8.15	7.86	0.20	0.949	0.941	0.760

Table 4 The Influence of Mannan-Oligosaccharide Supplementation on Serum Biochemical Indexes in Fattening Tan Sheep.

Items*	Control group	Mannan-Oligosaccharide added levels (%)			SEM	*P*-value		
		1	2	3		ANOVA	Linear	Quadratic
TP/(g/L)	39.55	40.85	40.82	40.60	0.50	0.789	0.507	0.472
ALB/(g/L)	36.48	36.38	36.53	36.50	0.40	0.999	0.958	0.963
GLB/ (g/L)	5.83	5.91	5.82	6.07	0.60	0.474	0.277	0.497
TG/(mmol/L)	2.48^a	1.98^b	1.94^b	1.89^b	0.60	≤0.001	≤0.001	0.005
TC/(mmol/L)	41.75	36.30	36.05	35.92	0.92	0.053	0.024	0.118
GLU/(mmol/L)	7.43	7.89	7.96	8.09	0.14	0.398	0.121	0.565
UN/(mmol/L)	21.89	21.90	23.74	24.92	0.53	0.104	0.020	0.553
ALP/(mg/dL)	1.94^c	2.51^b	2.69^{ab}	2.90^a	0.08	≤0.001	≤0.001	0.035

[abc]Values in a row with different superscripts differ significantly ($P≤0.05$).
TP: total protein; **ALB**: albumin; **GLB**: globulin; **TG**: triglyceride; **TC**: total cholesterol; **GLU**: glucose; **UN**: urea nitrogen; **ALP**: alkaline phosphatase

Table 5 The Influence of Mannan-Oligosaccharide Supplementation on Antioxidant Performance in Fattening Tan Sheep.

Items*	Control group	Mannan-Oligosaccharide added levels (%)			SEM	*P*-value		
		1	2	3		ANOVA	Linear	Quadratic
MDA/(nmol/mL)	1.83	1.75	1.73	1.75	0.42	0.880	0.520	0.631
SOD/(U/mL)	89.81	90.23	90.34	92.54	1.49	0.928	0.564	0.783
T-AOC/(mM)	3.23^c	3.95^b	4.46^a	4.48^a	0.12	≤0.001	≤0.001	0.017
GSH-Px/(U/mL)	264.59	272.06	279.05	280.08	4.94	0.691	0.256	0.756
CAT/(U/mL)	0.68^b	0.80^a	0.79^a	0.81^a	0.01	≤0.001	≤0.001	0.015

[ab]Values in a row with different superscripts differ significantly ($P≤0.05$).
*__MDA__: malondialdehyde; **SOD**: superoxide dismutase; **T-AOC**: total antioxidant capacity; **GSH-Px**: glutathione peroxidase; **CAT**: catalase activity

Table 6 The Influence of Mannan-Oligosaccharide Supplementation on Immune Function in Fattening Tan Sheep.

Items*	Control group	Mannan-Oligosaccharide added levels (%)			SEM	*P*-value		
		1	2	3		ANOVA	Linear	Quadratic
IgG/(mg/mL)	33.69^{c}	36.81^{bc}	38.27^{ab}	41.36^{a}	0.81	0.003	≤0.001	0.991
IgA/(μg/mL)	221.63^{b}	237.68^{a}	235.79^{a}	240.59^{a}	2.52	0.026	0.010	0.207
IgM/(μg/mL)	1899.06	2066.75	2155.78	2163.37	40.61	0.064	0.014	0.286
IL-1β/(pg/mL)	36.66	41.58	40.77	41.98	1.04	0.258	0.111	0.372
IL-2/(pg/mL)	729.08	738.37	780.58	759.93	11.26	0.392	0.195	0.513
IL-6/(pg/mL)	65.61^{b}	72.98^{a}	74.61^{a}	75.62^{a}	1.10	0.001	≤0.001	0.059
IL-10/(pg/mL)	106.03^{b}	125.83^{a}	129.96^{a}	120.27^{a}	2.63	0.002	0.015	0.001
IL-12/(pg/mL)	1275.21	1222.33	1240.21	1279.35	15.71	0.537	0.834	0.135
TNF-α/(pg/mL)	477.39^{c}	515.36^{bc}	545.03^{ab}	569.94^{a}	9.63	0.001	≤0.001	0.640
IFN-γ/(pg/mL)	491.34^{b}	619.99^{a}	655.78^{a}	606.99^{a}	15.88	≤0.001	≤0.001	≤0.001

[ab]Values in a row with different superscripts differ significantly (*P*≤0.05).

*IgG: immunoglobulin G; **IgA**: immunoglobulin A; **IgM**: immunoglobulin M; **IL-1β**: interleukin-β; **IL-2**: interleukin-2; **IL-6**: interleukin-6; **IL-10**: interleukin-10; **IL-12**: interleukin-12; **TNF-α**: tumor necrosis factor; **IFN-γ**: interferon-γ

Table 7 The Influence of Mannan-Oligosaccharide Supplementation on Intestinal Morphology in Fattening Tan Sheep.

Items	Control group	Mannan-Oligosaccharide added levels (%)			SEM	*P*-value		
		1	2	3		ANOVA	Linear	Quadratic
Duodenum								
Villus height (VC)/(μm)	821.43	820.86	841.67	850.21	7.61	0.445	0.133	0.769
Crypt depth (CD)/(μm)	247.24	243.41	242.09	235.27	2.49	0.410	0.110	0.767
Muscle thickness/(μm)	321.89	333.07	329.19	325.38	4.28	0.833	0.871	0.414
VC/CD	3.33	3.39	3.49	3.62	0.06	0.294	0.064	0.716
Jejunum								
Villus height (VC)/(μm)	607.65^{b}	684.85^{b}	682.91^{b}	693.57^{a}	8.96	≤0.001	≤0.001	0.008
Crypt depth (CD)/(μm)	237.57^{a}	215.74^{b}	212.54^{b}	207.71^{b}	2.85	≤0.001	≤0.001	0.019
Muscle thickness/(μm)	268.58	251.98	279.73	266.65	4.51	0.189	0.575	0.841
VC/CD	2.56^{b}	3.18^{a}	3.22^{a}	3.34^{a}	0.07	≤0.001	≤0.001	0.003

DISCUSSION

Impact of Mannan-Oligosaccharides on Growth Performance and Meat Quality in Fattening Tan Sheep: The impact of MOS on the growth indices and slaughter performance of Tan sheep highlights its potential as a valuable feed additive. Growth performance, often measured by metrics such as ADG and overall feed efficiency, directly reflects the nutritional uptake and utilization. Previous research has demonstrated that MOS, as a prebiotic, enhances growth in various livestock including weaned piglets and calves by improving gut health and nutrient absorption (Dong *et al.* 2022;Yu *et al.* 2021). In this study, supplemental MOS significantly increased the ADG in Tan sheep, aligning with findings from other ruminant studies that highlight the role MOS in boosting growth efficiency and potentially enhancing disease resistance (Yang *et al.* 2021).

When it comes to slaughter performance and meat quality, while MOS has been observed to boost carcass weight in growing pigs, its impact on slaughter indices or meat quality in fattening Tan sheep was not significant (Edwards *et al.* 2014). This variation could suggest species-specific reactions to MOS or might be influenced by factors such as feeding duration, and inherent characteristics of the Tan sheep breed. Therefore, despite MOS supplementation evidently benefiting growth performance, more investigation is needed to understand its effects on meat quality and slaughter performance, aiming to identify optimal conditions and long-term consequences.

Effects of Mannan-Oligosaccharides on Serum Biochemical Indices in Fattening Tan Sheep: This study found that incorporating MOS into the diet of fattening Tan sheep significantly lowered serum TG levels and increased ALP levels, underscoring the significance of exploring metabolic effects of MOS as prebiotics on Tan sheep. Based on previous research, the significant reduction in serum TG levels in fattening Tan sheep may be related to MOS promoting lipid metabolism and enhancing fatty acid oxidation (Dev *et al.* 2021;Wang *et al.* 2022). Mannan-Oligosaccharides are thought to improve the gut microbial community structure of the host and increase the production of SCFAs (Chen *et al.* 2024). Short-chain fatty acids (SCFAs)are known activators of fatty acid oxidation, which can enhance fatty acid entry into mitochondria for β-oxidation by activating the peroxisome proliferator-activated receptor (PPARs) pathway, potentially leading to reduced serum TG levels (Wang *et al.* 2023). Additionally, SCFAs can participate in regulating liver lipid metabolism, inhibit fatty acid synthesis, and reduce fat accumulation. Therefore, the reduction in TG levels may reflect the changes in Tan sheep's gut microbial community structure due to MOS and the subsequent regulation of overall lipid metabolism. The significant increase in ALP levels might be related to its roles in bone metabolism and liver function. Alkaline phosphatase (ALP) is an enzyme associated with cell membranes, involved in phosphate transport and bone mineralization. In bone metabolism, an increase in ALP activity is often related to increased osteoblast activity during bone growth and repair processes (Zha *et al.* 2023). Therefore, the increase in ALP could indicate that MOS promotes bone development in Tan sheep. Moreover, ALP is also expressed in the liver, where its increased levels might be associated with enhanced metabolic activities or the self-healing capacity of liver cells. This could suggest a positive regulatory effect of MOS on liver function, although further research is needed to confirm this.

The Impact of Mannan-Oligosaccharides on the Antioxidant Capacity of Fattening Tan Sheep: Antioxidant capacity is a crucial indicator for assessing the healthful growth of animals during their developmental phase and the quality of meat post-slaughter. This study observed a significant enhancement in the activities of T-AOC and CAT in Tan sheep, indicative of improved overall systemic antioxidant potential. T-AOC, representing the body's total antioxidant capacity, encompasses both enzymatic and non-enzymatic antioxidant systems; an increase in T-AOC denotes a strengthened systemic response to oxidative stress, which is a key factor in maintaining cellular homeostasis and animal health (Surai and Earle-Payne 2022). CAT functions to reduce the formation of hydroxyl radicals and scavenge peroxides and superoxide radicals (Baker *et al.* 2023), and increased CAT activity suggests that MOS may particularly enhance this antioxidant pathway.

Regarding the specific mechanisms, Mannan-Oligosaccharides, as functional prebiotics, may improve the gut microbial environment and promote the growth of beneficial bacterial populations, thereby indirectly influencing the host's oxidative stress status. Such regulatory effects of prebiotics might be realized by enhancing intestinal barrier functions and lowering inflammation levels, playing a crucial role in mitigating oxidative damage (Cheng *et al.* 2019). Additionally, MOS can directly influence intestinal cells by activating signaling pathways and boosting antioxidant gene expression, thus enhancing CAT and other antioxidant enzymes' activity to mitigate oxidative stress (Cheng *et al.* 2019). Other antioxidant indicators, like MDA, SOD, and GSH-Px, did not show significant changes, which may seem inconsistent at first glance. However, this could be due to MOS selectively enhancing certain antioxidant mechanisms rather than others, related to the specific ways in which MOS affect the gut and other metabolic pathways.

Impact of Mannan-Oligosaccharides on the Immune Function of Fattening Tan Sheep: In this study, supplementation of MOS to fattening Tan sheep significantly elevated serum IgG and IgA levels, indicative of enhanced humoral immunity through stimulated antibody production. Additionally, marked increases in inflammatory cytokines such as IL-6, IL-10, TNF-α, and IFN-γ suggest MOS-induced activation of cellular immune responses in these animals. These findings corroborate the research by (Ren *et al.* 2020) on hybrid grouper juveniles, demonstrating that dietary MOS inclusion significantly upregulated immune-related gene expression in the intestines and liver of the juvenile fish, thereby supporting Mannan-Oligosaccharides' immunomodulatory role across species. However, no significant alterations were detected in other immune-related indices, indicating that the effects of Mannan-Oligosaccharides on these parameters might be selective or attributable to variations in dosage and duration of supplementation. The notable increase in serum IgG and IgA levels suggests a possible mechanism for the enhancement of humoral immune responses by MOS. IgG and IgA antibodies, produced by B cells, are regulated by type 2 helper T cells (Th2), which promote B cell differentiation into plasma cells and subsequent antibody secretion through cytokines like IL-4 (Zhu 2015; Yin *et al.* 2021).Therefore, Mannan-Oligosaccharides may indirectly enhance IgG and IgA synthesis by activating the Th2 cell pathway. This hypothesis is partially supported by the observed increase in levels of IL-6 and IL-10, which play significant roles in immune regulation. IL-6 is a multifunctional cytokine that can promote the growth and maturation of B cells, possibly contributing to the increased levels of IgG and IgA. Meanwhile, IL-6 also has pro-inflammatory effects, capable of activating T cells and macrophages involved in the acute phase response. However, excessive IL-6 can lead to uncontrolled inflammatory responses, suggesting that MOS may raise IL-6 levels within a beneficial physiological range to maintain immune balance. On the other hand, IL-10 is a key anti-inflammatory cytokine that can inhibit Th1 cell activity and limit the production of pro-inflammatory cytokines, thereby exerting an immune-regulatory effect (Seehus *et al.* 2017). The rise in IL-10 levels may indicate that while MOS activate immune responses, they also enhance the immune system's self-regulatory mechanisms to prevent excessive inflammation. Furthermore, the elevation of TNF-α and IFN-γ levels are noteworthy phenomena. TNF-α is a major pro-inflammatory mediator in the immune system, playing a significant role in infection resistance and cell signaling, while IFN-γ, produced by Th1 cells, is primarily involved in the activation of macrophages for enhanced microbial clearance (Akdis *et al.* 2016). Thus, the elevation of these cytokines may reflect the activation of cell-mediated immune responses by MOS. Linking these findings together, it can be inferred that MOS may optimize immune responses by modulating the Th1/Th2 balance. By promoting the production of IgG and IgA as well as activating cell-mediated immune responses, and simultaneously preventing excessive inflammation through the elevation of IL-10 levels, Mannan-Oligosaccharides provide comprehensive immune protection for Tan sheep. This synergistic effect could be the underlying mechanism through which MOS enhance health status and productive performance in animal husbandry. Nonetheless, these inferences require confirmation through further cellular and molecular level research, such as assessing the activation status of specific immune cell subgroups and detailed variations in the cytokines they secrete.

The Impact of Mannan-Oligosaccharides on the Intestinal Morphology of Fattening Tan Sheep: In our study, adding 1% to 3% MOS to the diet of fattening Tan sheep notably improved jejunum villus height, reduced crypt depth, and increased the villus-to-crypt ratio. These findings align with previous studies in poultry and swine (Teng *et al.* 2021; Yang *et al.* 2022), indicating MOS's potential to enhance intestinal morphology. This suggests MOS may boost nutrient absorption efficiency by promoting intestinal epithelial cell growth and differentiation. However, MOS didn't affect duodenal morphology significantly, hinting at region-specific responses or different MOS mechanisms. Increasing villus height and decreasing crypt depth typically indicate better intestinal health (Wang *et al.* 2020). The enhanced jejunum villus height may result from MOS stimulating epithelial cell proliferation, warranting validation through markers like Ki-67. Reduced crypt depth suggests slower cell turnover, which benefits intestinal barrier integrity. Moreover, the increased villus-to-crypt ratio suggests improved absorptive function, potentially enhancing nutrient absorption and growth in fattening Tan sheep. Mannan-Oligosaccharides may regulate the intestinal environment as a prebiotic, promoting beneficial bacteria like Bifidobacteria and Lactobacilli, which can lower pH, inhibit harmful bacteria growth, and reduce inflammatory damage to intestinal cells (Jahanian and Ashnagar 2015). Metabolic products like SCFAs, particularly butyrate, derived from these bacteria, serve as energy sources for intestinal cells, directly promoting proliferation and villus height (Wang *et al.* 2018).

Conclusion: This study demonstrates that dietary inclusion of MOS significantly enhances the ADG of fattening Tan sheep, with a dose-dependent linear increase observed. The MOS supplementation markedly improved lipid metabolism and liver function, as evidenced by decreased serum TG and TC levels and increased ALP levels. Immunologically, MOS enhanced serum T-AOC, CAT, and various cytokine levels (IgA, IgG, IgM, IL-6, IL-10, IFN-γ, TNF-α), indicating a strengthened immune response. Intestinal morphology was also positively influenced, with increased villus height, reduced crypt depth, and higher villus-to-crypt ratios, suggesting better nutrient absorption and gut health. Overall, the optimal results were achieved with 2% MOS supplementation, in fattening Tan sheep.

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