Effects of Yeast Mannan Oligosaccharides on Growth Performance, Serum Immunity, Inflammation, and Antioxidant Indices in Mongolian Sheep (Postprint)

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Abstract

This experiment aimed to investigate the effects of yeast mannan oligosaccharides on growth performance, serum immune and inflammatory indices, and antioxidant indices in Mongolian sheep. Eighteen Mongolian sheep with good body condition and similar body weight [(28.91±1.81) kg] were selected and randomly divided into 3 groups (6 sheep per group): a control group fed the basal diet, a mannan oligosaccharide group, and a monensin group, with the latter two groups receiving supplementation of 0.1% yeast mannan oligosaccharides and 0.015% monensin in the concentrate, respectively. The experimental period lasted 70 days, including a 10-day preliminary period and a 60-day formal experimental period. During days 1-30, the concentrate-to-forage ratio of the diet was 4:6, and during days 31-60, the concentrate-to-forage ratio was gradually transitioned to 7:3. On days 1, 30, and 60 of the formal experimental period, body weight was measured and serum samples were collected after fasting to determine the concentrations of serum immunoglobulin M (IgM), lipopolysaccharide binding protein (LBP), interleukin-6 (IL-6), serum amyloid A (SAA), and nitric oxide (NO), as well as the activities of total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), and glutathione peroxidase (GSH-Px). The results showed that: 1) Compared with the control group, dietary supplementation with mannan oligosaccharides and monensin both significantly improved the growth performance of Mongolian sheep under high-concentrate feeding mode, manifested by significantly increased body weight (on day 60) and average daily gain ($P<0.05$), and significantly decreased feed-to-gain ratio ($P<0.05$). Moreover, the stage daily gain of the mannan oligosaccharide group during days 1-30 was significantly higher than that of the control and monensin groups ($P<0.05$), while during days 31-60, the stage daily gain of the monensin group was significantly higher than that of the control group (P<0.05). 2) On day 30, the concentrations of IgM and LBP and the activity of T-SOD in the serum of sheep in the mannan oligosaccharide group were significantly higher than those in the control group (P<0.05). On day 60, compared with the control group, the concentration of SAA in the serum of sheep in the mannan oligosaccharide group was significantly increased (P<0.05), while the serum T-AOC and GSH-Px activities were significantly decreased (P<0.05); the serum T-AOC in the monensin group was also significantly decreased (P<0.05). In conclusion, supplementation with yeast mannan oligosaccharides can improve the growth performance, serum immune function, and antioxidant capacity of Mongolian sheep fed high-concentrate diets, showing similar effects to monensin supplementation, with better efficacy observed at a concentrate-to-forage ratio of 4:6.

Full Text

Abstract

This experiment was conducted to investigate the effects of yeast mannan oligosaccharides on growth performance, serum immune, inflammatory, and antioxidant indicators in Mongolian sheep. Eighteen Mongolian sheep with good body condition and similar body weight [(28.91±1.81) kg] were randomly allocated to three groups (n=6 per group). The control group was fed a basal diet, while the mannan oligosaccharides group and rumensin group received the basal diet supplemented with 0.1% yeast mannan oligosaccharides and 0.015% rumensin in the concentrate, respectively. The experiment lasted 70 days, including a 10-day pre-trial period and a 60-day formal trial period.

During days 1-30, the dietary concentrate-to-forage ratio was 4:6, which was gradually transitioned to 7:3 during days 31-60. On days 1, 30, and 60 of the formal trial period, sheep were weighed after overnight fasting and serum samples were collected to determine concentrations of immunoglobulin M (IgM), lipopolysaccharide binding protein (LBP), interleukin-6 (IL-6), serum amyloid A (SAA), and nitric oxide (NO), as well as total antioxidant capacity (T-AOC) and activities of total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px). The results showed that: (1) Compared with the control group, dietary supplementation with mannan oligosaccharides and rumensin both significantly improved growth performance of Mongolian sheep under high-concentrate feeding, as evidenced by increased body weight (on day 60) and average daily gain (P<0.05), and decreased feed-to-gain ratio (P<0.05). Moreover, stage daily gain in the mannan oligosaccharides group during days 1-30 was significantly higher than in the control and rumensin groups (P<0.05), while during days 31-60, stage daily gain in the rumensin group was significantly higher than in the control group (P<0.05). (2) On day 30, serum IgM concentration, LBP concentration, and T-SOD activity in the mannan oligosaccharides group were significantly higher than in the control group (P<0.05). On day 60, serum SAA concentration in the mannan oligosaccharides group was significantly elevated ($P<0.05$), while serum T-AOC and GSH-Px activity were significantly decreased ($P<0.05$); serum T-AOC in the rumensin group was also significantly reduced ($P<0.05$). In conclusion, supplementation with yeast mannan oligosaccharides can improve growth performance, serum immune function, and antioxidant capacity in Mongolian sheep fed high-concentrate diets, with effects similar to rumensin, and shows better efficacy when the dietary concentrate-to-forage ratio is 4:6.

Keywords: yeast mannan oligosaccharides; sheep; growth performance; immune; antioxidant

Introduction

With increasing public concern over livestock product safety, feed safety as the source of livestock product safety has attracted growing attention. The search for safe, reliable, residue-free, non-antibiotic-resistance-inducing, and environmentally friendly green feed additives has become a research hotspot in the feed industry. Yeast polysaccharides are carbohydrates widely present in yeast cell walls, with main components being glucans and mannan oligosaccharides [1-2]. They possess physiological functions including immune enhancement, disease resistance, growth promotion, antioxidant activity, and detoxification [3-4], and are characterized by safety, absence of residues, no induction of antibiotic resistance, and environmental friendliness [5]. Wu et al. [2] reported that adding different doses of yeast polysaccharides to basal diets significantly improved body weight gain and reduced feed-to-gain ratio in broilers, with optimal effects at 0.2% supplementation. Wu et al. [6] found that adding 50 or 75 mg/kg mannan oligosaccharides to broiler diets significantly increased feed intake and body weight. Li [7] discovered that Saccharomyces cerevisiae mannan oligosaccharides reduced harmful bacteria in animal intestines, improved growth performance, and regulated immune function; adding Pichia pastoris mannan oligosaccharides to weaned piglet diets increased weaning weight and colostral immunoglobulin G concentration, improving sow reproductive performance and health status. Yang [8] demonstrated that mannan oligosaccharides from yeast cell walls increased lamb growth rate. Collectively, previous research on yeast polysaccharides has focused primarily on monogastric animals, showing improvements in immunity and growth performance. However, for ruminants, due to their unique digestive tract structure, rumen microbial activity, and varying dietary compositions, further validation is needed regarding the effects of yeast polysaccharides on growth performance and immunity. Therefore, this study aimed to investigate the effects of yeast mannan oligosaccharides on growth performance, serum immune, inflammatory, and antioxidant indicators in Mongolian sheep fed high-concentrate diets, explore its potential as an antibiotic alternative, and provide scientific basis for its application in ruminants.

1.1 Experimental Animals and Dietary Design

Eighteen Mongolian sheep with good body condition and similar body weight [(28.91±1.81) kg] were randomly divided into three groups (control, mannan oligosaccharides, and rumensin) with six sheep per group. All experimental animals were dewormed and vaccinated uniformly, and housed individually. The three groups were fed the same basal diet; the mannan oligosaccharides group received 0.1% yeast mannan oligosaccharides [purchased from Alltech Bio-products (China) Co., Ltd., purity 14%] in the concentrate, while the rumensin group received 0.015% rumensin (gifted by Mengrui Feed Technology Co., Ltd., purity 20%) in the concentrate. Both additives were incorporated into the concentrate using a stepwise mixing method. The trial lasted 70 days (10-day pre-trial period and 60-day formal trial period). During the first 30 days of the formal trial, the dietary concentrate-to-forage ratio was 4:6, which was gradually transitioned to 7:3 for the remaining 30 days. The basal diet was formulated according to Chinese "Feeding Standard of Meat Sheep" (NY/T 816-2004). The forage component was mixed hay. The composition and nutrient levels of the concentrate and nutrient levels of mixed forages are presented in Table 1 . Nutrient levels were determined following methods described by Zhang [9]: crude protein (CP) by Kjeldahl method (K9840 automatic Kjeldahl nitrogen analyzer, Jinan Hanon Instruments), neutral detergent fiber (NDF) by Van Soest detergent fiber analysis, calcium (Ca) by potassium permanganate method, and phosphorus (P) by molybdenum yellow colorimetry (UV-1780 UV spectrophotometer, Shimadzu, Japan). Animals were fed twice daily at 08:00 and 18:00 with ad libitum access to feed and water.

1.2.1 Growth Performance Measurement

Daily feed intake was recorded individually during the formal trial period. Body weight was measured after overnight fasting on days 1, 30, and 60 of the formal trial period to calculate average daily feed intake (ADFI), stage daily feed intake (SDFI), stage daily gain (SDG), average daily gain (ADG), and feed-to-gain ratio (F/G) using the following formulas:

Average daily feed intake = Total feed intake / Total trial days
Stage daily feed intake = Stage feed intake / Stage days
Average daily gain = Total weight gain / Trial days
Stage daily gain = Total weight gain during stage / Stage days
Feed-to-gain ratio = Average daily feed intake / Average daily gain

1.2.2 Serum Index Determination

On days 1, 30, and 60 of the formal trial period, blood samples were collected from the jugular vein of fasted sheep to prepare serum, which was stored at -80°C. Serum IgM concentration was determined by immunoglobulin M antibody binding method; lipopolysaccharide binding protein (LBP), interleukin-6 (IL-6), and serum amyloid A (SAA) concentrations were measured by biotin double-antibody sandwich enzyme-linked immunosorbent assay (ELISA); total antioxidant capacity (T-AOC) by Fe^3 reduction method; nitric oxide (NO) concentration by nitrate colorimetry; total superoxide dismutase (T-SOD) activity by xanthine oxidase method; and glutathione peroxidase (GSH-Px) activity by reduced glutathione (GSH) consumption method. All indices were determined using kits from Nanjing Jiancheng Bioengineering Institute with a UV spectrophotometer (UV-1780, Shimadzu, Japan) and microplate reader (SYNERGY H11, BioTek, USA).

1.3 Statistical Analysis

Experimental data were calculated and organized using Excel 2007. Significance analysis was performed using ANOVA in SAS 9.0, with Duncan' s multiple comparison test. Differences were considered significant at $P<0.05$. Results are expressed as mean $\pm$ standard deviation.

2.1 Effects of Dietary Yeast Mannan Oligosaccharides on Sheep Growth Performance

As shown in Table 2 , there were no significant differences in body weight among groups on day 1 ($P>0.05$). On day 30, body weight in the mannan oligosaccharides group was significantly higher than in the control group ($P<0.05$), but did not differ significantly from the rumensin group ($P>0.05$). On day 60, body weight in both the mannan oligosaccharides and rumensin groups was significantly increased by over 10% compared with the control group ($P<0.05$), with no significant difference between these two groups ($P>0.05$).

During days 1–30, stage daily gain in the mannan oligosaccharides group was significantly higher than in both the control and rumensin groups ($P<0.05$), while the difference between rumensin and control groups was not significant ($P>0.05$). During days 31–60, stage daily gain in the rumensin group was significantly higher than in the control group ($P>0.05$). Over the entire 60-day period, average daily gain in both the mannan oligosaccharides and rumensin groups was significantly higher than in the control group ($P<0.05$), with no significant difference between the two supplemented groups ($P>0.05$). The mannan oligosaccharides and rumensin groups showed 31.25% and 37.50% improvements in average daily gain compared with the control group, respectively.

There were no significant differences in stage daily feed intake or average daily feed intake among groups ($P>0.05$); however, the mannan oligosaccharides and rumensin groups showed 9.50% and 8.11% higher average daily feed intake than the control group, respectively. The feed-to-gain ratio was significantly lower in both the mannan oligosaccharides and rumensin groups compared with the control group ($P<0.05$), with no significant difference between the two supplemented groups ($P>0.05$).

2.2 Effects of Dietary Yeast Mannan Oligosaccharides on Serum Immune and Inflammatory Indicators in Sheep

As shown in Table 3 , on day 30, there were no significant differences in serum SAA concentration among the three groups (P>0.05). Serum IgM and LBP concentrations in the mannan oligosaccharides group were significantly higher than in the control group (P<0.05), while other inter-group differences were not significant (P>0.05). On day 60, serum SAA concentrations in both the mannan oligosaccharides and rumensin groups were significantly higher than in the control group (P<0.05), with no significant difference between the two supplemented groups (P>0.05). There were no significant differences in serum LBP and IgM concentrations among groups on day 60 (P>0.05). On both day 30 and day 60, serum IL-6 concentration in the rumensin group was significantly higher than in the control group (P<0.05), while the mannan oligosaccharides group showed no significant differences in IL-6 concentration compared with either the control or rumensin groups (P>0.05).

2.3 Effects of Dietary Yeast Mannan Oligosaccharides on Serum Antioxidant Indicators in Sheep

As shown in Table 4 , on day 30, there were no significant differences in serum NO concentration, T-AOC, or GSH-Px activity among the three groups (P>0.05). Serum T-SOD activity in both the mannan oligosaccharides and rumensin groups was significantly higher than in the control group (P<0.05), with no significant difference between the two supplemented groups (P>0.05). On day 60, there were no significant differences in serum NO concentration or T-SOD activity among groups (P>0.05). Serum T-AOC showed a ranking of mannan oligosaccharides group < rumensin group < control group, with significant differences among groups (P<0.05). Serum GSH-Px activity in the mannan oligosaccharides group was significantly lower than in the control group (P<0.05), but did not differ significantly from the rumensin group (P>0.05).

3.1 Effects of Yeast Mannan Oligosaccharides on Sheep Growth Performance

Yeast polysaccharides can regulate intestinal microflora, promote proliferation of beneficial bacteria, and bind pathogenic microorganisms to establish appropriate microbial communities in the intestine, thereby improving animal growth performance [10]. Numerous studies have demonstrated that adding yeast cell wall polysaccharides to piglet diets significantly increases average daily gain and feed intake, tends to reduce feed-to-gain ratio, and significantly increases jejunal villus height-to-crypt depth ratio, improving small intestinal mucosal morphology [11-12]. Dietary yeast polysaccharide supplementation in broilers also increased average daily gain and reduced feed-to-gain ratio [13]. Ma et al. [14] proved that dietary supplementation with 75 mg yeast -glucan improved average daily gain and feed utilization in Holstein calves, and significantly increased villus height in intestinal crypts. Rumensin is one of the few antibiotic additives approved for ruminant diets, capable of reducing protein degradation in the rumen, increasing rumen-bypass protein quantity, improving energy utilization, and enhancing weight gain and feed conversion [15]. The present results indicate that compared with the control group, supplementation with 0.1% yeast mannan oligosaccharides or 0.015% rumensin in the concentrate significantly improved average daily feed intake, body weight (on day 60), and average daily gain, while significantly reducing feed-to-gain ratio in Mongolian sheep. Notably, sheep in the mannan oligosaccharides group showed faster growth during days 1–30, whereas the rumensin group exhibited better growth during days 31–60. However, overall 60-day data revealed no significant difference in weight gain between the two supplemented groups, suggesting both additives enhanced feed intake and promoted animal growth. Yeast mannan oligosaccharides demonstrated better growth-promoting effects under a 4:6 concentrate-to-forage ratio, while rumensin required longer feeding duration to achieve similar effects.

3.2 Effects of Yeast Mannan Oligosaccharides on Serum Immune and Inflammatory Indicators in Sheep

Serum IgM, LBP, IL-6, and SAA are important indicators of immune and inflammatory responses. IgM is the primary immunoglobulin produced during primary immune responses, possessing antibacterial, antiviral, and antitoxic activities. Jin et al. [16] demonstrated that adding 2.5 g/kg mannan oligosaccharides to calf starter significantly increased serum IgM concentrations at 21 and 56 days of age. Xiao [17] reported that supplementing goat diets with 1% mannan oligosaccharides at a 3:7 concentrate-to-forage ratio significantly increased serum IgM concentrations on days 7 and 14 (by 37.13% and 32.21%, respectively), without affecting serum IL-6 concentration. He et al. [18] found that dietary 0.5% rumensin tended to increase serum IgM concentration in beef cattle. In the present study, serum IgM concentration in the mannan oligosaccharides group was significantly higher than in the control group on day 30, consistent with previous findings, but showed no significant differences among groups on day 60. This suggests that mannan oligosaccharides have stronger immunomodulatory effects when supplemented in diets with a 4:6 concentrate-to-forage ratio.

LBP binds to lipopolysaccharide (LPS) and acts on membrane receptors, activating target cells through intracellular signal transduction to release proinflammatory cytokines and immune regulatory factors (such as IL-1, IL-6, IL-8, etc.) [19]. Shu et al. [20] demonstrated that high LBP concentration (1,000 ng/mL) combined with 10 ng/mL LPS decreased inflammatory cytokine secretion from mononuclear macrophages, confirming the negative regulatory effect of LBP at high concentrations. The LBP-LPS complex may bind to proteins other than membrane CD14, promoting LPS inactivation. Jin [21] found that high-concentrate diets increased LPS concentrations in blood and feces of beef cattle. Chang [22] proved that long-term feeding of high-concentrate diets (6:4) to goats induced subacute ruminal acidosis (SARA), increasing LPS accumulation in the liver and concentrations of pro-inflammatory cytokines and acute-phase proteins in peripheral blood. In the present study, serum LBP concentration increased while IL-6 concentration decreased in the mannan oligosaccharides group on day 30 compared with day 1, consistent with Shu et al. [20]. However, no significant differences in serum LBP concentration were observed among groups on day 60, suggesting that both additives may help alleviate LPS stress through alternative pathways under high-concentrate conditions, though this requires further investigation.

IL-6 is a pleiotropic pro-inflammatory cytokine with complex functions in the interleukin family, capable of promoting T and B cell activation while stimulating hepatocytes to produce acute-phase proteins. It effectively promotes synthesis and secretion of inflammatory cytokines such as tumor necrosis factor- (TNF-), interleukin-1 (IL-1), and interleukin-10 (IL-10) during infection and immune responses, serving as an important effector molecule in acute-phase inflammatory reactions [23]. Studies have shown that yeast polysaccharides from yeast cell walls possess immunomodulatory effects, promoting release of inflammatory cytokines TNF- and IL-6 [24]; however, Xiao [17] found no significant change in serum IL-6 concentration when supplementing goat diets with 1% mannan oligosaccharides. In the present study, serum IL-6 concentration in the mannan oligosaccharides group did not differ significantly from the control or rumensin groups on either day 30 or 60, consistent with Xiao [17].

SAA is a highly specific acute-phase protein with high sensitivity and specificity, beginning to increase approximately 8 hours after inflammatory responses and rapidly decreasing when inflammation is controlled and stimulating factors subside [25]. SAA is primarily derived from the liver and circulates throughout the body [26]. Che et al. [27] demonstrated that dietary mannan oligosaccharide supplementation in pigs significantly increased blood immune and inflammatory indicators, playing an important role in promoting innate and cellular immune mediators, and increased acute-phase proteins (including heparin proteins and SAA) in pigs. In the present study, serum SAA concentrations showed no significant changes among groups on day 30, but were significantly higher in both the mannan oligosaccharides and rumensin groups compared with the control group on day 60, potentially helping the body rapidly clear LPS.

3.3 Effects of Yeast Mannan Oligosaccharides on Serum Antioxidant Indicators in Sheep

Free radicals exist in animals during growth, and their generation, utilization, and clearance maintain a dynamic balance in healthy animals. When this balance is disrupted by internal or external factors, oxidative stress occurs [28]. NO is a marker of oxidative stress in vivo. Two antioxidant systems coexist in animals: an enzymatic system including SOD and GSH-Px, and a non-enzymatic system including vitamin C, vitamin E, and trace elements zinc and selenium. These two systems jointly maintain redox homeostasis [29]. This study investigated serum T-AOC, NO concentration, and T-SOD and GSH-Px activities to reflect the effects of yeast mannan oligosaccharides on antioxidant capacity in Mongolian sheep.

Zheng et al. [30] proved that exogenous mannan oligosaccharides improved antioxidant performance in sheep, significantly increasing serum GSH-Px and T-SOD activities and T-AOC when added to diets with a 4:6 concentrate-to-forage ratio, without significantly affecting serum NO concentration. Xiao [17] found that supplementing goat diets with 1% mannan oligosaccharides at a 3:7 concentrate-to-forage ratio had no significant effect on blood T-SOD and GSH-Px activities on days 14 and 28. In the present study, on day 30, serum NO concentration showed no significant change while T-SOD activity was significantly higher in the mannan oligosaccharides group compared with the control group, consistent with Zheng et al. [30]; however, the lack of significant changes in serum T-AOC and GSH-Px activity aligned with Xiao [17]. Liu et al. [31] found that adding 25 mg/kg rumensin to sheep diets with a 4:6 concentrate-to-forage ratio did not significantly increase blood superoxide dismutase (SOD) concentration, whereas in the present study, serum T-SOD activity in the rumensin group was significantly higher than in the control group on day 30. On day 60, serum T-AOC and GSH-Px activity were significantly decreased in the mannan oligosaccharides group, and T-AOC was also significantly reduced in the rumensin group. These results differ from previous studies on low-concentrate diets supplemented with mannan oligosaccharides, possibly because mannan oligosaccharide supplementation enhanced immune capacity, enabling more rapid LPS clearance and consequently elevating oxidative stress levels. The detailed mechanisms require further investigation. The present study demonstrated better regulatory effects of mannan oligosaccharides under a 4:6 concentrate-to-forage ratio, though the appropriate dosage and efficacy under a 7:3 ratio require continued research.

Conclusion

Supplementation with 0.1% yeast mannan oligosaccharides in the concentrate significantly improved growth performance in Mongolian sheep under high-concentrate feeding conditions, achieving effects similar to rumensin supplementation. When the dietary concentrate-to-forage ratio was 4:6, supplementation with 0.1% yeast mannan oligosaccharides improved serum immune function and antioxidant capacity in sheep.

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Note: Figure translations are in progress. See original paper for figures.