

## Effects of Dietary Supplementation with Different Biological Agents on Production Performance and Slaughter Performance of Duhan Hybrid Meat Sheep (Postprint)

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**Date:** 2017-10-23T00:00:00+00:00

### Abstract

This experiment aimed to compare the effects of dietary supplementation with different biological preparations on growth performance, slaughter performance, tissue organ development, and meat quality of Dorper × Han crossbred mutton sheep. A single-factor experimental design was adopted, and 160 Dorper × Han crossbred F1 mutton sheep with an average body weight of approximately 32 kg were selected and randomly divided into 5 groups, with 4 replicates per group and 8 sheep per replicate. The control group was fed a basal diet, while the experimental groups were supplemented with 21 mg/kg monensin,  $4 \times 10^9$  CFU/kg *Bacillus licheniformis*,  $3.2 \times 10^9$  CFU/kg *Saccharomyces cerevisiae*, and 1.1 g/kg compound biological preparation (containing *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and alkaline protease), respectively. The experiment consisted of a 10-day pre-trial period and a 56-day formal trial period. Feed intake was recorded every 2 days, and body weight was measured every 20 days. When the average body weight of sheep in the compound biological preparation group reached approximately 50 kg, 10 sheep from each group were selected for slaughter to determine their slaughter performance, tissue organ weights, and meat quality indices. The results showed that: 1) The average daily gain and dressing percentage of the compound biological preparation group were significantly higher than those of the control group ( $P < 0.05$ ); the feed conversion ratio of the *Bacillus licheniformis* group and the compound biological preparation group was significantly lower than that of the control group ( $P < 0.05$ ). 2) The complex stomach weight of the *Bacillus licheniformis* group, *Saccharomyces cerevisiae* group, and compound biological preparation group was significantly higher than that of the control group ( $P < 0.05$ ), and the proportion of complex stomach weight to pre-slaughter live weight in the compound biological prepara-

tion group was significantly higher than that in the control group and monensin group ( $P < 0.05$ ); the small intestine weight and its proportion to pre-slaughter live weight in the *Bacillus licheniformis* group, *Saccharomyces cerevisiae* group, and compound biological preparation group were significantly higher than those in the control group and monensin group ( $P < 0.05$ ). 3) The proportion of kidney weight to pre-slaughter live weight in the *Saccharomyces cerevisiae* group was significantly higher than that in the control group ( $P < 0.05$ ), while the proportions of other visceral organ weights to pre-slaughter live weight showed no significant differences among groups ( $P > 0.05$ ). 4) No significant differences were observed in meat quality indices among groups ( $P > 0.05$ ). In conclusion, as feed additives for improving mutton sheep production performance, *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and compound biological preparation can replace monensin, with *Bacillus licheniformis* being superior to *Saccharomyces cerevisiae*, and the compound biological preparation being the best.

## Full Text

### Effects of Dietary Supplementation of Different Biological Agents on Growth and Slaughter Performance of Dorper×Thin-Tailed Han Crossbred Mutton Lambs

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## Abstract

This experiment was conducted to compare the effects of dietary supplementation of different biological agents on growth performance, slaughter performance, tissue and organ development, and meat quality of Dorper×thin-tailed Han crossbred mutton lambs. Using a single-factor experimental design, 160 Dorper×thin-tailed Han crossbred F1 mutton lambs with an average body weight of approximately 32 kg were randomly allocated into 5 groups, with 4 replicates per group and 8 lambs per replicate. The control group received a basal diet, while the experimental groups were supplemented with 21 mg/kg monensin,  $4 \times 10^8$  CFU/kg *Bacillus licheniformis*,  $3.2 \times 10^8$  CFU/kg *Saccharomyces cerevisiae*, or 1.1 g/kg compound biological agents (containing *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and alkaline proteinase). The pre-trial period lasted 10 days, followed by a 56-day formal trial period. Feed

intake was recorded every 2 days, and body weight was measured every 20 days. When the average body weight of lambs in the compound biological agents group reached approximately 50 kg, 10 lambs from each group were selected for slaughter to determine slaughter performance, tissue and organ weights, and meat quality indicators. The results showed: (1) The compound biological agents group exhibited significantly higher average daily gain and dressing percentage compared to the control group ( $P < 0.05$ ), while the *Bacillus licheniformis* and compound biological agents groups showed significantly lower feed-to-gain ratio than the control group ( $P < 0.05$ ). (2) The complex stomach weight in the *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and compound biological agents groups was significantly higher than that in the control group ( $P < 0.05$ ), with the compound biological agents group showing a significantly higher proportion of complex stomach weight to pre-slaughter live weight compared to the control and monensin groups ( $P < 0.05$ ). Additionally, the small intestine weight and its proportion to pre-slaughter live weight in these three treatment groups were significantly higher than those in both the control and monensin groups ( $P < 0.05$ ). (3) The proportion of kidney weight to pre-slaughter live weight in the *Saccharomyces cerevisiae* group was significantly higher than that in the control group ( $P < 0.05$ ), though other internal organ weight proportions showed no significant differences among groups ( $P > 0.05$ ). (4) No significant differences were observed in meat quality indicators among all groups ( $P > 0.05$ ). In conclusion, as feed additives for mutton lambs, *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and compound biological agents can effectively replace monensin, with *Bacillus licheniformis* showing superior effects to *Saccharomyces cerevisiae*, and the compound biological agents demonstrating the best overall efficacy.

**Keywords:** mutton lamb; biological agents; growth performance; slaughter performance; organ index

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## Introduction

With rising living standards, consumer demand for meat products continues to grow. However, bacterial resistance and veterinary drug residues pose serious threats to meat safety and ecological balance, ultimately affecting human health. Consequently, many countries have enacted legislation prohibiting antibiotic supplementation in animal feed, with regulations on antibiotic use in livestock production becoming increasingly stringent [1]. This has led to growing interest in new, non-polluting, residue-free probiotic additives. Numerous livestock and poultry operations have adopted microecological preparations as feed additives with considerable success. Probiotics, as a component of microecological preparations, are non-pathogenic and non-toxic microorganisms found in nature. When ingested by animals through the digestive tract, they improve gastrointestinal microflora and morphology, enhance immune function, increase feed utilization and animal performance, reduce disease incidence, and improve

the ecological environment, thereby ensuring both profitability and sustainable development of animal husbandry [2-4]. According to the Ministry of Agriculture Announcement No. 2045, "Catalogue of Feed Additive Varieties (2013)," 33 probiotic strains are approved for use as feed additives [5].

Monensin is commonly used as a growth promoter in animal diets, but its use as an antibiotic inevitably leads to drawbacks such as microbial imbalance, resistance development, and drug residues. Previous studies have demonstrated that *Bacillus licheniformis* (a bacterium) and *Saccharomyces cerevisiae* (a fungus) can individually improve animal performance [6-7], though the efficacy of combining these organisms with enzymes into compound biological preparations remains unclear. This experiment investigated the effects of supplementing Dorper×thin-tailed Han crossbred mutton lamb diets with monensin, *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and compound biological agents on fattening lamb growth and slaughter performance. The objective was to provide a theoretical basis for replacing antibiotics with probiotic preparations and to promote healthy, green development of the sheep industry.

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## Materials and Methods

### 1.1 Time and Location

The experiment was conducted from September to November 2016 at the modern mutton sheep farm of Inner Mongolia Fuchuan Feed Science and Technology Co., Ltd., with a total duration of 66 days.

### 1.2 Experimental Design

This study employed a single-factor experimental design using Dorper×thin-tailed Han crossbred mutton lambs as experimental animals. One hundred sixty healthy male lambs aged 4-6 months with an initial body weight of approximately 32 kg were randomly divided into 5 groups, each consisting of 4 replicates with 8 lambs per replicate. The control group received a basal diet, while the experimental groups were supplemented with monensin, *Bacillus licheniformis*, *Saccharomyces cerevisiae*, or compound biological agents in their basal diets. When the average body weight of lambs in the compound biological agents group reached approximately 50 kg, 10 healthy lambs with body weights close to the group average were selected from each group (50 lambs total) for slaughter to determine slaughter performance, tissue and organ development, and meat quality indicators. The pre-trial period lasted 10 days, followed by a 56-day formal trial period.

### 1.3 Sources and Characteristics of Additives

Monensin (90% active ingredient content) was added at 21 mg/kg and purchased from Wudi Ruilikang Bioengineering Co., Ltd. *Bacillus licheniformis* ( $2 \times 10^{11}$

CFU/g viable count) was added at  $4 \times 10^1$  CFU/kg. *Saccharomyces cerevisiae* ( $1 \times 10^1$  CFU/g viable count) was added at  $3.2 \times 10^1$  CFU/kg. The compound biological agents (containing *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and alkaline proteinase;  $1 \times 10^1$  CFU/g viable count) were added at 1.1 g/kg. The *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and compound biological agents were all provided by Beijing Xindayang Technological Development Co., Ltd.

#### 1.4 Basal Diet

The basal diet was formulated according to the nutritional requirements for a 300 g daily gain in 30-40 kg and 41-50 kg mutton sheep proposed by our research group [8-12]. Biological agents were mixed uniformly with dietary ingredients to produce pelleted feed in the form of total mixed rations. During the 30-40 kg period, Chinese leymus grass was supplemented at 20% of total feed intake. The basal diet was self-formulated, with premix provided by Beijing Precision Animal Nutrition Research Center. The composition and nutrient levels of the basal diet are presented in Table 1 .

#### 1.5 Management

The experimental sheep were housed in semi-open pens. Prior to the trial, all lambs were sheared and ear-tagged, and followed the farm's standard immunization program. The pens, floors, fences, and exercise areas were disinfected with Qiangli Xiaoduling solution before the experiment. Each replicate was housed in a separate pen. Experimental lambs were fed twice daily at 08:00 and 18:00. Throughout the trial, lambs had free access to feed and water. Starting from the formal trial period, residual feed was collected every 2 days to calculate average feed intake, and feeding amounts were adjusted according to ad libitum feeding requirements (residual feed accounting for 10% of feed offered).

#### 1.6 Measurements

**1.6.1 Growth Performance** Feed intake was recorded daily before feeding, and residual feed was collected and recorded every 2 days. Based on the proportion of residual feed to feed offered, subsequent feeding amounts were calculated to ensure ad libitum intake. Strict records of feed intake and feeding amounts were maintained to calculate dry matter intake for each group throughout the experimental period. Lambs were weighed every 20 days to record body weight changes.

**1.6.2 Slaughter Performance** On the day before slaughter, experimental lambs were weighed at 16:00, then fasted from feed and water for 16 hours. They were weighed again at 07:00 the next day before being stunned with carbon dioxide and slaughtered by jugular venesection. Pre-slaughter live weight was recorded for all lambs. After slaughter, the head, hooves, and viscera were

removed, and the carcass weight and individual organ weights were measured after skinning. The digestive tract was emptied of contents, rinsed clean, and the weights of rumen, reticulum, omasum, abomasum, small intestine, and large intestine were recorded separately.

The eye muscle (longissimus dorsi) contour between the last and second-to-last ribs on the vertebra was traced with sulfuric acid paper for subsequent eye muscle area determination. Tissue thickness at a point 11 cm from the dorsal midline between the 12th and 13th ribs was measured using vernier calipers to determine the GR value. Key indicators were calculated as follows:

- Carcass weight (kg) = Pre-slaughter live weight - weight of head, hooves, skin, tail, reproductive organs and surrounding fat, and viscera (retaining kidneys and perirenal fat)
- Dressing percentage (%) =  $100 \times \text{carcass weight} / \text{pre-slaughter live weight}$

**1.6.3 Meat Quality Cooked meat percentage:** Two longissimus dorsi muscle samples (6 cm × 3 cm × 3 cm) were weighed (m), placed in cooking bags, heated in an 80°C water bath for 30 minutes, then stored overnight at 4°C. After removal, surface moisture was absorbed with filter paper and the samples were reweighed (m). Cooked meat percentage was calculated as:  $100 \times m / m$  [13].

**Drip loss:** Two eye muscle samples (5 cm × 3 cm × 2 cm) were weighed initially, then hung in sealed paper cups without contacting the cup walls. After storage at 4°C for 24 hours, surface moisture was absorbed with filter paper and final weight was recorded. Drip loss was calculated as:  $100 \times (\text{initial weight} - \text{final weight}) / \text{initial weight}$  [14].

**Meat color parameters:** Brightness (L), redness (a), and yellowness (b\*) values of longissimus dorsi muscle at identical locations were measured on-site using a Konica Minolta CR-10 colorimeter (each sample was measured three times and averaged for final results).

## 1.7 Data Analysis

Experimental data were organized using Excel 2010 and analyzed using the ANOVA procedure in SAS 9.1 statistical software for one-way analysis of variance. When significant differences were detected, Duncan's multiple range test was applied for post-hoc comparisons.

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## Results

### 2.1 Effects of Different Biological Agents on Growth Performance

As shown in Table 2, no significant differences were observed in dry matter intake among groups ( $P > 0.05$ ). Average daily gain in the monensin, *Bacillus*

*licheniformis*, and *Saccharomyces cerevisiae* groups was not significantly different from the control group ( $P>0.05$ ), though a numerical increasing trend was evident. However, the compound biological agents group exhibited significantly higher average daily gain compared to the control group ( $P<0.05$ ), with no significant differences among treatment groups ( $P>0.05$ ). Both the *Bacillus licheniformis* and compound biological agents groups showed significantly lower feed-to-gain ratio than the control group ( $P<0.05$ ), while the monensin and *Saccharomyces cerevisiae* groups were numerically lower than the control.

## 2.2 Effects of Different Biological Agents on Slaughter Performance

As shown in Table 2, dressing percentage ranged from 40.67% to 44.30% across groups, with the compound biological agents group achieving the highest value of 44.30%, which was significantly different from the control group ( $P<0.05$ ). Other biological agent groups showed numerical increases compared to the control but without statistical significance ( $P>0.05$ ). The compound biological agents group also exhibited significantly higher GR values than the control group ( $P<0.05$ ). Eye muscle area ranged from 16.90 to 17.90 cm<sup>2</sup> among groups, with no significant differences ( $P>0.05$ ), though all treatment groups were numerically higher than the control, with the compound biological agents group showing the highest value.

## 2.3 Effects of Different Biological Agents on Internal Organ Development

As shown in Table 4, no significant differences were observed in heart and liver weights or their proportions to pre-slaughter live weight among groups ( $P>0.05$ ). While the proportion of lung weight to pre-slaughter live weight did not differ significantly among groups ( $P>0.05$ ), both the *Bacillus licheniformis* and compound biological agents groups showed significantly higher absolute lung weights compared to the control group ( $P<0.05$ ), with no significant differences among treatment groups. The *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and compound biological agents groups all exhibited significantly higher kidney weights than the control group ( $P<0.05$ ), though no significant differences were detected among treatment groups for either absolute kidney weight or its proportion to pre-slaughter live weight. No significant differences were found in perirenal fat weight or its proportion to pre-slaughter live weight among all groups ( $P>0.05$ ).

## 2.4 Effects of Different Biological Agents on Gastrointestinal Development

As shown in Table 5, the proportion of rumen weight to pre-slaughter live weight in the *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and compound biological agents groups was significantly higher than that in the monensin group ( $P<0.05$ ). The compound biological agents group demonstrated significantly

higher rumen weight than all other groups ( $P < 0.05$ ), while the *Bacillus licheniformis* and *Saccharomyces cerevisiae* groups were significantly higher than both the control and monensin groups ( $P < 0.05$ ). The compound biological agents group also showed significantly higher reticulum and omasum weights compared to the control and monensin groups ( $P < 0.05$ ). Complex stomach weight in the compound biological agents group was significantly higher than all other groups ( $P < 0.05$ ), while the *Bacillus licheniformis* and *Saccharomyces cerevisiae* groups were significantly higher than the control group ( $P < 0.05$ ). The proportion of complex stomach weight to pre-slaughter live weight in the compound biological agents group was significantly higher than that in the control and monensin groups ( $P < 0.05$ ).

Both the *Saccharomyces cerevisiae* and compound biological agents groups exhibited significantly higher small intestine weights than all other groups ( $P < 0.05$ ), while the *Bacillus licheniformis* group was significantly higher than the control and monensin groups ( $P < 0.05$ ). The proportions of small intestine weight to pre-slaughter live weight in the *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and compound biological agents groups were all significantly higher than those in the control and monensin groups ( $P < 0.05$ ). The *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and compound biological agents groups showed significantly higher large intestine weights compared to the control group ( $P < 0.05$ ), though no significant differences were observed among groups for the proportion of large intestine weight to pre-slaughter live weight ( $P > 0.05$ ).

## 2.5 Effects of Different Biological Agents on Meat Quality

As shown in Table 6, the compound biological agents group showed a numerical increasing trend for both cooked meat percentage and drip loss, though no significant differences were detected among groups ( $P > 0.05$ ). Similarly, no significant differences were observed among groups for any of the three meat color parameters ( $P > 0.05$ ).

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## Discussion

### 3.1 Effects of Different Biological Agents on Growth Performance

Probiotics improve animal performance by competitively excluding pathogens, enhancing immunity, reducing pathogen colonization, and increasing beneficial bacteria populations to improve intestinal microbial balance [15-16]. They also promote nutrient absorption by synthesizing various digestive enzymes, improving digestive tract physiology, and producing organic acids that lower intestinal pH [16]. In this study, no significant differences in dry matter intake were observed among groups, indicating that none of the biological agents adversely affected diet palatability. These findings align with previous research showing

that monensin [17], yeast culture [18], probiotics in forage [19], and composite enzyme-probiotic preparations [20] did not significantly affect feed intake in lambs or goats. However, some studies have reported increased feed intake with probiotic supplementation [21], while others have shown variable effects depending on probiotic strain and dosage [22]. These discrepancies likely result from differences in probiotic species, supplementation levels, and experimental conditions. Under the conditions of this experiment, none of the biological agents significantly affected dry matter intake in mutton lambs.

All biological agents improved average daily gain and reduced feed-to-gain ratio, with the compound biological agents group showing significant differences from the control. These results demonstrate that dietary biological agents promote lamb growth. Mirzaei-Alamouti et al. [17] reported that monensin increased average daily gain and reduced feed-to-gain ratio in lambs, though differences were not significant, consistent with our findings. *Bacillus licheniformis*, as an aerobic bacterium, produces substantial amounts of amylase, cellulase, and proteinase when colonizing the animal gut, reducing antinutritional factors in feed while generating bacteriocins and volatile fatty acids (acetic, propionic, and butyric acid) that inhibit pathogen growth, enhance immunity, and ultimately improve average daily gain and feed utilization [23-24]. *Saccharomyces cerevisiae*, a facultative anaerobic fungus, produces lactic acid to improve gastrointestinal environment and microflora structure, contains superoxide dismutase and glutathione to enhance antioxidant capacity, facilitates rumen microbial ammonia utilization, increases microbial protein content [23,25], and possesses strong enzymatic activity that increases fiber-degrading bacteria populations in the rumen, promoting nutrient digestion, absorption, and utilization [26-27].

Liu [23] demonstrated that dietary supplementation with either *Bacillus* or yeast improved average daily gain in lambs, while Shan et al. [28] showed that composite *Bacillus*-yeast preparations increased average daily gain and reduced feed-to-gain ratio. In our study, the *Bacillus licheniformis* and *Saccharomyces cerevisiae* groups showed numerical improvements in average daily gain and feed-to-gain ratio compared to the control and monensin groups, though differences were not statistically significant. This may be attributed to insufficient dosage for stable colonization and complete improvement of gastrointestinal environment and microflora structure. Exogenous proteinase preparations can eliminate antinutritional factors, reduce inflammation, supplement endogenous enzyme deficiencies, stimulate endogenous enzyme secretion, and improve nutrient digestibility, ultimately promoting animal growth and feed utilization [29-31]. In this study, the compound biological agents containing *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and alkaline proteinase produced the best results for average daily gain and feed-to-gain ratio. Composite probiotic preparations more readily produce lactic acid bacteria, adapt to diverse conditions and hosts, and promote livestock growth more effectively than single-strain preparations [32]. Additionally, proteinase preparations produced by probiotics exhibit synergistic effects. E Muqazhe et al. [33] reported that enzyme-probiotic preparations significantly improved average daily gain and feed utilization in 4-month-old lambs, while

Li et al. [20] demonstrated that composite enzyme-probiotic preparations significantly increased average daily gain in Inner Mongolia cashmere goats. The combination of enzyme and probiotic preparations has shown promising results in livestock production [34], consistent with our findings. Regarding growth performance, the ranking of treatments from best to worst was: compound biological agents > *Bacillus licheniformis* > *Saccharomyces cerevisiae* > monensin > control.

### 3.2 Effects of Different Biological Agents on Slaughter Performance and Meat Quality

Slaughter performance is a crucial indicator of animal production efficiency, primarily evaluated through dressing percentage, eye muscle area, and GR value [35]. This study demonstrated that dietary biological agents significantly improved carcass weight and increased dressing percentage, with only the compound biological agents group showing statistically significant effects. Previous research has established that pre-slaughter live weight most significantly affects carcass weight and dressing percentage [35], with sheep dressing percentage increasing with age and live weight [36]. Our results confirmed this trend, as groups with higher pre-slaughter live weight exhibited correspondingly higher carcass weight and dressing percentage. No significant effects on eye muscle area were observed among groups. The GR value represents an important indicator of carcass fat content, and the compound biological agents group showed significantly increased GR values. Lambs in the compound biological agents group had higher pre-slaughter live weight and carcass weight than the other four groups, along with improved carcass fat content. These results indicate that compound biological agents can significantly improve the slaughter performance of Dorper×thin-tailed Han crossbred mutton lambs.

Lamb carcass color determines consumer acceptability [37], while drip loss affects meat juiciness [38]. Cooked meat percentage measures water retention during cooking, with higher values indicating stronger water-holding capacity, more tender meat, and better overall quality [39]. In this study, no significant differences were observed among groups in cooked meat percentage, drip loss, or meat color parameters of the longissimus dorsi muscle, though the compound biological agents group showed numerically higher values for all indicators. Previous research has shown that lamb feeding management and dietary energy and protein levels are important factors affecting carcass quality [39-40]. Under our experimental conditions, dietary supplementation with monensin, *Bacillus licheniformis*, *Saccharomyces cerevisiae*, or compound biological agents did not significantly affect meat quality.

### 3.3 Effects of Different Biological Agents on Internal Organ and Gastrointestinal Development

Changes in tissue and organ weights reflect animal growth and development and indicate physiological function status, holding important significance for both

theoretical research and production practice [41-42]. In this study, the proportions of internal organ weights to pre-slaughter live weight in all groups fell within normal ranges [43-45]. No significant differences were observed among groups in heart, liver, or perirenal fat weights or their proportions to pre-slaughter live weight. Although the *Bacillus licheniformis* and compound biological agents groups significantly increased lung weight, they did not significantly affect the proportion of lung weight to pre-slaughter live weight, indicating coordinated development of organs with overall body growth.

The kidney's primary function is excreting metabolic waste through urine [44]. The *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and compound biological agents groups significantly increased kidney weight, but only the *Saccharomyces cerevisiae* group significantly increased the proportion of kidney weight to pre-slaughter live weight. This may be because the *Saccharomyces cerevisiae* group had higher dry matter intake, increasing metabolic waste and promoting kidney development, while having lower pre-slaughter live weight compared to the *Bacillus licheniformis* and compound biological agents groups, resulting in a higher proportion. Under our experimental conditions, dietary supplementation with different biological agents did not adversely affect normal internal organ development, consistent with animals' innate ability to regulate organ growth in coordination with body development [46].

The degree of gastrointestinal development in ruminants directly affects feed intake and digestion capacity, with rumen development being particularly critical for adult production performance [47]. Our results showed that the monensin group did not differ significantly from the control group in rumen, reticulum, omasum, abomasum, or complex stomach weights or their proportions to pre-slaughter live weight, indicating coordinated stomach development with body growth. However, the *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and compound biological agents groups showed significantly higher rumen and complex stomach weights than the control group, with all stomach compartments and complex stomach weight proportions exceeding those of the control and monensin groups. These findings demonstrate that dietary supplementation with *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and compound biological agents promotes stomach development. Probiotics can improve diet digestion and metabolism [48], and the resulting metabolic stimulation can increase rumen weight and promote rumen muscle development. Therefore, probiotics may enhance stomach development by improving diet digestion and metabolism.

In this study, the *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and compound biological agents groups significantly increased both small intestine weight and its proportion to pre-slaughter live weight compared to the control and monensin groups. Probiotics promote intestinal microflora proliferation, and short-chain fatty acids produced through microbial fermentation provide energy to intestinal mucosa, improve local blood supply, promote intestinal epithelial cell repair, and increase pancreatic enzyme secretion, thereby facilitating intestinal development [49]. Thus, probiotics may promote small

intestine development by indirectly increasing short-chain fatty acid content.

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## Conclusions

Under the conditions of this experiment: (1) Compound biological agents comprising probiotics and enzymes exerted superior effects on average daily gain and feed-to-gain ratio compared to single probiotics and monensin; (2) The ranking of single biological agents for improving growth and slaughter performance was: *Bacillus licheniformis* > *Saccharomyces cerevisiae* > monensin > no supplementation; and (3) Biological agents did not significantly affect the meat quality of Dorper×thin-tailed Han crossbred mutton lambs.

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