

Effects of *Bacillus subtilis* and Echinacea Extract on Growth Performance, Immune Function, and Meat Quality of Finishing Sheep: Postprint

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Abstract

This experiment aimed to investigate the effects of probiotic *Bacillus subtilis* and herbal Echinacea extract on growth performance, nutrient apparent digestibility, diarrhea rate, serum biochemical indices, organ indices, slaughter performance, and meat quality of fattening sheep. Twenty-seven weaned Saanen male lambs aged 3-4 months with similar body condition were selected and randomly divided into control group, *Bacillus subtilis* group, and Echinacea group (3 replicates per group, 3 sheep per replicate), and fed basal diet (control group), basal diet + 100 mg/(kg BW · d) *Bacillus subtilis* (viable count 5×10^8 CFU/g) (*Bacillus subtilis* group), and basal diet + 100 mg/(kg BW · d) Echinacea extract (Echinacea group), respectively. The pre-trial period was 10 days, and the formal trial period was 60 days. The results showed: 1) There were no significant differences in average daily gain, dry matter intake, and feed conversion ratio among groups ($P > 0.05$). Compared with the control group and Echinacea group, the *Bacillus subtilis* group reduced dry matter intake by 12.07% and 8.87%, respectively, during the entire fattening period. 2) Compared with the control group, the *Bacillus subtilis* group significantly improved apparent digestibility of dry matter, crude protein, ether extract, neutral detergent fiber, and acid detergent fiber ($P < 0.05$), while the Echinacea group significantly improved apparent digestibility of acid detergent fiber ($P < 0.05$). 3) Compared with the control group, the *Bacillus subtilis* group significantly reduced diarrhea rate, serum alkaline phosphatase activity, and concentrations of serum urea nitrogen, albumin, triglyceride, and glucose ($P < 0.05$), and significantly increased serum total protein and globulin concentrations and spleen and lung indices ($P < 0.05$). The Echinacea group significantly increased serum alkaline phosphatase activity and globulin concentration ($P < 0.05$), and significantly decreased serum albumin and urea nitrogen concentrations ($P < 0.05$). 4) Compared with the control group, the *Bacillus subtilis* group showed no significant

changes in dressing percentage, GR value, loin eye area, and mutton pH, crude protein, and ether extract content ($P > 0.05$), but significantly reduced mutton shear force and moisture content ($P < 0.05$), and significantly increased mutton cooking yield ($P < 0.05$). The Echinacea group showed no significant changes in conventional nutrient content of mutton ($P > 0.05$). In conclusion, *Bacillus subtilis* can significantly improve nutrient apparent digestibility and immune performance, and enhance meat quality of fattening sheep; Echinacea extract can improve acid detergent fiber digestibility and has certain beneficial effects on serum biochemical indices.

Full Text

Effects of *Bacillus subtilis* and Echinacea Extract on Growth Performance, Immune Function and Meat Quality of Fattening Sheep

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Abstract

This study investigated the effects of probiotic *Bacillus subtilis* and herbal *Echinacea* extract on growth performance, apparent nutrient digestibility, diarrhea rate, serum biochemical indicators, organ indexes, carcass traits and meat quality of fattening sheep. Twenty-seven weaned male Suffolk×small tailed Han lambs aged 3–4 months with similar body condition were randomly assigned to three groups (3 replicates per group, 3 sheep per replicate). The groups were fed: (1) a basal diet (control group), (2) basal diet + 100 mg/(kg BW·d) *Bacillus subtilis* (viable count 5×10^8 CFU/g) (*Bacillus subtilis* group), and (3) basal diet + 100 mg/(kg BW·d) *Echinacea* extract (*Echinacea* group). The pre-test lasted 10 days, followed by a 60-day formal test period. The results showed: (1) No significant differences in average daily gain, dry matter intake or feed/gain ratio were observed among groups ($P > 0.05$). However, dry matter intake during the entire fattening period decreased by 12.07% and 8.87% in the *Bacillus subtilis* group compared with the control and *Echinacea* groups, respectively. (2) The *Bacillus subtilis* group exhibited significantly higher apparent digestibility of dry matter, crude protein, ether extract, neutral detergent fiber and acid detergent fiber compared with the control group ($P < 0.05$). The *Echinacea* group showed significantly improved apparent digestibility of acid detergent fiber ($P < 0.05$). (3) Compared with the control group, the *Bacillus subtilis* group had significantly lower diarrhea rate, serum alkaline phosphatase activity, and serum concentrations of urea nitrogen, albumin, triglycerides and glucose ($P < 0.05$), while serum

total protein and globulin concentrations, spleen index and lung index were significantly higher ($P < 0.05$). The Echinacea group showed significantly higher serum alkaline phosphatase activity and globulin concentration ($P < 0.05$), and significantly lower serum albumin and urea nitrogen concentrations ($P < 0.05$). (4) No significant changes were observed in slaughter rate, GR value, eye muscle area, or pH and contents of crude protein and crude fat of meat in the *Bacillus subtilis* group ($P > 0.05$). However, shear force and moisture content of meat decreased significantly ($P < 0.05$), while cooked meat rate increased significantly ($P < 0.05$). The Echinacea group showed no significant changes in conventional nutrient composition of meat ($P > 0.05$). These results indicate that *Bacillus subtilis* can significantly improve nutrient apparent digestibility, immune function and meat quality of fattening sheep, while *Echinacea* extract can improve apparent digestibility of acid detergent fiber and has beneficial effects on serum biochemical indicators.

Keywords: *Bacillus subtilis*; *Echinacea* extract; fattening sheep; growth performance; immunity

1 Materials and Methods

1.1 Experimental Animals and Design

Twenty-seven healthy weaned male Suffolk \times small tailed Han lambs aged 3–4 months with medium body condition and normal development were selected, with an average body weight of (19.02 ± 0.46) kg. The lambs were randomly divided into three groups with three replicates per group and three sheep per replicate. The control group received a basal diet formulated according to NRC (2007) nutrient requirements for meat sheep, with composition and nutrient levels shown in Table 1. The *Bacillus subtilis* group received the basal diet supplemented with $100 \text{ mg}/(\text{kg BW} \cdot \text{d})$ *Bacillus subtilis* (viable count 5×10^8 CFU/g). The Echinacea group received the basal diet supplemented with $100 \text{ mg}/(\text{kg BW} \cdot \text{d})$ *Echinacea* extract (extracted from aboveground stems, leaves and flowers, containing 4% polyphenols). The pre-test period lasted 10 days, followed by a 60-day test period.

1.2 Experimental Materials

Bacillus subtilis (embedded, viable count 5×10^8 CFU/g) was obtained from Shandong Baolai-Lilai Bioengineering Co., Ltd. *Echinacea* extract (powdered extract from aboveground stems, leaves and flowers, containing 4% polyphenols) was obtained from Xi'an Ruibo Biotechnology Co., Ltd.

1.3 Feeding Management

The experimental lambs were sourced from Dingxi Wangsheng Breeding Co., Ltd. and raised in pens under indoor feeding conditions. One week before the

experiment, the sheep house, pens and feed troughs were thoroughly cleaned and disinfected. Prior to the experiment, each lamb was weighed, ear-tagged, dipped and dewormed (with the sheep house thoroughly cleaned within 48 h after deworming). The lambs were then grouped and housed individually in pens measuring 3.0 m × 2.5 m, equipped with integrated water and feed troughs. Feed was provided twice daily at 07:30 and 16:30, with free access to water. The experimental facility was a semi-open color steel structure with consistent environmental conditions for temperature, lighting and ventilation.

1.4 Measurements

1.4.1 Growth Performance During the experiment, daily feed allocation and residual feed were recorded by replicate, along with diarrhea incidence. Fecal consistency was observed daily at 08:00 and classified into five grades: normal, soft, viscous, watery and bloody, with the latter four grades considered diarrhea. Individual fasting live weight was measured every 20 days during the test period (with two consecutive days of weighing at the start and end). Average daily gain (ADG), average daily feed intake (ADFI), dry matter intake (DMI), feed/gain ratio (F/G) and diarrhea ratio were calculated using the following formula:

Diarrhea ratio (%) = [number of diarrhea cases / (total number of sheep × experimental days)] × 100.

1.4.2 Nutrient Apparent Digestibility From days 31–35 of the test period, a 5-day digestion trial was conducted. Daily fecal samples were collected using fecal bags and weighed. After thorough mixing, 150 g was divided into three portions: one portion was dried at 105 °C to determine dry matter content; one portion was preserved with 10% sulfuric acid for ammonia nitrogen analysis and frozen; the final portion was dried at 65 °C to constant weight in an aluminum box, ground through a 40-mesh sieve and stored. The 5-day fecal samples were then combined, mixed thoroughly and subsampled (200 g) by quartering. Daily feed intake and dry matter intake were recorded, and 200 g feed samples were collected daily from each group. Dry matter, calcium, total phosphorus, crude protein, neutral detergent fiber and acid detergent fiber contents in fecal and feed samples were determined using methods described in *Feed Analysis and Feed Quality Detection Technology* [20].

1.4.2 Serum Biochemical Indicators On the final day of the test period at 08:00, 10 mL of fasting venous blood was collected from all sheep into centrifuge tubes without anticoagulant. After coagulation, serum was separated by centrifugation at 2,312×g for 10 min, and the supernatant was transferred to EP tubes and stored at -80 °C. For analysis, frozen serum was thawed in a 37 °C water bath. Serum alkaline phosphatase activity and concentrations of total protein, albumin, globulin, urea nitrogen, triglycerides and glucose were measured using an automatic biochemical analyzer (BX-3010, Sysmex Medi-

cal Electronics Co., Ltd., Japan) according to kit instructions. Alkaline phosphatase activity was measured using the kinetic method recommended by the International Federation of Clinical Chemistry (IFCC). Total protein concentration was determined by the biuret colorimetric method. Albumin concentration was measured by the bromocresol green (BCG) colorimetric method. Globulin concentration was calculated as the difference between total protein and albumin. Urea nitrogen concentration was determined by the enzyme-linked rate method. Triglyceride concentration was measured by the glucose oxidase-peroxidase (GOD-POD) enzyme colorimetric method. Glucose concentration was determined by the glucose oxidase method. All procedures followed kit instructions, using universal biochemical analysis kits from Guangzhou Panyu Huaxin Technology Co., Ltd.

1.4.3 Organ Indexes, Slaughter Performance and Meat Quality After the experiment, three healthy lambs with similar body weight were randomly selected from each group for slaughter. Feed was withheld for 24 h and water for 2 h before slaughter. After fasting weighing, lambs were slaughtered by the Islamic method (neck cutting). Following skinning and removal of head, limbs and viscera (retaining kidneys and perirenal fat), carcasses were weighed after 30 min of static rest to calculate slaughter rate:

$$\text{Slaughter rate (\%)} = (\text{carcass weight} / \text{pre-slaughter live weight}) \times 100.$$

Liver, spleen, heart and lungs were dissected, surface moisture was absorbed with filter paper, and organs were weighed to calculate organ indexes:

$$\text{Organ index (\%)} = (\text{organ weight} / \text{pre-slaughter live weight}) \times 100.$$

Following Zhao Youzhang' s method [21], the cross-sectional area of the eye muscle between the 12th and 13th ribs was traced with sulfuric acid paper and measured with a planimeter. GR value (tissue thickness 11 cm from the dorsal midline between the 12th and 13th ribs) was measured with a vernier caliper. The right longissimus dorsi muscle from ribs 5-10 was transported to the laboratory. pH and shear force were measured within 2 h (average of three measurements). A circular sampler (diameter 2.532 cm) was used to obtain a 1 cm thick longissimus dorsi sample, which was weighed, placed between 18 layers of neutral filter paper on each side, pressed between two plastic plates at 35 kg for 5 min, and reweighed to calculate water loss rate:

$$\text{Water loss rate (\%)} = [(\text{sample weight before pressing} - \text{sample weight after pressing}) / \text{sample weight before pressing}] \times 100.$$

Approximately 100 g of psoas major muscle was weighed, cooked in a 2,000 W electric stove for 45 min, hung to cool for 30 min and reweighed to calculate cooked meat rate:

$$\text{Cooked meat rate (\%)} = (\text{sample weight after cooking} / \text{sample weight before cooking}) \times 100.$$

The right longissimus dorsi muscle from ribs 11-13 was vacuum-packaged and stored at -20 °C for routine nutrient composition analysis according to national standards for meat and meat products.

1.5 Data Processing and Statistical Analysis

Experimental data were analyzed using SPSS 19.0. One-way ANOVA was used for variance analysis, and the least significant difference (LSD) method was used for multiple comparisons. The significance level was set at $P < 0.05$. Data are expressed as mean \pm standard deviation.

2 Results

2.1 Effects of *Bacillus subtilis* and Echinacea Extract on Growth Performance

As shown in Table 2, the fastest weight gain occurred during the middle fattening period (days 21-40) with average daily gain exceeding 250 g/d, followed by the early period (days 1-20) with average daily gain exceeding 228 g/d, while growth was slower during the late period (days 41-60). No significant differences in average daily gain were observed among the control, Echinacea and *Bacillus subtilis* groups throughout the experiment ($P > 0.05$).

The *Bacillus subtilis* group had the lowest dry matter intake during the entire fattening period (days 1-60), decreasing by 12.07% and 8.87% compared with the control and Echinacea groups, respectively, though differences among groups were not significant ($P > 0.05$). Dietary supplementation with *Bacillus subtilis* and Echinacea extract numerically decreased feed/gain ratio. During the entire fattening period, feed/gain ratio decreased by 13.64% and 6.99% in the *Bacillus subtilis* and Echinacea groups, respectively, compared with the control group, but differences were not significant ($P > 0.05$). No significant differences in feed/gain ratio were observed among groups during the early (days 1-20), late (days 41-60) or entire fattening period ($P > 0.05$). During the middle period (days 21-40), feed/gain ratio in the Echinacea group was significantly higher than in the *Bacillus subtilis* group ($P < 0.05$), but not significantly different from the control group ($P > 0.05$).

2.2 Effects on Nutrient Apparent Digestibility

As shown in Table 3, *Bacillus subtilis* significantly affected nutrient apparent digestibility ($P < 0.05$). Compared with the control and Echinacea groups, the *Bacillus subtilis* group showed significantly higher apparent digestibility of dry matter, crude protein, ether extract, neutral detergent fiber and acid detergent fiber ($P < 0.05$). The Echinacea group exhibited significantly higher apparent digestibility of acid detergent fiber compared with the control group ($P < 0.05$), while other nutrients showed no significant differences ($P > 0.05$).

2.3 Effects on Diarrhea Rate

As shown in Table 4 , dietary supplementation with *Bacillus subtilis* and Echinacea extract reduced diarrhea rate in fattening sheep. Diarrhea rate decreased by approximately 50.00% and 11.03% in the *Bacillus subtilis* and Echinacea groups, respectively, compared with the control group. However, the difference between the Echinacea and control groups was not significant ($P>0.05$), while the *Bacillus subtilis* group was significantly lower than the control group ($P<0.05$).

2.4 Effects on Serum Biochemical Indicators

As shown in Table 5 , the *Bacillus subtilis* group had significantly lower serum alkaline phosphatase activity and concentrations of urea nitrogen, albumin, triglycerides and glucose compared with the control group ($P<0.05$), while serum total protein and globulin concentrations were significantly higher ($P<0.05$). The Echinacea group showed significantly higher serum alkaline phosphatase activity and globulin concentration ($P<0.05$), and significantly lower serum albumin and urea nitrogen concentrations ($P<0.05$). Compared with the Echinacea group, the *Bacillus subtilis* group had significantly lower serum alkaline phosphatase activity and triglyceride and glucose concentrations ($P<0.05$), and significantly higher total protein and globulin concentrations ($P<0.05$), while other indicators showed no significant differences ($P>0.05$).

2.5 Effects on Organ Indexes

As shown in Table 6 , spleen index in both the *Bacillus subtilis* and Echinacea groups was significantly higher than in the control group ($P<0.05$). Lung index in the *Bacillus subtilis* group was significantly higher than in both the control and Echinacea groups ($P<0.05$). No significant differences were observed in liver index, heart index or kidney index among groups ($P>0.05$).

2.6 Effects on Slaughter Performance and Meat Quality

As shown in Table 7 , no significant differences were observed in slaughter rate, GR value, eye muscle area, or pH and contents of crude protein and crude fat of meat among groups ($P>0.05$). Shear force of meat in the *Bacillus subtilis* group was significantly lower than in the control and Echinacea groups ($P<0.05$). Water loss rate of meat in both the *Bacillus subtilis* and Echinacea groups was significantly lower than in the control group ($P<0.05$). Cooked meat rate in both the *Bacillus subtilis* and Echinacea groups was significantly higher than in the control group ($P<0.05$). Moisture content of meat in the *Bacillus subtilis* group was significantly lower than in the Echinacea and control groups ($P<0.05$). No significant differences in conventional nutrient composition of meat were observed between the Echinacea and control groups ($P>0.05$).

3 Discussion

3.1 Effects on Growth Performance

Qiu Wusong et al. [22] reported that adding 0.72% *Bacillus subtilis* to dietary concentrate had no significant effect on average daily gain of Hu sheep but significantly decreased feed intake and feed/gain ratio. Zhang Zhiyan et al. [23] found that adding 1% enzyme preparation containing *Bacillus subtilis* to fattening sheep concentrate significantly increased average daily gain by 12.88% and decreased feed/gain ratio by 6.50% compared with the control group, with no significant effect on average daily feed intake. Upadhaya et al. [24] demonstrated in pigs that dietary *Bacillus subtilis* supplementation significantly increased average daily gain and decreased feed/gain ratio in piglets within 6 weeks of age, but had no significant effect on growth performance in later stages (7-16 weeks). Jørgensen et al. [25] reported that *Bacillus subtilis* had no effect on growth performance of 28-42 day-old piglets, significantly increased average daily gain and decreased feed/gain ratio in 43-120 day-old piglets, and significantly decreased average daily gain and increased feed/gain ratio in 121-182 day-old piglets. Over the entire experimental period, *Bacillus subtilis* significantly improved average daily gain and feed/gain ratio. Aly et al. [26] found that *Echinacea* significantly increased body weight gain, specific growth rate and survival rate of tilapia during summer. Oskoi et al. [27] studied the effects of dietary *Echinacea* on rainbow trout and found that all supplementation levels increased body weight gain and specific growth rate and decreased feed coefficient, with 0.5% supplementation showing the best effect. The present study showed that *Bacillus subtilis* and *Echinacea* extract had no significant effect on average daily gain of fattening sheep, consistent with Qiu Wusong et al. [22]. Both additives only numerically decreased feed/gain ratio, which differs from previous studies, possibly due to variations in supplementation dosage or animal species, warranting further dosage screening experiments.

3.2 Effects on Nutrient Apparent Digestibility

Microbial additives can improve feed digestibility [28-29]. Reports indicate that adding probiotics to dairy cow diets can alter microbial populations in the rumen and hindgut, modify rumen fermentation patterns, increase nutrient outflow rate, and improve nutrient digestibility and feed utilization [30-32]. Xiao Yi et al. [33] supplemented meat sheep diets with *Bacillus* at three levels (2.4×10^7 , 2.4×10^8 and 2.4×10^9 CFU/sheep · d) and found that 2.4×10^8 CFU/sheep · d significantly decreased dry matter intake while improving apparent digestibility of dry matter, organic matter, neutral detergent fiber, acid detergent fiber and nitrogen, and significantly increased dietary digestible and metabolizable energy. Zhou Meng [34] reported that *Bacillus subtilis* significantly improved nutrient apparent digestibility in calves. Noh et al. [35] demonstrated that dietary supplementation with 5% *Bacillus subtilis* significantly improved apparent digestibility of dry matter, gross energy and crude ash. These results align with the present study. The *Bacillus subtilis* group showed significantly higher apparent

digestibility of dry matter, crude protein, ether extract, neutral detergent fiber and acid detergent fiber compared with the control and Echinacea groups. This may be because *Bacillus subtilis* colonization in the gastrointestinal tract supplements the animal with highly active enzymes including protease, lipase, amylase and cellulase, degrading proteins, triglycerides, non-starch polysaccharides and structural carbohydrates, and reducing anti-nutritional factors, thereby assisting digestion, promoting nutrient absorption and improving feed utilization [36]. Most studies on *Echinacea* have focused on immune function, with no reports on nutrient digestibility. The present study found that the Echinacea group had significantly higher apparent digestibility of acid detergent fiber than the control group, though the mechanism requires further investigation.

3.3 Effects on Diarrhea Rate, Serum Biochemical Indicators and Organ Indexes

Bacillus subtilis can antagonize pathogenic bacteria and enhance immune capacity. Under adverse conditions, it exists in spore form, grows rapidly and produces abundant enzymes, demonstrating beneficial effects on growth performance, intestinal flora balance, antioxidant capacity and immune function [37-38]. *Echinacea* contains heteroxylan and arabinose that stimulate mononuclear lymphocyte proliferation and macrophage activity, inducing release of tumor necrosis factor- α , interleukin-1 and interferon- γ , enhancing non-specific T cell activity and lymphocyte secretion, thereby strengthening immune function. *Echinacea* has significant immunostimulatory effects related to enhanced macrophage and lymphocyte function and stimulated cytokine and antibody production. *Bacillus subtilis* has preventive and therapeutic effects on diarrhea in cattle and sheep. Liang Jinqiong et al. [12] reported that oral administration of *Bacillus* preparation to diarrheal calves and lambs for 3 days achieved antidiarrheal rates of 93.33% and 86.66%, respectively. Yao Weiping et al. [39] found that dietary supplementation with 1.5% *Echinacea* preparation decreased diarrhea rate in weaned piglets. In this study, dietary *Bacillus subtilis* supplementation reduced diarrhea rate in fattening sheep by 50.00% compared with the control group, possibly by producing various digestive enzymes that supplement endogenous enzyme deficiency, establishing gastrointestinal mucosal microbial defense barriers, maintaining microecological balance, and producing physiologically active and antimicrobial substances [40-41] that inhibit bacteria, viruses and fungi, thereby improving immune function and reducing diarrhea. The Echinacea group showed a numerical decrease in diarrhea rate compared with the control group, but the difference was not significant, differing from previous piglet studies, possibly due to the low dosage used. Yuan Caihong [42] reported that the minimum inhibitory concentrations of *Echinacea* volatile oil against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* were 2.50, 1.25 and 0.63 mg/mL, respectively, with antibacterial effects resulting from polysaccharides, caffeic acid and other bioactive compounds that alter macrophage activity.

Guo Junrui et al. [9] showed that dietary *Bacillus subtilis* supplementation in laying hens significantly decreased serum glucose concentration at weeks 2, 3, 4, 12, 16 and 20 and serum urea concentration at weeks 2, 3, 8, 12 and 20, while significantly increasing serum immunoglobulin A at week 16, immunoglobulin G at weeks 1 and 4, and immunoglobulin M at weeks 2, 4 and 8. Li Weifen et al. [43] reported that *Bacillus subtilis* significantly increased serum alkaline phosphatase activity and decreased uric acid, cholesterol and triglyceride concentrations. Qadis et al. [44] found that compound probiotic preparations containing *Bacillus subtilis* increased immune factor levels and enhanced cellular immune function in weaned calves. Sun et al. [38] showed that *Bacillus subtilis* natto increased serum immunoglobulin and interferon- (IFN-) concentrations in weaned calves. However, some reports indicate that compound probiotics of *Bacillus licheniformis* and *Bacillus subtilis* had no significant effect on serum biochemical indicators in calves [45]. Dai Xueli et al. [19] reported that dietary supplementation with 1% *Echinacea* compound preparation in laying hens significantly increased serum glucose and total protein concentrations and alkaline phosphatase activity, while decreasing triglyceride and cholesterol concentrations. In this study, the *Bacillus subtilis* group showed significantly lower serum alkaline phosphatase activity and concentrations of urea nitrogen, albumin, triglycerides and glucose, and significantly higher total protein and globulin concentrations. The *Echinacea* group showed significantly higher serum alkaline phosphatase activity and globulin concentration, and significantly lower serum albumin and urea nitrogen concentrations. Serum total protein maintains tissue protein homeostasis, repairs tissues, stabilizes blood pH and can provide energy through oxidation when needed, thus reflecting nutritional and immune status. Serum urea nitrogen accurately reflects protein metabolism and dietary amino acid balance, with decreased levels indicating increased nitrogen deposition and improved protein utilization. Serum triglycerides reflect lipid metabolism, and decreased concentrations promote normal lipid metabolism and healthy growth. Alkaline phosphatase, synthesized primarily by osteoblasts and liver, is an important bone metabolism indicator that plays an active role in bone mineralization.

Organ development is mainly determined by nutritional level and breed [46]. Compound *Bacillus* preparations promote intestinal development in meat rabbits. Sun Huanlin [47] found that dietary *Bacillus subtilis* supplementation in broilers significantly increased spleen and bursa of Fabricius indexes. No reports exist on *Echinacea* effects on organ indexes. This study showed that dietary *Bacillus subtilis* supplementation significantly increased spleen and lung indexes, possibly because antimicrobial substances secreted by *Bacillus subtilis* promote immune organ maturation, maintain tissues in a highly reactive state, increase T and B lymphocyte numbers, and improve humoral and cellular immunity. *Echinacea* extract significantly increased spleen index, likely because heteroxylan and arabinose stimulate mononuclear lymphocyte proliferation and macrophage activity, enhance non-specific T cell activity, strengthen immune function and promote immune organ enlargement.

3.4 Effects on Slaughter Performance and Meat Quality

Li Weifen et al. [43] showed that dietary *Bacillus subtilis* supplementation in broilers decreased breast muscle water loss rate by 22.66% and numerically improved muscle lightness, redness and yellowness values. Other reports indicate that dietary supplementation with *Bacillus subtilis* and *Bacillus licheniformis* had no significant effect on broiler muscle water loss rate [48]. No reports exist on *Echinacea* extract effects on slaughter performance and meat quality. This study found that dietary *Bacillus subtilis* supplementation decreased shear force and water loss rate while increasing cooked meat rate of mutton, thereby improving meat quality, while *Echinacea* extract increased cooked meat rate of mutton.

4 Conclusion

Dietary supplementation with 100 mg/(kg BW · d) *Bacillus subtilis* can numerically decrease daily feed intake and feed/gain ratio of fattening sheep, while significantly improving apparent digestibility of dry matter, crude protein, ether extract, neutral detergent fiber and acid detergent fiber.

Dietary *Bacillus subtilis* supplementation can improve immune function of fattening sheep, decrease diarrhea rate and serum alkaline phosphatase activity and concentrations of urea nitrogen, albumin, triglycerides and glucose, while increasing lung index, spleen index and serum concentrations of total protein and globulin.

Dietary *Bacillus subtilis* supplementation has no significant effect on slaughter performance of fattening sheep but can improve meat quality.

Dietary supplementation with 100 mg/(kg BW · d) *Echinacea* extract significantly increases apparent digestibility of acid detergent fiber and serum alkaline phosphatase activity and globulin concentration, while significantly decreasing serum concentrations of albumin and urea nitrogen.

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