

## Effects of *Allium mongolicum* Flavonoids on Production Performance and $\alpha$ -Defensin Gene Expression in Intestinal Tissue of Meat Sheep (Postprint)

**Authors:** Chen Shengyang, Ao Changjin, Zheng Yankai, Renwei Chen, Liu Wangjing, Muqier, Bai Chen

**Date:** 2018-12-24T00:00:00+00:00

### Abstract

This study aimed to investigate the effects of *Allium mongolicum* flavonoids on production performance and the gene expression of intestinal immune factors  $\alpha$ -defensin-1 (sBD-1) and  $\alpha$ -defensin-2 (sBD-2) in meat sheep. Sixty healthy, 6-month-old Small-tailed Han wether sheep with similar body weight [(39.9±3.2) kg] were selected and allocated into 4 groups (n=15) using a single-factor completely randomized block design. The control group was fed a basal diet, while the experimental groups were fed experimental diets supplemented with 11 (low-flavonoid group), 22 (medium-flavonoid group), and 33 mg/kg *Allium mongolicum* flavonoids (high-flavonoid group) in the basal diet, respectively, for a total of 70 days, including a 15-day pre-trial period and a 60-day formal trial period. Daily feed intake was recorded for each group, and body weight was measured once every 15 days before morning feeding after overnight fasting. At the end of the trial, 3 sheep were randomly selected from each group for slaughter, and tissue samples from the duodenum, jejunum, and ileum were collected. Real-time quantitative PCR was applied to determine the relative expression levels of sBD-1 and sB-2 genes. The results showed: 1) During days 30-45 and 45-60 of the trial, the average daily feed intake of meat sheep in all experimental groups was significantly higher than that in the control group ( $P<0.05$ ), with the highest value observed in the high-flavonoid group; during days 45-60, compared with the control group, the average daily gain of meat sheep in all experimental groups was significantly increased ( $P<0.05$ ), and the feed-to-gain ratio was significantly decreased ( $P<0.05$ ), with the highest average daily gain observed in the medium-flavonoid group and the lowest feed-to-gain ratio in the high-flavonoid group. 2) Compared with the control group, dietary supplementation with 33 mg/kg *Allium mongolicum* flavonoids significantly increased the

relative expression levels of sBD-1 gene in the jejunum and ileum and sBD-2 gene in the jejunum ( $P < 0.05$ ), while dietary supplementation with 22 mg/kg *Allium mongolicum* flavonoids significantly increased the relative expression level of sBD-2 gene in the duodenum ( $P < 0.05$ ). In conclusion, dietary supplementation with 22-33 mg/kg *Allium mongolicum* flavonoids can significantly improve production performance and intestinal gene expression of  $\alpha$ -defensins (sBD-1, sBD-2) in meat sheep.

## Full Text

### Effects of *Allium mongolicum* Regel Flavonoids on Performance and $\alpha$ -Defensins Gene Expression in Intestinal Tissue of Meat Sheep

\*\*CHEN Shengyang, AO Changjin\*, ZHENG Yankai, CHEN Renwei, LIU Wangjing, MU Qier, BAI Chen\*\* (College of Animal Science, Inner Mongolia Agricultural University, Hohhot 010018, China)

#### Abstract

This study investigated the effects of *Allium mongolicum* Regel flavonoids on the performance and expression of intestinal immune factors  $\alpha$ -defensin-1 (sBD-1) and  $\alpha$ -defensin-2 (sBD-2) genes in meat sheep. Sixty healthy, six-month-old thin-tailed Han wethers with similar body weight [(39.9±3.2) kg] were randomly allocated to four groups (n=15) using a single-factor completely randomized block design. The control group received a basal diet, while experimental groups received the basal diet supplemented with 11 (low-dose flavonoid group), 22 (medium-dose flavonoid group), and 33 mg/kg *Allium mongolicum* Regel flavonoids (high-dose flavonoid group), respectively. The 70-day feeding trial consisted of a 15-day pre-feeding period followed by a 60-day formal experimental period. Daily feed intake was recorded, and body weight was measured every 15 days before morning feeding. At the end of the trial, three sheep from each group were randomly selected for slaughter to collect duodenal, jejunal, and ileal tissues for determination of sBD-1 and sBD-2 gene relative expression levels using real-time quantitative PCR.

The results showed: (1) During days 30-45 and 45-60, average daily feed intake in all experimental groups was significantly higher than in the control group ( $P < 0.05$ ), with the highest intake observed in the high-dose flavonoid group. During days 45-60, experimental groups exhibited significantly higher average daily gain and lower feed-to-gain ratio compared to the control group ( $P < 0.05$ ), with the medium-dose group showing the highest average daily gain and the high-dose group showing the lowest feed-to-gain ratio. (2) Compared with the control group, dietary supplementation with 33 mg/kg *Allium mongolicum* Regel flavonoids significantly increased the relative expression of sBD-1 gene in jejunum and ileum and sBD-2 gene in jejunum ( $P < 0.05$ ), while 22

mg/kg supplementation significantly enhanced sBD-2 gene expression in duodenum ( $P < 0.05$ ). In conclusion, dietary supplementation with 22-33 mg/kg *Allium mongolicum* Regel flavonoids significantly improved meat sheep performance and upregulated  $\alpha$ -defensin (sBD-1, sBD-2) gene expression in intestinal tissues.

**Keywords:** meat sheep; performance; immunity;  $\alpha$ -defensins; gene expression

---

## Introduction

*Allium mongolicum* Regel, commonly known as “Mongolian chive,” is a Liliaceae *Allium* species widely distributed in northwestern China and central-western Inner Mongolia. As a high-quality forage rich in various nutrients, *Allium mongolicum* and its extracts exhibit strong antioxidant activity and immunomodulatory functions in animals. Flavonoids represent one of the primary active components in the liposoluble extracts of *Allium mongolicum* and play crucial roles in immune responses. Zhao et al. [1] reported that *Allium mongolicum* flavonoids enhanced non-specific immune function in mice by increasing immune organ indices, acid phosphatase and lysozyme activities in blood and liver, and carbon clearance index. In vitro studies have also demonstrated that *Allium mongolicum* isoflavones possess free radical scavenging capacity [2]. Furthermore, *Allium mongolicum* flavonoids promote proliferation of peripheral blood lymphocytes and upregulate interleukin-2 (IL-2) and interferon- $\gamma$  (IFN- $\gamma$ ) gene expression while downregulating IL-4 expression, thereby enhancing immunomodulatory effects [3].

Antimicrobial peptides are small molecules widely distributed in animals and plants that constitute the first line of innate immune defense against foreign antigen invasion [4].  $\alpha$ -defensins are cysteine-rich cationic antimicrobial peptides primarily produced by epithelial cells, containing 6-8 cysteine residues connected by 3-4 disulfide bonds that form stable molecular conformations. These peptides play vital immunomodulatory roles in epithelial tissues of the respiratory and digestive tracts [5-6]. Compared with antibiotics,  $\alpha$ -defensins offer numerous advantages including small molecular mass, high thermal stability, good water solubility, and low risk of resistance development [7-8]. Enhancing  $\alpha$ -defensin expression to improve intestinal immune function represents a novel strategy for controlling and preventing intestinal diseases, making  $\alpha$ -defensin research highly significant for livestock disease prevention, treatment, and healthy development of the animal production industry.

Numerous studies have shown that dietary supplementation with *Allium mongolicum* flavonoids in confined meat sheep enhances non-specific immune function, reduces disease incidence, and accelerates growth rate [9-12]. While the extraction processes, composition, structure, and immunological activities of *Allium mongolicum* flavonoids have been elucidated, their effects on performance and expression of intestinal immune factors  $\alpha$ -defensin-1 (sBD-1) and  $\alpha$ -defensin-

2 (sBD-2) genes in confined meat sheep remain poorly documented. Therefore, this experiment investigated the effects of different dietary levels of *Allium mongolicum* flavonoids on meat sheep performance and intestinal immune factor -defensin (sBD-1, sBD-2) gene expression to provide a theoretical basis for further application of *Allium mongolicum* flavonoids in meat sheep production.

---

## Materials and Methods

### 1.1 Experimental Materials

The following equipment was used: Elx800 multi-functional microplate reader (BioTek H4, USA), fluorescence quantitative PCR instrument (Bio-Rad, USA), gradient PCR instrument (Veriti Thermal Cycler 4375786, USA), autoclave (Panasonic MLS-375, Japan), electric blast drying oven (CiMo DHG-9073BS-III, Shanghai Xinmiao Medical Instrument Manufacturing Co., Ltd.), -20°C refrigerator (DW-40W255, Qingdao Haier Co., Ltd.), -80°C ultra-low temperature freezer (Thermo Forma-8600, USA), pipettes (Eppendorf Research Plus, Germany), low-temperature high-speed centrifuge (Sigma 3K30, Germany), mini centrifuge (Jiangsu Haimen Qilinbeier Instrument Manufacturing Co., Ltd.), and liquid nitrogen tank (YDS-10B, East Asia Mechanical and Electrical Industry and Trade Co., Ltd.).

### 1.2 Experimental Design

Sixty healthy, six-month-old thin-tailed Han wethers with similar body weight [(39.9±3.2) kg] were randomly divided into four groups (n=15) using a single-factor completely randomized design. The control group received a basal diet, while experimental groups received the basal diet supplemented with 11 (low-dose flavonoid group), 22 (medium-dose flavonoid group), and 33 mg/kg *Allium mongolicum* flavonoids (high-dose flavonoid group), respectively. The composition and nutrient levels of the basal diet are presented in Table 1.

**Table 1** Composition and nutrient levels of the basal diet (air-dry basis)

| Items               | Content |
|---------------------|---------|
| <b>Ingredients</b>  |         |
| Chinese wildrye     |         |
| Alfalfa             |         |
| Corn                |         |
| Wheat bran          |         |
| Sunflower seed meal |         |
| Pea stalk           |         |
| Pomace              |         |
| CaHPO               |         |
| NaCl                |         |

| Items                  | Content |
|------------------------|---------|
| Premix                 |         |
| <b>Total</b>           |         |
| <b>Nutrient levels</b> |         |
| ME/(MJ/kg)             |         |
| CP                     |         |
| NDF                    |         |
| ADF                    |         |

Premix provided per kilogram of diet: Fe (as ferrous sulfate) 25 mg, Zn (as zinc sulfate) 29 mg, Cu (as copper sulfate) 8 mg, Mn (as manganese sulfate) 30 mg, I (as potassium iodide) 0.04 mg, Co (as cobaltous sulfate) 0.1 mg, VA 3,200 IU, VD 1,200 IU, VE 20 IU.

### 1.3 Preparation of *Allium mongolicum* Flavonoids

*Allium mongolicum* was collected from a greenhouse in Alxa Left Banner, Alxa League, Inner Mongolia Autonomous Region. The flavonoids used in this study were prepared in our laboratory using the method reported by Saruli et al. [13].

### 1.4 Feeding Management

The sheep pen was uniformly disinfected one week before the trial. The experimental period lasted 75 days, including a 15-day pre-feeding period and a 60-day formal experimental period. Sheep were group-housed with free access to water. During the formal period, feeding occurred twice daily at 07:00 and 18:00, with each feeding lasting 1.5 hours. Residual feed was collected at 08:30 and 19:30 daily. All groups were maintained under consistent feeding, management, and environmental conditions throughout the trial.

### 1.5 Performance Measurement

During the formal experimental period, residual feed was weighed and recorded daily to calculate actual daily feed intake and average daily feed intake. Body weight was measured before morning feeding on days 1, 15, 30, 45, and 60. Feed-to-gain ratio and average daily gain were calculated based on feed intake and body weight changes using the following formulas:

$$\text{Average daily gain} = \text{Total weight gain (g)} / \text{Experimental days (d)}$$

$$\text{Feed-to-gain ratio} = \text{Average daily feed intake (g)} / \text{Average daily gain (g)}$$

### 1.6 Intestinal Tissue Sampling and Determination of sBD-1 and sBD-2 Gene Expression

On day 60, three sheep from each group were randomly selected and slaughtered after passing quarantine inspection. Duodenal, jejunal, and ileal tissues

were rapidly collected, wrapped in pre-prepared gauze, immediately frozen in liquid nitrogen, and subsequently stored at -80°C until analysis. Total RNA was extracted from samples, and its purity and integrity were assessed using spectrophotometry and gel electrophoresis. cDNA synthesis was performed according to the kit instructions (RR047A, TaKaRa). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the reference gene. Primers were designed and synthesized by BGI Genomics. The amplification protocol consisted of: step 1, 95°C for 30 s (1 cycle); step 2, 95°C for 5 s, 60°C for 30 s (40 cycles); step 3, 95°C for 15 s, 60°C for 30 s, 95°C for 30 s (1 cycle). Primer sequences and parameters are listed in Table 2.

**Table 2** Primer sequences and parameters

| Genes        | GeneBank accession number | Primer sequences (5' -3' )                                    | Length/bp |
|--------------|---------------------------|---|-----------|
| <b>GAPDH</b> | KY041634.1                | F: GGTCCGAGT-GAACGGATTTGR:<br>TGGCAACGATGTC-CACTTTG           |           |
| <b>sBD-1</b> | U75250                    | F: GTCTAAGCTGC-CATAGGAATAAAGCR:<br>ACTTCTTTCT-GCAGCATTTTACTGG |           |
| <b>sBD-2</b> | NM_001198545              | F: ACCTGCTCCTCGT-GCTCTTCR:<br>GCACACAGATGC-CTTTCTTCC          |           |

*F* = forward primer, *R* = reverse primer.

### 1.7 Statistical Analysis

Data were organized using Excel 2007 software. One-way ANOVA was performed using SAS 9.0 software. Results are expressed as “mean ± standard error.” Differences were considered significant at  $P < 0.05$ .

## Results

### 2.1 Effects of *Allium mongolicum* Flavonoids on Average Daily Feed Intake

As shown in Table 3, no significant differences in average daily feed intake were observed among groups during days 1-15 ( $P > 0.05$ ). During days 15-30, the high-dose flavonoid group exhibited the highest average daily feed intake,

significantly greater than the control and other experimental groups ( $P < 0.05$ ), while the low-dose group showed significantly lower intake than the control and other groups ( $P < 0.05$ ). During days 30–45 and 45–60, all experimental groups demonstrated significantly higher average daily feed intake compared to the control group ( $P < 0.05$ ), with the highest values observed in the high-dose flavonoid group.

**Table 3** Effects of *Allium mongolicum* Regel flavonoids on average daily feed intake of meat sheep

| Time       | Supplemental level of <i>Allium mongolicum</i> Regel flavonoids/(mg/kg) | P-value   |
|------------|---|-----------|
|            | 0   | 11        |
| Days 1-15  | 1,149±50  | 1,151±43  |
| Days 15-30 | 1,148±42  | 1,107±45  |
| Days 30-45 | 1,244±87  | 1,308±146 |
| Days 45-60 | 1,409±103   | 1,563±53  |

*In the same row, values with no letter or the same letter superscripts indicate no significant difference ( $P > 0.05$ ), while different letters indicate significant difference ( $P < 0.05$ ). The same applies below.*

## 2.2 Effects of *Allium mongolicum* Flavonoids on Average Daily Gain

Table 4 shows that initial body weight was similar across groups ( $P > 0.05$ ), with differences not exceeding 1.06%. During days 1–15, no significant differences in average daily gain were observed between the control and experimental groups ( $P > 0.05$ ). During days 15–30, all experimental groups exhibited significantly higher average daily gain than the control group ( $P < 0.05$ ), with the medium-dose group showing numerically higher values than other experimental groups, though not significantly different ( $P > 0.05$ ). During days 30–45, dietary flavonoid supplementation increased average daily gain compared to the control, with the high-dose group showing significantly higher gain ( $P < 0.05$ ) and the medium- and low-dose groups showing a trend toward improvement without significant differences ( $P > 0.05$ ). During days 45–60, all experimental groups demonstrated significantly higher average daily gain than the control group ( $P < 0.05$ ), with the medium-dose group achieving the highest values.

**Table 4** Effects of *Allium mongolicum* Regel flavonoids on average daily gain of meat sheep

| Items                    | Supplemental level of <i>Allium mongolicum</i> Regel flavonoids/(mg/kg) | P-value      |
|--------------------------|---|--------------|
|                          | 0   | 11           |
| Initial body weight      | 42.37±3.25  | 42.48±4.39   |
| Average daily gain (g/d) |   |              |
| Days 1-15                | 150.67±56.77  | 126.22±52.69 |
| Days 15-30               | 194.22±82.37  | 226.67±65.37 |
| Days 30-45               | 218.67±169.43   | 259.56±50.92 |
| Days 45-60               | 226.22±67.48  | 311.56±60.56 |

### 2.3 Effects of *Allium mongolicum* Flavonoids on Feed-to-Gain Ratio

As presented in Table 5, no significant differences in feed-to-gain ratio were observed among groups during days 1-15 ( $P>0.05$ ). During days 15-30, all experimental groups showed significantly lower feed-to-gain ratios compared to the control group ( $P<0.05$ ), with the high-dose group exhibiting significantly higher ratios than the low- and medium-dose groups ( $P<0.05$ ). During days 30-45, the control group displayed the highest feed-to-gain ratio, significantly greater than all experimental groups ( $P<0.05$ ), with the medium-dose group showing significantly lower values than the low- and high-dose groups ( $P<0.05$ ). During days 45-60, all experimental groups demonstrated significantly lower feed-to-gain ratios than the control group ( $P<0.05$ ), with the high-dose group showing significantly lower than the low-dose group ( $P<0.05$ ) and showing a trend toward lower values than the medium-dose group without significant difference ( $P>0.05$ ).

**Table 5** Effects of *Allium mongolicum* Regel flavonoids on feed-to-gain ratio of meat sheep

| Time | Supplemental level of <i>Allium mongolicum</i> Regel flavonoids/(mg/kg) | P-value |
|------|---|---------|
|      | 0   | 11      |

| Time       | Supplemental level of <i>Allium mongolicum</i> Regel flavonoids/(mg/kg) | P-value   |
|------------|---|-----------|
| Days 1-15  | 8.05±0.70   | 8.39±0.33 |
| Days 15-30 | 6.22±0.47   | 5.01±0.17 |
| Days 30-45 | 5.91±0.22   | 4.88±0.20 |
| Days 45-60 | 5.68±0.41   | 5.04±0.58 |

#### 2.4 Effects of *Allium mongolicum* Flavonoids on Relative Expression Levels of sBD-1 and sBD-2 Genes in Different Intestinal Tissues

Table 6 reveals that the high-dose flavonoid group exhibited significantly higher relative expression of sBD-1 gene in jejunum and ileum compared to the control and other experimental groups ( $P < 0.05$ ), while no significant difference was observed in duodenum ( $P > 0.05$ ). The medium-dose group (jejunum, ileum, duodenum) and low-dose group (duodenum, ileum) showed significantly lower sBD-1 expression than the control group ( $P < 0.05$ ), whereas the low-dose group in jejunum showed no significant difference from the control ( $P > 0.05$ ).

Regarding sBD-2 expression, the medium-dose group demonstrated significantly increased relative expression in duodenum compared to the control ( $P < 0.05$ ). The low-dose group showed numerically higher expression in duodenum than the control without significant difference ( $P > 0.05$ ), while the high-dose group exhibited significantly lower expression ( $P < 0.05$ ). In jejunum, both high- and medium-dose groups showed significantly higher sBD-2 expression than the control ( $P < 0.05$ ), with the high-dose group significantly exceeding the medium-dose group ( $P < 0.05$ ). No significant differences were observed in sBD-2 expression in ileum among all groups ( $P > 0.05$ ).

**Table 6** Effects of *Allium mongolicum* Regel flavonoids on relative expression levels of sBD-1 and sBD-2 genes in different intestinal tissues of meat sheep

| Genes | Supplemental level of <i>Allium mongolicum</i> Regel flavonoids/(mg/kg) | P-value   |
|-------|---|-----------|
| sBD-1 | 0   | 11        |
|       | Duodenum 1.00±0.12  | 0.83±0.19 |
|       | Jejunum 1.00±0.03   | 1.12±0.02 |

---

| Genes        | Supplemental level of <i>Allium mongolicum</i> Regel flavonoids/(mg/kg) | P-value   |
|--------------|---|-----------|
| Ileum        | 1.00±0.02   | 0.89±0.01 |
| <b>sBD-2</b> |   |           |
| Duodenum     | 1.00±0.03   | 1.02±0.02 |
| Jejunum      | 1.00±0.09   | 1.02±0.01 |
| Ileum        | 1.00±0.08   | 0.84±0.15 |

---

## Discussion

### 3.1 Effects of *Allium mongolicum* Flavonoids on Meat Sheep Performance

Feed cost is a critical factor determining economic efficiency in animal production. Enhancing daily weight gain while reducing feed-to-gain ratio represents a major challenge in animal husbandry. Our results indicate that dietary supplementation with *Allium mongolicum* flavonoids showed no significant effects on average daily gain during days 1-15. However, growth-promoting effects became evident after day 15, reaching optimal efficacy during days 45-60 when all experimental groups exhibited significantly increased average daily gain compared to the control. This suggests that the growth-promoting effects of *Allium mongolicum* flavonoids require a certain period to manifest, demonstrating time-dependent characteristics. The findings also indicate that supplementation at 22-33 mg/kg yielded higher average daily gain, suggesting that this dosage range significantly promotes meat sheep performance. Additionally, the lowest feed-to-gain ratio during days 45-60 at 22-33 mg/kg supplementation indicates maximized feed efficiency.

### 3.2 Effects of *Allium mongolicum* Flavonoids on Intestinal Immunity in Meat Sheep

The intestinal barrier constitutes a crucial component of the innate immune system and serves as the first defense against foreign antigens and toxins [14]. To resist microbial invasion, lymphocytes migrate to epithelial surfaces while intestinal cells produce immunoglobulins and cytokines that protect and repair the intestinal barrier [15]. Research demonstrates that intestinal cells generate endogenous immune defense factors with broad antimicrobial activity against bacteria, fungi, and viruses, while also regulating immune function by chemoattracting and activating immune cells [16]. Therefore, this study examined duodenal, jejunal, and ileal tissues to investigate flavonoid effects on intestinal immune factor (sBD-1, sBD-2) gene expression.

Flavonoids are primary active compounds in *Allium mongolicum* that play es-

sential roles in immune responses. Previous mouse studies showed that dietary bamboo leaf flavonoids at 0–80 g/mL promoted IFN- $\gamma$  gene expression in splenocytes and enhanced T and B lymphocyte proliferation and differentiation, significantly improving immune function [17]. Yin et al. [18] reported that daidzein and genistein improved immune function in mouse models of endometrial cancer by regulating TNF- $\alpha$ , IL-1, and IL-6 gene expression. Studies have also demonstrated that 11–33 mg/kg *Allium mongolicum* flavonoid supplementation in meat sheep upregulated IL-1, IL-4, IL-6, and TNF- $\alpha$  gene expression [11]. Our findings of significantly enhanced sBD-1 expression in jejunum and ileum and sBD-2 expression in jejunum and duodenum align with these previous results. As endogenous small-molecule antimicrobial peptides produced by intestinal cells,  $\alpha$ -defensins play important antibacterial and immunomodulatory roles. *Allium mongolicum* flavonoids enhance non-specific immune function and promote growth by upregulating  $\alpha$ -defensin gene expression, though the underlying mechanisms require further investigation.

*Allium mongolicum*, as a nutrient-rich forage, plays an important role in maintaining optimal immune status by regulating immune function at both organ and molecular levels through gene transcription and protein translation [19]. Animal immune function is influenced by nutritional status, environment, endocrine system, and stress factors. Dietary levels of protein, fat, vitamins, and other nutrients critically affect immune function; nutrient deficiencies can predispose animals to disease or pathogen invasion [20]. The immune system can also regulate feed intake and behavior through the central nervous system, thereby affecting growth and nutrient metabolism. These factors do not act independently but rather interact synergistically—average daily feed intake influences average daily gain, and both collectively affect feed-to-gain ratio. Immune status directly impacts animal health and consequently affects performance. Our results demonstrate that 22–33 mg/kg dietary supplementation with *Allium mongolicum* flavonoids significantly enhanced immune factor  $\alpha$ -defensin (sBD-1 and sBD-2) gene expression, effectively improved immune function in ileum, jejunum, and duodenum, and consequently increased feed intake and daily gain while reducing feed-to-gain ratio.

Current large-scale, high-density, intensive production systems and genetic selection for maximum performance have made animals increasingly sensitive to environmental conditions, where minor changes can trigger stress responses. Poor sanitary conditions directly impair performance, causing growth retardation regardless of clinical symptom manifestation. Immune factors affect performance by altering nutrient metabolism and distribution through neuro-endocrine and immune systems [21]. Therefore, nutritional modulation to mitigate or eliminate the impact of immune stress represents a promising research direction, though specific mechanisms warrant further investigation.

## Conclusion

1. Dietary supplementation with 11-33 mg/kg *Allium mongolicum* flavonoids significantly improved meat sheep performance by increasing average daily feed intake and average daily gain while decreasing feed-to-gain ratio.
  2. Supplementation with 22-33 mg/kg *Allium mongolicum* flavonoids significantly enhanced intestinal immune factor  $\alpha$ -defensin (sBD-1, sBD-2) gene expression in meat sheep.
  3. In summary, dietary inclusion of 22-33 mg/kg *Allium mongolicum* flavonoids improves meat sheep performance and enhances immune function in duodenum, jejunum, and ileum.
- 

## References

- [1] Zhao CY, Ao CJ, Zhang XF. Effects of flavonoids from *Allium mongolicum* on non-specific immune function in mice [J]. *Feed Industry*, 2010, 31(18): 11-14.
- [2] Miao YJ. Determination of isoflavone content in *Allium mongolicum* and its effects on antioxidant capacity and non-specific immunity [D]. Master's thesis. Hohhot: Inner Mongolia Agricultural University, 2009: 38-51.
- [3] Saruli. Optimization of flavonoid extraction process from *Allium mongolicum*, structural identification, and related biological activities [D]. Doctoral dissertation. Hohhot: Inner Mongolia Agricultural University, 2014: 18-26.
- [4] Zhang GL, Ross CR, Blecha F. Porcine antimicrobial peptides: new prospects for ancient molecules of host defense [J]. *Veterinary Research*, 2000, 31(3): 277-296.
- [5] Taylor K, Barran P, Dorin J. Review: structure-activity relationships in beta-defensin peptides [J]. *Biopolymers*, 2008, 90(1): 1-7.
- [6] Defensins: antimicrobial peptides innate immunity. *Nat. Rev. Immunol.* 3, 710-720 [J]. *Nature Reviews Immunology*, 2003, 3(9): 710-710.
- [7] Röhr J, Geissler EK, Hehlhans T. Friend or foe: a novel role of  $\alpha$ -defensins in tumor development [J]. *Oncimmunology*, 2012, 1(7): 1159-1160.
- [8] Ma WM, She RP, Peng FZ, et al. Extraction and partial biological activity study of antimicrobial peptides from porcine small intestine [J]. *Science Technology and Engineering*, 2004, 4(3): 202-205.
- [9] Mu Qier, Ao CJ, Saruli, et al. Effects of total flavonoids from *Allium mongolicum* on antioxidant capacity in meat sheep [J]. *Chinese Journal of Animal Nutrition*, 2016, 28(6): 1823-1831.

- [10] Chen RW. Effects of *Allium mongolicum* flavonoids on performance and meat quality in meat sheep [D]. Master' s thesis. Hohhot: Inner Mongolia Agricultural University, 2016: 19-22.
- [11] Mu Qier. Effects and mechanisms of *Allium mongolicum* flavonoids on antioxidant capacity and immune function in meat sheep [D]. Doctoral dissertation. Hohhot: Inner Mongolia Agricultural University, 2016: 32-38.
- [12] Saruli, Mu Qier, Wang CF, et al. Effects of *Allium mongolicum* flavonoids on peripheral blood lymphocyte proliferation and IL-2, IL-4, IFN- mRNA expression in sheep [J]. *Acta Veterinaria et Zootechnica Sinica*, 2015, 46(6): 1063-1070.
- [13] Saruli, Mu Qier, Wang CF, et al. Optimization of extraction process and in vitro antioxidant and antibacterial activities of total flavonoids from *Allium mongolicum* [J]. *Food Science*, 2014, 35(24): 1-8.
- [14] Wells JM, Loonen LM, Karczewski JM. The role of innate signaling in the homeostasis of tolerance and immunity in the intestine [J]. *International Journal of Medical Microbiology*, 2010, 300(1): 41-48.
- [15] Pomorska-Móí M, Markowska-Daniel I. Porcine cathelicidins defensins [J]. *Medycyna Weterynaryjna*, 2011, 67(1): 20-24.
- [16] Gallo RL, Kim KJ, Bernfield M, et al. Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse [J]. *Journal of Biological Chemistry*, 1997, 272(20): 13088-13093.
- [17] Tang HG, Wei XX, Li Y, et al. Molecular mechanism of bamboo leaf flavonoids on mouse splenocyte immunity [J]. *Food Science*, 2007, 28(9): 523-526.
- [18] Yin XH, Cheng Y, Cui SF. Effects of daidzein and genistein on TNF- , IL-1 and IL-6 expression in mouse models of endometrial cancer [J]. *China Journal of Modern Medicine*, 2010, 20(6): 832-835.
- [19] Gershwin ME, German JB, Keen CL. *Nutrition and Immunology: Principles and Practice* [M]. New York: Humana Press, 2000.
- [20] Babu US, Raybourne RB. Impact of dietary components on chicken immune system and Salmonella infection [J]. *Expert Review of Anti-Infective Therapy*, 2008, 6(1): 121-35.
- [21] Yang H, Zhang HJ, Wu XM, et al. Effects of microecological preparations on growth performance and immune function in meat sheep [J]. *Journal of Domestic Animal Ecology*, 2015, 36(10): 27-32.

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

*Source: ChinaXiv –Machine translation. Verify with original.*