

## Effects of Dietary Lycopene Supplementation on Growth and Development, Slaughter Performance, and Serum Antioxidant Indices in Sheep (Postprint)

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### Abstract

This experiment aimed to investigate the effects of dietary lycopene supplementation on growth and development, slaughter performance, and serum antioxidant indices in sheep. Thirty 3-month-old Hu ram lambs with a body weight of  $(22.0 \pm 0.5)$  kg were selected and randomly divided into 3 groups with 10 lambs per group, and raised in individual pens. 1) dietary supplementation of 200 and 400 mg/d lycopene tended to increase the averaged daily gain of sheep ( $0.05 < P < 0.10$ ), and significantly reduced the feed conversion ratio ( $P < 0.05$ ); 2) dietary supplementation of 200 and 400 mg/d lycopene tended to increase body length, body height, and chest girth ( $0.05 < P < 0.10$ ); 3) dietary supplementation of 200 and 400 mg/d lycopene tended to increase pre-slaughter liveweight, back muscle weight, kidney weight, and GR value ( $0.05 < P < 0.10$ ), and supplementation of 400 mg/d lycopene significantly increased skin weight ( $P < 0.05$ ), while other slaughter performance indices showed no significant differences among groups ( $P > 0.05$ ); 4) dietary supplementation of 200 and 400 mg/d lycopene tended to increase serum superoxide dismutase activity and supplementation of 200 mg/d lycopene significantly increased serum catalase and glutathione peroxidase activities ( $P < 0.05$ ). The results indicated that dietary lycopene supplementation had certain improving effects on growth and development, slaughter performance, and serum antioxidant capacity in sheep, and under the conditions of this experiment, the dosage of 200 mg/d showed superior effects.

## Full Text

# Effects of Lycopene Supplementation on Growth Development, Slaughter Performance and Serum Antioxidant Indices of Sheep

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## Abstract

This experiment was conducted to investigate the effects of dietary lycopene supplementation on growth development, slaughter performance, and serum antioxidant indices in sheep. Thirty male 3-month-old Hu lambs with an initial body weight of  $(22.0 \pm 0.5)$  kg were randomly allocated into three groups of ten animals each and housed individually. The 30-day adaptation period followed by a 100-day data collection period. The results showed that: (1) supplementation with 200 and 400 mg/d lycopene tended to increase averaged daily gain ( $0.05 P < 0.10$ ) and significantly decreased feed-to-gain ratio ( $P < 0.05$ ); (2) supplementation with 200 and 400 mg/d lycopene significantly increased body length, withers height, and chest circumference ( $0.05 P < 0.10$ ); (3) supplementation with 200 and 400 mg/d lycopene tended to increase pre-slaughter liveweight, Longissimus dorsi muscle weight, kidney weight, and GR value ( $0.05 P < 0.10$ ), and 400 mg/d lycopene significantly increased leather weight ( $P < 0.05$ ), though other slaughter performance parameters showed no significant differences among groups ( $P > 0.05$ ); (4) supplementation with 200 and 400 mg/d lycopene tended to increase serum superoxide dismutase activity while 200 mg/d lycopene significantly increased serum catalase and glutathione peroxidase activities ( $P < 0.05$ ). These findings indicate that dietary lycopene supplementation can improve growth development, slaughter performance, and serum antioxidant capacity in sheep, with 200 mg/d being the optimal dosage under the conditions of this study.

**Keywords:** lycopene; sheep; growth development; slaughter performance; serum antioxidant indices

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Lycopene (LP), also known as  $\beta$ -carotene, is a natural pigment found in plants and belongs to the carotenoid family, with the molecular formula  $C_{40}H_{56}$  and a relative molecular mass of 536.85. Its structure contains 11 conjugated double bonds and 2 non-conjugated double bonds [1]. Lycopene is widely present in fruits and vegetables and was named after its initial isolation from tomatoes [2], with content varying among tomato cultivars and reaching up to 89 mg/kg in some varieties [3]. Its primary biological function is potent antioxidant activity; studies have demonstrated that lycopene can directly scavenge reactive oxygen species (ROS) generated during cellular metabolism, with singlet oxygen quenching capacity twice that of  $\beta$ -carotene and ten times that of vitamin E [4].

Additionally, lycopene can stimulate gap junctional intercellular communication, facilitate signal transduction between cells, and influence cell proliferation and differentiation [5-6]; it also enhances immune function, improves overall health status, and helps prevent various diseases including cancer [7-9]. The Joint FAO/WHO Expert Committee on Food Additives (JECFA), along with the Food and Agriculture Organization (FAO) and World Health Organization (WHO), has recognized lycopene as a Class A nutrient, and it is currently being researched and utilized internationally as an important component in functional foods, pharmaceuticals, and cosmetics [10].

China is the world's largest tomato producer, with an annual output exceeding 50 million tons, accounting for approximately 30% of global production [11], thus providing abundant sources of lycopene. Current research on lycopene efficacy has primarily focused on humans and rats [12-14], with limited application reports in animal nutrition, and studies on its supplementation in sheep diets are particularly scarce. Our research team pioneered the investigation of lycopene supplementation in sheep diets, with preliminary results indicating that dietary lycopene improved production performance and meat quality in sheep under summer heat stress conditions [15], likely due to its antioxidant properties effectively mitigating heat stress effects. However, whether lycopene can promote sheep growth and development under optimal environmental conditions remains unreported. Therefore, this study further investigated the effects of dietary lycopene at different supplementation levels on growth development, slaughter performance, and serum antioxidant indices in sheep under normal environmental conditions, providing a scientific basis for lycopene application in animal husbandry.

### 1.1 Experimental Location and Materials

The experiment was conducted from December 2014 to March 2015 at the Runlin Animal Husbandry Farm in Linqing City, Shandong Province. Throughout the trial period, ambient temperature in the sheep barn ranged from -4 to 15 °C, which represents a suitable environmental temperature for sheep [16]. All feed ingredients were provided by the farm. Lycopene was purchased from Shaanxi Sciphar Biotechnology Co., Ltd. as a red powder that passed quality inspection.

### 1.2 Experimental Design

Thirty male 3-month-old weaned Hu lambs with consistent genetic background and an average body weight of  $(22.0 \pm 0.5)$  kg were randomly assigned to three groups of ten animals each using a completely randomized design, with individual pen housing. The control group (Group I) received the basal diet, while the experimental groups (Groups II and III) received the basal diet supplemented with 200 mg/d and 400 mg/d lycopene, respectively. The trial lasted 110 days, comprising a 10-day adaptation period and a 100-day experimental period. The composition and nutrient levels of the basal diet are presented in Table 1 .

### 1.3 Feeding Management

During the experimental period, the barn was well-ventilated, cleaned daily, and disinfected regularly. All experimental sheep had free access to water. The basal diet was fed in two equal portions at 08:00 and 16:00 daily, with roughage offered before concentrate. Lycopene was removed from 4 °C storage each morning before feeding, weighed according to supplementation level, and thoroughly mixed with the concentrate to ensure complete consumption. Roughage refusal was maintained at no more than 10%.

#### 1.4.1 Growth Development Indices

During the experimental period, daily feed offered and refusals were recorded accurately to calculate average daily dry matter intake (ADDMI). At the end of the trial, all animals were weighed before morning feeding after overnight fasting to record final body weight, which was used to calculate average daily gain (ADG) and feed-to-gain ratio (F/G). The formulas were as follows:

ADDMI = Total dry matter intake during experimental period / Number of experimental days

ADG = (Final body weight - Initial body weight) / Number of experimental days

F/G = ADDMI / ADG

Following the trial, body measurements were taken for all sheep using a measuring stick and tape, including body length, withers height, chest circumference, and chest width, following the Technical Specification for Performance Testing of Sheep and Goats (NY/T 1236–2006) [17]. Body length index, chest circumference index, and body trunk index were then calculated. Specific measurement methods and formulas were:

- **Withers height:** Vertical distance from the highest point of the scapula to the ground when the sheep stood upright on a firm, level surface, measured with a measuring stick.
- **Body length:** Straight-line distance from the chest prominence to the ischial tuberosity when the sheep stood upright on a firm, level surface, measured with a measuring stick.
- **Chest circumference:** Circumference around the chest from behind the scapula when the sheep stood upright on a firm, level surface, measured with a measuring tape.
- **Chest width:** Straight-line distance between the left and right sides at the widest point of the scapula when the sheep stood upright on a firm, level surface, measured with a measuring stick.

Body length index (%) =  $100 \times \text{Body length} / \text{Withers height}$

Chest circumference index (%) =  $100 \times \text{Chest circumference} / \text{Withers height}$

Body trunk index (%) =  $100 \times \text{Chest circumference} / \text{Body length}$

### 1.4.2 Slaughter Performance Indices

All animals were slaughtered according to halal methods at the end of the trial. Sheep were fasted for 12 hours and deprived of water for 2 hours before slaughter. After recording pre-slaughter live weight, sheep were slaughtered, fully bled, skinned, and eviscerated to determine slaughter performance indices including carcass weight, dressing percentage, meat weight, bone weight, meat percentage, carcass meat percentage, meat-to-bone ratio, loin muscle area, GR value, and fat weight from various depots. Measurement methods and formulas were:

- **Carcass weight:** Weight after slaughter and bleeding, with head, feet, skin, and internal organs removed, but kidneys and kidney fat retained.
- **Dressing percentage (%)** =  $100 \times (\text{Carcass weight} + \text{Internal fat weight}) / \text{Pre-slaughter live weight}$
- **Meat weight** = Carcass weight - Bone weight
- **Meat percentage (%)** =  $100 \times \text{Meat weight} / \text{Pre-slaughter live weight}$
- **Carcass meat percentage (%)** =  $100 \times \text{Meat weight} / \text{Carcass weight}$
- **Meat-to-bone ratio** = Meat weight / Bone weight
- **Loin muscle area:** The carcass was cross-sectioned posterior to the 12th rib, the loin muscle cross-section outline was traced on tracing paper, and the area was calculated using a planimeter.
- **GR value:** The carcass was cross-sectioned posterior to the 12th rib, and tissue thickness was measured at a point 11 cm from the dorsal midline between the 12th and 13th ribs using vernier calipers.

### 1.4.3 Serum Antioxidant Indices

Blood samples (10 mL) were collected from the jugular vein of all experimental sheep before slaughter and immediately centrifuged at 3,000 r/min for 10 minutes to obtain serum, which was stored at -20 °C. Serum antioxidant indices were measured using commercial kits, including total antioxidant capacity (T-AOC), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA). Kits were purchased from Nanjing Jiancheng Bioengineering Institute.

## 1.5 Statistical Analysis

Experimental data were initially processed using Excel 2007 and analyzed using SPSS 19.0 software via one-way ANOVA. Duncan's multiple range test was used for post-hoc comparisons. Data are expressed as mean  $\pm$  standard error, with  $P < 0.05$  considered statistically significant and  $0.05 \leq P < 0.10$  considered a significant trend.

### 2.1.1 Growth Performance

As shown in Table 2, compared with the control group, dietary supplementation with 200 and 400 mg/d lycopene tended to increase final body weight ( $P = 0.065$ )

and ADG during the experimental period ( $P=0.061$ ). No significant differences were observed in ADDMI among groups ( $P>0.05$ ). The F/G in Groups II and III was significantly lower than in Group I ( $P<0.05$ ), with Group II also lower than Group III, though not significantly ( $P>0.05$ ). Overall, dietary lycopene supplementation improved growth performance in sheep, with 200 mg/d being the more effective dosage.

### 2.1.2 Body Size Development

As shown in Table 3, compared with the control group, dietary supplementation with 200 and 400 mg/d lycopene significantly increased chest width ( $P<0.05$ ). Supplementation with 200 mg/d lycopene tended to increase body length ( $P=0.057$ ), withers height ( $P=0.072$ ), and chest circumference ( $P=0.069$ ). No significant differences were observed among groups in body length index, body trunk index, or chest circumference index ( $P>0.05$ ). Overall, dietary lycopene supplementation improved body size development indices in sheep, with 200 mg/d being the more effective dosage.

### 2.2 Effects of Lycopene on Slaughter Performance of Sheep

As shown in Table 4, compared with the control group, dietary supplementation with 200 and 400 mg/d lycopene tended to increase pre-slaughter live weight ( $P=0.062$ ), Longissimus dorsi muscle weight ( $P=0.072$ ), GR value ( $P=0.062$ ), and kidney weight ( $P=0.054$ ). Carcass weight and meat weight in Groups II and III were numerically higher than in the control group, though differences were not significant ( $P>0.05$ ). Leather weight in Group III was significantly higher than in Group I ( $P<0.05$ ). Except for leather weight, no significant differences were observed among groups in dressing percentage, meat percentage, loin muscle area, or fat deposition ( $P>0.05$ ). Overall, dietary lycopene supplementation had minor effects on slaughter performance in sheep, though these effects were not pronounced.

### 2.3 Effects of Lycopene on Serum Antioxidant Indices of Sheep

As shown in Table 5, compared with the control group, dietary supplementation with 200 and 400 mg/d lycopene tended to increase serum SOD activity ( $P=0.081$ ). Serum CAT and GSH-Px activities in Group II were significantly higher than in the control group ( $P<0.05$ ). No significant differences were observed among groups in serum T-AOC or MDA content ( $P>0.05$ ). Overall, dietary lycopene supplementation improved serum antioxidant capacity in sheep, with 200 mg/d being the more effective dosage.

### 3.1 Effects of Lycopene on Growth Development of Sheep

In recent years, many natural plant extracts have been widely developed and applied as feed additives in livestock and poultry diets due to their abundant

resources and high safety profiles. Lycopene, as a functional nutrient, has attracted increasing attention, and some researchers have begun investigating its effects in animal production. Sahin et al. [18] reported that under heat stress conditions, dietary supplementation with 50–200 mg/kg lycopene significantly improved feed intake and weight gain in quail while reducing F/G. Subsequently, the same researchers observed similar improvements in growth performance in rainbow trout, with 400 mg/kg being the optimal dosage [19]. Currently, few studies have reported on lycopene use in mutton sheep production. Wu Anquan et al. [20] supplemented sheep diets with high, medium, and low levels of tomato pomace (containing approximately 1.3% lycopene) to replace alfalfa hay, and found that sheep feed intake was significantly higher than in the control group, with ADG showing nearly a 10% increase compared to the control group, though not reaching statistical significance [15]. Collectively, previous research indicates that lycopene supplementation can improve animal feed intake. However, the present study did not observe changes in sheep feed intake following lycopene supplementation, possibly due to differences in experimental conditions. In most previous studies, animals were under various stress conditions that affected feeding behavior, and lycopene's antioxidant properties alleviated these negative stress effects, thereby restoring normal feeding behavior. In contrast, this experiment was conducted under optimal temperature conditions where animals already exhibited high feeding motivation, thus no significant differences were observed. The current results showed that dietary lycopene supplementation tended to increase ADG and significantly decreased F/G in sheep, which aligns with previous findings [21]. This may be attributed to lycopene's regulatory effects on gap junctional intercellular communication, thereby maintaining homeostasis and promoting animal growth [22]. Overall, lycopene supplementation improved growth performance in sheep, with 200 mg/d being the optimal dosage under this experimental condition.

Body measurements are important indicators reflecting whether animals have developed completely both overall and in specific parts. In sheep production, commonly monitored indices include body length, withers height, chest circumference, and chest width. Research has shown that 6-month-old twin male Hu lambs can reach 71.7 cm in body length, 62.0 cm in withers height, and 68.2 cm in chest circumference [23]. According to relevant industry standards [17], the body measurement indices determined in this experiment all fell within normal ranges, indicating adequate nutrient intake and good body condition. This study is the first to investigate the effects of lycopene on body measurement indices in sheep, finding that dietary lycopene supplementation significantly increased chest width and tended to increase chest circumference. Additionally, 200 mg/d lycopene tended to increase body length and withers height, demonstrating that lycopene supplementation promoted sheep development. Previous studies have reported significant positive correlations between sheep body weight and body measurement indices such as body length, withers height, and chest circumference [24–25]. Therefore, these results corroborate the positive effects of lycopene on sheep weight gain.

Body measurement indices represent quantitative relationships between different measurements, reflecting the relative development of various body regions and indicating animal conformation characteristics and potential production performance [26]. In this experiment, no significant differences were observed among groups in body length index, chest circumference index, or body trunk index, suggesting appropriate body proportions and good development.

### 3.2 Effects of Lycopene on Slaughter Performance of Sheep

Slaughter performance is a crucial evaluation index for meat-producing livestock and poultry. Currently, few reports exist on the effects of lycopene on slaughter performance in livestock. Sahin et al. [18] demonstrated that dietary lycopene supplementation increased carcass weight and meat yield in quail. In our team's previous study on Bamei mutton sheep, lycopene supplementation under heat stress conditions increased GR value but had no significant effects on other slaughter performance indices [15]. The present experiment, conducted under optimal temperature conditions, found that dietary lycopene supplementation tended to increase pre-slaughter live weight and similarly tended to increase GR value. Compared with the control group, Longissimus dorsi muscle weight in sheep supplemented with 200 and 400 mg/d lycopene increased by 11.35% and 17.77%, respectively. Although carcass weight and meat weight in the 200 mg/d group did not differ significantly from the control group, they increased by 6.2% and 6.9%, respectively, indicating that lycopene improved meat production performance. Furthermore, lycopene supplementation increased leather weight and kidney weight, suggesting it promotes animal growth and visceral organ development, possibly by enhancing disease resistance and environmental adaptability, though the specific mechanisms require further investigation. Overall, lycopene supplementation had minor effects on slaughter performance in sheep, though these effects were not pronounced.

### 3.3 Effects of Lycopene on Serum Antioxidant Indices of Sheep

The antioxidant enzyme system is one of the body's important defense systems against reactive oxygen species, capable of inhibiting free radical generation, balancing metabolism, and preventing various diseases. SOD, CAT, and GSH-Px are key enzymes in this system, and their activities reflect the strength of the body's antioxidant capacity. SOD and CAT work synergistically to form an antioxidant chain, first catalyzing superoxide anion radicals to hydrogen peroxide, then decomposing hydrogen peroxide to water. GSH-Px not only scavenges lipid peroxides but also reduces oxidation of polyunsaturated fatty acids in cell membranes, thereby protecting membrane structure and function integrity.

Lycopene possesses extremely strong reactive oxygen species quenching capacity due to its molecular structure containing 11 conjugated double bonds, while also exerting regulatory effects on the body's antioxidant enzyme system. Previous research demonstrated that lycopene supplementation significantly increased an-

tioxidant enzyme activities in rainbow trout liver under high stocking density conditions [19]. Another study found that dietary lycopene oil significantly increased SOD and CAT activities in crucian carp serum [21]. The present results showed that serum SOD activity in sheep tended to increase following lycopene supplementation, while CAT and GSH-Px activities in the 200 mg/d group were significantly higher than in the control group, which is consistent with our team's previous findings in Bamei mutton sheep [27]. The difference between these results lies in the generally lower numerical values of antioxidant enzyme activities in the current study, likely because the previous research was conducted under high temperature conditions where heat stress generated more reactive oxygen species and consequently elevated antioxidant enzyme activities, whereas this experiment was performed under more optimal environmental conditions resulting in relatively lower antioxidant enzyme activity values.

T-AOC is a comprehensive indicator of antioxidant capacity, representing the overall level of both enzymatic and non-enzymatic antioxidant systems. MDA is a final product of lipid peroxidation, and its content directly reflects free radical levels and the degree of lipid peroxidation in the body. In this experiment, lycopene supplementation did not significantly affect either parameter, possibly because fewer reactive oxygen species were produced under optimal conditions, metabolism remained normal, and lipid peroxidation was relatively low, resulting in minimal differences. However, comprehensive comparison indicated that 200 mg/d was the optimal dosage.

Under the conditions of this experiment, dietary lycopene supplementation in sheep: (1) tended to increase ADG and significantly decreased F/G; (2) had no effect on body measurement indices but significantly increased chest width and tended to affect withers height, body length, and chest circumference; (3) tended to increase Longissimus dorsi muscle weight and GR value, though overall effects on slaughter performance were minor; and (4) improved serum antioxidant capacity, particularly with significant effects on CAT and GSH-Px activities. Based on comprehensive evaluation of all indices, 200 mg/d was the optimal dosage.

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