Effects of Alfalfa Polysaccharide on Growth Performance, Slaughter Performance, Meat Quality, and Antioxidant Capacity in Broiler Chickens of Different Sexes: Postprint

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Abstract

The present study aimed to investigate the effects of alfalfa polysaccharides on growth performance, slaughter performance, meat quality, and antioxidant capacity of broiler chickens of different sexes. A total of 468 one-day-old Arbor Acres (AA) broiler chickens were randomly allocated into 3 groups with 12 replicates per group (6 male replicates and 6 female replicates), with 13 birds per replicate. Group 1 served as the control group and was fed a basal diet, while groups 2 and 3 were fed the basal diet supplemented with 1,000 and 2,000 mg/kg alfalfa polysaccharides, respectively. The experimental period lasted 42 days. The results showed that, compared with the control group: 1) Dietary supplementation with 1,000 and 2,000 mg/kg alfalfa polysaccharides had no significant effects ($P>0.05$) on average body weight, average daily feed intake, average daily gain, feed-to-gain ratio, dressing percentage, leg muscle percentage, and abdominal fat percentage in both male and female broilers, but significantly increased the eviscerated yield percentage and breast muscle percentage in female broilers ($P<0.05$), and supplementation with 2,000 mg/kg alfalfa polysaccharides significantly increased the breast muscle percentage in male broilers ($P<0.05$). 2) The 1,000 mg/kg alfalfa polysaccharide group (i.e., group 2) exhibited significantly increased pH45 min of breast muscle in male broilers ($P<0.05$), and significantly reduced drip loss percentage of breast muscle in both sexes and cooking loss percentage of breast muscle in male broilers ($P<0.05$); the 2,000 mg/kg alfalfa polysaccharide group (i.e., group 3) showed significantly reduced drip loss percentage of breast muscle in both sexes and cooking loss percentage of breast muscle in male broilers ($P<0.05$). The 1,000 mg/kg alfalfa polysaccharide group demonstrated significantly reduced drip loss percentage of leg muscle in both sexes and shear force of leg muscle in female broilers ($P<0.05$), and significantly increased pH24 h of leg muscle in female broilers ($P<0.05$);

the 2,000 mg/kg alfalfa polysaccharide group exhibited significantly reduced drip loss percentage and shear force of leg muscle in female broilers (P<0.05). 3) The 1,000 and 2,000 mg/kg alfalfa polysaccharide groups showed significantly increased serum total antioxidant capacity (T-AOC) in 21-day-old male broilers (P<0.05), the 2,000 mg/kg alfalfa polysaccharide group exhibited significantly increased serum T-AOC in 35- and 42-day-old female broilers (P<0.05), the 2,000 mg/kg alfalfa polysaccharide group demonstrated significantly increased serum glutathione peroxidase (GSH-Px) activity in 28-day-old male and female broilers (P<0.05), and the 1,000 and 2,000 mg/kg alfalfa polysaccharide groups showed significantly increased serum total superoxide dismutase (T-SOD) activity in 21- and 35-day-old female broilers (P<0.05); however, dietary supplementation with 1,000 and 2,000 mg/kg alfalfa polysaccharides had no significant effect (P>0.05) on malondialdehyde (MDA) content in serum of both sexes; moreover, dietary supplementation with 1,000 and 2,000 mg/kg alfalfa polysaccharides had no significant effects (P>0.05) on T-AOC, GSH-Px and T-SOD activities, or MDA content in liver and breast muscle of both male and female broilers. In summary, dietary supplementation with alfalfa polysaccharides had no significant effect on growth performance of male and female broilers, but could improve slaughter performance, meat quality, and serum antioxidant capacity in both sexes, with the optimal supplementation level being 1,000 mg/kg.

Full Text

Effects of Alfalfa Polysaccharide on Growth Performance, Slaughter Performance, Meat Quality and Antioxidant Capacity in Male and Female Broilers

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Abstract

This experiment was conducted to investigate the effects of alfalfa polysaccharide (AP) on growth performance, slaughter performance, meat quality, and antioxidant capacity in male and female broilers. A total of 468 one-day-old Arbor Acres (AA) broilers were randomly allocated into three groups, each with 12 replicates (six replicates of each sex) and 13 birds per replicate. Group 1 served as the control and received a basal diet, while groups 2 and 3 received the basal diet supplemented with 1,000 and 2,000 mg/kg AP, respectively. The experiment lasted 42 days.

The results showed that, compared with the control group: (1) Dietary supplementation with 1,000 and 2,000 mg/kg AP had no significant effects on average

body weight, average daily feed intake, average daily gain, feed/gain ratio, dressing percentage, leg muscle percentage, or abdominal fat rate in either male or female broilers (P>0.05). However, AP supplementation significantly increased the eviscerated percentage and breast muscle percentage in females (P<0.05), and the 2,000 mg/kg AP level also significantly increased breast muscle percentage in males (P<0.05).

(2) In breast muscle, the 1,000 mg/kg AP group showed significantly increased pH at 45 min postmortem (pH_{45}) in males (P<0.05), along with significantly reduced drip loss rate in both sexes and decreased cooking loss rate in males (P<0.05). The 2,000 mg/kg AP group also significantly reduced drip loss rate in both sexes and cooking loss rate in males (P<0.05). In leg muscle, the 1,000 mg/kg AP group significantly reduced drip loss rate in both sexes and shear force in females, while increasing pH at 24 h postmortem (pH_{24}) in females (P<0.05). The 2,000 mg/kg AP group significantly reduced drip loss rate and shear force in female leg muscle (P<0.05).

(3) The 1,000 and 2,000 mg/kg AP groups significantly increased serum total antioxidant capacity (T-AOC) in males at 21 days of age (P<0.05). In females, the 2,000 mg/kg AP group showed significantly increased serum T-AOC at 35 and 42 days of age (P<0.05). Serum glutathione peroxidase (GSH-Px) activity was significantly increased in both sexes at 28 days of age in the 2,000 mg/kg AP group (P<0.05). Serum total superoxide dismutase (T-SOD) activity was significantly increased in females at 21 and 35 days of age in both AP-supplemented groups (P<0.05). However, dietary AP supplementation had no significant effects on serum malondialdehyde (MDA) content in either sex (P>0.05). Furthermore, AP supplementation did not significantly affect T-AOC, GSH-Px activity, T-SOD activity, or MDA content in liver or breast muscle of either sex (P>0.05).

In conclusion, dietary AP supplementation did not significantly affect growth performance but improved slaughter performance, meat quality, and serum antioxidant capacity in both male and female broilers. The optimal supplemental level of AP appears to be 1,000 mg/kg.

Keywords: alfalfa polysaccharide; broilers; sex; growth performance; slaughter performance; meat quality; antioxidant capacity

Introduction

Plant polysaccharides possess multiple biological functions, including antioxidant, immunomodulatory, antiviral, lipid-lowering, and hypoglycemic activities, and are gaining attention as novel feed additives. Alfalfa polysaccharide (AP), extracted from alfalfa, exhibits bioactivity similar to other polysaccharides such as astragalus polysaccharide, achyranthes polysaccharide, ganoderma-astragalus

polysaccharide, and dandelion polysaccharide. Previous studies have demonstrated that dietary AP supplementation can enhance immune function and antioxidant capacity, promote growth, reduce viral infections, and lower blood glucose and lipid levels. Ouyang et al. reported that dietary supplementation with 1.0% water-soluble AP increased the expression of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) genes in broiler tissues, thereby promoting growth and improving slaughter performance. Meat quality is closely related to antioxidant status; higher antioxidant capacity reduces oxidative stress and improves meat quality. While most research on AP has focused on antioxidant and immune functions, its effects on broiler meat quality remain unreported. Therefore, this study investigated the effects of dietary AP supplementation at different levels on growth performance, slaughter performance, meat quality, and antioxidant capacity in male and female broilers to provide experimental evidence for developing and applying this novel feed additive.

Materials and Methods

1.1 Experimental Materials One-day-old Arbor Acres (AA) broilers were purchased from Beijing Huadu Broiler Company. Alfalfa polysaccharide (containing 22.71% polysaccharides) was provided by the Feed Additive Laboratory of the Institute of Animal Science, Chinese Academy of Agricultural Sciences.

1.2 Experimental Design A total of 468 one-day-old AA broilers were randomly divided into three groups with 12 replicates each (six male and six female replicates) and 13 birds per replicate. Group 1 served as the control and received a basal diet, while groups 2 and 3 received the basal diet supplemented with 1,000 and 2,000 mg/kg AP, respectively. The experimental period lasted 42 days.

1.3 Diets and Nutrient Levels A corn-soybean meal basal diet was formulated as powdered complete feed according to the "Feeding Standard of Broilers" (NY/T 33-2004). The composition and nutrient levels of the basal diet are presented in Table 1 .

1.4 Management The experiment was conducted at the Changping Experimental Base of the Institute of Animal Science, Chinese Academy of Agricultural Sciences, in September 2015. The chicken house was disinfected before the experiment. The trial consisted of two phases: a starter period (1-21 days) and a finisher period (22-42 days). Broilers were housed in three-tier cages with ad libitum access to feed and water. The lighting schedule provided 24 h of light during the first week, followed by 23 h light and 1 h dark from the second week onward. House humidity was maintained at approximately 60%. Temperature was set at 35°C initially and decreased by 1°C every two days during the first week, then maintained at 24-26°C from the third week onward. Management procedures followed the "AA Broiler Management Manual."

1.5 Measurements 1.5.1 Growth Performance

At 1, 21, and 42 days of age, broilers were fasted for 12 h (water provided) and weighed by replicate. Remaining feed was also weighed to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed/gain ratio (F/G) for days 1-21, 22-42, and 1-42. Mortality was recorded daily for each replicate.

1.5.2 Slaughter Performance

At 42 days of age, two broilers from each replicate were selected for slaughter. After weighing, birds were bled from the neck, scalded at 65°C for 1 min, and defeathered. Carcass weight, eviscerated weight, breast muscle weight (both sides), leg muscle weight (both sides), abdominal fat weight, and fat weight around the gizzard were recorded to calculate dressing percentage, eviscerated percentage, breast muscle percentage, leg muscle percentage, and abdominal fat rate using the following formulas:

- Dressing percentage (%) = (carcass weight/live weight) × 100
- Eviscerated percentage (%) = (eviscerated weight/live weight) × 100
- Breast muscle percentage (%) = (breast muscle weight/eviscerated weight) × 100
- Leg muscle percentage (%) = (leg muscle weight/eviscerated weight) × 100
- Abdominal fat rate (%) = [(abdominal fat weight + fat weight around gizzard)/eviscerated weight] × 100

1.5.3 Meat Quality Indices

At 42 days of age, two broilers per replicate were slaughtered, and breast and leg muscles were excised, packaged in sealed bags, and stored at 4°C for meat quality analysis.

Muscle pH: Breast and leg muscle pH was measured at 45 min postmortem (pH_{45}) and again after 24 h storage at 4°C (pH_{24}). Each sample was measured twice and averaged using a PH-STAR carcass muscle pH meter.

Drip loss rate: Approximately 10 g of breast or leg muscle stored at 4°C for 24 h was weighed (W_1), suspended vertically in a paper cup using a thin wire hook with muscle fibers oriented downward, placed in a sealed bag, and stored at 4°C for 24 h before reweighing (W_2). Drip loss rate was calculated as: $[(W_1\text{-}W_2)/W_1] \times 100$.

Cooking loss rate: Breast and leg muscle samples stored at 4°C for 24 h were cut into 3.5 cm × 1.0 cm × 0.5 cm blocks and weighed (W_1), then sealed in bags and heated in an 80°C water bath for 10 min until reaching a core temperature of 70°C. After cooling to room temperature, samples were reweighed (W_2). Cooking loss rate was calculated as: $[(W_1\text{-}W_2)/W_1] \times 100$.

Shear force: Cooked and cooled meat samples were used for shear force determination. Each sample was measured three times and averaged using a C-LM4 digital tenderness meter.

1.5.4 Antioxidant Indices

At 14, 21, 28, 35, and 42 days of age, three broilers per replicate were bled (approximately 4 mL) into tubes. After clot formation, serum was obtained by centrifugation at 3,000 rpm for 8 min, aliquoted into EP tubes, and stored at -20°C for analysis. At the end of the experiment, two broilers per replicate were slaughtered, and liver and breast muscle (fat removed) were collected, rinsed with cold saline, snap-frozen in liquid nitrogen, and stored at -80°C.

Serum, liver, and breast muscle glutathione peroxidase (GSH-Px) activity, total superoxide dismutase (T-SOD) activity, total antioxidant capacity (T-AOC), and malondialdehyde (MDA) content were determined using commercial assay kits (Nanjing Jiancheng Bioengineering Institute) following the manufacturer' s instructions.

1.6 Statistical Analysis Data were analyzed using the ANOVA procedure in SAS 9.3 software. Duncan' s multiple range test was used for post-hoc comparisons, with $P<0.05$ considered statistically significant.

Results

2.1 Effects of Alfalfa Polysaccharide on Growth Performance As shown in Figures 1 [Figure 1: see original paper] through 5 [Figure 5: see original paper], dietary supplementation with 1,000 and 2,000 mg/kg AP had no significant effects on average body weight at 21 and 42 days of age, nor on ADFI, ADG, or F/G during days 1-21, 22-42, or 1-42 in either male or female broilers ($P>0.05$).

2.2 Effects of Alfalfa Polysaccharide on Slaughter Performance Dietary AP supplementation at both levels had no significant effect on dressing percentage in either sex ($P>0.05$) (Figure 6 [Figure 6: see original paper]). However, both 1,000 and 2,000 mg/kg AP significantly increased eviscerated percentage in females ($P<0.05$) without affecting males ($P>0.05$) (Figure 7 [Figure 7: see original paper]).

Breast muscle percentage was significantly increased by 2,000 mg/kg AP in males ($P<0.05$) and by both AP levels in females ($P<0.05$) (Figure 8 [Figure 8: see original paper]). Neither AP level significantly affected leg muscle percentage or abdominal fat rate in either sex ($P>0.05$) (Figures 9 [Figure 9: see original paper] and 10 [Figure 10: see original paper]).

2.3 Effects of Alfalfa Polysaccharide on Meat Quality In breast muscle, 1,000 mg/kg AP significantly increased pH_{45} in males ($P<0.05$) but not in females ($P>0.05$), while neither AP level significantly affected pH_{24} in either sex ($P>0.05$) (Figure 11 [Figure 11: see original paper]). Both AP levels significantly reduced drip loss rate in breast muscle of both sexes ($P<0.05$) (Figure 12 [Figure 12: see original paper]). Cooking loss rate in male breast muscle was significantly

reduced by both AP levels ($P<0.05$), while female breast muscle cooking loss was unaffected ($P>0.05$) (Figure 13 [Figure 13: see original paper]). Shear force in breast muscle was not significantly affected by AP supplementation in either sex ($P>0.05$), though numerical reductions were observed (Figure 14 [Figure 14: see original paper]).

In leg muscle, 1,000 mg/kg AP significantly increased pH_{24} in females ($P<0.05$) without significantly affecting pH_{45} or pH_{24} in males ($P>0.05$) (Figure 15 [Figure 15: see original paper]). Drip loss rate in leg muscle was significantly reduced by 1,000 mg/kg AP in males and by both AP levels in females ($P<0.05$) (Figure 16 [Figure 16: see original paper]). Neither AP level significantly affected cooking loss rate in leg muscle of either sex ($P>0.05$) (Figure 17 [Figure 17: see original paper]). Shear force in leg muscle was significantly reduced by both AP levels in females ($P<0.05$) but not in males ($P>0.05$) (Figure 18 [Figure 18: see original paper]).

2.4 Effects of Alfalfa Polysaccharide on Antioxidant Capacity 2.4.1 Serum Antioxidant Capacity

At 21 days of age, serum T-AOC was significantly increased in males by both AP levels ($P<0.05$). In females, serum T-AOC was significantly increased at 35 and 42 days of age only in the 2,000 mg/kg AP group ($P<0.05$) (Figure 19 [Figure 19: see original paper]). Serum GSH-Px activity was significantly increased at 28 days of age in both sexes by the 2,000 mg/kg AP level ($P<0.05$) (Figure 20 [Figure 20: see original paper]). Serum T-SOD activity in females was significantly increased at 21 and 35 days of age by both AP levels ($P<0.05$), while in males it was significantly decreased at 21 days of age in the 2,000 mg/kg AP group ($P<0.05$) (Figure 21 [Figure 21: see original paper]). Serum MDA content was not significantly affected by AP supplementation in either sex at any age ($P>0.05$) (Figure 22 [Figure 22: see original paper]).

2.4.2 Liver Antioxidant Capacity

Dietary AP supplementation had no significant effects on T-AOC, GSH-Px activity, T-SOD activity, or MDA content in liver of either male or female broilers ($P>0.05$) (Figures 23 [Figure 23: see original paper] through 26 [Figure 26: see original paper]).

2.4.3 Breast Muscle Antioxidant Capacity

Similarly, AP supplementation did not significantly affect T-AOC, GSH-Px activity, T-SOD activity, or MDA content in breast muscle of either sex ($P>0.05$) (Figures 27 [Figure 27: see original paper] through 30 [Figure 30: see original paper]).

Discussion

3.1 Effects on Growth Performance Reports on the effects of plant polysaccharides on broiler growth performance have been inconsistent. Chen

found that dietary supplementation with 1% and 2% AP significantly increased body weight and ADG while reducing F/G in AA broilers. Similarly, Liu et al. reported that 1% AP significantly improved body weight and ADG while reducing F/G. Guo et al. demonstrated that mushroom polysaccharides (lentinan and tremella polysaccharide) or astragalus polysaccharide improved growth performance, with optimal lentinan supplementation at 0.2% showing effects comparable to antibiotics. These growth-promoting effects may be attributed to enhanced immune function and antioxidant capacity, which reduce viral infections and improve nutrient utilization. However, Chen et al. reported that 200 mg/kg achyranthes polysaccharide or astragalus polysaccharide had no significant effects on growth performance. Our results align with Chen et al., showing no significant effects of 1,000 or 2,000 mg/kg AP on body weight, ADFI, ADG, or F/G. These discrepancies may be related to polysaccharide source, supplementation level, or rearing environment.

3.2 Effects on Slaughter Performance Liu et al. reported that AP supplementation had no significant effects on dressing percentage, semi-eviscerated percentage, eviscerated percentage, breast muscle percentage, or leg muscle percentage. Li et al. found that 500 mg/kg watermelon peel polysaccharide significantly increased breast muscle percentage at 21 days of age and tended to reduce abdominal fat and leg muscle percentage, but had no significant effects on carcass quality at 42 days. Ouyang et al. reported that AP supplementation did not affect dressing percentage or leg muscle percentage, but 1.0% and 1.5% AP significantly increased breast muscle percentage and reduced abdominal fat rate, thereby improving slaughter performance. Our study found that 1,000 and 2,000 mg/kg AP did not affect dressing percentage, leg muscle percentage, or abdominal fat rate, but significantly increased eviscerated percentage and breast muscle percentage in females, with 2,000 mg/kg AP also increasing breast muscle percentage in males. These effects may be related to AP' s ability to increase protein metabolism and reduce crude fat metabolism.

3.3 Effects on Meat Quality Muscle pH, drip loss rate, cooking loss rate, and shear force are important physical indicators of meat quality. Muscle pH reflects postmortem glycogenolysis rate, with pH decreasing over time as lactate accumulates. Our results showed that breast muscle pH_{24} was lower than pH_{45} , consistent with this pattern, while leg muscle pH_{24} remained similar to pH_{45} , likely due to lower glycogen content and less lactate production in leg muscle. As pH approaches the isoelectric point of myoglobin, protein-water binding capacity decreases, reducing water-holding capacity and increasing drip loss. Therefore, higher muscle pH correlates with better water-holding capacity and longer shelf life. Lower drip loss and cooking loss rates indicate higher water-holding capacity and better tenderness. Drip loss rate is associated with cell membrane integrity; better integrity reduces fluid loss. Plant polysaccharides possess antioxidant capacity that prevents membrane lipid oxidation, preserves membrane integrity, and blocks fluid leakage from the sarcoplasm, thereby re-

ducing drip loss. El-Rammouz et al. reported negative correlations between muscle pH and drip loss, cooking loss, and water loss rate, while water loss rate positively correlated with drip loss and cooking loss positively correlated with shear force. Huo et al. found that 1.12 and 2.24 g/kg ganoderma-astragalus polysaccharide significantly reduced water loss rate and improved meat color without affecting shear force or pH. Hanczakowska et al. reported that 1,000 mg/kg purple coneflower extract significantly improved water-holding capacity and pH_{45} in pork. Wen et al. demonstrated that 4,000 mg/kg tremella polysaccharide reduced drip loss rate and increased loin pH in growing pigs. Our results showed that AP supplementation increased pH_{45} in male breast muscle, reduced drip loss rate in both muscles of both sexes, decreased cooking loss rate in male breast muscle, increased pH_{24} in female leg muscle, and reduced shear force in female leg muscle. Overall, AP supplementation tended to increase pH while reducing drip loss, cooking loss, and shear force, consistent with El-Rammouz et al. and indicating improved meat quality in both sexes. The optimal AP supplementation level appears to be 1,000 mg/kg.

3.4 Effects on Antioxidant Capacity Under normal physiological conditions, free radical production and elimination maintain dynamic equilibrium. When this balance is disrupted, free radicals competitively utilize reducing hydrogen required for cellular metabolism, causing cellular damage. GSH-Px and SOD are crucial antioxidant enzymes; GSH-Px decomposes hydrogen peroxide, while SOD eliminates hydroxyl and oxygen radicals, protecting membrane structure and function while reducing lipid peroxide formation. T-AOC is a comprehensive indicator of systemic antioxidant capacity, while MDA, the end product of lipid peroxidation, causes membrane lipid-protein cross-linking and loss of cellular function, with higher levels indicating greater oxidative damage. Therefore, enhancing antioxidant enzyme activity and reducing MDA content are important for healthy broiler growth.

Xu reported that 500 mg/kg AP significantly increased serum T-AOC, T-SOD, and GSH-Px activities while inhibiting free radical production. Chen found that 25 and 50 mg/kg acanthopanax polysaccharide significantly increased serum T-SOD and GSH-Px activities, with 50 mg/kg also reducing serum MDA content. Yao et al. demonstrated that 1,000 and 3,000 mg/kg hericium polysaccharide extract significantly increased serum and liver T-SOD and catalase activities while reducing MDA content. Wang et al. reported that 5 g/L dandelion polysaccharide in drinking water significantly increased breast muscle SOD activity and reduced MDA content, with effects comparable to vitamin E. Lai et al. found that mung bean polysaccharide exhibited strong in vitro free radical scavenging capacity. Wang et al. showed that AP increased SOD and catalase activities and T-AOC in laying hen hepatocytes while preserving membrane integrity, confirming AP's antioxidant properties. Meat quality is closely related to lipid oxidation, particularly post-slaughter when antioxidant capacity declines, causing rapid oxidation of unsaturated fatty acids, membrane damage, fluid loss, nutrient loss, and ultimately reduced meat quality. Li et al. reported that higher SOD activity

and lower MDA content correlate with better water-holding capacity and tenderness. Plant polysaccharides may enhance antioxidant capacity by increasing endogenous antioxidant enzyme activity and directly scavenging oxygen radicals. Our study found that AP supplementation significantly increased serum T-AOC in males at 21 days and in females at 35-42 days, increased serum GSH-Px activity in both sexes at 28 days, and increased serum T-SOD activity in females at 21 and 35 days, without significantly affecting serum or tissue MDA content. This suggests that broilers did not experience significant oxidative stress during the experiment, resulting in minimal changes in lipid peroxidation end products, though the specific mechanisms require further investigation.

Conclusion

Dietary supplementation with alfalfa polysaccharide did not significantly affect growth performance in male or female broilers but improved slaughter performance, meat quality, and serum antioxidant capacity. The optimal supplementation level appears to be 1,000 mg/kg.

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