

## Effects of Alfalfa Saponins on Production Performance, Egg Quality and Antioxidant Capacity of Laying Hens (Postprint)

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### Abstract

This experiment aimed to investigate the effects of alfalfa saponins on the production performance, egg quality, and antioxidant capacity of laying hens. A total of 150 Hy-Line Brown laying hens aged 190 days were selected and randomly divided into 5 groups, with 5 replicates per group and 6 hens per replicate. The control group was fed a basal diet, while groups I-IV were fed the basal diet supplemented with 60, 120, 240, and 480 mg/kg alfalfa saponins, respectively. The experimental period lasted 77 days. The results showed that: 1) The average daily feed intake and feed-to-egg ratio of group II were significantly lower than those of the control group ( $P < 0.05$ ), the egg weight of groups II and III was significantly lower than that of the control group ( $P < 0.05$ ), and there was no significant difference in laying rate among all groups ( $P > 0.05$ ). 2) On day 30, the yolk color of group IV was significantly lower than that of the control group ( $P < 0.05$ ); on day 45, the yolk color of groups II and III was significantly lower than that of the control group ( $P < 0.05$ ); on day 60, the yolk color of groups I, II, III, and IV was significantly lower than that of the control group ( $P < 0.05$ ). 3) The activities of glutathione peroxidase and superoxide dismutase and the content of malondialdehyde in the liver, breast muscle, and leg muscle of groups I, II, III, and IV showed no significant differences compared with the control group ( $P > 0.05$ ). Based on a comprehensive analysis of all indicators, under the conditions of this experiment, supplementation of 120 mg/kg alfalfa saponins in the diet yielded better results.

## Full Text

### Effects of Alfalfa Saponins on Performance, Egg Quality and Antioxidant Capacity of Laying Hens

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**Abstract:** This experiment was conducted to investigate the effects of alfalfa saponins on the performance, egg quality, and antioxidant capacity of laying hens. One hundred and fifty 190-day-old Hy-Line brown laying hens were randomly allocated into five groups, with five replicates per group and six hens per replicate. The control group was fed a basal diet, while groups I-IV were fed the basal diet supplemented with 60, 120, 240, and 480 mg/kg alfalfa saponins, respectively. The experimental period lasted 77 days. The results showed: (1) The average daily feed intake and feed-to-egg ratio of group II were significantly lower than those of the control group ( $P < 0.05$ ). The egg weight of groups II and III was significantly lower than that of the control group ( $P < 0.05$ ). No significant differences in laying rate were observed among all groups ( $P > 0.05$ ). (2) On day 30, the yolk color of group IV was significantly lower than that of the control group ( $P < 0.05$ ). On day 45, the yolk color of groups II and III was significantly lower than that of the control group ( $P < 0.05$ ). On day 60, the yolk color of groups I, II, III, and IV was significantly lower than that of the control group ( $P < 0.05$ ). (3) No significant differences were found in glutathione peroxidase activity, superoxide dismutase activity, or malondialdehyde content in the liver, breast muscle, or leg muscle between the treatment groups and the control group ( $P > 0.05$ ). Based on comprehensive analysis of all indicators, supplementation of 120 mg/kg alfalfa saponins in the diet showed the best results under the conditions of this experiment.

**Keywords:** alfalfa saponin; laying hens; performance; egg quality; antioxidant capacity

**Classification Code:** S831.5

Saponins have traditionally been considered anti-nutritional factors due to their role in causing rumen bloating in ruminants. However, numerous studies have demonstrated that this view is one-sided. Appropriate levels of saponins can not only improve the growth performance of monogastric animals but also play an important role in reducing ammonia emissions and improving livestock housing environmental hygiene. For monogastric animals, saponins serve as active beneficial compounds. Alfalfa saponins are natural bioactive substances extracted from alfalfa with potent biological activities and functions. Research has shown that saponins possess various bioactive functions including anti-tumor,

immune-enhancing, cardiovascular protective, and hypoglycemic, hypolipidemic, and cholesterol-lowering effects. This study investigated the effects of different dietary supplementation levels of alfalfa saponins on the performance, egg quality, and antioxidant capacity of laying hens to provide a theoretical basis for the application of alfalfa saponins in laying hen diets.

## 1 Materials and Methods

### 1.1 Experimental Materials

The alfalfa saponin extract used in this experiment was purchased from Cangzhou, Hebei Province. The alfalfa saponin content was determined to be [value missing in original text] using UV spectrophotometry.

### 1.2 Experimental Design

A single-factor completely randomized design was adopted. One hundred and fifty healthy Hy-Line brown laying hens at 190 days of age with similar body weights were randomly divided into five groups, with five replicates per group and six hens per replicate. The control group was fed a basal diet, while groups I-IV were fed the basal diet supplemented with 60, 120, 240, and 480 mg/kg alfalfa saponins, respectively. The experimental period lasted 77 days.

### 1.3 Basal Diet and Management

The nutrient levels of the basal diet for laying hens were formulated according to the NRC (1994) nutrient requirements for laying hens. The composition and nutrient levels of the basal diet are shown in Table 1. A three-tier step-cage system was used. The lighting schedule consisted of 16 hours of light per day (05:00-21:00) with supplementary artificial lighting in the morning and evening. Feed and water were provided ad libitum. Routine vaccination and disinfection procedures were followed. Feeding was conducted at 06:00 and 15:00 daily, and eggs were collected at 18:00.

### 1.4 Measurement Indicators and Methods

Daily egg production and egg weight were recorded by replicate to calculate laying rate. Feed intake was recorded every 7 days to calculate average daily feed intake. On days 15, 30, 45, and 60, four eggs per replicate were selected to determine egg quality parameters. At the end of the experiment, two hens per replicate were randomly selected, and liver, leg muscle, and breast muscle tissues were collected and stored at -20 °C for antioxidant index determination.

#### 1.4.1 Performance Indicators

Average daily feed intake = total feed intake / experimental days; feed-to-egg ratio = feed intake / egg weight; laying rate = actual number of eggs / total number of hens.

#### 1.4.2 Egg Quality Indicators

Haugh unit and yolk color were measured using a multifunctional egg analyzer. Eggshell strength was measured using an eggshell force gauge, and eggshell thickness was measured using an eggshell thickness gauge. Egg shape index = egg longitudinal diameter / egg transverse diameter.

#### 1.4.3 Antioxidant Indicators

Malondialdehyde (MDA) content and total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activities in the liver, leg muscle, and breast muscle were determined using assay kits provided by the Nanjing Jiancheng Bioengineering Institute.

#### 1.5 Statistical Analysis

Data were analyzed using one-way ANOVA with SPSS 13.0. Results are expressed as “mean  $\pm$  standard deviation.” Duncan’ s multiple comparison test was used for pairwise comparisons among groups.

## 2 Results

### 2.1 Effects of Alfalfa Saponins on Performance of Laying Hens

As shown in Table 2 , the average daily feed intake of hens in groups I, II, III, and IV showed a decreasing trend compared with the control group, with group II showing a significant reduction ( $P < 0.05$ ). No significant differences in laying rate were observed among all groups ( $P > 0.05$ ). The egg weight of groups II and III was significantly lower than that of the control group ( $P < 0.05$ ). The feed-to-egg ratio of group II was significantly lower than that of the control group ( $P < 0.05$ ), while the other three groups showed no significant differences compared with the control group ( $P > 0.05$ ).

### 2.2 Effects of Alfalfa Saponins on Egg Quality of Laying Hens

As shown in Table 3 , on day 30, the yolk color of group IV was significantly lower than that of the control group ( $P < 0.05$ ), while group II showed a significantly lower yolk ratio ( $P < 0.05$ ). On day 45, the yolk color of groups II and III was significantly lower than that of the control group ( $P < 0.05$ ). On day 60, the yolk color of groups I, II, III, and IV was significantly lower than that of the control group ( $P < 0.05$ ), and the eggshell thickness of group III was significantly higher than that of the control group ( $P < 0.05$ ).

### 2.3 Effects of Alfalfa Saponins on Liver and Muscle Antioxidant Indices of Laying Hens

As shown in Table 4 , GSH-Px activity followed the order: leg muscle  $>$  breast muscle  $>$  liver. No significant differences in GSH-Px activity were observed in the liver, breast muscle, or leg muscle among all groups ( $P > 0.05$ ). Similarly, no significant differences in SOD activity were found in the liver, breast muscle, or

leg muscle among all groups ( $P>0.05$ ). The MDA content in the liver and breast muscle of groups I, II, III, and IV showed a decreasing trend compared with the control group, but the differences were not significant ( $P>0.05$ ). The leg muscle MDA content of groups I, II, and III also decreased compared with the control group, but without significant differences ( $P>0.05$ ). Group II, supplemented with 120 mg/kg alfalfa saponins, showed increased GSH-Px and SOD activities and decreased MDA content in the liver, breast muscle, and leg muscle, but none reached significant levels ( $P>0.05$ ).

### 3 Discussion

#### 3.1 Effects of Alfalfa Saponins on Performance of Laying Hens

The average daily feed intake of laying hens fed diets supplemented with alfalfa saponins was slightly lower than that of the control group, possibly due to the bitter taste of alfalfa saponins and its irritating effect on the oral cavity and digestive tract, leading to reduced feed intake. As early as 1959, Heywang et al. added alfalfa saponins to laying hen diets and found that when saponin supplementation levels reached 0.26% or 0.40%, egg production and average daily feed intake were significantly reduced. Hou Yonggang et al. reported that adding 30, 60, or 90 mg/kg alfalfa saponins to the diet of 400-day-old Hy-Line brown laying hens for 60 days improved laying rate and egg quality compared with the control group, while the feed-to-egg ratio decreased by 1.8%, 3.7%, and 3.1%, respectively. Dong et al. fed alfalfa extract to 1-day-old broiler chicks and found that it reduced abdominal fat percentage and enhanced immunity without affecting performance. Most studies suggest that adding alfalfa saponins to diets does not decrease livestock performance. Anderson found that dietary alfalfa saponins could improve laying rate in hens. Güçlü et al. reported that adding 9% alfalfa meal to quail diets improved eggshell quality without adverse effects on performance. Zhang Lina noted that alfalfa extract at supplementation levels of 500 and 1000 mg/kg significantly improved laying rate and feed-to-egg ratio in laying hens.

Laying rate in hens is influenced by many factors, among which serum very low-density lipoprotein (VLDL) and hormones are two important indicators. When serum VLDL content is low, laying rate decreases rather than maintaining production by reducing yolk volume. Follicle-stimulating hormone (FSH) can stimulate follicle maturation and ovulation under the synergistic action of luteinizing hormone (LH), inhibit follicular atresia, increase follicle number, and improve laying rate. The decline in laying rate in aging hens is associated with decreased serum FSH and LH levels. Studies have shown that serum VLDL content was reduced in groups fed diets supplemented with alfalfa saponins compared with the unsupplemented control group. The results of this experiment indicate that dietary alfalfa saponins reduced the feed-to-egg ratio, which is related to both feed intake and egg weight.

### 3.2 Effects of Alfalfa Saponins on Egg Quality

Egg quality encompasses not only external appearance but also internal indicators such as Haugh unit, yolk color, and yolk ratio, which determine egg quality. The Haugh unit is an important indicator reflecting egg freshness; the fresher the egg, the thicker and more viscous the albumen, resulting in a higher Haugh unit. The normal range for fresh eggs is 75–85, though some may reach 90. Yolk color influences consumer perception, as consumers often associate deeper yolk color with better egg quality. Since laying hens cannot synthesize lutein, the pigment responsible for yolk color, ideal yolk color depends on the quality and quantity of dietary lutein. In the later stage of this experiment, most Haugh units were within the normal range, and the saponin-supplemented groups showed slightly higher values than the control group, suggesting that alfalfa saponins may help maintain egg freshness to some extent.

Eggshell thickness is one of the earliest and primary indicators used to evaluate eggshell quality. Deng et al. reported that eggshell thickness was improved when alfalfa extract was added to high-producing laying hen diets. Eggshell thickness reflects the compactness of the shell and is an important indicator of eggshell quality and a key factor affecting egg breakage rate. Tyler reported a significant non-linear relationship between eggshell thickness and breakage rate. Wang Xiuqi et al. noted that normal eggshell thickness ranges from 0.20 to 0.48 mm, with minor variations having substantial impacts on breakage rate. Advanced hen age, high summer temperatures, and disease infection often lead to thinner eggshells and increased breakage rates. In this experiment, all eggshell thickness values were within the normal range, though the alfalfa saponin groups showed an increasing trend in eggshell strength throughout the experimental period.

Higher yolk ratio indicates greater nutritional value of eggs. The results of this experiment showed that alfalfa saponins had no significant effect on egg color, as alfalfa saponins themselves do not contain lutein. However, in the later stage of the experiment (day 60), the control group showed significantly higher yolk color than the saponin-supplemented groups, with yolk color decreasing as alfalfa saponin supplementation level increased.

Egg shape index is related to hatching value and breakage rate. The normal range for egg shape index is 1.30–1.35. Elongated eggs ( $>1.35$ ) or spherical eggs ( $<1.30$ ) are more susceptible to breakage during transport and packaging. The egg shape indices in this experiment ranged from 1.25 to 1.27, which are slightly lower than normal and approximate spherical eggs, indicating that alfalfa saponins had no effect on egg breakage rate.

### 3.3 Effects of Alfalfa Saponins on Liver and Muscle Antioxidant Indices

Lipid peroxidation reactions *in vivo* can cause the occurrence and development of coronary heart disease and atherosclerosis. Under caged conditions and laying stress, laying hens produce large amounts of free radicals. With increasing age,

the ability to scavenge free radicals weakens, particularly in metabolically active tissues such as the ovary and liver. Free radicals are atoms or atomic groups containing an unpaired electron and are highly reactive, capable of reacting with polyunsaturated fatty acids on membranes and causing lipid peroxidation, leading to damage to cell membranes and submicroscopic structures. Lipid peroxidation damage to hepatocyte organelle membranes such as the endoplasmic reticulum and Golgi apparatus can affect VLDL synthesis and transport in the liver, causing triglyceride (TG) accumulation. Ovarian damage can reduce the sensitivity of oocyte vitellogenesis receptors (OVR) and FSH and LH receptors, causing lipid metabolism disorders.

MDA is a metabolite of lipid peroxidation reactions, and its content can reflect the degree of lipid peroxidation in vivo. MDA can also oxidize low-density lipoprotein (LDL) to oxidized LDL (OX-LDL), ultimately forming thrombi in blood vessels. SOD can scavenge superoxide anion radicals ( $O_2^-$ ) by dismutating them into hydrogen peroxide ( $H_2O_2$ ) and oxygen ( $O_2$ ), protecting cells from damage. It is a crucial protein in maintaining oxidative balance in animals; deficiency can disrupt cellular oxidative metabolism and cause cell damage. GSH-Px can scavenge  $H_2O_2$  in mitochondria, cytoplasm, and lipids, converting lipid peroxidation products into stable hydroxy compounds (RHO) and blocking the free radical chain reaction of lipid peroxidation, thereby protecting the liver from damage. Wang Chengzhang et al. reported that alfalfa saponins reduced serum MDA content in weaned piglets. Luo Ailing also found that alfalfa saponins increased SOD activity and decreased MDA content in broiler thigh and breast muscles. The results of this study similarly found that appropriate levels of dietary alfalfa saponins tended to increase GSH-Px and SOD activities and decrease MDA content in the liver, breast muscle, and leg muscle of laying hens.

#### 4 Conclusion

1. Dietary supplementation with 120 mg/kg alfalfa saponins reduced average daily feed intake and feed-to-egg ratio in laying hens without decreasing laying rate, though egg weight was reduced.
2. Dietary alfalfa saponins showed no clear pattern of effects on egg quality. In the later stage of the experiment (day 60), groups I-IV supplemented with alfalfa saponins showed significantly reduced yolk color.
3. Dietary supplementation with 120 mg/kg alfalfa saponins tended to improve antioxidant indices in the liver, breast muscle, and leg muscle.
4. Based on comprehensive analysis, dietary supplementation with 120 mg/kg alfalfa saponins showed the best results for laying hens.

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