Effects of Astaxanthin Composite Additive on Production Performance, Egg Quality, Yolk Antioxidant Indices, and Yolk Astaxanthin Content in Laying Hens (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary astaxanthin complex additive on production performance, egg quality, yolk antioxidant indices, and astaxanthin content in egg yolk of laying hens. A total of 360 Jinghong laying hens aged 43 weeks were selected and randomly divided into 5 groups with 6 replicates per group and 12 hens per replicate. Group I served as the control group fed a basal diet without astaxanthin complex additive; Groups II–V were treatment groups fed experimental diets supplemented with 0.6, 1.2, 2.4, and 3.6 g/kg astaxanthin complex additive, respectively. The preliminary period lasted for 4 weeks, followed by a 6-week formal experimental period. The results showed: 1) Dietary supplementation of astaxanthin complex additive had no significant effects on average daily feed intake, laying rate, or feed-to-egg ratio of laying hens ($P > 0.05$). 2) Dietary supplementation of astaxanthin complex additive had no significant effects on average egg weight, yolk percentage, shell thickness, or Haugh unit of laying hens ($P > 0.05$). The yolk color of all treatment groups was significantly higher than that of the control group ($P < 0.05$); yolk color gradually deepened with increasing supplementation levels of astaxanthin complex additive. 3) With increasing supplementation levels of astaxanthin complex additive, the malondialdehyde content in egg yolk gradually decreased, while the activities of superoxide dismutase and glutathione peroxidase in egg yolk gradually increased; the malondialdehyde content in egg yolk of Groups III, IV, and V was significantly lower than that of the control group ($P < 0.05$), and the activities of superoxide dismutase and glutathione peroxidase in egg yolk of all treatment groups were significantly higher than those of the control group ($P < 0.05$). 4) No astaxanthin was detected in egg yolk of the control group, while the astaxanthin content in egg yolk of treatment groups increased significantly with increasing supplementation levels of astaxanthin complex additive ($P < 0.05$). It was concluded that dietary supplementation of astaxanthin complex additive had no significant effect on production performance of laying hens, but could deepen yolk color, improve yolk antioxidant capacity, and increase astaxanthin content in egg yolk, with the optimal effect observed at the supplementation level of 3.6 g/kg.

Full Text

Effects of Astaxanthin Compound Additive on Performance, Egg Quality, Yolk Antioxidant Indexes and Yolk Astaxanthin Content of Laying Hens

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Abstract: This experiment investigated the effects of dietary astaxanthin compound additive on production performance, egg quality, yolk antioxidant indexes, and yolk astaxanthin content in laying hens. Three hundred and sixty 43-week-old Jinghong hens were randomly allocated into five groups with six replicates of 12 hens each. Group I served as the control group receiving a basal diet without astaxanthin compound additive, while groups II–V were experimental groups fed the basal diet supplemented with 0.6, 1.2, 2.4, and 3.6 g/kg astaxanthin compound additive, respectively. The study consisted of a 4-week pre-trial period followed by a 6-week formal experimental period. The results showed that: (1) dietary supplementation with astaxanthin compound additive had no significant effects on average daily feed intake, laying rate, or feed-to-egg ratio ($P > 0.05$); (2) supplementation did not significantly affect average egg weight, yolk percentage, eggshell thickness, or Haugh unit ($P > 0.05$), though yolk color in all experimental groups was significantly higher than in the control group ($P < 0.05$), deepening progressively with increasing additive levels; (3) as additive levels increased, yolk malondialdehyde content decreased while superoxide dismutase and glutathione peroxidase activities increased, with groups III, IV, and V showing significantly lower malondialdehyde content than the control ($P < 0.05$), and all experimental groups exhibiting significantly higher antioxidant enzyme activities ($P < 0.05$); (4) no astaxanthin was detected in control group yolks, while experimental groups showed significantly increased yolk astaxanthin content with rising additive levels ($P < 0.05$). In conclusion, dietary astaxanthin compound additive supplementation did not significantly affect laying hen performance but enhanced yolk color, improved yolk antioxidant capacity, and increased yolk astaxanthin content, with the optimal effect

observed at 3.6 g/kg supplementation.

Keywords: laying hens; astaxanthin; performance; egg quality; antioxidant

Astaxanthin, also known as astaxanthin, belongs to the carotenoid family. It provides powerful antioxidant protection, preventing oxidation of vitamin A, vitamin E, and other carotenoids. As the most potent antioxidant among all carotenoids—10 times more effective than β-carotene and 100 times more effective than vitamin E—astaxanthin exhibits antioxidant, anti-tumor, and cardiovascular protective properties. Its vivid red pigmentation can alter the color of livestock products when added to feed, enhancing animal immunity while providing nutritional benefits to humans through residual astaxanthin in animal tissues and eggs. Naturally produced by algae, bacteria, and phytoplankton, astaxanthin can be obtained through artificial synthesis or biological extraction. Synthetic astaxanthin is not only expensive but also differs significantly from natural astaxanthin in structure, function, application, and safety. Natural sources include seafood processing waste, *Phaffia rhodozyma* yeast, and *Haematococcus pluvialis* algae. Previous studies demonstrated that dietary astaxanthin supplementation deepens skin and meat coloration in broilers and enhances egg nutritional value and yolk color. However, limited research exists on its effects on yolk antioxidant indexes. Therefore, this study evaluated different dietary levels of astaxanthin compound additive to provide reference data for its application in laying hen production.

1.1 Experimental Materials

The astaxanthin compound additive was provided by Jilin Xima Biotechnology Co., Ltd., extracted from *Phaffia rhodozyma* yeast with an astaxanthin content of 0.40%.

1.2 Experimental Design and Diets

A single-factor experimental design was employed using 360 healthy 43-week-old Jinghong hens with similar body weight, body size, and laying rate, randomly divided into five groups with six replicates of 12 hens each. The study included a 4-week pre-trial period and a 6-week formal experimental period. During pre-trial, all groups received the basal diet, with laying rates recorded by group for appropriate adjustments. Group I served as the control, while groups II–V received the basal diet supplemented with 0.6, 1.2, 2.4, and 3.6 g/kg astaxanthin compound additive, respectively. The basal diet was formulated according to NRC (1994), Chinese Feeding Standard of Chickens (NY/T 33-2004), and the Jinghong Hen Management Manual, with composition and nutrient levels shown in .

1.3 Management Practices

Hens were housed in a semi-open three-tier cage system (47 cm × 37 cm × 47 cm, 3 hens per cage) with natural ventilation combined with longitudinal negative pressure ventilation and natural light supplemented with artificial lighting. Powdered feed was provided twice daily at 08:00 and 14:00, with egg collection and manure removal performed twice daily. Hens had ad libitum access to feed and water, with weekly disinfection and routine immunization.

1.4 Measurement Indicators

1.4.1 Production Performance During the experimental period, daily records were maintained for mortality, feed intake, egg number, and egg weight by replicate to calculate average daily feed intake, laying rate, and feed-to-egg ratio.

1.4.2 Egg Quality Metrics On day 42 of the formal period, six eggs per replicate were randomly selected for quality assessment including average egg weight, eggshell thickness, Haugh unit, yolk color, and yolk percentage. Egg weight, shell weight, and yolk weight were measured using an electronic analytical balance to calculate average egg weight and yolk percentage. Eggshell thickness was measured at the large end, small end, and middle using a vernier caliper, with values averaged. Yolk color was determined using a Roche color fan, and Haugh unit was calculated from albumen height measured with a protein height gauge (NFN381/NFN382, FHK Company, Japan) using the formula: Haugh unit = 100 × lg(H - 1.7W^0.37 + 7.6), where H represents albumen height (mm) and W represents egg weight (g).

1.4.3 Yolk Antioxidant Indexes On day 42, six eggs per replicate were randomly selected to determine yolk malondialdehyde content, superoxide dismutase activity, and glutathione peroxidase activity using assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.4 Yolk Astaxanthin Content On day 42, six eggs per replicate were randomly selected for yolk astaxanthin analysis. Yolk samples (0.5 g) were accurately weighed into 50 mL centrifuge tubes, mixed with 5 mL n-hexane and vortexed until completely dissolved. After adding 10 mL sodium hydroxide-methanol solution (4 mol/L) and vortexing for 2 minutes, samples were reacted at 30 °C for 30 minutes in the dark. The reaction was terminated with 5 g sodium bisulfate, followed by low-temperature centrifugation (4 °C, 5000 r/min) for 5 minutes. The lower methanol-aqueous phase was filtered through a 0.45 m microporous membrane for detection. Chromatographic conditions: VanusilASB C18 column (250 mm × 4.6 mm, 5 m); column temperature: 30 °C; detector: DAD UV detector; detection wavelength: 476 nm; reference wavelength: 600 nm; injection volume: 20 L; flow rate: 1.0 mL/min; mobile phase: pure methanol with isocratic elution for 20 minutes.

1.5 Statistical Analysis

Data were processed using Excel 2017 and analyzed by one-way ANOVA using SPSS 17.0. Results are expressed as mean ± standard deviation, with $P < 0.05$ considered statistically significant.

2.1 Effects on Production Performance

As shown in , dietary astaxanthin compound additive supplementation had no significant effects on average daily feed intake, laying rate, or feed-to-egg ratio ($P > 0.05$).

2.2 Effects on Egg Quality

As shown in , dietary supplementation had no significant effects on average egg weight, yolk percentage, eggshell thickness, or Haugh unit ($P > 0.05$). However, yolk color in all experimental groups was significantly higher than in the control group ($P < 0.05$), deepening progressively with increasing additive levels, reaching 12.28 in group V, which differed significantly from other experimental groups ($P < 0.05$).

2.3 Effects on Yolk Antioxidant Indexes

As shown in , increasing astaxanthin compound additive levels progressively decreased yolk malondialdehyde content while increasing superoxide dismutase and glutathione peroxidase activities. Yolk malondialdehyde content in groups III, IV, and V was significantly lower than in the control group ($P < 0.05$), while yolk superoxide dismutase and glutathione peroxidase activities in all experimental groups were significantly higher than in the control group ($P < 0.05$). Additionally, group V showed significantly higher glutathione peroxidase activity than other experimental groups ($P < 0.05$).

2.4 Effects on Yolk Astaxanthin Content

As shown in , no astaxanthin was detected in control group yolks, while experimental groups exhibited significantly increased yolk astaxanthin content with rising additive levels ($P < 0.05$).

3.1 Effects on Production Performance

Akiba et al. [10] reported that dietary supplementation with 15, 20, and 30 mg/kg astaxanthin in 4-week-old chicks, and Waldenstedt et al. [11] found that 0, 7, 36, and 179 mg/kg astaxanthin in broiler diets did not improve growth performance or feed conversion. Conversely, Yang et al. [12] suggested that astaxanthin may maintain and promote beneficial microbial communities in rapidly growing broilers, potentially reducing maintenance energy requirements and improving nutrient utilization efficiency. In this study, dietary astaxanthin compound additive supplementation did not significantly affect average daily feed intake, laying rate, or feed-to-egg ratio, possibly because the additive did not substantially impact nutrient digestibility or feed palatability, though specific mechanisms require further investigation.

3.2 Effects on Egg Quality

As shown in , dietary astaxanthin compound additive supplementation did not significantly affect average egg weight, yolk percentage, eggshell thickness, or Haugh unit, consistent with Ross et al. [13]. Yolk color represents an important egg quality trait, with deeper-colored yolks preferred by consumers. In this study, yolk color deepened progressively with increasing additive levels, with the most intense coloration observed at 3.6 g/kg supplementation. Mammershoj [14] reported that dietary astaxanthin significantly deepened yolk color compared to controls. This pigmentation effect occurs because astaxanthin, as a carotenoid, exists as a dipalmitate ester that combines with low-density lipoproteins in the digestive tract for absorption. In blood, it circulates in free form via lipoprotein carriers into yolk and other tissues, where it deposits as dipalmitate esters, producing deep yellow to red coloration [15-16].

3.3 Effects on Yolk Antioxidant Indexes

Antioxidants are defined as substances that "delay, prevent, or remove oxidative damage to target molecules" [17] or "directly scavenge reactive oxygen species (ROS) or indirectly modulate antioxidant defense or inhibit ROS production" [18]. These compounds work synergistically against various free radicals and reactive species, with the most effective enzymatic antioxidants being glutathione peroxidase, catalase, and superoxide dismutase [19]. Astaxanthin' s red coloration derives from conjugated double bonds at its molecular center, which function as potent antioxidants by donating electrons and reacting with free radicals to form stable products, thereby terminating radical chain reactions in organisms [20]. Astaxanthin exhibits superior bioactivity compared to other antioxidants [21] due to its ability to associate with cell membranes from interior to exterior. It provides antioxidant protection during yolk lipid oxidation, with Kobayashi et al. [22], Kurashige et al. [23], and Palozza et al. [24] demonstrating that astaxanthin improves lipid stability by increasing superoxide dismutase and glutathione peroxidase activities. Chen et al. [25] reported that free-form astaxanthin powder reduced plasma and liver malondialdehyde content while significantly increasing liver catalase activity and glutathione levels in mice. In this study, increasing astaxanthin compound additive levels progressively decreased yolk malondialdehyde content and increased antioxidant enzyme activities, with the most pronounced effects observed at 3.6 g/kg supplementation. These results indicate that astaxanthin compound additive can be absorbed by hens, deposit in yolk, enhance antioxidant capacity, and maintain redox balance, with antioxidant performance improving as supplementation levels increase.

3.4 Effects on Yolk Astaxanthin Content

Ren [26] reported that supplementing broiler diets with 45 mg/kg β-carotene resulted in 137.3 g/kg astaxanthin deposition in muscle tissue. In this study, astaxanthin compound additive levels of 0.06, 0.12, 0.24, and 0.36 g/kg produced a dose-dependent increase in yolk astaxanthin content. Current research on carotenoid deposition has focused on tissue, cellular, and organelle levels, though the selective distribution in specific tissues, transport mechanisms, and resulting physiological effects require further investigation.

Conclusions: 1. Dietary astaxanthin compound additive supplementation did not significantly affect laying hen production performance. 2. Supplementation did not significantly affect eggshell thickness, eggshell percentage, Haugh unit, or yolk percentage, but significantly influenced yolk color, which deepened progressively with increasing additive levels, with optimal effects at 3.6 g/kg. 3. Dietary astaxanthin compound additive supplementation reduced yolk malondialdehyde content and increased yolk superoxide dismutase and glutathione peroxidase activities, with optimal effects at 3.6 g/kg. 4. Dietary astaxanthin compound additive supplementation increased yolk astaxanthin content, with optimal effects at 3.6 g/kg.

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