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Effects of Coated Acidifier and Small Peptide Chelated Iron on Production Performance, Trace Element Content in Egg Yolk, and Serum Immune and Antioxidant Indices of Laying Hens in Summer (Postprint)

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Abstract

This experiment was conducted to investigate the effects of coated acidifier and small peptide chelated iron and their interaction on production performance, trace element content in egg yolk, and serum immune and antioxidant indices of laying hens during the peak laying period in summer. A 2$×$4 two-factor completely randomized design was employed, with two supplemental levels of coated acidifier and four supplemental levels of small peptide chelated iron in the diets. A total of 576 healthy Roman pink-shell laying hens aged 38 weeks were randomly divided into 8 groups with 6 replicates per group and 12 hens per replicate. Groups A, B, C, and D were fed basal diets supplemented with 0 mg/kg coated acidifier and 0 (control), 0.04%, 0.08%, and 0.12% small peptide chelated iron (iron contents of 0, 60, 120, and 180 mg/kg), respectively; groups E, F, G, and H were fed basal diets supplemented with 300 mg/kg coated acidifier and 0, 0.04%, 0.08%, and 0.12% small peptide chelated iron (iron contents of 0, 60, 120, and 180 mg/kg), respectively. The preliminary period was 1 week, and the formal experimental period was 6 weeks. The results showed that: 1) Coated acidifier, small peptide chelated iron, and their interaction had no significant effects on average daily feed intake, laying rate, and feed-to-egg ratio of laying hens in week 3 and week 6 ($P>0.05$). The average daily feed intake of group G in week 3 and week 6 was significantly higher than that of the control group and groups C and D ($P<0.05$); the laying rate of group B in week 6 was significantly higher than that of the control group and groups D and E ($P<0.05$), and its feed-to-egg ratio was significantly lower than that of group D ($P<0.05$). Coated acidifier had significant effects on egg weight at the end of week 3, and yolk index and Haugh unit at the end of week 6 ($P<0.05$); small peptide chelated iron had a significant effect on yolk index at the end

of week 6 (P<0.05); the interaction between small peptide chelated iron and coated acidifier had an extremely significant effect on yolk index at the end of week 6 (P<0.01), and a significant effect on Haugh unit at the end of week 6 (P<0.05). At the end of week 3, the egg weight of group G was significantly higher than that of group B (P<0.05), and the yolk index of groups G and H was significantly higher than that of group C (P<0.05); at the end of week 6, the egg weight of groups C, D, and G was significantly higher than that of the control group (P<0.05), the yolk index of group F was significantly higher than that of groups C, D, and E (P<0.05), and the Haugh unit of groups E, F, and G was significantly higher than that of group D (P<0.05). 2) Small peptide chelated iron and the interaction between coated acidifier and small peptide chelated iron had extremely significant effects on iron content in egg yolk at the end of week 3 and week 6 (P<0.01). The iron content in yolk of groups B, C, D, F, G, and H at the end of week 3 and week 6 was extremely significantly higher than that of the control group and group E (P<0.01), and the iron content in yolk of group H at the end of week 3 was extremely significantly higher than that of groups B and F (P<0.01); the zinc content in yolk of group H at the end of week 3 was significantly higher than that of group F (P<0.05), and the zinc content in yolk of group B at the end of week 6 was significantly higher than that of group D (P<0.05). 3) Coated acidifier had an extremely significant effect on total superoxide dismutase (T-SOD) activity in serum (P<0.01) and a significant effect on serum malondialdehyde (MDA) content (P<0.05); the interaction between coated acidifier and small peptide chelated iron had an extremely significant effect on serum T-SOD activity (P<0.01). The serum immunoglobulin M (IgM) content of group C was significantly higher than that of the control group (P<0.05), and the serum T-SOD activity of groups D, E, F, G, and H was extremely significantly higher than that of the control group (P<0.01); the serum MDA content of groups B, F, and G was significantly lower than that of the control group and group E (P<0.05). In conclusion, dietary supplementation of 0.04% and 0.08% small peptide chelated iron alone or in combination with 300 mg/kg coated acidifier was beneficial for maintaining and prolonging the peak laying period of laying hens in summer, improving egg quality, increasing iron enrichment in eggs, and enhancing the antioxidant capacity of the body.

Full Text

Effects of Coated Acidifier and Small Peptide Chelate Iron on Performance, Contents of Trace Elements in Egg Yolk and Immune and Antioxidant Indices in Serum of Laying Hens in Summer

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Abstract: This experiment was conducted to investigate the effects of coated acidifier, small peptide chelate iron, and their interaction on production performance, trace element content in egg yolk, and serum immune and antioxidant indices of laying hens during peak production in summer. A 2$×$4 factorial completely randomized design was employed, with two supplementation levels of coated acidifier and four supplementation levels of small peptide chelate iron in the basal diets. A total of 576 healthy 38-week-old Roman pink-shell laying hens were randomly allocated to eight groups, each consisting of six replicates with twelve hens per replicate. Groups A, B, C, and D received basal diets supplemented with 0 mg/kg coated acidifier and 0 (control), 0.04%, 0.08%, and 0.12% small peptide chelate iron (providing 0, 60, 120, and 180 mg/kg Fe, respectively). Groups E, F, G, and H received basal diets supplemented with 300 mg/kg coated acidifier and 0, 0.04%, 0.08%, and 0.12% small peptide chelate iron (providing 0, 60, 120, and 180 mg/kg Fe, respectively). The pre-test period lasted one week, followed by a six-week experimental period.

The results showed that: (1) Coated acidifier, small peptide chelate iron, and their interaction had no significant effects on average daily feed intake (ADFI), laying rate, or feed-to-egg ratio at weeks 3 and 6 ($P>0.05$). However, ADFI in group G was significantly higher than in the control and groups C and D at both weeks 3 and 6 ($P<0.05$). Laying rate in group B was significantly higher than in the control and groups D and E at week 6 ($P<0.05$), with a significantly lower feed-to-egg ratio compared to group D ($P<0.05$). Coated acidifier significantly affected egg weight at week 3 and egg yolk index and Haugh unit at week 6 ($P<0.05$). Small peptide chelate iron significantly affected egg yolk index at week 6 ($P<0.05$), while the interaction between the two additives had highly significant effects on egg yolk index ($P<0.01$) and significant effects on Haugh unit ($P<0.05$) at week 6. At week 3, egg weight in group G was significantly higher than in group B ($P<0.05$), and egg yolk index in groups G and H was significantly higher than in group C ($P<0.05$). At week 6, egg weight in groups C, D, and G was significantly higher than in the control ($P<0.05$), egg yolk index in group F was significantly higher than in groups C, D, and E ($P<0.05$), and Haugh unit in groups E, F, and G was significantly higher than in group D ($P<0.05$).

(2) Small peptide chelate iron and its interaction with coated acidifier had highly significant effects on iron content in egg yolk at weeks 3 and 6 ($P<0.01$). Iron content in groups B, C, D, F, G, and H was significantly higher than in the control and group E at both time points ($P<0.01$). At week 3, iron content in group H was significantly higher than in groups B and F ($P<0.01$), while zinc content in group H was significantly higher than in group F ($P<0.05$). At week 6, zinc content in group B was significantly higher than in group D ($P<0.05$).

(3) Coated acidifier had highly significant effects on serum total superoxide dismutase (T-SOD) activity ($P<0.01$) and significant effects on serum malondialdehyde (MDA) content ($P<0.05$). The interaction between the two additives had highly significant effects on serum T-SOD activity ($P<0.01$). Serum immunoglobulin M (IgM) content in group C was significantly higher than in the control ($P<0.05$). Serum T-SOD activity in groups D, E, F, G, and H was significantly higher than in the control ($P<0.01$). Serum MDA content in groups B, F, and G was significantly lower than in the control and group E ($P<0.05$). In conclusion, dietary supplementation with 0.04% and 0.08% small peptide chelate iron alone or combined with 300 mg/kg coated acidifier is beneficial for maintaining and extending the peak laying period in summer, improving egg quality, enriching iron content in eggs, and enhancing antioxidant capacity.

Keywords: laying hens; coated acidifier; small peptide chelate iron; performance; trace element content in egg yolk; immune and antioxidant indices

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Introduction

Nutrient interactions in diets, namely synergistic or antagonistic effects, have long been a research focus in animal nutrition. Numerous studies have confirmed that rational combination of acidifiers with enzymes, probiotics, oligosaccharides, and other additives produces desirable outcomes. Acidifiers improve palatability, reduce dietary pH, promote gastric enzyme activation, regulate intestinal microflora balance, prevent pathogenic intestinal diseases, enhance mineral and vitamin absorption, boost immunity, and alleviate stress. However, studies on the combination of acidifiers and small peptide chelate iron have not been reported.

Coated acidifiers feature sustained-release and continuous acidification properties, making them more stable and effective than uncoated acidifiers. Laying hens have relatively high iron requirements, primarily to meet the needs of maternal tissues and embryonic development during incubation. Dietary iron can be effectively deposited in eggs without affecting other nutritional components or causing negative effects in hens. The NRC (1994) recommends 50-120 mg/kg iron in poultry diets, with a tolerance level of 2,000 mg/kg. Iron supplements have evolved from inorganic to organic forms, with small peptide chelate iron offering superior stability and safety. Iron in this form is rapidly absorbed by intestinal mucosa as small peptides, achieving higher absorption rates and biological efficacy than organic iron, which is significant for improving livestock performance and reducing environmental pollution.

This experiment investigated the effects of dietary supplementation with small peptide chelate iron and coated acidifier, individually and in combination, on production performance, trace element content in egg yolk, and serum immune and antioxidant indices of laying hens during summer peak production. The

study explored iron enrichment patterns in eggs and interactions with coated acidifiers to accumulate experience for further research on small peptide chelate iron application in livestock production and nutrient interactions, while providing scientific evidence for developing healthy laying hen production technologies and efficient, safe functional iron-enriched eggs.

1. Materials and Methods

1.1 Experimental Materials The coated acidifier was provided by Shenzhen Weiluwei Biotechnology Co., Ltd., containing $ $10% moisture with active ingredients of $ $200 g/kg lactic acid, $ $150 g/kg fumaric acid, $ $150 g/kg citric acid, and $ $20 g/kg L-malic acid. Small peptide chelate iron was provided by Omega Biotechnology Co., Ltd., as a soybean small peptide chelated trace element containing 2-3 amino acids (chelation rate $ $95%), with $ $15% iron content and $ $10% moisture.

1.2 Experimental Design and Management The trial was conducted at Hunan Tianxin Yellow Chicken Breeding Co., Ltd. from June to July 2015. A total of 576 healthy 38-week-old Roman pink-shell laying hens with similar body weight and performance were randomly divided into eight groups with six replicates of twelve hens each. Groups A, B, C, and D received basal diets supplemented with 0 mg/kg coated acidifier and 0 (control), 0.04%, 0.08%, and 0.12% small peptide chelate iron (providing 0, 60, 120, and 180 mg/kg Fe, respectively). Groups E, F, G, and H received basal diets supplemented with 300 mg/kg coated acidifier and 0, 0.04%, 0.08%, and 0.12% small peptide chelate iron (providing 0, 60, 120, and 180 mg/kg Fe, respectively). The pre-test period lasted one week, followed by a six-week experimental period.

The basal diet was formulated according to the *Feeding Standard of Chicken* (NY/T 33-2004) and NRC (1994) nutrient requirements for laying hens at peak production, with composition and nutrient levels shown in Table 1 . Hens were housed in three-tier step cages (upper, middle, lower) with replicates evenly distributed across all tiers in the same row. The house temperature was maintained at 24-32°C with 75-85% relative humidity. Feed and water were provided ad libitum with 16 hours of daily light (natural plus artificial). Routine management procedures were followed.

1.3 Measurement Indices and Methods 1.3.1 Production Performance

During the experimental period, daily records were kept by replicate for feed intake, egg number, egg weight, soft and broken eggs, and surviving hens. Weekly calculations were made for average daily feed intake, laying rate, and feed-to-egg ratio. At the ends of weeks 3 and 6, twelve eggs per group were collected for quality determination. Egg weight was measured using an analytical balance, yolk index with vernier calipers, and Haugh unit with an Egg Analyzer (Orka Technology Ltd.).

1.3.2 Trace Element Content in Egg Yolk

At the ends of weeks 3 and 6, twelve eggs per group were collected and stored at 4°C for trace element analysis. Iron, zinc, copper, and manganese contents in egg yolk were determined by flame atomic absorption spectrometry according to GB/T 5009.90-2003 and GB/T 9695.20-2008. Yolk samples were pretreated by wet digestion. Standard solutions were prepared following the operating instructions for the flame atomic absorption instrument (SP-AA3800) to establish calibration curves before measurement.

1.3.3 Serum Immune and Antioxidant Indices

At the end of the experiment, 5 mL blood samples were collected from the wing vein of twelve hens per group (two per replicate) at 08:00. After 30 minutes of inclined 静置, samples were centrifuged at 3,000 r/min for 10 minutes. The supernatant (0.5-1.0 mL) was transferred to 1.5 mL centrifuge tubes, labeled with group and date, and stored at -20°C for analysis. Serum glutathione peroxidase (GSH-Px), total superoxide dismutase (T-SOD) activity, total antioxidant capacity (T-AOC), and malondialdehyde (MDA) content were measured using a microplate reader (Multiskan GO), centrifuge, constant temperature water bath, and rapid mixer with kits from Nanjing Jiancheng Bioengineering Institute. Serum immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) contents were determined using an automatic biochemical analyzer (Mindray BS-200) with 配套试剂.

1.4 Statistical Analysis Experimental data were analyzed using the GLM procedure in SAS 9.2 software. Duncan' s multiple range test was used for inter-group comparisons. Results are expressed as means.

2. Results

2.1 Effects on Production Performance As shown in Table 2 , coated acidifier, small peptide chelate iron, and their interaction had no significant effects on ADFI, laying rate, or feed-to-egg ratio at weeks 3 and 6 ($P>0.05$). However, ADFI in group G was significantly higher than in the control and groups C and D at both weeks 3 and 6 ($P<0.05$). Laying rate in group B was significantly higher than in the control and groups D and E at week 6 ($P<0.05$), with a significantly lower feed-to-egg ratio compared to group D ($P<0.05$).

Coated acidifier significantly affected egg weight at week 3 and yolk index and Haugh unit at week 6 ($P<0.05$). Small peptide chelate iron significantly affected yolk index at week 6 ($P<0.05$). Their interaction had highly significant effects on yolk index ($P<0.01$) and significant effects on Haugh unit ($P<0.05$) at week 6. At week 3, egg weight in group G was significantly higher than in group B ($P<0.05$), while yolk index in groups G and H was significantly higher than in group C ($P<0.05$). At week 6, egg weight in groups C, D, and G was significantly higher than in the control ($P<0.05$), yolk index in group F was significantly

higher than in groups C, D, and E ($P<0.05$), and Haugh unit in groups E, F, and G was significantly higher than in group D ($P<0.05$).

2.2 Effects on Trace Element Content in Egg Yolk As shown in Table 3 , small peptide chelate iron and its interaction with coated acidifier had highly significant effects on iron content in egg yolk at weeks 3 and 6 ($P<0.01$). Iron content in groups B, C, D, F, G, and H was significantly higher than in the control and group E at both time points ($P<0.01$). At week 3, iron content in group H was significantly higher than in groups B and F ($P<0.01$), while zinc content in group H was significantly higher than in group F ($P<0.05$). At week 6, zinc content in group B was significantly higher than in group D ($P<0.05$).

2.3 Effects on Serum Immune and Antioxidant Indices As shown in Table 4 , coated acidifier had highly significant effects on serum T-SOD activity ($P<0.01$) and significant effects on serum MDA content ($P<0.05$). The interaction between the two additives had highly significant effects on serum T-SOD activity ($P<0.01$). Serum IgM content in group C was significantly higher than in the control ($P<0.05$). Serum T-SOD activity in groups D, E, F, G, and H was significantly higher than in the control ($P<0.01$). Serum MDA content in groups B, F, and G was significantly lower than in the control and group E ($P<0.05$).

3. Discussion

3.1 Effects on Production Performance As a healthy, residue-free feed additive, acidifiers improve gastrointestinal morphology and function, thereby maintaining intestinal health and production performance. Wei et al. found that dietary supplementation with 500 and 1,000 mg/kg coated compound acidifier significantly increased laying rate and decreased feed-to-egg ratio in Dongxiang blue-shell laying hens, while 250 mg/kg significantly improved yolk index. Chen et al. reported that 1.5 g/kg acidifier significantly reduced feed intake and feed-to-egg ratio in "Jinghong No. 1" laying hens at peak production on day 49, without affecting egg quality. However, Gül et al. found no significant effects of organic acids on performance in Roman laying hens. In this study, coated acidifier significantly affected egg weight at week 3 and yolk index and Haugh unit at week 6, indicating that it promoted nutrient deposition in Roman pink-shell eggs, improved egg freshness, and favored long-term egg storage in summer, consistent with Wei et al. and Kaya et al.

Amino acid chelated iron, as a new iron supplement, provides both amino acids and iron, improving poultry performance and feed utilization. Inkee et al. studied 65-week-old Hy-Line Brown hens and found that 100 mg/kg Fe-methionine significantly decreased daily laying rate and increased feed-to-egg ratio after 35 days, while 100 and 200 mg/kg Fe-soybean peptide significantly increased egg weight and Haugh unit. Bess et al. found no significant effects of meat-bone meal and amino acid chelated iron on broiler breeder egg production. Our re-

sults showed that small peptide chelate iron significantly affected yolk index at week 6, and its interaction with coated acidifier significantly affected yolk index and Haugh unit at week 6, improving egg freshness and quality, with the best results from combined supplementation of 300 mg/kg coated acidifier and 0.04% small peptide chelate iron. Supplementation with 0.08% and 0.12% small peptide chelate iron alone or combined with 300 mg/kg coated acidifier significantly increased egg weight at week 6, consistent with Inkee et al., possibly due to synergistic promotion of protein secretion and nutrient deposition without affecting egg quality. While no significant effects were observed on overall laying performance, there were positive trends, with 0.04% small peptide chelate iron alone significantly increasing laying rate and decreasing feed-to-egg ratio at week 6, suggesting this level is appropriate for summer diets to maintain and extend peak production. Additionally, combined supplementation of 300 mg/kg coated compound acidifier and 0.08% small peptide chelate iron significantly improved ADFI by enhancing diet palatability.

3.2 Effects on Trace Element Content in Egg Yolk Mineral metabolism in chickens is complex, influenced by dietary and non-nutritional factors that affect egg nutritional value, edibility, hatchability, and chick quality. While many studies have investigated iron enrichment in eggs, few have examined enrichment patterns. Bess et al. found that amino acid chelated iron significantly increased iron content in yolk of 33-38 and 39-42 week-old broiler breeders, but no further increase occurred at 43-46 weeks. Inkee et al. reported that 100 mg/kg Fe-soybean peptide significantly increased iron content at week 5, with both Fe sources significantly increasing zinc content without affecting iron content. Tang et al. also found that glycine chelated iron significantly increased yolk iron content. Our results showed that small peptide chelate iron and its interaction with coated acidifier had highly significant effects on yolk iron content at weeks 3 and 6, without significantly affecting zinc, copper, or manganese content, consistent with Bess et al. and Inkee et al. At week 3, yolk iron content increased linearly with dietary small peptide chelate iron level, while at week 6, iron content increased significantly then plateaued. This may occur because small peptide chelate iron is rapidly absorbed by intestinal mucosa as small peptides without interfering with other trace elements. However, as supplementation increases, iron deposition stabilizes, possibly due to homeostatic regulation in hens, with increased iron storage in liver and other tissues affecting egg enrichment. Alternatively, intestinal mucosal mechanisms may limit small peptide chelate iron transport, though the molecular mechanisms regulating transporter gene expression and function remain unclear and require further investigation. From the perspective of iron enrichment, dietary supplementation with 0.04% and 0.08% small peptide chelate iron synergistically promotes iron accumulation in yolk when combined with coated acidifier.

3.3 Effects on Serum Immune and Antioxidant Indices The three major immunoglobulins in poultry are IgA, IgG, and IgM. GSH-Px, T-SOD, and cata-

lase (CAT) are intracellular antioxidant enzymes that synergistically scavenge oxygen free radicals, reduce lipid peroxidation, and protect cellular structure and function. MDA is a lipid peroxidation degradation product that reflects the degree of lipid peroxidation and indirectly indicates cell damage. This experiment was conducted during hot, rainy summer conditions with house temperatures exceeding 30°C and high humidity and ammonia concentrations, which affected hen health and performance. Coated acidifier had highly significant effects on serum T-SOD activity and significant effects on serum MDA content, consistent with Wei et al. in Dongxiang blue-shell laying hens. Supplementation with 0.12% small peptide chelate iron alone significantly increased serum T-SOD activity, similar to Shi et al. who reported increased serum CAT activity in broilers fed glycine chelated iron, and Ma et al. who found increased liver CuZn-SOD activity in broilers fed glycine chelated iron, all enhancing antioxidant capacity through increased antioxidant enzyme activity. Supplementation with 0.04% small peptide chelate iron alone and combined with 300 mg/kg coated acidifier and 0.04% or 0.08% small peptide chelate iron significantly reduced serum MDA content, indicating that the combination improved antioxidant stress levels in summer laying hens. Supplementation with 0.08% small peptide chelate iron alone significantly increased serum IgM content, while no significant differences were observed in IgA or IgG among groups, suggesting that coated acidifier and small peptide chelate iron and their interaction do not affect normal immune levels in laying hens.

Conclusions

1. Dietary supplementation with 300 mg/kg coated acidifier alone improves egg freshness and enhances antioxidant capacity in summer laying hens.
2. Comprehensive evaluation indicates that dietary supplementation with 0.04% and 0.08% small peptide chelate iron alone or combined with 300 mg/kg coated acidifier is beneficial for maintaining and extending the peak laying period in summer, improving egg quality, enriching iron content in eggs, and enhancing antioxidant status.

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