Effects of Coated Acidifier and Small Peptide Chelated Iron and Their Interaction on Laying Performance, Egg Quality, and Serum Biochemical Indices of 38-44-Week-Old Laying Hens During Summer (Postprint)

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Date: 2017-10-23T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of coated acidifiers and small peptide chelated iron and their interaction on laying performance, egg quality, and serum biochemical indices of 38-44-week-old laying hens during summer. A 2$×$4 completely randomized experimental design was adopted, with two supplementation levels of coated acidifier (0 and 300 mg/kg) and four supplementation levels of small peptide chelated iron (0, 0.04%, 0.08%, and 0.12%) in the basal diet. A total of 576 healthy 38-week-old Roman pink-shell laying hens were selected and randomly divided into 8 groups, with 6 replicates per group and 12 hens per replicate. Groups A, B, C, and D were fed diets supplemented with 0 mg/kg coated acidifier and 0 (control group), 0.04%, 0.08%, or 0.12% small peptide chelated iron (iron contents of 0, 60, 120, and 180 mg/kg, respectively) in the basal diet, while groups E, F, G, and H were fed diets supplemented with 300 mg/kg coated acidifier and 0, 0.04%, 0.08%, or 0.12% small peptide chelated iron (iron contents of 0, 60, 120, and 180 mg/kg, respectively) in the basal diet. The preliminary period lasted for 1 week, and the formal experimental period lasted for 6 weeks.

The results showed: 1) Coated acidifier and the interaction between coated acidifier and small peptide chelated iron had significant effects on Haugh unit ($P < 0.05$). The total egg number in groups B and H was increased by 4.57% and 4.45% compared with the control group ($P > 0.05$), eggshell thickness in group F was significantly greater than that in the control group ($P < 0.05$), the feed-to-egg ratio in groups B and H was significantly lower than that in group

G ($P < 0.05$), and Haugh units in groups E, F, and G were significantly higher than that in group D ($P < 0.05$).

2) Coated acidifier had no significant effect on various biochemical indices in serum ($P > 0.05$), small peptide chelated iron had significant effects on serum calcium, phosphorus, and iron contents ($P < 0.05$), and the interaction between coated acidifier and small peptide chelated iron had significant effects on serum total cholesterol, calcium, and phosphorus contents ($P < 0.05$). Serum alkaline phosphatase activity in group D was significantly lower than that in the control group ($P < 0.05$), serum total cholesterol content in group H was significantly lower than that in groups B and C ($P < 0.05$) and decreased by 15.48% compared with the control group ($P > 0.05$), serum calcium content in groups B and F was significantly higher than that in group E ($P < 0.05$), serum phosphorus content in group C was significantly higher than that in group E ($P < 0.05$), and serum iron content in group C was significantly higher than that in the control group and groups E and H ($P < 0.05$).

In conclusion, supplementing 0.04% small peptide chelated iron alone in the diet or its combined use with 300 mg/kg coated acidifier is beneficial for improving laying performance, egg quality, and serum biochemical indices of 38-44-week-old laying hens during summer.

Full Text

Effects of Coated Acidifier and Small Peptide Chelated Iron and Their Interaction on Laying Performance, Egg Quality and Serum Biochemical Parameters of 38- to 44-Week-Old Laying Hens in Summer

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Abstract

This experiment was conducted to evaluate the effects of coated acidifier and small peptide chelated iron and their interaction on laying performance, egg quality, and serum biochemical parameters of 38- to 44-week-old laying hens during summer. A 2$×$4 factorial completely randomized design was adopted, with two supplemental levels of coated acidifier (0 and 300 mg/kg) and four supplemental levels of small peptide chelated iron (0, 0.04%, 0.08%, and 0.12%) in the basal diet. A total of 576 healthy 38-week-old Lohmann pink-shell laying

hens were randomly allocated to 8 groups with 6 replicates per group and 12 hens per replicate. Groups A, B, C, and D were fed the basal diet supplemented with 0 mg/kg coated acidifier and 0 (control), 0.04%, 0.08%, or 0.12% small peptide chelated iron (providing 0, 60, 120, and 180 mg/kg iron, respectively). Groups E, F, G, and H were fed the basal diet supplemented with 300 mg/kg coated acidifier and 0, 0.04%, 0.08%, or 0.12% small peptide chelated iron (providing 0, 60, 120, and 180 mg/kg iron, respectively). The adjustment period lasted for 1 week, followed by a 6-week experimental period.

The results showed: (1) Coated acidifier and the interaction between coated acidifier and small peptide chelated iron had significant effects on Haugh unit ($P<0.05$). Total egg number in groups B and H increased by 4.57% and 4.45%, respectively, compared with the control group ($P>0.05$). Eggshell thickness in group F was significantly greater than in the control group ($P<0.05$). Feed-to-egg ratio in groups B and H was significantly lower than in group G ($P<0.05$), while group B showed a 4.29% reduction compared with the control ($P>0.05$). Haugh units in groups E, F, and G were significantly higher than in group D ($P<0.05$). (2) Coated acidifier had no significant effects on serum biochemical parameters ($P>0.05$). Small peptide chelated iron significantly affected serum calcium, phosphorus, and iron contents ($P<0.05$). The interaction between coated acidifier and small peptide chelated iron significantly affected serum total cholesterol, calcium, and phosphorus contents ($P<0.05$). Serum alkaline phosphatase activity in group D was significantly lower than in the control group ($P<0.05$). Serum total cholesterol content in group H was significantly lower than in groups B and C ($P<0.05$), and decreased by 15.48% compared with the control group ($P>0.05$). Serum calcium content in groups B and F was significantly higher than in group E ($P<0.05$). Serum phosphorus content in group C was significantly higher than in group E ($P<0.05$). Serum iron content in group C was significantly higher than in the control group and groups E and H ($P<0.05$).

In conclusion, dietary supplementation with 0.04% small peptide chelated iron alone or in combination with 300 mg/kg coated acidifier is beneficial for improving laying performance, egg quality, and serum biochemical parameters of 38- to 44-week-old laying hens during summer.

Keywords: laying hens; coated acidifier; small peptide chelated iron; laying performance; egg quality; serum biochemical parameters

Introduction

Animal nutritional status influences immune function and disease resistance, while health status also affects nutritional requirements [1-2]. Research has confirmed that acidifiers can reduce feed pH and acid-binding capacity, activate gastric proenzymes, regulate intestinal microecological balance, prevent enteric pathogenic microbial diseases, promote nutrient digestion and absorp-

tion, enhance immunity, and alleviate stress [3-4]. Coated acidifiers feature sustained-release and continuous acidification properties, demonstrating more stable and effective application than uncoated inorganic or organic acids. Iron is one of the most important trace elements for livestock and poultry, serving as a component of hemoglobin, myoglobin, cytochromes, and various oxidases. It participates in normal oxygen transport within body tissues and directly affects protein and energy metabolism, indirectly influencing immune function. Laying hens primarily obtain iron from their diet, and both excessive and insufficient iron intake can affect their health. The NRC (1994) [5] recommends dietary iron content of 50-120 mg/kg for poultry, with a tolerance level of 2,000 mg/kg. As a novel nutritional iron additive, small peptide chelated iron offers higher stability, safety, absorption efficiency, and biological value compared with inorganic and organic iron sources, showing great potential and economic benefits for improving livestock performance, health status, and reducing environmental pollution [6-8].

This experiment leveraged the advantages of coated acidifier and small peptide chelated iron by supplementing them in laying hen diets to investigate their individual and interactive effects on laying performance, egg quality, and serum biochemical parameters of 38- to 44-week-old laying hens during summer. The study aims to accumulate experience for further research on the production application of small peptide chelated iron in laying hens and nutrient interactions in feed, while providing a scientific basis for developing healthy laying hen production technologies.

1.1 Test Materials

Coated acidifier: Provided by Shenzhen Wellhope Bio-Tech Co., Ltd., containing $ $200 g/kg lactic acid, $ $150 g/kg fumaric acid, $ $150 g/kg citric acid, $ $20 g/kg L-malic acid, and $ $10% moisture.

Small peptide chelated iron: Provided by Omega Biotech Co., Ltd., 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 experiment was conducted from June to July 2015 at the Hunan Tianxin Yellow Chicken Breeding Co., Ltd. farm. A total of 576 healthy 38-week-old Lohmann pink-shell laying hens at peak production [laying rate (92.16$±$2.54)%] with similar body weight and performance were randomly divided into 8 groups with 6 replicates per group and 12 hens per replicate. Groups A, B, C, and D were fed the basal diet supplemented with 0 mg/kg coated acidifier and 0 (control), 0.04%, 0.08%, or 0.12% small peptide chelated iron (providing 0, 60, 120, and 180 mg/kg iron, respectively). Groups E, F, G, and H were fed the basal diet supplemented with 300 mg/kg coated acidifier and 0, 0.04%, 0.08%, or 0.12% small peptide chelated iron (providing 0, 60,

120, and 180 mg/kg iron, respectively). The adjustment period lasted 1 week, followed by a 6-week experimental period.

The basal diet was formulated according to the *Feeding Standard of Chicken* (NY/T 33-2004) [9] and NRC (1994) [5] nutrient requirements for poultry. The composition and nutrient levels are shown in Table 1 . The experimental hens were housed in three-tier step cages (upper, middle, lower), with replicates evenly distributed across all tiers in the same row of the chicken house. The house temperature ranged from 24 to 32°C with relative humidity of 75%-85%. Hens had free access to feed and water, with 16 hours of daily lighting combining natural and artificial light. Cleaning and disinfection followed routine farm management procedures.

1.3.1 Laying Performance and Egg Quality

During the experimental period, daily records were kept for each replicate regarding feed intake, egg number, egg weight, soft-shelled and broken eggs, and surviving hens. Average egg weight, total egg number, average daily feed intake, feed-to-egg ratio, and mortality rate were calculated for the entire experimental period.

On the final day of the experiment, 12 eggs per group were collected for egg quality determination. Eggshell thickness was measured using an eggshell thickness gauge from Orka Food Technology. Egg weight and yolk weight were measured using an analytical balance to calculate yolk ratio (100 × yolk weight/egg weight). Haugh unit was determined using an egg quality tester from Orka Food Technology.

Table 1 Composition and nutrient levels of the basal diet (air-dry basis) %

Items	Content
Ingredients	
Corn	65.00
Soybean meal	23.00
Limestone	8.00
Premix[1]	4.00
Total	**100.00**
Nutrient levels[2]	
ME/(MJ/kg)	11.10
CP	16.50
Ca	3.50
AP	0.35
Lys	0.75
Met	0.35
Met+Cys	0.65

[1]The premix provided the following per kg of diet: VA 6,000 IU, VD_3 2,500 IU, VE 25 IU, VK_3 2.25 mg, VB_{12} 0.18 mg, VB_6 4 mg, VB_2 5.5 mg, VB_1 1.75 mg, pantothenate 12 mg, phytase 400 IU, biotin 0.14 mg, nicotinic acid 34 mg, folic acid 0.8 mg, chloride 350 mg, NaCl 3.7 g, Ca 5 g, P 1 g, Cu 7.5 mg, Se 0.15 mg, Fe 75 mg, Zn 60 mg, Mn 60 mg, I 0.35 mg.
[2]Nutrient levels were calculated values.

1.3.2 Serum Biochemical Parameters

On the final experimental day at 08:00, blood samples were collected from fasting hens. Twelve hens per group were randomly selected by replicate for wing vein blood collection (5 mL). Blood collection tubes were tilted and left to stand for 30 minutes, then centrifuged at 3,000 rpm 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 alkaline phosphatase (ALP) activity and contents of total cholesterol (TC), triglycerides (TG), uric acid (UA), glucose (Glu), calcium, phosphorus, and iron were determined using an automatic biochemical analyzer (Mindray BS-200) with corresponding reagents.

1.4 Statistical Analysis

Experimental data were analyzed using the GLM procedure of SAS 9.2 statistical software. Duncan's multiple range test was used for inter-group comparisons. Results are expressed as means and standard error of the mean (SEM).

2.1 Effects of Coated Acidifier and Small Peptide Chelated Iron and Their Interaction on Laying Performance and Egg Quality of Laying Hens in Summer

As shown in Table 2 , except for the significant effects of coated acidifier and the interaction between coated acidifier and small peptide chelated iron on Haugh unit ($P<0.05$), neither coated acidifier nor small peptide chelated iron nor their interaction significantly affected average egg weight, total egg number, average daily feed intake, feed-to-egg ratio, mortality rate, eggshell thickness, or yolk ratio ($P>0.05$). Total egg number in groups B and H increased by 4.57% and 4.45%, respectively, compared with the control group ($P>0.05$). Eggshell thickness in group F was significantly greater than in the control group ($P<0.05$). Feed-to-egg ratio in groups B and H was significantly lower than in group G ($P<0.05$), with group B showing a 4.29% reduction compared with the control ($P>0.05$). Haugh units in groups E, F, and G were significantly higher than in group D ($P<0.05$).

Table 2 Effects of coated acidifier and small peptide chelated iron and their interaction on laying performance and egg quality of laying hens in summer

Groups	Small peptide chelated iron/%	Coated acidifier/(mg/kg)	Average egg weight/g	Total egg number	Average daily intake/g	Feed to egg ratio	Death culling rate/%	Egg shell thickness/mm	Yolk ratio/%	Haugh unit
A	0	0	58.70	539.52	118.42	2.10abc	2.22	0.442b	25.10	67.47ab
B	0.04	0	58.73	564.16	119.95	2.01c	2.22	0.471ab	25.80	68.01ab
C	0.08	0	58.57	517.76	119.15	2.09abc	6.67	0.472ab	26.40	70.25ab
D	0.12	0	58.75	512.96	119.47	2.19ab	4.44	0.477ab	25.60	61.93b
E	0	300	58.55	510.08	120.10	2.14abc	6.67	0.473ab	26.50	73.80a
F	0.04	300	58.73	547.20	123.48	2.12abc	4.44	0.492a	27.70	74.84a
G	0.08	300	58.73	538.56	124.91	2.22a	2.22	0.483ab	24.10	73.61a
H	0.12	300	58.63	563.52	116.62	2.04bc	0.00	0.466ab	27.00	67.96ab
Main effects										
Small peptide chelated iron/%										
0			58.63	524.80	119.26	2.12	4.44	0.458	25.80	70.64
0.04			58.73	555.68	121.72	2.07	3.33	0.482	26.75	71.43
0.08			58.65	528.16	122.03	2.16	4.44	0.478	25.25	71.93
0.12			58.69	538.24	118.05	2.12	2.22	0.472	26.30	64.95
Coated acidifier/(mg/kg)										
0			58.69	533.60	119.25	2.10	3.89	0.466	25.73	66.92b
300			58.66	539.84	121.28	2.13	3.33	0.479	26.42	72.55a
P-value of two-way ANOVA										
Coated acidifier			0.923	0.728	0.406	0.648	0.637	0.286	0.558	0.002

Groups	Small peptide chelated iron/%	Coated acidifier/(mg/kg)	Average egg weight/g	Total egg number	Average daily intake/g	Feed to egg ratio	Death culling rate/%	Egg shell thickness/mm	Yolk ratio/%	Haugh unit
Small peptide chelated iron			0.985	0.628	0.532	0.719	0.679	0.326	0.596	0.061
Small peptide chelated iron × coated acidifier			0.999	0.543	0.589	0.234	0.593	0.520	0.178	0.045

In the same column, values with different small letter superscripts indicate significant differences (P<0.05), and different capital letter superscripts indicate highly significant differences (P<0.01), while the same or no letter superscripts indicate no significant difference (P>0.05). The same applies below.

2.2 Effects of Coated Acidifier and Small Peptide Chelated Iron and Their Interaction on Serum Biochemical Parameters of Laying Hens in Summer

As shown in Table 3 , coated acidifier had no significant effects on serum biochemical parameters (P>0.05). Small peptide chelated iron significantly affected serum calcium, phosphorus, and iron contents (P<0.05) but showed no significant effects on other serum biochemical parameters (P>0.05). Serum triglyceride, calcium, phosphorus, and iron contents exhibited a trend of initial increase followed by decrease with increasing levels of small peptide chelated iron. The interaction between coated acidifier and small peptide chelated iron significantly affected serum total cholesterol, calcium, and phosphorus contents (P<0.05) but showed no significant effects on other serum biochemical parameters (P>0.05). Serum alkaline phosphatase activity in group D was significantly lower than in the control group (P<0.05). Serum total cholesterol content in group H was significantly lower than in groups B and C (P<0.05) and decreased by 15.48% compared with the control group (P>0.05). Serum calcium content in groups B and F was significantly higher than in group E (P<0.05). Serum phosphorus content in group C was significantly higher than in group E (P<0.05). Serum

iron content in group C was significantly higher than in the control group and groups E and H (P<0.05).

Table 3 Effects of coated acidifier and small peptide chelated iron and their interaction on serum biochemical parameters of laying hens in summer

Groups	Small peptide chelated iron/%	Coated acidifier/(mg/kg)	ALP/(U/L)	TC/(mmol/L)	TG/(mmol/L)	UA/(μmol/L)	Glu/(mmol/L)	Ca/(mmol/L)	P/(mmol/L)	Fe/(mg/L)
A	0	0	728.53	2.52ab	4.01ab	321.25	9.91b	2.41ab	3.57b	4.91a
B	0.04	0	454.65	2.97a	4.74a	326.50	14.68ab	3.04ab	4.19ab	1.83b
C	0.08	0	513.24	2.99a	4.57ab	328.75	20.98a	3.76a	4.52a	2.12b
D	0.12	0	318.13	2.55ab	4.42ab	318.75	12.14ab	2.48ab	4.83a	3.08a
E	0	300	699.10	2.32ab	4.42ab	319.50	10.50b	2.59ab	3.13ab	15.92ab
F	0.04	300	453.13	2.90ab	4.91a	327.25	15.92ab	3.57b	4.47ab	2.43ab
G	0.08	300	577.12	2.87ab	4.47ab	329.00	14.68ab	2.59ab	4.19ab	10.94b
H	0.12	300	576.74	2.13b	4.83a	328.50	10.20b	2.42ab	4.31ab	11.54b
Main effects										
Small peptide chelated iron/%										
0			713.82	2.42	4.22	320.38	10.21	2.50	3.35	10.42
0.04			453.89	2.94	4.73	326.88	15.30	3.31	4.33	2.13
0.08			545.18	2.63	4.52	328.88	17.83	3.18	4.36	6.53
0.12			447.44	2.34	4.63	323.63	11.17	2.45	4.57	7.31
Coated acidifier/(mg/kg)										
0			503.64	2.76	4.44	323.81	14.43	2.92	4.28	2.99
300			576.52	2.41	4.66	326.06	12.83	2.79	4.03	10.21
P-value of two-way ANOVA										
Coated acidifier			0.426	0.110	0.521	0.783	0.517	0.677	0.528	0.101

Groups	Small peptide chelated iron/%	Coated acidifier/(mg/kg)	ALP/(U/L)	TC/(mmol/L)	TG/(mmol/L)	UA/(μmol/L)	Glu/(mmol/L)	Crea/(μmol/L)	BUN/(mmol/L)	Ca/(mg/L)
Small peptide chelated iron			0.069	0.082	0.528	0.699	0.005	0.001	0.001	0.001
Small peptide chelated iron × coated acidifier			0.426	0.049	0.521	0.783	0.517	0.001	0.001	0.101

3.1 Effects of Coated Acidifier and Small Peptide Chelated Iron and Their Interaction on Laying Performance and Egg Quality of Laying Hens in Summer

Research indicates that hens at peak production experience production stress, exhibit the most vigorous reproductive performance and metabolic levels, have relatively weak resistance, and are susceptible to environmental and nutritional factors. During summer, elevated house temperature, humidity, and ammonia concentration can lead to decreased health and production levels and increased mortality [10]. As a green feed additive, appropriate dietary supplementation of acidifiers in summer can improve acid-base balance and gastrointestinal morphology and function, enhancing stress resistance [11]. Small peptide chelated iron, as a novel nutritional iron additive, can simultaneously supply amino acids and iron, potentially improving laying performance and health status. This experiment found that although differences in mortality rate and total egg number among groups were not statistically significant, the group receiving combined supplementation of 300 mg/kg coated acidifier and 0.12% small peptide chelated iron showed 0% mortality and a 4.45% increase in total egg number compared with the control group, indicating that combined supplementation is beneficial for reducing mortality and maintaining or improving laying performance during peak production in summer.

Dietary supplementation with 0.04% small peptide chelated iron alone reduced feed-to-egg ratio by 4.29% and increased total egg number by 4.57% compared with the control, suggesting improved production efficiency and feed conversion

during peak production in summer. These results align with Bess et al. [12], who supplemented broiler breeder diets with meat and bone meal and amino acid chelated iron, and Tang et al. [13], who supplemented Dongxiang black-shell laying hens with ferrous glycinate. The significant interaction between coated acidifier and small peptide chelated iron on Haugh unit observed in this study is consistent with Paik et al. [14], who reported that supplementing 65-week-old Hy-Line Brown laying hens with 100 or 200 mg/kg iron chelated soy peptide (Fe-SP) significantly improved egg weight and Haugh unit compared with a basal diet containing 52.5 mg/kg inorganic iron. This demonstrates that nutritional measures can improve egg quality to some extent. Coated acidifier and small peptide chelated iron may synergistically enhance gastrointestinal nutrient absorption capacity and health status, thereby improving egg freshness, though the underlying mechanisms require further investigation.

In this study, combined supplementation of 300 mg/kg coated acidifier and 0.04% small peptide chelated iron significantly improved eggshell thickness, consistent with findings by Wei et al. [15] in Dongxiang green-shell laying hens supplemented with coated compound acidifier and Liu et al. [16] in Lohmann brown laying hens supplemented with acidifier (main components: butyrin, clove oil, cinnamon oil, and oregano oil). This improvement may be attributed to enhanced dietary calcium and phosphorus absorption and deposition. However, Chen et al. [17] found that dietary supplementation with 1.5 g/kg organic acidifier had no significant effects on egg quality in peak-production "Jinghong No. 1" laying hens. These discrepancies may result from differences in basal diet, breed, production stage, and acidifier type.

3.2 Effects of Coated Acidifier and Small Peptide Chelated Iron and Their Interaction on Serum Biochemical Parameters of Laying Hens in Summer

Serum biochemical parameters are closely related to poultry metabolism, nutritional status, and disease, serving as sensitive indicators of physiological condition. Serum alkaline phosphatase is a zinc-containing enzyme produced by osteoblasts that reflects liver and bone physiological function. Serum total cholesterol and triglyceride contents reflect lipid metabolism levels. Uric acid, the final product of purine metabolism in poultry, is widely present in chicken plasma and tissues, scavenging approximately two-thirds of plasma free radicals. It is associated with gout and serves as a sensitive indicator of kidney damage [18]. Serum glucose, regulated by insulin and glucagon, reflects carbohydrate metabolism status. Serum calcium and phosphorus contents reflect their absorption and utilization, while serum iron content is a sensitive indicator of iron deficiency.

This study showed that coated acidifier had no significant effects on serum biochemical parameters, consistent with Li et al. [19] in Wenchang chickens supplemented with compound organic acids, but inconsistent with Wei et al. [15], who reported that coated compound acidifier significantly increased serum uric

acid, glucose, and calcium contents in Dongxiang green-shell laying hens. These differences may be attributed to breed, season, and acidifier dosage. Small peptide chelated iron significantly affected serum calcium, phosphorus, and iron contents, and its interaction with coated acidifier significantly influenced serum total cholesterol, calcium, and phosphorus contents. The combined supplementation of 300 mg/kg coated acidifier and 0.12% small peptide chelated iron reduced serum total cholesterol by 15.48% compared with the control, indicating enhanced lipid metabolism. Supplementation with 0.08% small peptide chelated iron alone significantly increased serum iron content compared with the control, demonstrating improved dietary iron absorption. Supplementation with 0.12% small peptide chelated iron alone significantly reduced serum alkaline phosphatase activity, suggesting alleviated bone damage. Overall, dietary supplementation with coated acidifier and small peptide chelated iron promoted the absorption and metabolism of calcium, phosphorus, iron, and other nutrients, improved serum biochemical parameters, and enhanced health status, consistent with results from Shi et al. [20] and Ma et al. [21] in broilers supplemented with iron glycine chelate.

Conclusions

1. Dietary supplementation with 0.04% small peptide chelated iron alone or combined with 300 mg/kg coated acidifier is beneficial for maintaining and improving laying performance and health status of laying hens at peak production.
2. Supplementation with small peptide chelated iron at 0.04% and 0.08% levels promotes calcium, phosphorus, and iron absorption. Combined supplementation with 300 mg/kg coated acidifier further improves egg freshness and eggshell thickness.
3. Considering all factors, dietary supplementation with 0.04% small peptide chelated iron alone or in combination with 300 mg/kg coated acidifier is beneficial for improving laying performance, egg quality, and serum biochemical parameters of 38- to 44-week-old laying hens during summer.

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