Effects of Pyrroloquinoline Quinone Disodium Salt on Production Performance, Antioxidant Status, and Plasma Biochemical Parameters in Laying Hens (Postprint)

Authors: Wang Jing, Yang Linlin, Zhang Haijun, Wu Shugeng, Qi Guanghai

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Abstract

This study investigated the effects of different doses of pyrroloquinoline quinone disodium (PQQ · Na2) on production performance, antioxidant status, and plasma biochemical indices in laying hens, aiming to evaluate the biological safety of PQQ · Na2 in laying hens. A total of 540 healthy 25-week-old Hy-Line Gray laying hens were selected and randomly divided into 5 groups, with 6 replicates per group and 18 hens per replicate. The five groups of laying hens were fed five experimental diets supplemented with 0 (control), 0.04, 0.08, 0.12 mg/kg PQQ · Na2, and 200 mg/kg vitamin E, respectively. The experiment consisted of a 1-week pre-trial period and a 24-week formal trial period. The results showed: 1) Compared with the control group, dietary supplementation with 0.04~0.12 mg/kg PQQ · Na2 had no significant effect on the production performance of laying hens ($P<0.05$); 2) At week 24 of the experiment, compared with the control group, dietary supplementation with 0.04~0.12 mg/kg PQQ · Na2 significantly increased the Haugh unit of eggs ($P<0.05$), and supplementation with 0.08 mg/kg PQQ · Na2 also significantly increased eggshell thickness ($P<0.05$); 3) Compared with the control group, dietary supplementation with 0.08 and 0.12 mg/kg PQQ · Na2 significantly increased plasma glutathione peroxidase (at week 12) and total superoxide dismutase activities (at week 2 and week 24) ($P<0.05$); 4) Compared with the control group, dietary supplementation with 0.08 mg/kg PQQ · Na2 significantly decreased cardiac carbonyl content ($P<0.05$) and significantly increased hepatic total antioxidant capacity ($P<0.05$), and supplementation with 0.12 mg/kg PQQ · Na2 also significantly decreased hepatic malondialdehyde content ($P<0.05$). These experimental results suggest that dietary supplementation with 0.08~0.12 mg/kg PQQ · Na2 had no adverse effects on the production performance of laying hens and could improve antioxidant function. Taking all

factors into consideration, the appropriate supplementation level of PQQ · Na2 in laying hen diets was determined to be 0.08 mg/kg.

Full Text

Effects of Pyrroloquinoline Quinone Disodium on Performance, Antioxidant Status, and Plasma Biochemical Parameters of Laying Hens

WANG Jing, YANG Linlin, ZHANG Haijun, WU Shugeng, QI Guang-hai*

(Feed Research Institute, Chinese Academy of Agricultural Sciences; Key Laboratory of Feed Biotechnology, Ministry of Agriculture; National Engineering Research Center of Biological Feed, Beijing 100081, China)

Abstract

This study investigated the effects of different dietary levels of pyrroloquinoline quinone disodium (PQQ · Na) on production performance, antioxidant status, and plasma biochemical parameters of laying hens to evaluate the biological safety of PQQ · Na in laying hens. A total of 540 healthy 25-week-old Hyline Grey laying hens were randomly divided into 5 groups with 6 replicates of 18 birds each. The five groups were fed five experimental diets supplemented with 0 (control), 0.04, 0.08, 0.12 mg/kg PQQ · Na , and 200 mg/kg vitamin E, respectively. The trial consisted of a 1-week adaptation period followed by a 24-week experimental period. The results showed that: 1) dietary supplementation with 0.04–0.12 mg/kg PQQ · Na did not significantly affect the production performance of laying hens compared with the control group ($P>0.05$); 2) at week 24, dietary supplementation with 0.04–0.12 mg/kg PQQ · Na significantly increased Haugh unit ($P<0.05$), and supplementation with 0.08 mg/kg PQQ · Na also significantly increased eggshell thickness ($P<0.05$); 3) compared with the control group, dietary supplementation with 0.08 and 0.12 mg/kg PQQ · Na significantly increased plasma glutathione peroxidase (GSH-Px) activity (week 12) and total superoxide dismutase (T-SOD) activity (week 2 and 24) ($P<0.05$); 4) compared with the control group, dietary supplementation with 0.08 mg/kg PQQ · Na significantly decreased heart carbonyl content and significantly increased hepatic total antioxidant capacity (T-AOC) ($P<0.05$), and supplementation with 0.12 mg/kg PQQ · Na also significantly decreased hepatic malondialdehyde (MDA) content ($P<0.05$). These results suggest that dietary supplementation with 0.08–0.12 mg/kg PQQ · Na had no adverse effects on production performance but improved antioxidant function. Based on comprehensive consideration, the appropriate supplemental level of PQQ · Na in laying hen diets is 0.08 mg/kg.

Keywords: pyrroloquinoline quinone disodium; laying hens; antioxidant; plasma biochemical parameters; egg quality

In production practice, laying hens are frequently subjected to various stressors that disrupt the redox balance. Additionally, oxidative stress contributes to secondary damage from multiple pathogenic factors, thereby affecting bird health, production performance, egg quality, and safety. Currently, antioxidants commonly used in diets, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are primarily chemically synthesized compounds. The development and application of safe and efficient natural antioxidants have become an important research direction in poultry production, holding significant meaning for safeguarding laying hen health and egg quality while achieving nutritional regulation of laying hens.

Pyrroloquinoline quinone (PQQ), chemically known as 4,5-dihydro-4,5-dioxo-1H-pyrrolo[2,3-f]quinoline-2,7,9-tricarboxylic acid, is a redox enzyme cofactor discovered in microorganisms following niacinamide and riboflavin, and is widely distributed in various biological tissues. PQQ exhibits multiple biological functions including neurotrophic and protective effects, prevention of cardiac and hepatic injury, cataract prevention, anti-cancer, anti-inflammatory, and anti-aging activities. These functions are primarily associated with its unique antioxidant properties and activation of cell signaling pathways. Cellular and animal studies have demonstrated that PQQ can alleviate and inhibit oxidative damage caused by free radicals, such as carbon tetrachloride-induced liver injury [1], radiation damage [2], and hypoxia/glucose-induced cardiomyocyte apoptosis [3]. Brain injury and neurodegenerative diseases are related to excessive reactive oxygen species (ROS) production, and the neuroprotective effects of PQQ are associated with ROS scavenging and mitigation of mitochondrial oxidative stress [4-5]. The potential mechanisms of PQQ' s antioxidant action include: 1) donating electrons to reduce free radicals directly; 2) reacting with harmful substances through its electrophilic properties to form stable products; and 3) activating signaling pathways to induce antioxidant enzyme production, thereby enhancing cellular resistance to oxidative stress and protecting tissues from oxidative damage [6]. In North America, PQQ has been approved as a natural antioxidant dietary supplement, with its disodium salt form—pyrroloquinoline quinone disodium (PQQ · Na)—being the most widely used.

Our research group has conducted several studies on PQQ · Na in laying hen production in recent years. The results demonstrated that PQQ · Na can alleviate lipid metabolism and hormone secretion disorders induced by high-energy, low-protein diets, and mitigate associated production performance declines [7]. Under oxidized oil stress conditions, PQQ · Na prevents liver damage by scavenging free radicals, inhibiting lipid peroxidation, and enhancing the body' s antioxidant defense system [6,8]. In hepatic steatosis and oxidative stress cell models, PQQ · Na improves cell survival and reduces hepatocyte steatosis or oxidative stress damage by alleviating intracellular oxidative stress and promoting mitochondrial synthesis while maintaining mitochondrial function [9]. Additionally, PQQ · Na can improve albumen quality during egg storage, delay

egg oxidation, and extend shelf life. Under artificial oxygen-enriched heating conditions, the rapid oxidation time was delayed by 115 minutes compared with the control group [10]. As a novel antioxidant product, it is necessary to clarify the effects of long-term PQQ·Na use on target animal physiological status and production performance. Therefore, this 24-week production trial was conducted to observe the effects of dietary PQQ·Na supplementation on laying hen performance and antioxidant status, monitor changes in plasma biochemical parameters, and determine the appropriate supplemental level of PQQ·Na in laying hen diets, thereby providing a reference basis for the application of PQQ·Na as a safe and effective antioxidant in laying hen production.

1.1 Experimental Material

The PQQ·Na preparation was provided by Shanghai Medical Life Science Research Center Co., Ltd., produced through microbial fermentation and purification, with a PQQ·Na content of 1‰.

1.2 Experimental Design and Diets

A total of 540 healthy 25-week-old Hyline Grey laying hens were selected and randomly divided into 5 groups with 6 replicates of 18 birds each. Three hens were housed per cage (45 cm × 45 cm × 45 cm). Statistical analysis revealed no significant differences among groups in body weight, laying rate, average egg weight, etc. ($P>0.05$). The trial consisted of a 1-week adaptation period followed by a 24-week experimental period. Based on NRC (1994) and NY/T 33-2004 standards, combined with the "Hyline Grey Laying Hen Feeding Manual," a corn-soybean meal basal diet was formulated. The composition and nutrient levels are shown in Table 1 . Five experimental diets were prepared by supplementing the basal diet with 0, 0.04, 0.08, 0.12 mg/kg PQQ·Na , and 200 mg/kg vitamin E, respectively.

1.3 Housing and Management

The hens were raised in a semi-open four-tier cage system. Lighting consisted of natural light supplemented with artificial light for 16 h/d at an intensity of 20 lx. Relative humidity was maintained at 50%–90%. Ventilation combined natural ventilation with longitudinal negative pressure ventilation. Temperature was controlled at (22±3)°C. Diets were provided as dry powder, fed twice daily with four redistributions. Feed and water were provided ad libitum. Dedicated personnel managed the facility, with eggs collected twice daily. Chickens were disinfected once weekly, manure was removed twice daily, and routine disease prevention and immunization protocols were followed.

1.4.1 Measurement of Production Performance

Egg number, egg weight, and mortality were recorded daily by replicate, while feed consumption was recorded weekly. These data were used to calculate weekly

and overall laying rate, average daily feed intake, average egg weight, feed-to-egg ratio, and mortality rate.

1.4.2 Measurement of Egg Quality

At the end of weeks 2, 12, and 24 of the trial, 10 eggs per replicate were collected to determine eggshell strength, eggshell thickness, Haugh unit, and yolk color. Yolk color and Haugh unit were measured using an egg quality analyzer (ORKA Food Technology Ltd., Israel). Eggshell thickness was measured with an eggshell thickness gauge (ESTG-1, ORKA Food Technology Ltd., Israel), and eggshell strength was determined using an eggshell force analyzer (Egg Force Reader, ORKA Food Technology Ltd., Israel).

1.4.3 Measurement of Plasma and Tissue Antioxidant Indices

At the end of weeks 2, 12, and 24 of the trial, two laying hens with similar body weight were randomly selected from each group. Blood was collected from the wing vein under fasting and sterile conditions into heparin sodium anticoagulant vacuum tubes. Plasma was prepared by centrifugation at 3,000 r/min for 10 min and stored at -20°C for subsequent antioxidant index analysis. At the end of week 24, one hen per replicate was slaughtered. After bleeding, the heart and liver were removed to prepare tissue homogenates for antioxidant index determination.

Preparation of tissue homogenates: The liver or heart was rinsed with physiological saline to remove blood, blotted dry with filter paper, and weighed (0.5 g). Nine volumes of physiological saline were added, and the tissue was minced and homogenized to prepare a 10% tissue homogenate. The homogenate was centrifuged at 3,000 r/min for 10 min, and the supernatant was collected and stored at -80°C for analysis.

Total superoxide dismutase (T-SOD) activity was determined using the xanthine oxidase method. Malondialdehyde (MDA) content was measured using the thiobarbituric acid method. Glutathione peroxidase (GSH-Px) activity was determined using a colorimetric method. Carbonyl content was measured using the dinitrophenylhydrazine method (5,5' -dithiobis-2-nitrobenzoic acid). Total antioxidant capacity (T-AOC) was determined using the ferric ion reducing/antioxidant power method. Tissue protein content was measured using the bicinchoninic acid (BCA) method. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.4 Measurement of Plasma Biochemical Parameters

Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities, as well as total protein (TP), albumin (ALB), total bilirubin (TBIL), creatinine (CRE), and uric acid (UA) contents were determined using a ZY-300 automatic biochemical analyzer (Shanghai Kehua) with assay kits purchased from Shanghai Kehua Bioengineering Co., Ltd.

1.5 Statistical Analysis

Data were analyzed using the one-way ANOVA procedure of SPSS 16.0 statistical software. Duncan' s multiple range test was used for intergroup comparisons, with $P<0.05$ as the criterion for statistical significance. Results are expressed as "mean ± standard deviation."

2.1 Effects of Dietary PQQ·Na Supplementation on Production Performance and Egg Quality

Dietary supplementation with 0.04–0.12 mg/kg PQQ·Na or 200 mg/kg vitamin E did not significantly affect production performance during any week of the trial (data not shown) or over the entire 24-week period (Table 2) ($P>0.05$). As shown in Table 3 , compared with the control group, Haugh unit was significantly increased in all PQQ·Na groups and the vitamin E group at week 24 ($P<0.05$), with no significant differences among PQQ·Na groups and the vitamin E group ($P>0.05$). Haugh unit increased linearly with increasing dietary PQQ·Na supplementation ($P=0.050$; results not shown). At week 24, eggshell thickness in the 0.04 mg/kg PQQ·Na group was significantly higher than that in the control and 0.12 mg/kg PQQ·Na groups ($P<0.05$), but did not differ significantly from the vitamin E and 0.08 mg/kg PQQ·Na groups ($P>0.05$). Dietary PQQ·Na supplementation showed a quadratic relationship with eggshell thickness ($P=0.003$; results not shown). Dietary supplementation with 0.04–0.12 mg/kg PQQ·Na or 200 mg/kg vitamin E had no significant effect on yolk color or eggshell strength ($P>0.05$).

2.2 Effects of Dietary PQQ·Na Supplementation on Plasma Antioxidant Indices of Laying Hens

The effects of dietary PQQ·Na supplementation on plasma antioxidant indices of laying hens are presented in Table 4 . Regarding plasma T-AOC, at week 12, all PQQ·Na groups were significantly higher than the control group ($P<0.05$), and the 0.08 mg/kg PQQ·Na group was also significantly higher than the vitamin E group ($P<0.05$). At week 24, the 0.04 and 0.08 mg/kg PQQ·Na groups were significantly higher than the control, vitamin E, and 0.12 mg/kg PQQ·Na groups ($P<0.05$). For plasma T-SOD activity, at week 2, the 0.08 and 0.12 mg/kg PQQ·Na groups were significantly higher than the control and 0.04 mg/kg PQQ·Na groups ($P<0.05$), with no significant difference from the vitamin E group ($P>0.05$). At week 12, the 0.12 mg/kg PQQ·Na group did not differ significantly from the vitamin E group, but both were significantly higher than other groups ($P<0.05$). At week 24, the 0.08 and 0.12 mg/kg PQQ·Na groups were significantly higher than the control group ($P<0.05$), and the 0.12 mg/kg PQQ·Na group was also significantly higher than the vitamin E group ($P<0.05$). For plasma GSH-Px activity, at week 2, the 0.08 and 0.12 mg/kg PQQ·Na groups and vitamin E group were significantly higher than the control group ($P<0.05$). At week 12, all PQQ·Na groups and the vitamin E group were significantly higher than the control group ($P<0.05$). No significant

differences were observed between PQQ·Na groups and the vitamin E group at any sampling time point. Dietary supplementation with 0.04–0.12 mg/kg PQQ·Na or 200 mg/kg vitamin E had no significant effect on plasma MDA content (P>0.05).

2.3 Effects of Dietary PQQ·Na Supplementation on Tissue Antioxidant Indices of Laying Hens

As shown in Figure 1 [Figure 1: see original paper], heart (P=0.034; results not shown) and liver (P=0.009; results not shown) MDA content decreased linearly with increasing dietary PQQ·Na supplementation. Compared with the control group, heart and liver MDA content in the 0.12 mg/kg PQQ·Na group decreased by 17.81% (P>0.05) and 21.67% (P<0.05), respectively. No significant differences in heart and liver MDA content were observed between PQQ·Na groups and the vitamin E group (P>0.05). Heart carbonyl content showed a quadratic decrease with increasing dietary PQQ·Na supplementation (P=0.031; results not shown), with the 0.08 mg/kg PQQ·Na group being significantly lower than the control group (P<0.05). Dietary supplementation with 0.04–0.12 mg/kg PQQ·Na or 200 mg/kg vitamin E had no significant effect on liver carbonyl content (P>0.05).

Value columns with different letters differ significantly (P<0.05).

Fig. 1 Effects of PQQ·Na supplementation on heart MDA content (A), heart carbonyl group content (B), hepatic MDA content (C) and hepatic carbonyl group content (D) of laying hens

As shown in Table 5 , heart GSH-Px activity in the 0.12 mg/kg PQQ·Na group was significantly higher than that in the control and vitamin E groups (P<0.05), and also significantly higher than the 0.04 and 0.08 mg/kg PQQ·Na groups (P<0.05). Compared with the control group, heart T-SOD activity was significantly increased in the 0.04 mg/kg PQQ·Na group and vitamin E group (P<0.05), but dietary PQQ·Na supplementation had no significant effect on heart T-AOC (P>0.05). Liver T-AOC in the 0.08 mg/kg PQQ·Na group was significantly higher than that in the control group (P<0.05), with no significant differences from the vitamin E group and other PQQ·Na groups (P>0.05). Dietary PQQ·Na supplementation had no significant effect on liver T-SOD activity (P>0.05).

2.4 Effects of Dietary PQQ·Na Supplementation on Plasma Biochemical Parameters of Laying Hens

Two weeks after dietary PQQ·Na supplementation, plasma CRE content in the 0.08 and 0.12 mg/kg PQQ·Na groups and vitamin E group was significantly lower than that in the control group (P<0.05). Additionally, plasma UA content in the 0.08 and 0.12 mg/kg PQQ·Na groups was significantly higher than that in the control group (P<0.05). At week 12, plasma CRE content in all PQQ·Na groups was significantly higher than that in the control group (P<0.05),

but did not differ significantly from the vitamin E group (P>0.05). At week 24 of the trial, plasma ALP activity and ALB and UA contents were significantly affected by PQQ·Na supplementation level (P<0.05). Specifically, plasma ALP activity in all PQQ·Na groups was significantly lower than that in the control and vitamin E groups (P<0.05), with no significant differences among PQQ·Na groups (P>0.05). Plasma ALB content in the 0.08 and 0.12 mg/kg PQQ·Na groups was significantly higher than that in the control, vitamin E, and 0.02 mg/kg PQQ·Na groups (P<0.05). Plasma UA content in the 0.12 mg/kg PQQ·Na group was significantly higher than that in all other groups (P<0.05).

3.1 Effects of Dietary PQQ·Na Supplementation on Production Performance and Egg Quality of Laying Hens

PQQ is an essential nutritional factor for animal growth, development, and reproduction. When mice are fed purified diets deficient in PQQ, growth retardation, developmental arrest, and poor reproductive capacity can be observed [11-13]. PQQ supplementation improves reproductive performance and growth when mice are fed purified diets; however, no nutritional effects of PQQ are observed when mice are fed complete diets [13]. In this experiment, no significant effects of PQQ or vitamin E on overall production performance of laying hens were observed. PQQ is a micronutrient, and the dietary PQQ content required for optimal growth in newborn mice is 300 ng/g [12]. The PQQ content in food ranges from 0.19 to 7.02 ng/g [14]. When mice are fed complete diets, PQQ deficiency may not occur even without additional PQQ supplementation. No reports have been published regarding PQQ requirements and optimal supply levels for laying hens.

Both producers and consumers are highly concerned about egg quality; however, albumen and shell quality indices decline as hen age increases. Xu et al. [15] demonstrated that dietary PQQ·Na supplementation for 6 weeks tended to improve egg albumen quality (albumen height and Haugh unit), though not reaching statistical significance. The present results indicate that dietary supplementation with 0.08–0.12 mg/kg PQQ·Na for 24 weeks significantly improved Haugh unit of eggs in the late laying period (50 weeks of age). The beneficial effects of PQQ·Na on albumen quality may be related to treatment duration and dosage. Ovomucin accounts for 1.5%–3.5% of total egg white protein, and its content determines albumen height [16]. Reportedly, ovomucin content is associated with energy metabolism rate in the magnum of the oviduct [17] and shell gland activity [18]. The beneficial effects of PQQ·Na on albumen quality may be related to its ability to improve mitochondrial function and promote energy metabolism. PQQ can promote mitochondrial biogenesis and improve cellular energy metabolism through multiple cell signaling pathways [19-20]. Furthermore, albumen quality may also be affected by egg antioxidant status. Studies have shown that egg Haugh unit is negatively correlated with MDA content, a lipid oxidation product in egg yolk [21]. Dietary antioxidant supplementation can increase egg Haugh unit and reduce lipid oxidation product content dur-

ing egg storage [22]. In this trial, yolk MDA content in all PQQ·Na groups was significantly lower than that in the control group, while T-AOC was significantly higher (data not shown). Therefore, the beneficial effects of PQQ·Na on albumen quality may be related to its antioxidant properties.

3.2 Effects of Dietary PQQ·Na Supplementation on Antioxidant Status of Laying Hens

MDA and carbonyl groups are lipid and protein peroxidation products generated when free radicals attack polyunsaturated fatty acids and proteins in vivo, serving as specific markers of oxidative damage. PQQ possesses electrophilic properties and can react with carbonyl-containing substances, o-phenylenediamine, sulfite, and malononitrile complexes to form stable products [23]. In this trial, heart and liver MDA content decreased linearly with increasing PQQ·Na supplementation, while carbonyl content showed a quadratic decreasing trend only in the heart, suggesting tissue-specific antioxidant characteristics of PQQ. Additionally, PQQ can induce antioxidant enzyme production, enhance intracellular oxidative defense capacity, and thereby reduce oxidation product formation. PQQ can increase antioxidant enzyme expression in *Escherichia coli* cells [24] and enhance antioxidant enzyme activity in nerve cells (PC12 cells) under methylmercury treatment [25]. Our previous research demonstrated that PQQ can increase antioxidant enzyme activity by stimulating peroxisome proliferator-activated receptor- coactivator-1 (PGC-1) and Nrf2-ARE signaling pathways, thereby eliminating the adverse effects of oxidized oil in laying hens [6]. In this experiment, dietary supplementation with 0.04–0.12 mg/kg PQQ·Na exerted varying degrees of influence on antioxidant enzyme activities in plasma, heart, and liver of laying hens. Evidently, PQQ·Na acts as an antioxidant that promotes free radical scavenging and improves antioxidant capacity in blood and tissues of laying hens to varying degrees. However, the optimal supplemental level of PQQ·Na varied among different organs and tissues and for different antioxidant enzyme indices. Based on plasma antioxidant enzyme activities and MDA content, the optimal dietary PQQ·Na level was 0.08–0.12 mg/kg. According to hepatic antioxidant enzyme activities, the optimal dietary PQQ·Na level was 0.08 mg/kg, as antioxidant enzyme activity tended to decrease at 0.12 mg/kg supplementation. Liver MDA content decreased linearly with increasing dietary PQQ·Na supplementation, indicating that decreased antioxidant enzyme activity may be related to other antioxidant mechanisms of PQQ rather than inhibitory effects. Akaike et al. [26] reported that PQQ can donate electrons to reduce free radicals directly, with stronger scavenging capacity than other water-soluble antioxidants (such as vitamin C, glutathione, and uric acid). Therefore, PQQ·Na may exert antioxidant effects through non-enzymatic antioxidant systems, thereby sparing antioxidant enzymes in hens.

3.3 Effects of Dietary PQQ·Na Supplementation on Plasma Biochemical Parameters of Laying Hens

The liver and kidney are the primary sites for xenobiotic transformation and metabolism in the body. Plasma enzyme activities such as AST, ALT, and ALP can reflect the degree and severity of hepatocyte damage. ALT and AST are primarily located in the cytoplasmic soluble fraction or mitochondria of hepatocytes. Elevated activities of these two enzymes in blood often indicate hepatocyte destruction, increased cell membrane permeability, or mitochondrial damage. CRE is the end-product of creatine phosphate metabolism in muscle, cannot be reabsorbed, and is excreted after glomerular filtration. Changes in plasma CRE content reflect glomerular filtration capacity. UA is the end-product of amino acid metabolism in poultry and reflects dietary amino acid balance and protein utilization efficiency in chickens. Analysis of plasma biochemical parameter changes throughout the entire trial period revealed that plasma ALP activity was significantly affected by dietary PQQ·Na supplementation, with consistent trends across all sampling time points. The effects of dietary PQQ·Na supplementation on other indices reached statistical significance only at specific sampling time points. For example, plasma CRE content in the 0.08 and 0.12 mg/kg groups was significantly lower than that in the control group at the early stage of the experiment (week 2), but significantly higher than the control group at week 12. Plasma UA content in the 0.08 and 0.12 mg/kg PQQ·Na groups was significantly higher than that in the control group at week 2, showed no significant difference from the control group at week 12, but was significantly higher than the control group again in the 0.12 mg/kg PQQ·Na group at week 24. These unstable changes in plasma biochemical parameters suggest the transient nature of PQQ·Na effects. Additionally, individual hen variations at different stages may also contribute to these unstable changes in plasma biochemical parameters.

ALP is an enzymatic indicator that reflects liver damage and cholestasis, with clinical significance primarily associated with increased activity. Furthermore, ALP plays an important role in calcium and phosphorus metabolism and bone mineral deposition in vivo, being closely related to animal growth and development. Plasma ALP activity can indirectly reflect ALP activity derived from osteoblasts in bone and shows a strong negative correlation with bone mineral deposition [27]. In this trial, dietary supplementation with 0.04–0.12 mg/kg PQQ decreased plasma ALP activity (week 24), potentially improving eggshell quality in laying hens during the late laying period. Plasma ALP activity is negatively correlated with plasma calcium concentration and bone strength in laying hens [28] and may be involved in the expression of osteopontin and shell matrix protein 116 genes in the shell gland [29]. However, whether changes in ALP activity are related to PQQ regulation of eggshell quality requires further investigation.

4 Conclusions

1. Dietary supplementation with 0.08-0.12 mg/kg PQQ·Na had no adverse effects on production performance or major plasma biochemical parameters of laying hens.
2. Dietary supplementation with 0.04-0.12 mg/kg PQQ·Na improved albumen and shell quality of laying hens during the late laying period, with the best results observed at 0.08 mg/kg supplementation.
3. Dietary supplementation with 0.04-0.12 mg/kg PQQ·Na improved plasma and tissue antioxidant status of laying hens, with better effects observed at 0.08 and 0.12 mg/kg supplementation.
4. Based on comprehensive results from this trial, the appropriate supplemental level of PQQ·Na in laying hen diets is 0.08 mg/kg.

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Effects of Pyrroloquinoline Quinone Disodium Supplementation on Performance, Antioxidant Status and Plasma Biochemical Parameters of Laying Hens

WANG Jing YANG Linlin ZHANG Haijun WU Shugeng QI Guanghai1
(National Engineering Research Center of Biological Feed, Key Laboratory of Feed Biotechnology of Ministry of Agriculture, Feed Research Institute of Chinese Academy of Agricultural Sciences, Beijing 100081, China)

Abstract: The current study aimed to evaluate the biological safety of pyrroloquinoline quinone disodium (PQQ·Na) for laying hens and investigated the effects of dietary PQQ·Na levels on laying performance, egg quality, plasma biochemical parameters and antioxidant indices. Five hundred and forty 25-week-old Hyline Grey laying hens were randomly divided into 5 groups with each group consisted of 6 replicates of 18 birds each. The laying hens in the 5 groups were fed 5 experimental diets with 0, 0.04, 0.08, 0.12 mg/kg PQQ·Na and 200 mg/kg vitamin E, respectively. All hens were fed the diets for a 1-week adaptation followed by a 24-week trial period. The result showed as follows: 1) diet supplemented with 0.04 to 0.12 mg/kg PQQ·Na did not affect the performance of laying hens ($P>0.05$). 2) Compared with the control group, the Haugh unit was significantly increased by diet supplemented with 0.04 to 0.12 mg/kg PQQ·Na ($P<0.05$), and the eggshell thickness was significantly increased by diet supplemented with 0.8 mg/kg PQQ·Na at the week 24 of trial ($P<0.05$). 3) Compared with the control group, the activities of plasma glutathion peroxidase (GSH-Px) (week 12) and total superoxide dismutase (T-SOD) (week 2 and 24) were significantly increased by diet supplemented with 0.08 and 0.12 mg/kg PQQ·Na ($P<0.05$). 4) Compared with the control group, diet supplemented with PQQ·Na at 0.08 mg/kg significantly decreased the content of heart carbonyl group and significantly increased the hepatic total antioxidant capacity (T-AOC) ($P<0.05$), and diet supplemented with 0.12 mg/kg PQQ·Na significantly decreased the hepatic malondialdehyde (MDA) content ($P<0.05$). Above results indicate that the supplementation of PQQ·Na at 0.08 to 0.12

mg/kg in laying hens' diet do not have negative effects, but can enhance the laying hens' antioxidant function. The suitable supplemental level of PQQ·Na in laying hens' diet is 0.08 mg/kg by comprehensive considering of the influence factors.

Key words: pyrroloquinoline quinone disodium; laying hens; antioxidant; plasma biochemical parameters; egg quality

1 Corresponding author, professor, E-mail: qiguanghai@caas.cn

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