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Postprint: Biosafety Evaluation of Chromium Picolinate in Laying Hens

Authors: Gang Wang, Sun Xiaoming, Liao Xiudong, Zhang Liyang, Lü Lin, Lan Yunxian, Luo Xugang

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with different levels of chromium picolinate on laying performance, blood physiological and biochemical indices, organ development, and histopathological changes in major organs of Beijing Red commercial laying hens, and thereby evaluate the biosafety of chromium picolinate for laying hens. A single-factor completely randomized design was adopted, and 360 23-week-old Beijing Red commercial laying hens were selected and randomly divided into 4 groups with 6 replicates per group and 15 hens per replicate, based on the principle of consistent laying rate and body weight. Each group was supplemented with 0, 0.6, 4.0, and 6.0 mg/kg (as chromium) of chromium picolinate in a corn-soybean meal basal diet, respectively. The pre-trial period was 21 days, and the formal trial period was 70 days. The results showed: 1) Dietary chromium supplementation level had no significant effect on laying performance and organ index of laying hens throughout the entire experimental period ($P>0.05$). 2) Except for significantly increasing plasma aspartate aminotransferase activity in laying hens on day 35 of the experiment ($P<0.05$), dietary chromium supplementation level had no significant effect on other plasma biochemical indices and routine blood physiological indices ($P>0.05$). 3) Dietary chromium supplementation level had no obvious effect on the development of major visceral organs in laying hens, and no obvious histopathological changes were observed in the heart, liver, kidney, ovary, and oviduct tissues of laying hens. The above results indicate that when the recommended supplementation level of chromium picolinate in corn-soybean meal diets for laying hens is 0.6 mg/kg, it possesses a 10-fold safety margin, meaning that chromium supplementation in the form of chromium picolinate in diets is safe for laying hens.

Full Text

Evaluation of Biological Safety of Chromium Picolinate for Laying Hens

WANG Gang¹, SUN Xiaoming^{2*}, LIAO Xiudong², ZHANG Liyang², LV Lin², LAN Yunxian¹, LUO Xugang²

¹ Southwest University Rongchang Campus, Chongqing 402460, China

² Mineral Nutrition Research Division, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Abstract

This experiment was conducted to investigate the effects of dietary supplementation with different levels of chromium picolinate (Cr-Pic) on laying performance, blood physiological and biochemical parameters, organ development, and histopathological changes in major organs of Beijing Red commercial laying hens, thereby evaluating the biological safety of Cr-Pic for laying hens. A single-factor completely randomized design was employed, utilizing 360 twenty-three-week-old Beijing Red commercial laying hens selected for consistent laying rate and body weight. The hens were randomly divided into 4 groups with 6 replicates per group and 15 hens per replicate. Each group received a corn-soybean meal basal diet supplemented with 0, 0.6, 4.0, or 6.0 mg/kg chromium (as Cr-Pic). The preliminary period lasted 21 days, followed by a 70-day experimental period. The results showed: (1) Dietary chromium supplementation level had no significant effect on laying performance or organ indices throughout the entire experimental period ($P>0.05$). (2) Dietary chromium supplementation level significantly increased plasma glutamic-oxalacetic transaminase activity on day 35 ($P<0.05$), but had no significant effect on other plasma biochemical parameters or routine blood physiological indices ($P>0.05$). (3) Dietary chromium supplementation level showed no obvious influence on the development of major visceral organs, and no significant histopathological changes were observed in the heart, liver, kidney, ovary, or oviduct of laying hens. These results indicate that a 10-fold safety margin exists when the recommended dietary chromium level is 0.6 mg/kg for laying hens fed corn-soybean meal diets, meaning that chromium supplementation as Cr-Pic is safe for laying hens.

Keywords: chromium picolinate; biological safety evaluation; laying hens

Introduction

Chromium (Cr) is one of the essential trace elements in animals, and its physiological functions have attracted increasing attention from researchers. Chromium exists in three biologically active forms in vivo: glucose tolerance factor (GTF), chromium picolinate (Cr-Pic), and low molecular weight

chromium-binding substance (LMWCr) [1]. As an organic trivalent chromium compound, Cr-Pic plays an important role in the biological activity of GTF and is widely used as a nutritional additive in food and livestock production. Several studies have demonstrated that supplementation with appropriate concentrations of Cr-Pic can promote growth, improve carcass quality, alleviate environmental stress, enhance disease resistance, and reduce blood glucose and lipid levels in livestock and poultry, thereby mitigating symptoms related to glucose and lipid metabolism disorders [2-6].

However, current research on the safety of Cr-Pic has yielded some data but with inconsistent conclusions. For instance, Anderson et al. [7] reported acute toxicity when supplementing mouse diets with 100 mg/kg Cr-Pic for 24 weeks. Other studies have documented cytotoxicity and genotoxicity resulting from excessive Cr-Pic supplementation. Cr-Pic cytotoxicity primarily manifests as mitochondrial damage and apoptosis. Manygoats et al. [8] demonstrated that supplementation with 1.0 mmol/L Cr-Pic (125 times the appropriate level) caused mitochondrial damage and apoptosis. Genotoxicity is mainly characterized by DNA base oxidation, DNA strand breakage, and gene mutation. Bagchi et al. [9] found that treating macrophage J774A.1 cells with 50 mg/mL (120 mmol/L) Cr-Pic for 24 hours caused DNA strand breakage and the presence of DNA fragments in cells.

Nevertheless, no studies have been reported on the feed safety evaluation of Cr-Pic for laying hens. This experiment evaluated the biological safety of Cr-Pic supplementation in corn-soybean meal diets for laying hens by measuring laying performance, blood physiological and biochemical parameters, organ development, and histopathological changes in major organs, providing a scientific basis for determining the safety margin of maximum supplemental levels of Cr-Pic in laying hen diets and ensuring its safe application in egg production.

Materials and Methods

1.1 Experimental Design

A single-factor completely randomized design was adopted. Based on domestic and international experimental data on chromium supplementation in laying hens, the effective dose range of chromium under conventional feeding conditions is 0.4-1.6 mg/kg [10-11]. Therefore, 0.4 mg/kg was considered the minimum effective recommended level for laying hen diets. According to the “Guidelines for the Evaluation of Tolerance of Feed and Feed Additives in Target Livestock (Trial)” issued by the Ministry of Agriculture, four groups were established: a control group without chromium supplementation, a chromium use-level group (1.5 times the minimum effective recommended level), and two multi-level dose groups at 10 times the minimum effective recommended level and the use level, respectively. The Cr-Pic supplementation levels were 0, 0.6, 4.0, and 6.0 mg/kg (as chromium).

1.2 Experimental Animals and Diets

Four hundred 20-week-old Beijing Red commercial laying hens were selected, from which 360 hens with similar laying rate and body weight were chosen and randomly divided into 4 groups with 6 replicates per group and 15 hens per replicate. Each group contained 90 hens, housed in 6 replicate cages with 15 hens per cage (each replicate cage consisted of 5 small unit cages with 3 hens per unit). A 21-day preliminary period was initiated. At the end of week 23, the laying rate had reached peak production (>85%). Fine adjustments were made to ensure similar laying rates and body weights among groups before commencing the 70-day formal experimental period, which concluded at week 33.

Management practices and routine immunization followed the “Beijing Red Parent Stock and Commercial Stock Management Manual.” Hens had free access to feed and water. Clinical symptoms, morbidity, and mortality were observed and recorded daily. Any dead hens were necropsied to observe pathological changes. At day 35 (week 28) and day 70 (week 33) of the experiment, after overnight fasting, hens were weighed on a per-unit-cage basis.

A corn-soybean meal basal diet was formulated according to the nutrient levels recommended by NRC (1994) [12] for brown-egg laying hens (Table 1). Three experimental diets were prepared according to the experimental design. Cr-Pic was diluted with cornstarch before being mixed into the basal diet. Cr-Pic was provided by Sichuan Shangyuan Company with a measured chromium content of 12.24%. Diets were fed as mash.

1.3 Sample Collection and Preparation

During diet preparation, samples were collected on-site, ground to pass through a 200-mesh sieve, placed in self-sealing bags, and stored at low temperature for subsequent analysis of crude protein, calcium, and chromium content. At day 35 (week 28) and day 70 (week 33) of the experiment, all hens were fasted for 12 hours (with water available). At 08:00 the following day, hens were weighed on a per-unit-cage basis, and 2 hens were selected from each replicate cage based on average cage body weight. After individual weighing, 3-4 mL of blood was collected from the wing vein. One portion of whole blood (with sodium heparin anticoagulant) was used to determine routine blood physiological indices, while another portion was centrifuged (3,000 r/min) to obtain plasma, which was stored at -20°C for plasma biochemical analysis. Whole blood or plasma from 2 hens in each replicate cage was pooled as one analytical sample.

At the end of the experiment on day 70 (week 33) after blood collection, one hen from each replicate cage was slaughtered. The abdomen was quickly opened to remove the heart, liver, kidney, ovary, and oviduct, which were weighed separately to calculate organ indices, and external abnormalities were observed and recorded. Organ index (%) = (organ weight/live body weight) × 100.

After weighing, portions of heart, liver, kidney, ovary, and uterus part of the oviduct were collected from one hen per replicate cage in the 0, 4.0, and 6.0 mg/kg groups, fixed in brown bottles containing 4% formalin solution for paraffin sectioning to observe histopathological changes.

1.4 Analytical Methods

1.4.1 Crude Protein Content Crude protein content in feed ingredients and diets was determined using the Kjeldahl method [13].

1.4.2 Calcium Content Calcium content in feed ingredients and diets was determined using a full-spectrum direct-reading plasma emission spectrometer (IRIS Intrepid II, Thermal Jarrell Ash, Waltham, MA, USA).

1.4.3 Chromium Content Total chromium content in Cr-Pic and experimental diets was determined using inductively coupled plasma mass spectrometry (ICP-MS) [14].

1.4.4 Blood Indices Whole blood red blood cell count (RBC), hemoglobin (HGB) content, and hematocrit (HCT) were determined using an automatic blood analyzer (Sysmex XE-2100). Plasma glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and alkaline phosphatase (AKP) activities, as well as albumin (ALB), total protein (TP), glucose (GLU), triglyceride (TG), total cholesterol (TCHO), urea nitrogen (UN), and creatinine (CRE) contents were measured using a Unicel DxC 800 Synchron automatic biochemical analysis system (Beckman Coulter, USA).

1.4.5 Histopathological Examination Paraffin sections of heart, liver, kidney, ovary, and uterus part of the oviduct were prepared for histopathological observation. The paraffin sectioning process included: sampling, fixation, rinsing, dehydration (70% ethanol for 4 h → 80% ethanol for 4 h → 90% ethanol for 4 h → 100% ethanol for 1 h), clearing (absolute ethanol:xylene 1:1 for 15 min → xylene for 15 min), paraffin infiltration, embedding, sectioning, mounting, baking, hematoxylin-eosin staining (deparaffinization: baked sections in xylene I for 10 min → xylene II for 10 min; rehydration: deparaffinized sections in 100% ethanol for 5 min → 90% ethanol for 5 min → 80% ethanol for 5 min → 70% ethanol for 5 min → distilled water for 5 min), microscopic observation, and image acquisition of corresponding tissues.

1.5 Statistical Analysis

All data were analyzed using the General Linear Model (GLM) procedure in SAS 9.0 software [15]. Significant differences were further compared using the Least Significant Difference (LSD) method. The significance level for all data was set at $P < 0.05$. Data are presented as means \pm standard deviation.

Results

2.1 Effects of Dietary Chromium Supplementation Level on Laying Performance

As shown in Table 2 , dietary chromium supplementation level had no significant effect on overall laying rate during peak production, average egg weight, daily egg mass, soft-shell and abnormal egg rate, average daily feed intake, or feed-to-egg ratio ($P>0.05$). Throughout the experimental period, no hens in any group exhibited toxic reactions or mortality.

These results indicate that under the conditions of this experiment, dietary supplementation with various levels of Cr-Pic had no significant effect on the laying performance of laying hens.

2.2 Effects of Dietary Chromium Supplementation Level on Routine Blood Physiological Indices

As shown in Table 3 , dietary chromium supplementation level had no significant effect on blood red blood cell count, hemoglobin content, or hematocrit at 28 and 33 weeks of age ($P>0.05$).

These results indicate that dietary supplementation with various levels of Cr-Pic did not affect the main routine blood physiological indices of laying hens.

2.3 Effects of Dietary Chromium Supplementation Level on Organ Indices

As shown in Table 4 , dietary chromium supplementation level had no significant effect on heart index, liver index, kidney index, ovary index, or oviduct index on day 70 ($P>0.05$), and no abnormal external changes were observed in these organs.

These results indicate that under the conditions of this experiment, dietary supplementation with various levels of Cr-Pic had no adverse effects on the development of major visceral organs in laying hens.

2.4 Effects of Dietary Chromium Supplementation Level on Plasma Biochemical Indices

As shown in Table 5 , dietary chromium supplementation level had a significant effect on plasma glutamic-oxalacetic transaminase activity on day 35 ($P<0.05$), but had no significant effect on other plasma biochemical indices ($P>0.05$). Compared with the control group, dietary supplementation with 0.6, 4.0, and 6.0 mg/kg Cr-Pic significantly increased plasma glutamic-oxalacetic transaminase activity on day 35 ($P<0.05$), but there were no significant differences among the supplemented groups ($P>0.05$).

These results indicate that dietary supplementation with various levels of Cr-Pic had no significant effect on most plasma biochemical indices of laying hens.

2.5 Effects of Dietary Chromium Supplementation Level on Histopathology of Major Organs

As shown in Figure 1 [Figure 1: see original paper], no histopathological changes were observed in the hearts of laying hens in the chromium-supplemented groups. All groups exhibited smooth epicardium, tightly and neatly arranged myocardial cells with uniform staining, no inflammatory cell infiltration in intercalated discs and striated interstitium, and round or oval nuclei located centrally within muscle cells.

As shown in Figure 2 [Figure 2: see original paper], no histopathological changes were observed in the livers of laying hens in the chromium-supplemented groups. All groups showed intact polyhedral hepatic lobules, hepatocytes radiating from the central vein with clear boundaries and uniform reticular structure, and intact hepatic cords and sinusoids.

As shown in Figure 3 [Figure 3: see original paper], no histopathological changes were observed in the kidneys of laying hens in the chromium-supplemented groups. All groups exhibited normal glomeruli of moderate size with clear capsules in the cortical region, homogeneously red-stained cytoplasm in renal tubular epithelial cells, and relatively homogeneous cytoplasm in collecting duct epithelial cells of medullary bodies.

As shown in Figure 4 [Figure 4: see original paper], no histopathological changes were observed in the ovaries of laying hens in the chromium-supplemented groups. All groups showed well-organized, intact ovarian structure with normal interstitial cells and growing follicles at various developmental stages among interstitial cells.

As shown in Figure 5 [Figure 5: see original paper], no histopathological changes were observed in the oviducts of laying hens in the chromium-supplemented groups. All groups exhibited relatively intact mucosal cells, muscular layer cells, and serosal layer cells.

These results indicate that under the conditions of this experiment, no histopathological changes in microstructure or morphology were observed in the major organs of laying hens in any chromium-supplemented group.

Discussion

3.1 Effects of Dietary Chromium Supplementation Level on Laying Performance

During this experiment, dietary chromium supplementation level had no significant effect on laying rate, average daily feed intake, or feed-to-egg ratio of laying hens. Current research findings on the effects of dietary chromium supplementation on laying performance are inconsistent. Wang et al. [16] found that supplementation with 1.0 mg/kg chromium (as chromium nicotinate) increased laying rate by 7% and average daily feed intake by 6.61%, and improved net protein

utilization by 12.93%. Su et al. [14] supplemented corn-soybean meal basal diets (containing 0.25 mg/kg chromium) with 0.4, 0.8, 1.6, and 3.2 mg/kg chromium as Cr-Pic, and reported that 0.4-1.6 mg/kg chromium reduced feed-to-egg ratio and soft-shell egg rate, while 3.2 mg/kg chromium significantly increased feed-to-egg ratio and soft-shell egg rate. Kim et al. [17] found that supplementation with 0.8 mg/kg chromium (as Cr-Pic) significantly improved laying rate and egg production in 36-week-old brown laying hens under high protein levels (16%), suggesting that appropriate dietary chromium levels may improve reproductive performance in poultry, possibly through the hypothalamic-pituitary-gonadal axis, similar to mammals. Uyanik et al. [18] supplemented basal diets with high doses (20 mg/kg) of chromium chloride and observed decreased feed intake and a 4.28% increase in feed-to-egg ratio, but no effects on body weight, egg weight, or total egg production. The inconsistent results regarding chromium supplementation effects on laying performance may be related to breed, dosage, and feeding conditions. The present experiment indicates that dietary Cr-Pic supplementation had no effect on laying performance and did not cause obvious toxic reactions.

3.2 Effects of Dietary Chromium Supplementation Level on Routine Blood Indices

Routine blood indices are important measures of nutritional balance and health status in animals. Under normal conditions, certain blood chemical components maintain dynamic balance, changing only when metabolic dysfunction or tissue lesions occur. The present results show that dietary Cr-Pic supplementation did not affect red blood cell count, hemoglobin content, or hematocrit, indicating that Cr-Pic had no significant effect on blood physiological indices of laying hens.

3.3 Effects of Dietary Chromium Supplementation Level on Plasma Biochemical Indices

Serum total protein and albumin contents reflect protein synthesis and metabolism, while urea nitrogen, as the main end product of protein catabolism, reflects protein utilization efficiency. Research indicates that serum urea nitrogen content is related to protein metabolism and amino acid balance, and can reflect protein metabolism and dietary amino acid balance [20]. In this experiment, dietary Cr-Pic supplementation had no significant effect on plasma total protein, albumin, or urea nitrogen contents, suggesting that Cr-Pic had no adverse effects on protein metabolism in laying hens.

Serum total cholesterol and triglyceride contents reflect lipid metabolism status, with serum cholesterol approximating cholesterol synthesis and triglyceride reflecting lipid absorption and utilization. In this experiment, dietary supplementation with 0.6, 4.0, and 6.0 mg/kg chromium had no significant effect on serum total cholesterol or triglyceride contents, indicating that Cr-Pic had no adverse effects on lipid metabolism in laying hens.

Glutamic-pyruvic transaminase is the most sensitive indicator of liver function damage and reflects liver function. Hepatocyte damage increases plasma glutamic-pyruvic transaminase activity and reduces metabolic and detoxification capacity, impairing toxin elimination. Glutamic-oxalacetic transaminase, the most active aminotransferase in liver cells participating in amino acid metabolism, also serves as an indicator of liver function [21]. In this experiment, dietary supplementation with 0.6, 4.0, and 6.0 mg/kg chromium significantly increased plasma glutamic-oxalacetic transaminase activity on day 35, while showing a trend toward decreased plasma glutamic-pyruvic transaminase activity in the early stage, suggesting that Cr-Pic may have protective effects on the liver in the early stage, though this effect was not observed in the later stage, requiring further investigation. These results indicate that dietary Cr-Pic supplementation had no adverse effects on the liver of laying hens.

Total bilirubin, a product of hemoglobin catabolism, is commonly used to diagnose liver and gallbladder diseases. Plasma creatinine concentration accurately reflects kidney parenchymal damage, while alkaline phosphatase plays an important role in phosphate metabolism and is used as a diagnostic indicator for liver diseases. In this experiment, dietary supplementation with 0.6, 4.0, and 6.0 mg/kg chromium had no significant effect on plasma total bilirubin, creatinine, or alkaline phosphatase, indicating that Cr-Pic had no toxic effects on liver, gallbladder, or kidney, and no adverse effects on liver and kidney function.

3.4 Effects of Dietary Chromium Supplementation Level on Organ Indices

Under normal conditions, the ratio of organs to body weight remains relatively constant, changing only when tissues become diseased or damaged. In this experiment, dietary supplementation with 0.6, 4.0, and 6.0 mg/kg chromium had no significant effect on heart, liver, kidney, ovary, or oviduct indices, indicating that Cr-Pic had no adverse effects on visceral or reproductive organs of laying hens.

3.5 Effects of Dietary Chromium Supplementation Level on Histopathological Changes in Major Tissues

Some studies have reported that high doses of Cr-Pic can cause aberrations in Chinese hamster ovary cells, clearly pointing out that trivalent chromium accumulation in diets can cause DNA damage in humans or animals [22]. However, Ding et al. [23] fed Wistar rats with Cr-Pic and found no significant effects on maternal reproductive function or fetal growth and development, and no teratogenic effects on fetuses. Anderson et al. [7] fed rats diets containing 5, 25, 50, and 100 g/g chromium as Cr-Pic for 20 weeks to observe Cr-Pic toxicity, and found that rats in all treatment groups grew well with no abnormal histopathological findings. Similarly, this experiment observed no histopathological changes in microstructure or morphology of major internal organs (liver and kidney) or reproductive organs (ovary) in laying hens fed Cr-Pic-supplemented diets, indi-

cating that dietary Cr-Pic supplementation does not cause pathological changes in major tissues of laying hens.

Conclusion

Dietary supplementation with 0.6, 4.0, and 6.0 mg/kg chromium as Cr-Pic had no adverse effects on laying performance, major blood physiological indices, most plasma biochemical indices, or major organ development in laying hens. No histopathological changes were observed in major organs of hens in the 4.0 and 6.0 mg/kg chromium groups, and no toxic reactions occurred in any hens. Therefore, a 10-fold safety margin exists when the recommended dietary chromium level is 0.6 mg/kg for laying hens fed corn-soybean meal diets, indicating that chromium supplementation as Cr-Pic is safe for laying hens.

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