Effects of Complex Organic Acid Calcium on Growth Performance, Tibial Development and Serum Parameters in Broilers: Postprint

Authors: He Junna, Guo Yang, Chen Nana, Liu Bing, Xiong Pingwen, Yu Dongyou

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Abstract

This study aimed to investigate the effects of dietary supplementation of compound organic acid calcium on growth performance, tibia development, and serum indices in broiler chickens, and to evaluate the feeding efficacy of compound organic acid calcium in broilers. Nine hundred healthy 1-day-old Cobb 500 broiler chicks with similar body weight were selected and randomly divided into 3 groups with 6 replicates per group and 50 birds per replicate. The control group was fed a corn-soybean meal basal diet, while experimental groups 1 and 2 were fed test diets supplemented with 0.4% and 0.8% compound organic acid calcium (replacing part of the inorganic calcium source) in the basal diet, respectively, with consistent calcium and phosphorus levels across all groups; the experimental period lasted 42 days. The results showed: 1) Compared with the control group, the average daily gain (ADG) of broilers in experimental groups 1 and 2 during days 1-21, days 22-42, and days 1-42 was significantly increased ($P<0.05$), and the feed-to-gain ratio (F/G) was significantly decreased ($P<0.05$). 2) Compared with the control group, at day 21, the tibia calcium content of broilers in experimental groups 1 and 2 was significantly increased ($P<0.05$); at day 42, the tibia length and calcium content in experimental groups 1 and 2 were significantly increased ($P<0.05$), and the tibia ash content in experimental group 1 was significantly higher than that in the control group and experimental group 2 ($P<0.05$). 3) At day 42, the serum glutamic-oxaloacetic transaminase (GOT) activity of broilers in experimental groups 1 and 2 was significantly lower than that in the control group ($P<0.05$), but there was no significant difference in serum GOT activity between experimental group 1 and experimental group 2 ($P>0.05$). Dietary supplementation of compound organic acid calcium had no significant effects on serum parathyroid hormone (PTH), calcitonin (CT), calcium and phosphorus contents, as well as glutamic-pyruvic transaminase

(GPT) and alkaline phosphatase (AKP) activities in broilers at days 21 and 42 (P>0.05). 4) Dietary supplementation of compound organic acid calcium had no significant effects on serum total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activities, or malondialdehyde (MDA) content in broilers at days 21 and 42 (P>0.05). In conclusion, dietary supplementation of compound organic acid calcium can improve growth performance and promote tibia development in broiler chickens, with the 0.4% supplementation level showing better feeding efficacy.

Full Text

Effects of Compound Organic Acid Calcium on Growth Performance, Tibia Development and Serum Indices of Broilers

HE Junna, GUO Yang, CHEN Nana, LIU Bing, XIONG Pingwen, YU Dongyou*

(Key Laboratory of Animal Nutrition and Feed in East China of Ministry of Agriculture, College of Animal Science, Zhejiang University, Hangzhou 310058, China)

Abstract: This experiment was conducted to investigate the effects of dietary compound organic acid calcium on growth performance, tibia development and serum indices of broilers, and to evaluate its feeding efficacy. A total of 900 one-day-old healthy Cobb-500 male broilers with similar body weight were randomly allocated into three groups with six replicates per group and 50 broilers per replicate. The control group was fed a corn-soybean meal basal diet, while experimental groups 1 and 2 were fed the basal diet supplemented with 0.4% and 0.8% compound organic acid calcium (replacing part of the inorganic calcium source), respectively. Calcium and phosphorus levels were consistent across all groups, and the experimental period lasted 42 days. The results showed: 1) Compared with the control group, the average daily gain (ADG) of broilers in groups 1 and 2 was significantly increased (P<0.05) and the feed-to-gain ratio (F/G) was significantly decreased (P<0.05) during days 1-21, 22-42, and 1-42. 2) At 21 days of age, the tibia calcium content in groups 1 and 2 was significantly higher than that in the control group (P<0.05). At 42 days of age, tibia length and calcium content in groups 1 and 2 were significantly increased (P<0.05), and the ash content of tibia in group 1 was significantly higher than that in both the control group and group 2 (P<0.05). 3) At 42 days of age, serum glutamic-oxaloacetic transaminase (GOT) activity in groups 1 and 2 was significantly lower than that in the control group (P<0.05), though no significant difference was observed between groups 1 and 2 (P>0.05). Dietary compound organic acid calcium had no significant effects on serum parathyroid hormone (PTH), calcitonin (CT), calcium and phosphorus contents, or the activities of glutamic-pyruvic transaminase (GPT) and alkaline phosphatase (AKP) in broilers at 21 and 42 days of age (P>0.05). 4) Dietary compound organic acid calcium

had no significant effects on serum total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activities, or malondialdehyde (MDA) content in broilers at 21 and 42 days of age (P>0.05). In conclusion, dietary supplementation with compound organic acid calcium can improve growth performance and promote tibia development in broilers, with 0.4% being the more appropriate supplementation level.

Key words: compound organic acid calcium; broilers; growth performance; tibia development; antioxidant capacity

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The commonly used calcium source in broiler diets is limestone (mainly $CaCO_3$), which contains about 35% calcium but has relatively high heavy metal content [1-2], poor palatability, and strong acid-binding capacity that can disrupt the acidic environment in the stomach [3], thereby reducing nutrient digestion and absorption. Organic acid calcium sources such as calcium formate, calcium lactate, and calcium citrate not only provide high-quality calcium but also supply organic acids, with minimal gastrointestinal irritation [4-5] and high safety, making them suitable feed additives for calcium supplementation in livestock and poultry.

Calcium formate, calcium citrate, and calcium lactate have been included in the Ministry of Agriculture' s Announcement No. 2045 "Catalogue of Permitted Feed Additives" [6], indicating broad application prospects. However, the use of compound organic acid calcium in broiler diets remains exploratory, and its effects require further investigation. This study used broilers as experimental subjects to explore the effects of compound organic acid calcium on growth performance, tibia development, and serum indices, aiming to provide a scientific basis for its application in livestock production.

1.1 Experimental Material

The compound organic acid calcium used in this experiment was provided by Hangzhou Guogu Biological Technology Co., Ltd., with active ingredients of calcium formate, calcium citrate, and calcium lactate, and total calcium content $ $26%.

1.2 Experimental Design

A single-factor randomized design was employed. Nine hundred one-day-old Cobb-500 male broilers with similar body weight were randomly divided into three groups with six replicates per group and 50 broilers per replicate. The control group received a corn-soybean meal basal diet, while experimental groups 1 and 2 received the basal diet supplemented with 0.4% and 0.8% compound organic acid calcium (replacing part of the inorganic calcium source), respectively. Calcium and phosphorus levels were consistent across all groups, and the experiment lasted 42 days.

The corn-soybean meal basal diet was formulated in two phases (days 1-21 and days 22-42) according to the NRC (1994) "Nutrient Requirements of Poultry" and NY/T 33-2004 "Feeding Standard of Chickens" and prepared as mash feed. The composition and nutrient levels of experimental diets are shown in Table 1 and Table 2 .

1.3 Animal Management

The feeding trial was conducted at the research base of the Key Laboratory of Animal Nutrition and Feed in East China of Ministry of Agriculture during the summer season. All experimental broilers were housed in the same building. Floor rearing was adopted with free access to feed and water, continuous lighting, natural ventilation, and regular cleaning to maintain hygiene. Immunization was performed according to the routine farm program.

1.4 Measurements

1.4.1 Growth Performance At 08:00 on days 21 and 42, broilers were weighed by replicate after fasting. Feed intake and residual feed were recorded for each replicate to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

1.4.2 Tibia Indices On days 21 and 42, one healthy broiler with body weight close to the average was selected from each replicate, slaughtered, and both left and right tibias were collected. After removing surface fascia, tibias were weighed and measured for length using vernier calipers, and strength was determined using a tenderness meter (TMS-Pro texture analyzer). Tibias were then dried, defatted, and ground to determine ash, calcium, and phosphorus contents.

1.4.3 Serum Indices On days 21 and 42, one healthy broiler with body weight close to the average was selected from each replicate, slaughtered, and jugular vein blood was collected. After standing in a 37°C water bath, blood samples were centrifuged at 3,000 r/min for 15 min at 4°C, and the supernatant was collected for biochemical and antioxidant index analysis. Serum calcium content was determined by methylthymol blue (MTB) colorimetry, phosphorus by phosphomolybdic acid method, parathyroid hormone (PTH) and calcitonin (CT) by enzyme-linked immunosorbent assay (ELISA), glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) by Reitman-Frankel method, alkaline phosphatase (AKP) by 4-aminoantipyrine colorimetry, malondialdehyde (MDA) by thiobarbituric acid (TBA) method, glutathione peroxidase (GSH-Px) activity and total antioxidant capacity (T-AOC) by colorimetry, and total superoxide dismutase (T-SOD) by hydroxylamine method. All kits were purchased from Nanjing Jiancheng Bioengineering Institute and used strictly according to the manufacturer' s instructions.

1.5 Statistical Analysis

Data were initially processed using Excel 2003 and then analyzed by one-way ANOVA using SPSS 16.0 statistical software. Duncan' s multiple range test was used for post-hoc comparisons, with $P<0.05$ considered statistically significant.

2.1 Effects of Compound Organic Acid Calcium on Growth Performance of Broilers

As shown in Table 3 , compared with the control group, ADG of broilers in groups 1 and 2 increased by 7.22% and 6.13% ($P<0.05$), respectively, and F/G decreased by 3.47% and 4.05% ($P<0.05$) during days 1-21. During days 22-42, ADG increased by 7.84% and 7.86% ($P<0.05$), and F/G decreased by 5.24% and 4.76% ($P<0.05$) in groups 1 and 2, respectively. Throughout the entire growth period (days 1-42), ADG increased by 7.60% and 7.23% ($P<0.05$), and F/G decreased by 5.08% and 4.57% ($P<0.05$) in groups 1 and 2, respectively. Dietary compound organic acid calcium had no significant effect on ADFI during any experimental period ($P>0.05$).

2.2 Effects of Compound Organic Acid Calcium on Tibia Indices of Broilers

As shown in Table 4 , compared with the control group, tibia calcium content in groups 1 and 2 increased by 8.44% and 7.06% ($P<0.05$) at 21 days of age. At 42 days of age, tibia length increased by 10.05% and 8.74% ($P<0.05$), and calcium content increased by 8.20% and 1.86% ($P<0.05$) in groups 1 and 2, respectively. At 42 days of age, tibia ash and calcium contents in group 1 were significantly higher than those in group 2 ($P<0.05$), while tibia phosphorus content in group 1 was 3.73% and 3.03% higher than that in the control group and group 2, respectively ($P>0.05$).

2.3 Effects of Compound Organic Acid Calcium on Serum Biochemical Indices of Broilers

As shown in Table 5 , dietary compound organic acid calcium had no significant effects on serum PTH, CT, calcium and phosphorus contents, or GPT and AKP activities in broilers at 21 and 42 days of age ($P>0.05$). Compared with the control group, serum GOT activity decreased by 46.32% and 45.61% ($P<0.05$) in groups 1 and 2, respectively, at 42 days of age.

2.4 Effects of Compound Organic Acid Calcium on Serum Antioxidant Indices of Broilers

As shown in Table 6 , dietary compound organic acid calcium had no significant effects on serum T-AOC, T-SOD and GSH-Px activities, or MDA content in broilers at 21 and 42 days of age ($P>0.05$).

3.1 Effects of Compound Organic Acid Calcium on Growth Performance of Broilers

Compound organic acid calcium possesses dual nutritional functions, providing both organic acids and high-quality calcium sources. Supplementing broiler diets with acidifiers or organic acid salts can improve diet palatability, participate in metabolic reactions after entering the body, accelerate nutrient absorption [7-9], and consequently promote weight gain, reduce F/G, and improve growth performance.

Chowdhury et al. [10] and Adil et al. [11] reported that organic acids can significantly improve daily weight gain and feed conversion ratio in broilers. Liu et al. [12] found that supplementing broiler diets with organic acids at 0.375%, 0.500%, and 0.625% increased body weight to varying degrees, with 0.500% showing the best effect. The present results demonstrate that dietary supplementation with 0.4% and 0.8% compound organic acid calcium significantly increased ADG and decreased F/G, consistent with previous reports. The growth-promoting effect of compound organic acid calcium is also closely related to its biological properties. Organic acid calcium exhibits high biological absorption characteristics, with calcium citrate absorption rate being 2.6 times that of inorganic calcium [5,13], which also contributes to weight gain in broilers.

In this experiment, dietary supplementation with 0.4% or 0.8% compound organic acid calcium had no significant effect on ADFI, consistent with findings by Sultan et al. [14] and Gunal et al. [15]. However, Chowdhury et al. [10] and Haque et al. [16] reported that dietary organic acids increased feed intake in broilers, while other studies found that organic acids decreased feed intake [9,17]. These discrepancies may be related to differences in organic acid types, supplementation levels, rearing environments, and management practices [14,16,18].

3.2 Effects of Compound Organic Acid Calcium on Tibia Development of Broilers

Chowdhury et al. [10], Snow et al. [19], and Liem et al. [20] demonstrated that dietary organic acids significantly increased tibia ash content in broilers. Brenes et al. [21] also reported that dietary citric acid increased tibia ash, calcium, and phosphorus contents and improved mineral utilization. Thus, organic acid supplementation can enhance mineral utilization and improve tibia development, possibly because minerals combined with organic acids are more readily absorbed. In this study, dietary compound organic acid calcium significantly affected tibia length and calcium and ash contents, with similar promoting effects as reported above. Soluble calcium (primarily in Ca^{2+} form) is the main form for effective animal absorption. Studies have reported that organic acid calcium has high solubility and absorption rates, can promote bone growth and development, increase calcium content in bone tissue, and serves as an excellent calcium nutritional fortifier [5,22]. The calcium source used in this experiment was a

compound organic acid calcium synthesized from calcium formate, calcium citrate, and calcium lactate at a specific ratio, which provides both organic acids and high-quality calcium sources (Ca^{2+}). Currently, few studies have reported on the application of compound organic acid calcium in broilers, and the mechanism underlying its tibia-promoting effects warrants further investigation.

3.3 Effects of Compound Organic Acid Calcium on Serum Indices of Broilers

GOT and GPT are two important transaminases in animals. Within normal ranges, decreased transaminase activity indicates improved protein utilization [23], while tissue damage increases biological membrane permeability, leading to elevated serum GOT and GPT activities [24]. The present results showed that dietary compound organic acid calcium reduced serum GOT activity in broilers, indirectly indicating improved protein utilization. Serum AKP activity represents the combined activity of several isoenzymes primarily from bone, liver, and small intestine. Since AKP activity from liver and small intestine is relatively stable, serum AKP activity can indirectly reflect osteoblast AKP activity and bone metabolism status [25]. This study found no significant effect of dietary compound organic acid calcium on serum AKP activity, whereas Xu et al. [7] reported that 0.1% coated acidifier significantly affected serum AKP activity. These different results may be related to acid types and rearing environments [26].

Calcium metabolism is regulated by multiple hormones, with PTH and CT being the two primary hormones that regulate extracellular Ca^{2+} concentration and maintain stable blood calcium through antagonistic effects. PTH, produced by the parathyroid gland, reduces bone calcium transfer and promotes osteoclast generation, while CT inhibits osteolysis and osteoclast activity, promotes osteoclast transformation into osteoblasts, and reduces serum calcium [27]. This study showed that dietary compound organic acid calcium had no significant effects on serum PTH and CT contents, and no significant differences were observed in serum calcium and phosphorus contents compared with the control group, possibly due to the regulatory effects of PTH and CT on calcium and phosphorus. The mechanism of action of compound organic acid calcium on PTH and CT requires further investigation.

The antioxidant defense system in the body (antioxidant substances and enzymes) possesses strong antioxidant capacity [7]. T-SOD and GSH-Px are important antioxidant enzymes that effectively scavenge superoxide radicals and peroxides and reduce hydroxyl radical formation, while MDA is an indicator of lipid peroxidation mediated by oxygen radicals [28]. Liu et al. [12] and Xu et al. [7] reported that dietary organic acids and acidifiers improved antioxidant capacity in broilers. In this experiment, dietary compound organic acid calcium had no significant effects on serum T-AOC, T-SOD and GSH-Px activities, or MDA content in broilers at 21 and 42 days of age.

Conclusion

Dietary supplementation with appropriate levels of compound organic acid calcium can effectively improve growth performance and promote tibia development in broilers, with 0.4% being the more suitable supplementation level.

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