Relative Biological Availability of Monocalcium Phosphate in Broiler Chickens (Postprint)

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Abstract

This experiment aimed to investigate the effects of monodicalcium phosphate (MDCP) and dicalcium phosphate (DCP) on growth performance, tibial indices, and serum biochemical indices in broiler chickens, and to evaluate the biological utilization rate of MDCP relative to DCP. A 2×6 two-factor randomized block design was employed, utilizing two phosphorus sources (MDCP and DCP) and six phosphorus supplementation levels (0, 0.05%, 0.10%, 0.15%, 0.20%, and 0.25%). A total of 990 1-day-old healthy Cobb male broilers were randomly allocated into 11 groups (with a shared control group) according to the principle of similar body weight, with 6 replicates per group and 15 birds per replicate. The experimental period lasted 21 days. The results showed that the interaction between phosphorus source and phosphorus supplementation level had no significant effects on growth performance, apparent nutrient utilization rate, tibial indices, or serum biochemical indices in broiler chickens ($P>0.05$). Broilers in the MDCP supplementation group exhibited significantly higher feed intake, body weight gain, tibial ash content, and serum phosphorus content compared to those in the DCP supplementation group ($P<0.05$), while serum alkaline phosphatase activity was significantly lower ($P<0.05$). Moreover, as the phosphorus supplementation level increased, feed intake and body weight gain in broilers increased significantly ($P<0.05$), whereas feed conversion ratio and mortality and culling rate decreased significantly ($P<0.05$). The 0.25% supplementation group demonstrated significantly higher apparent calcium utilization rate than the control group and the 0.05% supplementation group ($P<0.05$), and its apparent phosphorus utilization rate, tibial calcium content, tibial phosphorus content, and tibial strength were significantly higher than those of the control group ($P<0.05$). With increasing phosphorus supplementation levels, serum phosphorus content increased significantly ($P<0.05$), while serum alkaline phosphatase activity decreased significantly ($P<0.05$). Taking into comprehensive consideration of body weight gain, tibial indices, and serum biochemical indices in broiler chickens, the biological utilization rate of MDCP for broilers was

112.5% relative to DCP (100%).

Full Text

A Study on the Relative Bioavailability of Mono-Dicalcium Phosphate in Broiler Chickens

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Abstract

This experiment was conducted to investigate the effects of mono-dicalcium phosphate (MDCP) and dicalcium phosphate (DCP) on growth performance, tibia indices, and serum biochemical indices of broiler chickens, and to evaluate the relative bioavailability of MDCP compared to DCP. A 2×6 factorial randomized block design was employed, incorporating two phosphorus sources (MDCP and DCP) and six supplemental phosphorus levels (0, 0.05%, 0.10%, 0.15%, 0.20%, and 0.25%). A total of 990 one-day-old healthy Cobb male broilers were randomly allocated to 11 groups (with a shared control group) according to the principle of similar average body weight, with 6 replicates per group and 15 broilers per replicate. The experimental period lasted 21 days. The results indicated that the interaction between phosphorus source and supplemental level had no significant effect on growth performance, nutrient apparent metabolic rate, tibia indices, or serum biochemical indices ($P>0.05$). Broilers in the MDCP supplementation group exhibited significantly higher feed intake, body weight gain, tibia ash content, and serum phosphorus content compared to the DCP group ($P<0.05$), while serum alkaline phosphatase activity was significantly lower ($P<0.05$). As the phosphorus supplementation level increased, feed intake and body weight gain increased significantly ($P<0.05$), whereas the feed-to-gain ratio and mortality rate decreased significantly ($P<0.05$). The calcium apparent metabolic rate in the 0.25% supplementation group was significantly higher than that in the control and 0.05% groups ($P<0.05$), and its phosphorus apparent metabolic rate, tibia calcium content, tibia phosphorus content, and tibia strength were significantly higher than those in the control group ($P<0.05$). With increasing phosphorus supplementation levels, serum phosphorus content increased significantly ($P<0.05$), while serum alkaline phosphatase activity decreased significantly ($P<0.05$). Based on comprehensive consideration of body weight gain, tibia indices, and serum biochemical indices, the biological utilization rate of MDCP for broilers relative to DCP (100%) was 112.5%.

Keywords: broilers; mono-dicalcium phosphate; dicalcium phosphate; bioavailability

Introduction

Phosphorus is a major mineral element in poultry nutrition and a primary constituent of bone tissue. Approximately 80% of the phosphorus in chickens is present in the skeleton, with phosphorus accounting for over 70% of bone ash [1]. During the rearing period, chickens experience rapid bone growth and require adequate phosphorus intake from the diet to maintain health. Phosphorus participates in energy metabolism and various nutrient metabolic processes within the body, while also comprising the phosphate buffer system in blood that plays a crucial role in maintaining acid-base balance. Calcium and phosphorus deficiency or an imbalanced ratio in broiler diets can lead to rickets, characterized by long bone curvature, reduced bone calcification, leg weakness, impaired growth and development, low feed utilization, and increased mortality [2]. Dietary phosphorus sources are diverse, including dicalcium phosphate (DCP) (anhydrous and dihydrate forms), monocalcium phosphate (MCP), calcium phosphate, among others, with DCP being the most widely used. Annual DCP consumption in China approaches 4 million tons, exceeding 10 million tons globally. However, the relatively high content of citrate-soluble phosphorus in DCP results in low phosphorus utilization efficiency [3-4]. Mono-dicalcium phosphate (MDCP) is a co-crystalline compound of MCP and DCP. Compared to DCP, MDCP contains water-soluble phosphorus as high as 17.5%, substantially exceeding the national standard of 10% [5]. Early research has confirmed that using highly water-soluble phosphorus sources in animal diets results in phosphate excreted through feces and urine that is more readily absorbed and utilized by plants [6]. Currently, MDCP, as a novel phosphorus source product distinct from both MCP and DCP, has steadily increased its market share. However, existing literature on the biological utilization evaluation of MDCP in broiler diets is relatively scarce and dated, necessitating a re-evaluation of its biological availability. This study employed feeding and metabolic trials to measure growth performance, tibia indices, and serum biochemical indices, aiming to assess the relative bioavailability of MDCP compared to DCP and to provide reference data for MDCP application in broiler diets.

Materials and Methods

1.1 Experimental Materials

The high water-soluble MDCP (feed grade) contained 14.7% calcium and 21.4% inorganic phosphorus. The DCP had a purity of 99%, with calcium and inorganic phosphorus contents of 23.0% and 17.7%, respectively.

1.2 Experimental Design

A 2×6 factorial randomized block design was adopted, incorporating two phosphorus sources (MDCP and DCP) and six supplemental phosphorus levels (0, 0.05%, 0.10%, 0.15%, 0.20%, and 0.25%). The basal diet contained 0.15% nonphytate phosphorus. A total of 990 one-day-old healthy Cobb male broilers

were randomly divided into 11 groups (with a shared control group) based on the principle of similar average body weight among groups, with 6 replicates per group and 15 broilers per replicate. The experimental period was 21 days.

1.3 Experimental Diets

The experimental diets were corn-soybean meal-miscellaneous meal based, formulated according to the nutrient recommendations (except for available phosphorus) in the Ministry of Agriculture of the People' s Republic of China standard "Feeding Standard of Chicken" (NY/T 33-2004) [7]. The crude protein, calcium, and phosphorus contents in feed ingredients were measured values, while other indices referenced the "Chinese Feed Composition and Nutritional Value Table" (26th edition) from the Chinese Feed Database 2015. The experimental diets were in powder form, with 0.40% titanium dioxide added as an exogenous indicator to all diets. The composition and nutrient levels of experimental diets are presented in Table 1 .

1.4 Management

The experimental chicken house was fully enclosed, with three-tier stacked cage rearing and a single cage area of 0.93 m^2. Positional effects were considered in the arrangement of replicates among groups. The house temperature was maintained at 33–36°C during days 1–3, then reduced by 1°C daily until reaching 24°C at the end of day 14, which was maintained until the experiment concluded. Relative humidity was 70–75% during days 1–3, then maintained at 50–65%. Mechanical ventilation and artificial lighting were provided, with 23 hours of continuous light followed by 1 hour of darkness. Birds had free access to water and feed. The chicken house was cleaned of manure promptly, with sweeping and disinfection performed twice weekly. The immunization program and methods followed commercial company recommendations.

1.5 Sample Collection and Preparation

A digestion and metabolism trial was conducted from days 17–20 of the feeding experiment without a separate preliminary period. Plastic sheets were placed in the manure trays of each replicate, and all fresh fecal samples were collected at a fixed time each afternoon (with feathers, feed, dander, and other debris removed). After mixing, 20% of the sample was placed in self-sealing bags, sprayed with 10% hydrochloric acid solution, and stored at 4°C. Finally, the fecal samples collected over four days were thoroughly mixed by replicate, dried to constant weight in a 65°C oven, equilibrated at room temperature for 24 hours, ground to pass through a 40-mesh sieve, and stored for analysis. On day 21 of the experimental period, two broilers from each replicate were selected, and 5 mL of blood was collected from the wing vein. The blood was immediately centrifuged at 3,000 r/min for 10 minutes, and the serum was stored at -20°C for subsequent determination of calcium and phosphorus contents and alkaline phosphatase activity. After blood collection, broilers were euthanized by cervical dislocation,

and the left tibia was removed. Muscles and connective tissue were removed, and the tibia was dried at 105°C for 48 hours for analysis of compressive strength. The bone was then defatted for 8 hours and ashed in a muffle furnace at 580°C for 24 hours to determine ash, calcium, and phosphorus contents.

1.6 Measurements

1.6.1 Growth Performance At the end of day 21, broilers were weighed by replicate, feed consumption was recorded, and feed intake, body weight gain, mortality rate, and feed-to-gain ratio were calculated.

1.6.2 Nutrient Apparent Metabolic Rate Both experimental diets and metabolic excreta were analyzed for calcium, phosphorus, and titanium dioxide contents, and dietary acid-binding capacity was measured to calculate apparent metabolic rates of dry matter, calcium, and phosphorus. Calcium and phosphorus contents were determined according to Zhang [8], titanium dioxide content was measured using the method described by Short et al. [9], and acid-binding capacity was determined following the method recommended by Tu et al. [10].

1.6.3 Tibia Indices and Serum Biochemical Indices Tibia ash content was determined according to GB 6438–1986. Tibia compressive strength was measured using a compressive strength tester (Model ZN7-16A). Serum calcium and phosphorus contents and alkaline phosphatase activity were determined using assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.7 Statistical Analysis

All experimental data were subjected to two-way analysis of variance using the GLM procedure of SAS 9.2 software, with the statistical model including phosphorus source, phosphorus level, and their interaction. When significant differences were detected by analysis of variance, means were compared using the LSD method. Multiple linear regression equations were generated using the GLM procedure of SAS:

$$Y = b_0 + b_1 X(s) + b_2 X(t)$$

where Y represents the measured index; X(s) and X(t) represent DCP and MDCP, respectively; and b , b , and b are equation coefficients. Using DCP as the standard (100%), the relative bioavailability of MDCP compared to DCP was calculated using the slope-ratio method of multiple linear regression [11].

Results

2.1 Effects of Different Phosphorus Sources and Levels on Growth Performance of Broilers

The effects of different phosphorus sources and supplementation levels on broiler growth performance are presented in Table 2 . As shown, feed intake and body weight gain of broilers in the MDCP supplementation group were significantly higher than those in the DCP group ($P<0.05$). With increasing phosphorus supplementation levels, feed intake and body weight gain increased significantly ($P<0.05$), while feed-to-gain ratio and mortality rate decreased significantly ($P<0.05$). The interaction between phosphorus source and supplementation level had no significant effect on any growth performance indices ($P>0.05$).

2.2 Effects of Different Phosphorus Sources and Levels on Nutrient Apparent Metabolic Rate of Broilers

The effects of different phosphorus sources and supplementation levels on nutrient apparent metabolic rate are shown in Table 3 . Phosphorus source had no significant effect on calcium or phosphorus apparent metabolic rates ($P>0.05$). The calcium apparent metabolic rate in the 0.25% supplementation group was significantly higher than that in the control and 0.05% groups ($P<0.05$), and its phosphorus apparent metabolic rate was significantly higher than that in the control, 0.05%, and 0.10% groups ($P<0.05$).

2.3 Effects of Different Phosphorus Sources and Levels on Tibia Indices of Broilers

The effects of different phosphorus sources and supplementation levels on tibia indices are presented in Table 4 . Tibia ash content in the MDCP supplementation group was significantly higher than that in the DCP group ($P<0.05$). With increasing phosphorus supplementation levels, tibia ash, calcium, phosphorus contents, and tibia strength all improved. The 0.25% supplementation group showed significantly higher tibia ash content and tibia strength compared to the control, 0.05%, and 0.10% groups ($P<0.05$), and significantly higher tibia phosphorus content compared to the control group ($P<0.05$). Both the 0.20% and 0.25% groups exhibited significantly higher tibia calcium content than the control and 0.05% groups ($P<0.05$). The control and 0.05% groups had significantly lower tibia ash content than the 0.15% and 0.20% groups ($P<0.05$). The 0.20% group demonstrated significantly higher tibia strength than the control, 0.05%, and 0.10% groups ($P<0.05$).

2.4 Effects of Different Phosphorus Sources and Levels on Serum Calcium, Phosphorus Contents and Alkaline Phosphatase Activity of Broilers

The effects of different phosphorus sources and supplementation levels on serum calcium, phosphorus contents, and alkaline phosphatase activity are shown in

Table 5 . Serum phosphorus content and alkaline phosphatase activity in the MDCP supplementation group were significantly higher than those in the DCP group ($P<0.05$). With increasing phosphorus supplementation levels, serum phosphorus content increased significantly ($P<0.05$), while serum alkaline phosphatase activity decreased significantly ($P<0.05$).

Relative Bioavailability of MDCP

The relative bioavailability of MDCP compared to DCP is presented in Table 6 . Using body weight gain, calcium apparent metabolic rate, phosphorus apparent metabolic rate, tibia ash, tibia calcium content, tibia phosphorus content, tibia strength, serum phosphorus content, and serum alkaline phosphatase activity of 1–21 day-old broilers as response criteria, the relative bioavailability values of MDCP compared to DCP were 124.7%, 96.7%, 131.2%, 106.7%, 108.7%, 118.1%, 105.3%, 114.5%, and 106.4%, respectively. Verification tests showed that the coefficients of the multiple linear regression equations for all response criteria were significant ($P<0.05$), indicating their suitability for determining biological value. Therefore, the average biological utilization rate (112.5%) was established as the relative bioavailability of MDCP compared to DCP.

Discussion

3.1 Effects of Different Phosphorus Sources and Levels on Broiler Growth Performance

Global phosphorus resources are limited and non-renewable [12]. Statistics indicate that 33% of phosphorus in the United States is discharged into soil through livestock manure, causing severe environmental pollution [13]. Therefore, reducing phosphorus emissions from livestock is imperative. Currently, two main strategies exist for reducing phosphorus excretion in animals: first, adding exogenous phytase to improve utilization of dietary phytate phosphorus, thereby reducing inorganic phosphorus supplementation to achieve phosphorus reduction [14-15]; second, selecting phosphorus sources with higher biological value or employing nutritional regulation techniques to improve phosphorus utilization efficiency and animal growth rate [16]. The former approach is limited by dietary phytate phosphorus content, and phytase can reduce dietary available phosphorus to 0.20–0.25% without affecting livestock performance. However, seeking highly efficient phosphorus sources is essential for maximizing phosphorus reduction in waste. DCP is currently the most widely used phosphorus source in the feed industry, belonging to citrate-soluble phosphates that are poorly water-soluble but dissolve in hydrochloric or citric acid, resulting in low animal utilization. MDCP is a co-crystalline compound of DCP and MCP, combining water-soluble and citrate-soluble phosphate feed additives, with MCP (a water-soluble phosphate) accounting for approximately 60%. Early research demonstrated that MDCP as a phosphorus source offers advantages over DCP. Wu et al. [17] reported that MDCP improved broiler body weight and weight gain compared to DCP. Similar results were documented by Wan et al. [18]

and Xia et al. [19]. Furthermore, Chen et al. [20] demonstrated in laying hens that, using potassium dihydrogen phosphate (MPP) as the standard, the relative biological value of phosphorus sources ranked as follows: MDCP > DCP > MCP > MPP, indicating MDCP is a superior phosphorus source for poultry diets compared to DCP. The present study also observed that broilers in the MDCP supplementation group exhibited significantly higher body weight gain and feed intake during days 1–21, reaffirming the application value of MDCP.

Phosphorus deficiency can cause slow bone growth, rickets, and osteomalacia in livestock [2]. Chinese national standards specify that dietary total phosphorus and non-phytate phosphorus contents for broiler chickens should be 0.68% and 0.50% at 1–2 weeks, and 0.65% and 0.40% at 3–6 weeks, respectively [7]. Previous reports on dietary phosphorus levels affecting broiler growth performance are numerous. Liu et al. [21] investigated dietary non-phytate phosphorus requirements in Arbor Acres broilers fed corn-soybean meal diets containing eight non-phytate phosphorus levels (0.10%, 0.15%, 0.25%, 0.30%, 0.35%, 0.40%, 0.45%, and 0.50%) using CaHPO ·2H O as the phosphorus source (basal diet contained 0.08% non-phytate phosphorus and 1.00% calcium). Results showed that phosphorus level significantly affected broiler body weight gain in a linear increasing trend, recommending an appropriate dietary non-phytate phosphorus content of 0.39% for 1–21 day-old broilers. These findings are consistent with the present study. Using 21-day body weight gain as a response criterion, the biological utilization rate of MDCP relative to DCP was determined to be 112.5%, which is close to the 113.2% reported by Wu et al. [17], further confirming the advantages of MDCP as a phosphorus source for poultry diets.

3.2 Effects of Different Phosphorus Sources and Levels on Broiler Tibia Indices

Calcium and phosphorus are the primary constituents of bones and teeth, with normal bone storing large amounts of phosphorus and calcium, accounting for 65–70% of total mineral weight in chickens [22]. Bone tissue, like other organ tissues, undergoes continuous metabolism and regulates blood calcium and phosphorus concentrations. When dietary calcium and phosphorus cannot meet physiological requirements, they must be mobilized from bone. Within certain limits, calcium and phosphorus depletion from storage does not endanger life [23]; however, clinical tibial symptoms are evident, making tibia quality an important target for assessing calcium and phosphorus status and balance. Wang et al. [24] demonstrated that dietary calcium and phosphorus levels significantly affect tibia ash and calcium and phosphorus deposition in broilers, and that increasing dietary calcium and phosphorus levels can increase tibia ash content. Increased non-phytate phosphorus levels significantly improve tibia strength, consistent with findings by Liu et al. [21], Wilkinson et al. [25], Bradbury et al. [26], and the present study. Analysis suggests that low dietary calcium and phosphorus levels lead to unmet requirements or severely imbalanced calcium-to-phosphorus ratios, forming insoluble calcium-phosphorus complexes

in the animal digestive tract that reduce ash deposition in bone. Additionally, bone grows rapidly during the first three weeks, requiring substantial calcium and phosphorus. When dietary phosphorus cannot meet broiler developmental needs, bone-stored phosphorus must be mobilized to compensate for dietary deficiency, reducing bone mineralization and ultimately decreasing bone strength [27].

3.3 Effects of Different Phosphorus Sources and Levels on Broiler Serum Biochemical Indices

Alkaline phosphatase is a class of non-specific phosphomonoesterases that hydrolyze phosphomonoester compounds to generate inorganic phosphate, sugars, alcohols, and phenols in alkaline environments. Localized on biological membranes, it serves as a marker enzyme for matrix vesicles and plasma membranes, and is widely distributed in bacteria, fungi, and animal organisms. In animals, it is broadly distributed across various tissues, primarily in liver, bone, kidney, placenta, small intestine, blood cells, mammary epithelial cells, and bile [28]. Typically, bone contains 50% of serum alkaline phosphatase [21]. This study also found that serum alkaline phosphatase activity decreased linearly with increasing dietary phosphorus levels, possibly because broiler bones obtained phosphorus from other metabolic reactions such as energy metabolism, cell signal transduction, and cell membrane repair. Alkaline phosphatase is positively correlated with tissue-nonspecific alkaline phosphatase (TNAP), and TNAP activity induced by low phosphorus levels directly affects bone mineralization [29-31]. Furthermore, phosphorus homeostasis is regulated by intestinal phosphorus absorption, renal phosphorus reabsorption, and exchange between extracellular fluid and bone [32]. Low dietary phosphorus levels can downregulate expression of type IIb sodium-phosphate transporter (NaPi-IIb) and type IIa sodium-phosphate transporter (NaPi-IIa), two sodium-phosphate cotransporters [33-34], which may be the fundamental cause of phosphorus balance disruption.

Conclusion

Based on comprehensive evaluation of body weight gain, tibia quality, and serum biochemical indices in 1–21 day-old broilers, the biological utilization rate of MDCP relative to DCP (100%) was determined to be 112.5%.

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