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Original Research Article

# Determination of optimal dietary calcium levels under different zinc sources in Jing Tint 6 layer chicks from hatch to day 14

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ABSTRACT

An experiment was conducted to investigate the optimal dietary calcium (Ca) levels in Jing Tint 6 layer chicks fed different sources of zinc (Zn). A total of 1440 day-old birds with similar body weight (38 ± 0.5 g) were selected and divided into 10 groups with 6 replicates per group, each containing 24 chicks. The experimental period lasted from hatch to d 14. Diets were formulated to contain 2 sources of Zn: organic Zn (80 mg/kg Zn as hydroxy methionine Zn, HMZn) and inorganic Zn (80 mg/kg Zn as Zn sulfate). For each Zn source, 5 diets were formulated with Ca levels of 0.80%, 0.90%, 1.03%, 1.10%, and 1.20%, respectively. The results showed that dietary Ca significantly increased the average daily gain (ADG) ($P < 0.001$). In addition, ADG was enhanced by the supplementation of organic Zn compared to diets containing inorganic Zn ($P = 0.009$). Tibia length was significantly increased by the interaction of Ca and Zn ($P = 0.038$). Serum Ca and P levels were significantly higher in the group supplemented with organic Zn compared to the group fed inorganic Zn ($P < 0.05$). The apparent total tract retention coefficients (ATTRC) of Ca increased quadratically with the increase of Ca level ($P < 0.001$). The excreta Ca levels decreased in the group supplemented with organic Zn compared to the group fed inorganic Zn ($P < 0.001$). The optimal dietary Ca levels were estimated as 0.90%, 0.86% and 0.96% for birds fed organic Zn and 0.91%, 0.92% and 1.06% for birds fed inorganic Zn using non-linear models based on the criteria of ADG, tibial length, and ATTRC of Ca, respectively. In conclusion, supplementing with organic Zn enhanced the growth performance of birds and reduced their calcium requirements from hatch to d 14.



## 1. Introduction

Calcium (Ca), as an essential mineral involved in a wide range of physiological activities, is the basis for the formation and growth of poultry skeleton and is critical for muscle contraction, enzyme activation and nerve impulse transmission (Ribeiro et al., 2019; David et al., 2021; Wan and Yin, 2023). The deficiency of Ca may affect the growth and development of chicks, resulting in rickets, gastrointestinal tract damage, and low immunity (Li et al., 2020; Bai et al., 2022; Landy and Toghyani, 2018). The optimum amount of Ca varies among different breeds of poultry at different stages in terms of skeletal development, growth performance and nutrient utilization (Jiang et al., 2016; Gong et al., 2024b). Bai et al. (2022) found that 1.0% Ca was sufficient for bone development and weight gain in birds on d 21. Walk et al. (2024) found that 0.40% to 0.53% Ca could meet the physiological needs of fast-growing broilers from 1 to 14 d of age. Wang et al. (2022) found that a dietary Ca level of 0.72% was sufficient for birds aged 22 to 42 d to grow healthily. Zhang et al. (2023b) recommended that dietary Ca of 0.66% benefits BYC growing pullets.

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Zinc (Zn) is also an important trace element involved in animal biological activities, and its deficiency may lead to slow growth, a weak immune system and reduced antioxidative ability of poultry (Selvaraj, 2020; Ma et al., 2023; Wang et al., 2023). High intake of one mineral has an interactive effect on other minerals (Lin et al., 2020). Recently, there has been a clear correlation in the absorption and metabolism of Zn with other trace elements in animals (Brzóska and Moniuszko-Jakoniuk, 2001). Mineral interactions are complex, and studies have shown that an increase in Zn intake may reduce the bioavailability of Ca in animals (Lin et al., 2020). It has also been found that Ca addition may lead to low Zn utilization and that excess Ca reduces Zn uptake due to competition for mineral-binding ligands (Ando et al., 2005). Furthermore, Suzuki et al. (2015) found that Zn deficiency reduced the efficiency of intestinal Ca absorption in rats. Nevertheless, it is possible to increase the bioavailability of minerals and to decrease their interactions with each other via the use of organic minerals (Yenice et al., 2015).

Jing Tint 6 layer chicks are a breed based on Rhode Island Red and White Leghorn with red featured and small pink eggs. It's a 3-way crossbreed with lower egg weight, high quality eggs, low mortality, modest body weight, displaying good adaptability and excellent production performance (Zhang et al., 2023a; Zhao et al., 2024). The non-phytate P requirements have been well assessed in Jing Tint 6 layer chicks from 1 to 42 d of age; however, the optimal dietary Ca requirements still require further evaluation (Gong et al., 2024a). Therefore, the purpose of the present study was to evaluate the optimal dietary Ca level under different Zn sources in Jing Tint 6 layer chicks from hatch (d 1) to d 14.

## 2. Materials and methods

### 2.1. Animal ethics statement

The experiment was conducted according to the guidelines of the National Act on the Use of Experimental Animals (People's Republic of China) and approved by the Animal Welfare Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences (IACUC # 201302).

### 2.2. Experimental diets

Every bird received one of 10 experimental diets, organized in a 2 × 5 factorial format, from the age of 1 to 14 d. Diets based on corn–soybean meal were developed using 2 types of Zn that contained organic Zn (80 mg/kg Zn as hydroxy methionine Zn [HMZn], Xing Jia Bio-engineering Co. Ltd., Changsha, Hunan, China) or inorganic Zn (80 mg/kg Zn as Zn sulfate, Merck, Darmstadt, Germany). For every Zn source, 5 distinct diets were developed to contain Ca concentrations of 0.80%, 0.90%, 1.03%, 1.10%, and 1.20% of the total diet. Diets based on corn–soybean meal were developed to cater to the dietary needs for birds aged from 1 to 14 d, as shown in Table 1. The metabolizable energy (ME) and other nutrient values in the diet were calculated according to the Feed Database in China (2020).

### 2.3. Birds and housing

A total of 1440 chickens with similar body weight (38 ± 0.5 g) were randomly placed in groups of 24 (90 cm deep × 600 cm

**Table 1**
The ingredients, calculated and measure composition of diets (%).

| Item | HMZn | | | | | Zn sulfate | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.80% Ca | 0.90% Ca | 1.03% Ca | 1.10% Ca | 1.20% Ca | 0.80% Ca | 0.90% Ca | 1.03% Ca | 1.10% Ca | 1.20% Ca |
| **Ingredients (as-fed basis)** | | | | | | | | | | |
| Corn | 56.00 | 56.00 | 56.00 | 56.00 | 56.00 | 56.00 | 56.00 | 56.00 | 56.00 | 56.00 |
| Soybean meal | 35.10 | 35.10 | 35.10 | 35.10 | 35.10 | 35.10 | 35.10 | 35.10 | 35.10 | 35.10 |
| Soybean oil | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Wheat bran | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 |
| Limestone | 0.22 | 0.52 | 0.85 | 1.06 | 1.36 | 0.22 | 0.52 | 0.85 | 1.06 | 1.36 |
| Zeolite powder | 1.31 | 1.01 | 0.68 | 0.47 | 0.17 | 1.31 | 1.01 | 0.68 | 0.47 | 0.17 |
| Mono-calcium phosphate | 2.55 | 2.55 | 2.55 | 2.55 | 2.55 | 2.55 | 2.55 | 2.55 | 2.55 | 2.55 |
| Salt | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| DL-Methionine | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 |
| Choline chloride | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| L-Lysine hydrochloride | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 |
| Premix[1] | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **Calculated nutrient level (dry matter basis)** | | | | | | | | | | |
| ME, Mcal/kg | 2.90 | 2.90 | 2.90 | 2.90 | 2.90 | 2.90 | 2.90 | 2.90 | 2.90 | 2.90 |
| Crude fat | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 | 5.6 |
| Crude protein | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Total calcium | 0.80 | 0.91 | 1.03 | 1.10 | 1.21 | 0.80 | 0.91 | 1.03 | 1.10 | 1.21 |
| Available P | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 |
| Lysine | 2.62 | 2.62 | 2.62 | 2.62 | 2.62 | 2.62 | 2.62 | 2.62 | 2.62 | 2.62 |
| Methionine | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 |
| Tryptophan | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Threonine | 1.64 | 1.64 | 1.64 | 1.64 | 1.64 | 1.64 | 1.64 | 1.64 | 1.64 | 1.64 |
| **Analyzed nutrient level (dry matter basis)** | | | | | | | | | | |
| Crude fat | 5.90 | 5.90 | 5.90 | 5.90 | 5.90 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| Crude protein | 19.60 | 19.60 | 19.60 | 19.60 | 19.60 | 19.60 | 19.60 | 19.60 | 19.60 | 19.60 |
| Total phosphorus | 0.87 | 0.87 | 0.87 | 0.87 | 0.87 | 0.84 | 0.84 | 0.84 | 0.84 | 0.84 |
| Calcium | 0.97 | 1.03 | 1.16 | 1.28 | 1.42 | 0.96 | 0.98 | 1.05 | 1.08 | 1.29 |
| Zinc, mg/kg | 106 | 106 | 106 | 106 | 106 | 103 | 103 | 103 | 103 | 103 |

Zn = zinc; HMZn = hydroxy methionine Zn; Ca = calcium; ME = metabolizable energy; P = phosphorus.
[1] Vitamin and mineral premix provided (per kilogram of diet): vitamin A 10,000 IU; vitamin $D_3$ 3000 IU; vitamin E 40 mg; vitamin K 33 mg; vitamin $B_1$ 2.5 mg; vitamin $B_2$ 8 mg; vitamin $B_6$ 5 mg; vitamin $B_{12}$ 0.025 mg; biotin 0.15 mg; folic acid 1.5 mg; niacinamide 50 mg; pantothenic acid 12 mg; Fe 60 mg; Cu 15 mg; Mn 80 mg; I 1.2 mg; Se 0.297 mg; Zn 80 mg.

wide × 40 cm high) from a supplier (Beijing Huadu Yukou Poultry Industry Co. Ltd., Beijing, China). Each cage was treated as a duplicate of the experiment, and 6 replicates were given for each diet. The duration of lighting gradually decreased from 24 to 12 h with increasing days of age. The temperature was slowly decreased from 35 to 34 °C for 1 to 7 d, and from 34 to 30 °C for 8 to 14 d. Maintaining a consistent 65% relative humidity was achieved through a 12-h cycle of light and darkness. Throughout the experiments, every bird had unrestricted access to feed and water, with weekly records of their body weight and feed consumption. Mortality rates were recorded to correct feed consumption and to adjust the data of feed:gain, and feed conversion ratio (FCR) was recorded accordingly.

### 2.4. Growth performance

Weekly records were completed for feed intake and body weight of every cage, followed by the calculation of FCR.

### 2.5. Serum biochemical analysis

On d 14, syringes were used to extract 3 mL of blood from a wing vein of 2 randomly selected birds in each cage, and serum was collected through centrifugation at 3000 × *g* and 4 °C for 10 min, followed by biochemical analysis using an automated analyzer (Roche Life Science, cobas c311, Basel, Switzerland), and commercial reagent kits (Lidman Biotech, Beijing, China). Biochemical indices in the serum included Ca, phosphorus (P), alkaline phosphatase (ALP), total protein (TP), and albumin (ALB).

### 2.6. Bone characteristics

The same birds used for blood sampling were sacrificed and then their left tibias were preserved at −20 °C pending further analysis. The strength of tibia was measured using an electronic universal testing machine (Weidu Analytical Instrument Manufacturing Ltd., WDS-2000, Wenzhou, Zhejiang, China).

### 2.7. Calcium utilization

Titanium (Ti) dioxide was added to diets (3 g/kg, as-fed) to calculate nutrient utilization via the index method. On d 10, trays for collecting excreta were set up, and the total excreta was collected over the final 3 d. The gathered excreta for Ca and Ti was analyzed to measure the apparent total tract retention coefficients (ATTRC), utilizing the ratios of Ti markers in feed and excreta (Liu et al., 2013).

### 2.8. Chemical analysis

Feathers and other debris in the excreta were removed, the excreta was sprayed with 10% hydrochloric acid, mixed well and preserved at −20 °C pending further analysis. The excreta samples were thawed, dried to constant weight at 56 °C and ground to pass through a 0.5-mm screen before analysis. For Ti analysis of the feed and fecal samples, inductively coupled plasma-mass spectrometry (Thermo Fisher Scientific, iCAPRQ, Schaumburg, IL, USA) was used according to China National Standard (GB 5009.268-2016). The Ca of the feed and fecal samples were determined using an inductively coupled plasma optical emission spectrometer (PerkinElmer, ICP-OES optima 8000, Waltham, MA, USA) according to China National Standard (GB/T 6436-2018). Diets were also analyzed for Zn, P, crude protein and crude fat according to the China National Standard GB/T 13885-2017, GB/T 6437-2018, GB/T6432-2018 and GB/T6433-2006, respectively.

### 2.9. Statistical analyses

All data were analyzed via a general linear model (GLM) procedure using IBM SPSS Statistics 22, followed by Duncan's multiple range tests, with the cage serving as the experimental unit for all statistical analyses. Orthogonal comparisons were used to analyze both linear and quadratic responses of the dependent variables to the independent variables (Ca level). The effects were regarded as significant when $P < 0.05$. If the interaction or main effects were significant, then a regression analyses of a straight-broken line and quadratic models were performed, respectively, using Origin 2023. The equation of the straight-broken line model is as follows:

$$Y_i = a + b \times (k - X_i) \times I,$$

Where $Y_i$ is the dependent variable, $a$ and $b$ are constants, $k$ is breakpoint (Ca requirements) and $X_i$ is the independent variable. $I = 1$ when $X_i \le k$, otherwise $I = 0$.

The equation of the quadratic model is as follows:

$$Y = c + b \times X + a \times X^2,$$

where $Y$ represents the dependent variable, $c$, $b$ and $a$ are constants, and $X$ is the independent variable.

Then the best-fit model correlating the response criteria with dietary Ca level (the quadratic model with a maximum response or broken line model with breakpoint) was used to determine the optimal dietary Ca requirements for birds.

## 3. Results

### 3.1. Growth performance

The influences of dietary Ca and Zn on growth performance are presented in Table 2. Dietary Ca had a significant influence on average daily gain (ADG) ($P < 0.001$), but did not influence the average daily feed intake (ADFI) ($P = 0.709$). In the group supplemented with organic zinc, the ADG showed a linear increasing trend with the increase of dietary Ca level ($P = 0.013$). However, in the group fed diets containing inorganic zinc, the ADG showed a quadratic trend with an increase in Ca intake ($P = 0.001$). Moreover, in comparison with those fed diets containing inorganic Zn, the ADG was significantly improved in the group supplemented with organic zinc from hatch to d 14 ($P = 0.009$).

### 3.2. Tibial characteristics

As presented in Table 3, there was a significant linear and quadratic trend in the tibial length as the level of Ca was increased for birds fed organic ($P = 0.047$ and $P = 0.019$, respectively) and inorganic Zn ($P < 0.001$ and $P = 0.003$, respectively) diets. Whereas, tibia breaking strength was not affected by Ca levels ($P = 0.414$). In addition, at a low Ca level (0.80%), tibial length in the group supplemented with organic zinc was significantly higher than those fed diets containing inorganic Zn ($P < 0.05$).

### 3.3. Serum parameters

As presented in Table 4, compared with the group fed diets containing inorganic Zn, the organic Zn diet increased the serum TP contents of birds on d 14 ($P < 0.001$), and the TP contents showed a quadratic trend with the increase of dietary Ca level ($P < 0.001$), while under the inorganic Zn diets, there was no significant effect on TP contents ($P > 0.05$). No significant effect was found on serum ALB contents by dietary Ca level ($P = 0.246$). However, in

**Table 2**
Effects of dietary calcium (Ca) and zinc (Zn) on growth performance of birds from 1 to 14 d.

| Item | | Final BW, g/bird | ADG, g/bird | ADFI, g/d | FCR, g/g |
|---|---|---|---|---|---|
| Zn source | Ca level | | | | |
| Organic Zn | 0.80% | 116.86$^{b}$ | 5.65$^{bcd}$ | 11.27 | 1.99 |
| | 0.91% | 128.21$^{a}$ | 6.46$^{a}$ | 11.49 | 1.90 |
| | 1.03% | 125.92$^{ab}$ | 6.36$^{a}$ | 11.26 | 1.90 |
| | 1.10% | 121.62$^{ab}$ | 5.94$^{abc}$ | 11.37 | 2.11 |
| | 1.21% | 129.18$^{a}$ | 6.35$^{a}$ | 11.22 | 1.84 |
| Inorganic Zn | 0.80% | 114.67$^{c}$ | 5.41$^{cd}$ | 11.20 | 1.90 |
| | 0.91% | 123.59$^{ab}$ | 6.13$^{ab}$ | 11.77 | 1.92 |
| | 1.03% | 125.68$^{ab}$ | 5.40$^{cd}$ | 11.37 | 2.10 |
| | 1.10% | 114.29$^{c}$ | 5.28$^{d}$ | 10.82 | 1.96 |
| | 1.21% | 113.63$^{c}$ | 5.26$^{d}$ | 11.16 | 2.12 |
| SEM | | 1.102 | 0.083 | 1.722 | 0.041 |
| Main effects | | | | | |
| Ca level | 0.80% | 115.77$^{b}$ | 5.54$^{b}$ | 11.23 | 1.94 |
| | 0.91% | 125.90$^{a}$ | 6.30$^{a}$ | 11.63 | 1.91 |
| | 1.03% | 125.80$^{a}$ | 5.88$^{b}$ | 11.32 | 2.00 |
| | 1.10% | 118.87$^{b}$ | 5.71$^{b}$ | 11.10 | 2.02 |
| | 1.21% | 121.41$^{ab}$ | 5.81$^{b}$ | 11.19 | 1.98 |
| SEM | | 0.804 | 0.052 | 1.341 | 0.032 |
| Zn source | Organic | 124.81$^{a}$ | 6.19$^{a}$ | 11.32 | 1.92 |
| | Inorganic | 118.29$^{b}$ | 5.50$^{b}$ | 11.27 | 2.02 |
| SEM | | 0.734 | 0.047 | 1.214 | 0.030 |
| *P*-value for main effects and interaction | | | | | |
| Zn source | | 0.001 | 0.009 | 0.422 | 0.977 |
| Ca level | | <0.001 | <0.001 | 0.709 | <0.001 |
| Zn source × Ca level | | 0.065 | 0.048 | 0.358 | 0.043 |
| *P*-value for contrasts of Ca level | | | | | |
| Organic Zn diets linear | | 0.010 | 0.013 | 0.931 | 0.530 |
| Organic Zn diets quadratic | | 0.075 | 0.025 | 0.888 | 0.995 |
| Inorganic Zn diets linear | | 0.002 | 0.027 | 0.675 | 0.148 |
| Inorganic Zn diets quadratic | | 0.001 | 0.001 | 0.829 | 0.748 |

BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.
a, b, c, d Values within a column with different superscripts differ significantly at $P < 0.05$.

**Table 3**
Effects of dietary calcium (Ca) and zinc (Zn) on tibial characteristics of birds on d 14.

| Item | | Length, mm | Breaking strength, kg |
|---|---|---|---|
| Zn source | Ca level | | |
| Organic Zn | 0.8% | 43.71$^{b}$ | 2.23 |
| | 0.91% | 45.45$^{ab}$ | 2.33 |
| | 1.03% | 45.2$^{ab}$ | 2.32 |
| | 1.1% | 44.71$^{ab}$ | 2.16 |
| | 1.21% | 45.06$^{ab}$ | 2.49 |
| Inorganic Zn | 0.8% | 41.86$^{c}$ | 2.28 |
| | 0.91% | 45.33$^{ab}$ | 2.50 |
| | 1.03% | 46.19$^{a}$ | 2.52 |
| | 1.1% | 45.03$^{ab}$ | 2.22 |
| | 1.21% | 45.81$^{a}$ | 2.45 |
| SEM | | 1.433 | 0.401 |
| Main effects | | | |
| Ca level | 0.80% | 42.79$^{b}$ | 2.25 |
| | 0.91% | 45.39$^{a}$ | 2.42 |
| | 1.03% | 45.70$^{a}$ | 2.42 |
| | 1.10% | 44.87$^{a}$ | 2.19 |
| | 1.21% | 45.43$^{a}$ | 2.47 |
| SEM | | 1.051 | 0.327 |
| Zn source | Organic | 44.83 | 2.31 |
| | Inorganic | 44.85 | 2.40 |
| SEM | | 1.125 | 0.415 |
| *P*-value for main effects and interaction | | | |
| Zn source | | 0.948 | 0.428 |
| Ca level | | <0.001 | 0.414 |
| Zn source × Ca level | | 0.038 | 0.956 |
| *P*-value for contrasts of Ca level | | | |
| Organic Zn diets linear | | 0.047 | 0.400 |
| Organic Zn diets quadratic | | 0.019 | 0.310 |
| Inorganic Zn diets linear | | <0.001 | 0.650 |
| Inorganic Zn diets quadratic | | 0.003 | 0.470 |

a, b, c Values within a column with different superscripts differ significantly at $P < 0.05$.

comparison with the group fed diets containing inorganic Zn, serum ALB content was significantly improved in organic Zn diets ($P < 0.001$). Both of Ca level and Zn source did not affect serum ALP contents ($P > 0.05$). Dietary Ca level did not affect serum Ca and P contents ($P > 0.05$), however, the levels of Ca and P in the group fed organic Zn diets were higher than those fed inorganic Zn diets ($P < 0.05$).

### 3.4. Apparent total tract retention coefficients

As shown in Table 5, dietary Ca level in both organic and inorganic Zn diets had a significant effect on excreta Ca output and total tract retention coefficient (ATTRC) of Ca ($P < 0.05$). With increasing dietary Ca levels, the excreta Ca output increased quadratically ($P = 0.002$ and $P = 0.007$ for organic Zn and inorganic diets, respectively), while ATTRC of Ca showed a quadratic trend ($P < 0.001$ and $P = 0.020$ for organic Zn and inorganic diets, respectively). The ATTRC of Ca initially raised and then declined with the increase of Ca levels. In general, diets supplemented with organic Zn reduced excreta Ca output and enhanced ATTRC of Ca in birds on d 14, in comparison with those fed inorganic Zn diets.

### 3.5. Estimations of dietary Ca requirements of birds supplemented with different sources of zinc

The results of dietary Ca needs for birds from hatch to d 14 supplemented with organic or inorganic Zn diets, as estimated from fitted regression models, are presented in Table 6. It was suggested that ADG, tibia length, and Ca ATTRC were appropriate criteria to assess the dietary Ca needs in birds. Based on the best-fitted non-linear model, the optimum dietary Ca levels were

**Table 4**
Effects of dietary calcium (Ca) and zinc (Zn) on serum parameters of birds on d 14.

| Item | | TP, g/L | ALB, g/L | ALP, U/L | Ca, mmol/L | P, mmol/L |
|---|---|---|---|---|---|---|
| Zn source | Ca level | | | | | |
| Organic Zn | 0.80% | 33.16$^{bcd}$ | 17.82$^{abc}$ | 6713.25 | 3.02$^{a}$ | 2.76$^{ab}$ |
| | 0.91% | 36.15$^{abc}$ | 19.98$^{ab}$ | 7770.40 | 3.22$^{a}$ | 2.80$^{ab}$ |
| | 1.03% | 39.85$^{a}$ | 21.00$^{a}$ | 11,469.83 | 3.24$^{a}$ | 3.11$^{a}$ |
| | 1.10% | 38.75$^{a}$ | 21.10$^{a}$ | 8826.25 | 3.16$^{a}$ | 2.74$^{ab}$ |
| | 1.21% | 31.98$^{bcd}$ | 17.33$^{abc}$ | 7247.83 | 2.81$^{ab}$ | 2.69$^{ab}$ |
| Inorganic Zn | 0.80% | 33.35$^{bcd}$ | 18.23$^{abc}$ | 10,285.60 | 2.79$^{ab}$ | 2.50$^{b}$ |
| | 0.91% | 28.65$^{d}$ | 28.65$^{d}$ | 10,176.50 | 2.41$^{c}$ | 2.33$^{b}$ |
| | 1.03% | 30.80$^{cd}$ | 16.72$^{bc}$ | 10,148.33 | 2.89$^{ab}$ | 2.54$^{ab}$ |
| | 1.10% | 30.62$^{cd}$ | 16.10$^{bc}$ | 8885.17 | 2.95$^{a}$ | 2.72$^{ab}$ |
| | 1.21% | 31.12$^{cd}$ | 16.67$^{bc}$ | 6351.00 | 2.84$^{ab}$ | 2.58$^{ab}$ |
| SEM | | 4.563 | 2.723 | 3504.664 | 0.371 | 0.352 |
| Main effect | | | | | | |
| Ca level | 0.80% | 33.26$^{ab}$ | 18.03 | 6970.97 | 2.91 | 2.63 |
| | 0.91% | 32.40$^{ab}$ | 17.86 | 6629.83 | 2.81 | 2.56 |
| | 1.03% | 35.33$^{a}$ | 18.86 | 8272.00 | 3.07 | 2.82 |
| | 1.10% | 34.68$^{ab}$ | 18.60 | 7384.67 | 3.06 | 2.73 |
| | 1.21% | 31.55$^{b}$ | 17.00 | 5211.67 | 2.83 | 2.64 |
| SEM | | 2.317 | 1.013 | 1303.361 | 0.141 | 0.135 |
| Zn source | Organic | 35.98$^{a}$ | 19.45$^{a}$ | 7289.55 | 3.09$^{a}$ | 2.82$^{a}$ |
| | Inorganic | 30.91$^{b}$ | 16.69$^{b}$ | 6498.10 | 2.78$^{b}$ | 2.53$^{b}$ |
| SEM | | 2.031 | 0.931 | 1148.361 | 0.132 | 0.121 |
| *P*-value for main effects and interaction | | | | | | |
| Zn source | | <0.001 | <0.001 | 0.518 | <0.001 | 0.001 |
| Ca level | | 0.025 | 0.246 | 0.597 | 0.114 | 0.312 |
| Zn source × Ca level | | 0.001 | 0.008 | 0.055 | 0.021 | 0.175 |
| *P*-value for contrasts of Ca level | | | | | | |
| Organic Zn diets linear | | 0.860 | 0.886 | 0.677 | 0.188 | 0.569 |
| Organic Zn diets quadratic | | <0.001 | 0.002 | 0.021 | 0.018 | 0.078 |
| Inorganic Zn diets linear | | 0.514 | 0.268 | 0.145 | 0.108 | 0.216 |
| Inorganic Zn diets quadratic | | 0.083 | 0.129 | 0.426 | 0.800 | 0.919 |

TP = total protein; ALB = albumin; ALP = alkaline phosphatase; P = phosphorus.
a, b, c, d Values within a column with different superscripts differ significantly at $P < 0.05$.

**Table 5**
Effects of dietary calcium (Ca) and zinc (Zn) on excreta Ca output and ATTRC of birds on d 14.

| Item | | Excreta Ca, g/kg DMI | ATTRC of Ca, % |
|---|---|---|---|
| Zn source | Ca level | 3.83$^{d}$ | 63.04$^{ab}$ |
| Organic Zn | 0.80% | 3.89$^{d}$ | 64.45$^{ab}$ |
| | 0.91% | 3.66$^{d}$ | 71.10$^{a}$ |
| | 1.03% | 4.95$^{cd}$ | 63.48$^{ab}$ |
| | 1.10% | 7.49$^{a}$ | 50.88$^{c}$ |
| | 1.21% | 6.22$^{bc}$ | 39.36$^{d}$ |
| Inorganic Zn | 0.80% | 4.89$^{cd}$ | 54.40$^{bc}$ |
| | 0.91% | 5.31$^{c}$ | 54.79$^{bc}$ |
| | 1.03% | 5.29$^{c}$ | 56.87$^{bc}$ |
| | 1.10% | 6.84$^{ab}$ | 49.59$^{c}$ |
| SEM | | 1.323 | 1.672 |
| Main effect | | | |
| Ca level | 0.80% | 4.78$^{b}$ | 53.57$^{b}$ |
| | 0.91% | 4.39$^{b}$ | 59.42$^{a}$ |
| | 1.03% | 4.65$^{b}$ | 61.31$^{a}$ |
| | 1.10% | 5.12$^{b}$ | 60.18$^{a}$ |
| | 1.21% | 7.17$^{a}$ | 50.37$^{b}$ |
| SEM | | 0.921 | 1.135 |
| Zn source | Organic | 4.76$^{b}$ | 62.59$^{a}$ |
| | Inorganic | 5.71$^{a}$ | 51.00$^{b}$ |
| SEM | | 0.827 | 0.984 |
| *P*-value for main effects and interaction | | | |
| Zn source | | <0.001 | <0.001 |
| Ca level | | <0.001 | <0.001 |
| Zn source × Ca level | | 0.002 | 0.005 |
| *P*-value for contrasts of Ca level | | | |
| Organic Zn diets linear | | <0.001 | <0.001 |
| Organic Zn diets quadratic | | 0.002 | <0.001 |
| Inorganic Zn diets linear | | 0.144 | 0.001 |
| Inorganic Zn diets quadratic | | 0.007 | 0.020 |

ATTRC = apparent total tract retention coefficients.
a, b, c, d Values within a column with different superscripts differ significantly at $P < 0.05$.

**Table 6**
Estimations of dietary calcium (Ca) requirements of birds supplemented with different sources of zinc (Zn) based on the best-fitted non-linear models.

| Zn source | Dependent variable | Regression equation | $R^2$ | *P*-value | Estimated Ca requirement, % |
|---|---|---|---|---|---|
| Organic Zn | ADG | $Y = 6.31 - 6.45\,(0.90 - X)$ | 0.448 | 0.002 | 0.90 |
| | Tibial length | $Y = 45.22 - 31.67\,(0.86 - X)$ | 0.386 | 0.004 | 0.86 |
| | ATTRC of Ca | $Y = -196.40 + 553.88X - 196.40X^2$ | 0.732 | <0.001 | 0.96 |
| Inorganic Zn | ADG | $Y = 0.61 + 11.42X - 6.38X^2$ | 0.195 | 0.025 | 0.91 |
| | Tibial length | $Y = 29.02 + 107.08\,(0.92 - X)$ | 0.551 | <0.001 | 0.92 |
| | ATTRC of Ca | $Y = -261.16 + 613.43X - 295.53X^2$ | 0.542 | 0.008 | 1.06 |

ADG = average daily gain; ATTRC = apparent total tract retention coefficients.

estimated at 0.90%, 0.86% and 0.96% for birds that received organic Zn supplementation from hatch to d 14, and 0.91%, 0.92%, and 1.06% for birds received inorganic Zn supplementation from hatch to d 14. For birds given an organic Zn supplementation, 0.90% Ca was sufficient to achieve optimal growth without reducing dietary Ca utilization. For birds fed inorganic Zn diets, ADG was relatively insensitive to dietary Ca level, which leads to a poorer model ($R^2$ = 0.195) than tibial length ($R^2$ = 0.551) or ATTRC of Ca ($R^2$ = 0.542). Thus, approximately 0.92% Ca was needed to meet Ca metabolism requirements without decreasing dietary Ca utilization for Jing Tint 6 layer chicks from hatch to d 14 of age.

## 4. Discussion

The research found that dietary Ca level influenced ADG of 1 to 14 d birds. Compared with birds supplied with 0.91% Ca, ADG decreased when they were provided with 1.03% Ca in the group fed inorganic Zn diets. Previous studies have widely evaluated the effects of Ca on bird growth performance. Bai et al. (2022) found that dietary Ca affected the ADG and FCR of birds from hatch to d 21 d. High Ca level in diets negatively affects bird growth (Gautier et al., 2017), and was comparable to our findings where 1.03% Ca led to a lower ADG of 1 to 14 d birds. The negative impact of excessive Ca on performance might be explained. For instance, excessive dietary Ca can affect the uptake and utilization of other minerals by chelation, thus decreasing the energy value (Gautier et al., 2017). Our results showed that the organic zinc diets alleviated the negative impact of high Ca on the ADG of birds from 1 to 14 d of age. In this research, when only growth parameters were considered, the recommended Ca level in the diets for birds from 1 to 14 d of age was 0.90% for those fed organic Zn diets and 0.91% for those fed inorganic Zn diets.

Tibial characteristics, such as tibial breaking strength and length, were traditionally used in the evaluation of bone mineralization in birds (Liu et al., 2017). Earlier studies have found that bone properties have improved significantly with the increase in dietary Ca levels (Driver et al., 2005). Hamdi et al. (2015) also showed a linear increase in tibial weight with the increase of Ca in diet from 0.50% to 0.90%. Moreover, adding organic trace elements to the diet at different levels could increase the tibial breaking strength of layer chicks (Manangi et al., 2015). In this research, we have discovered that organic Zn supplementation in diets improved tibia growth. Considering only the tibia parameters, the optimum amount of Ca in the diets of 1 to 14 d birds was 0.86% for those fed organic Zn diets and 0.92% for those fed inorganic Zn diets.

Serum Ca and P levels are considered conventional standards for evaluating Ca nutrition in birds (Manangi et al., 2015). Our data indicated that dietary Ca level had no effect on serum Ca and P levels of birds on d 14 which was similar to several studies (Bai et al., 2022; Wang et al., 2022). Modern chickens were able to maintain serum Ca and P levels within a certain range regardless of dietary Ca levels, Hurwitz et al. (1987) explained. This is primarily due to the important role played by calcitonin, parathyroid hormone and 1,25$(OH)_2D_3$ in the oscillatory regulatory system. However, earlier studies reported that organic Zn increased serum Ca levels compared with inorganic Zn, independent of dietary Zn level (Yenice et al., 2015), which was similar to our current study. Some studies have found that there is an interaction between Ca and Zn at the levels of absorption, distribution, and retention (Pappas et al., 2011; Lin et al., 2020). In addition, it was found that the regulation of Ca and Zn metabolism was controlled by the circadian time-keeping system. Circadian Zn supplement regimes affect the distribution of Ca in serum and in eggs via Ca and Zn interactions in laying hens (Lin et al., 2020). In this experiment, it was also found that the TP and ALB showed a quadratic trend with the dietary Ca levels for 1 to 14 d of age birds fed organic Zn diets. To a certain extent, the serum biochemical parameters could reflect the level of nutrition metabolism and health index of birds. TP and ALB can represent the status of dietary protein metabolism (Zhang et al., 2023b). Similarly, the study reported by Li et al. (2019) also found that dietary organic Zn can significantly increase the serum ALB level of laying hens compared with inorganic Zn.

Diet, physiology, and animal factors can affect the absorption and retention of Ca (David et al., 2021). In the present research, the ATTRC of Ca was affected by Ca level and Zn source, which indicates that it was important to make dietary supplements with appropriate Ca and Zn resources. The effect of Zn on the digestion and absorption of Ca has been widely evaluated (McDowell, 2003), but different sources of Zn have different effects on Ca. Some studies have shown that Zn from different sources is widely used as a dietary supplement, but it does not have a significant effect on Ca digestion (Tsai et al., 2016). However other research has found that dietary organic Zn can reduce fecal Ca level compared with inorganic Zn, and it is dependent on the additional level of organic Zn (Yenice et al., 2015). Our study also found that 80 mg/kg dietary organic Zn can reduce fecal Ca level and increase the ATTRC of Ca compared with inorganic Zn. In addition, ATTRC of Ca showed a quadratic trend with dietary Ca levels, suggesting ATTRC of Ca was heavily dependent on dietary Ca levels. A study showed that low-Ca diets showed a more significant advantage in promoting Ca retention than diets with higher levels of Ca (Gautier et al., 2017), suggesting that birds alleviate Ca deficiency by enhancing Ca absorption and utilization efficiency. Parathyroid hormone plays a crucial regulatory role in the physiological response to increased absorption (Gautier et al., 2017; Zhu et al., 2018). Generally, considering only the Ca utilization, the optimal level of Ca in the diets of 1 to 14 d birds was 0.96% for those fed organic Zn diets and 1.06% for those fed inorganic Zn diets.

## 5. Conclusion

This study demonstrated that supplementing organic Zn in diets with lower Ca (0.91%) was adequate to optimize bird growth and that Ca utilization was not significantly reduced. In addition, at the same dietary Ca level, the organic zinc diet was more effective than the inorganic zinc diet in improving the ADG, as well as serum Ca, P and protein levels of birds on d 14. Therefore, for birds fed organic Zn diets, 0.90% Ca was sufficient to achieve optimal growth rate and meet Ca metabolism needs without reducing dietary Ca utilization. For birds fed inorganic Zn diets, the Ca requirement would be approximately 0.92% to meet all Ca metabolism needs without reducing dietary Ca utilization.

## Credit Author Statement

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## Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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