

Postprint: Different Dietary Combinations of Copper, Iron, Zinc, and Manganese for 22-42-Day-Old Broilers

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Date: 2017-10-10T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary copper, iron, zinc, and manganese levels on growth performance and fecal excretion of copper, iron, zinc, and manganese in 22- to 42-day-old broilers, and to explore the balance pattern of trace elements. A total of 940 1-day-old Arbor Acres (AA) male broilers were fed a corn-soybean meal diet containing 16.96, 166.66, 46.01, and 60.26 mg/kg of copper, iron, zinc, and manganese, respectively, from 1 to 21 days of age. Nine hundred 22-day-old AA male broilers with good body condition and similar body weight were selected and randomly divided into 15 groups with 6 replicates per group and 10 birds per replicate. The control group was fed a basal diet containing 8, 125, 40, and 60 mg/kg of copper, iron, zinc, and manganese, respectively, while the experimental groups were fed 14 experimental diets formulated by adding 2-8 mg/kg copper, 10-35 mg/kg iron, 10-40 mg/kg zinc, and 15-60 mg/kg manganese to the basal diet according to the uniform design principle. Growth performance was measured during the experimental period, and fecal samples were collected from 38 to 42 days of age to determine copper, iron, zinc, and manganese contents. The results showed: 1) When dietary copper, iron, zinc, and manganese levels ranged from NRC recommendations to 2 times NRC recommendations, there were no significant effects on average daily gain (ADG) and feed-to-gain ratio (F/G) of 22- to 42-day-old broilers ($P>0.05$). 2) Optimal solution analysis revealed that when fecal copper excretion was minimized at 23.11 mg/kg, dietary copper, iron, zinc, and manganese levels were 7.15, 116.28, 82.82, and 60.95 mg/kg, respectively; when iron excretion was minimized at 438.34 mg/kg, dietary levels were 14.78, 116.81, 38.15, and 63.72 mg/kg, respectively; when zinc excretion was minimized at 102.60 mg/kg, dietary levels were 7.69, 127.40, 40.70, and 133.26 mg/kg, respectively; when manganese excretion was minimized at 159.37 mg/kg, dietary

levels were 11.07, 136.97, 46.13, and 59.05 mg/kg, respectively; and when total fecal copper, iron, zinc, and manganese excretion was minimized at 218.94 mg/kg, dietary levels were 7.36, 125.74, 38.23, and 62.76 mg/kg, respectively. In conclusion, dietary copper, iron, zinc, and manganese levels ranging from NRC recommendations to 2 times NRC recommendations had no significant effect on growth performance of 22- to 42-day-old broilers ($P>0.05$); fecal excretion of any single element among copper, iron, zinc, and manganese was affected by dietary levels of other trace elements, and different optimal combinations existed for minimizing excretion of different elements; when dietary copper, iron, zinc, and manganese levels were 7.36, 125.74, 38.23, and 62.76 mg/kg, respectively, the trace element requirements of broilers could be met while total fecal copper, iron, zinc, and manganese excretion was minimized.

Full Text

Research on Different Combination Levels of Copper, Iron, Zinc, and Manganese for Broilers Aged 22 to 42 Days

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Abstract

This experiment investigated the effects of dietary copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn) levels on growth performance and fecal excretion of these trace elements in broilers aged 22 to 42 days, and explored an optimal trace element balance model. A total of 940 one-day-old male Arbor Acres (AA) broilers were fed a corn-soybean meal diet containing 16.96, 166.66, 46.01, and 60.26 mg/kg of Cu, Fe, Zn, and Mn, respectively, from 1 to 21 days of age. At 22 days, 900 healthy male AA broilers with similar body weight were selected and randomly allocated to 15 groups with 6 replicates per group and 10 broilers per replicate. The control group received a basal diet containing 8, 125, 40, and 60 mg/kg of Cu, Fe, Zn, and Mn, respectively. The experimental groups were fed 14 experimental diets formulated by supplementing the basal diet with 2-8 mg/kg Cu, 10-35 mg/kg Fe, 10-40 mg/kg Zn, and 15-60 mg/kg Mn according to uniform design principles. Growth performance was measured during the experimental period, and fecal samples were collected from 38 to 42 days to determine Cu, Fe, Zn, and Mn concentrations.

The results showed: (1) Dietary Cu, Fe, Zn, and Mn levels ranging from NRC recommendations to twice the NRC recommendations had no significant effects on average daily gain (ADG) or feed-to-gain ratio (F/G) in 22-42 day-old broilers ($P>0.05$). (2) Optimal solution analysis revealed that when fecal Cu excretion reached its minimum of 23.11 mg/kg, dietary Cu, Fe, Zn, and Mn levels

were 7.15, 116.28, 82.82, and 60.95 mg/kg, respectively. When fecal Fe excretion reached its minimum of 438.34 mg/kg, dietary levels were 14.78, 116.81, 38.15, and 63.72 mg/kg, respectively. When fecal Zn excretion reached its minimum of 102.60 mg/kg, dietary levels were 7.69, 127.40, 40.70, and 133.26 mg/kg, respectively. When fecal Mn excretion reached its minimum of 159.37 mg/kg, dietary levels were 11.07, 136.97, 46.13, and 59.05 mg/kg, respectively. When total fecal excretion of Cu, Fe, Zn, and Mn reached its minimum of 218.94 mg/kg, dietary levels were 7.36, 125.74, 38.23, and 62.76 mg/kg, respectively. In conclusion, dietary Cu, Fe, Zn, and Mn levels between NRC recommendations and twice the NRC recommendations did not significantly affect growth performance of 22-42 day-old broilers ($P>0.05$). Excretion of any single trace element in feces was influenced by dietary levels of the other trace elements, and different optimal combinations existed for minimizing excretion of different elements. Dietary Cu, Fe, Zn, and Mn levels of 7.36, 125.74, 38.23, and 62.76 mg/kg, respectively, could satisfy broiler trace element requirements while minimizing total fecal excretion of Cu, Fe, Zn, and Mn.

Keywords: copper, iron, zinc, manganese; broilers; growth performance; trace element excretion; uniform design

Introduction

Trace elements are indispensable nutrients for animal life and production, performing crucial physiological functions, particularly copper, iron, zinc, and manganese. Copper serves as a component of metalloenzymes such as ceruloplasmin, directly participating in metabolism. Iron is a component of hemoglobin in red blood cells, ensuring normal oxygen transport. Zinc is an essential component of numerous enzymes, exerting biological functions by influencing enzymatic activity. Manganese is closely related to bone growth in livestock and poultry, and dietary manganese deficiency can cause skeletal deformities such as perosis. Different trace elements exhibit synergistic and antagonistic effects in broilers that are closely related to their absorption. For example, metallothionein formed by excessive zinc supplementation can hinder copper transport in intestinal epithelial cells, causing secondary copper deficiency. Some scholars believe that the trace element requirements established by NRC (1994) for poultry are too low to meet practical production needs. However, excessive supplementation leads to incomplete absorption and utilization, with excess elements excreted in feces, causing waste and environmental pollution. Consequently, researchers have proposed a trace element balance model, suggesting that trace elements, like amino acids, have an optimal proportional relationship that can meet animal growth requirements while significantly reducing excretion. This study aimed to investigate the effects of different Cu, Fe, Zn, and Mn ratios on broiler growth performance and fecal excretion, and to identify appropriate supplementation ratios to provide theoretical and technical support for applying trace element balance models in broiler diets.

Materials and Methods

1.1 Experimental Materials Inorganic trace elements used in this experiment were copper sulfate pentahydrate, ferrous sulfate monohydrate, zinc sulfate monohydrate, and manganese sulfate monohydrate (all analytical grade), with metal element contents of 25.22%, 30.11%, 34.39%, and 31.89%, respectively. The basal feed ingredients contained 8.00, 124.82, 36.11, and 27.07 mg/kg of Cu, Fe, Zn, and Mn, respectively.

1.2 Experimental Animals A total of 940 one-day-old male Arbor Acres (AA) broilers (purchased from Beijing Huadu Broiler Company) were fed a corn-soybean meal diet containing 16.96, 166.66, 46.01, and 60.26 mg/kg of Cu, Fe, Zn, and Mn, respectively, from 1 to 21 days of age. At 22 days, 900 healthy male AA broilers with similar body weight were selected and randomly divided into 15 groups with 6 replicates per group and 10 broilers per replicate. The control group received the basal diet, while experimental groups received test diets. The basal diet composition and nutrient levels are shown in Table 1. The experimental period lasted 21 days.

1.3 Experimental Diets and Design This experiment employed a uniform design method, using basal diet trace element contents as the minimum dose and twice the NRC (1994) recommendations as the maximum dose. Supplemental levels of Cu, Fe, Zn, and Mn are shown in Table 2, and coded values and actual dietary contents are presented in Table 3.

1.4 Management Practices Broilers were housed in cages with ad libitum access to feed and water. Lighting was provided for 23 hours daily with 1 hour of darkness (19:00–20:00). Daily feed intake per replicate was recorded, along with mortality, time of death, and body weight of dead birds. All dietary ingredients were ground using a stainless steel grinder. Routine hygiene management was practiced, and no medications were administered during the experiment. The trial was conducted at the Changping Nankou Pilot Base of the Feed Research Institute, Chinese Academy of Agricultural Sciences.

At 42 days, broilers were fasted for 8 hours and then weighed by replicate after fasting. Feed consumption was accurately recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) for each replicate during 22–42 days.

1.5.2 Metabolic Parameters At 24:00 on day 36, feed was withdrawn to empty gastrointestinal contents. At 08:00 on day 37, excreta were removed from collection trays, clean plastic sheets were placed under cages, and feed was provided with accurate recording of feed amount. From day 38, all excreta were collected daily by replicate. Feathers and feed spillage were removed, total fresh fecal weight was recorded, and samples were taken after mixing. A small amount of 10% hydrochloric acid solution was sprayed for nitrogen fixation.

Collection continued for 3 consecutive days. Fecal samples were placed in an oven at 105°C for 15 minutes to inactivate enzymes and microorganisms, then dried at 65°C. After drying, samples were equilibrated in air for 24 hours before weighing. Dried samples were ground using a stainless steel grinder, passed through a 40-mesh sieve, and stored in sealed bags for analysis.

Approximately 0.5 g of fecal sample was weighed into a digestion tube, and 7 mL of nitric acid was added for overnight digestion. Afterward, 1 mL of hydrogen peroxide was added and the digestion tube was assembled. The microwave digestion program was set as follows: 130°C for 10 min, 150°C for 5 min, and 180°C for 20 min. After digestion, the digest was transferred to a 50 mL conical flask with deionized water and heated on a temperature-controlled plate for acid removal ($T < 180^{\circ}\text{C}$) until evaporated to 1–2 mL. The solution was then transferred and diluted to 25 mL with 1% HNO_3 , mixed well, and analyzed for Cu, Fe, Zn, and Mn content using atomic absorption spectrophotometry.

1.6 Data Processing and Statistical Analysis Data were analyzed using one-way ANOVA and Duncan's multiple comparison in SPSS 17.0 software, and polynomial stepwise regression in DPS (v 9.05). The coefficient of determination (R^2) indicated the goodness of fit of the polynomial. Significance of regression coefficients was tested using t-tests, with $P < 0.05$ considered significant and $P < 0.01$ considered highly significant. Experimental data are expressed as mean \pm standard error ($M \pm \text{SE}$).

Results

2.1 Effects of Dietary Cu, Fe, Zn, and Mn Levels on Growth Performance of 22–42 Day-Old Broilers As shown in Table 4, dietary Cu, Fe, Zn, and Mn levels significantly affected ADFI ($P < 0.05$) but had no significant effects on ADG or F/G ($P > 0.05$).

2.2 Effects of Dietary Cu, Fe, Zn, and Mn Levels on Fecal Excretion in 22–42 Day-Old Broilers Fecal Cu, Fe, Zn, and Mn excretion data under different dietary trace element levels (Table 5) were subjected to polynomial stepwise regression using DPS (v 9.05) to derive regression equations (Table 6). Optimal solution analysis was performed to determine dietary Cu, Fe, Zn, and Mn levels that minimized excretion of each element.

Regression equation 1 (Table 6) showed that dietary Fe and Zn levels significantly affected fecal Cu excretion ($P < 0.05$), both exhibiting negative effects, while the $\text{Fe} \times \text{Zn}$ interaction showed a highly significant positive effect ($P < 0.01$). Optimal solution analysis indicated that minimum fecal Cu excretion of 23.11 mg/kg occurred at dietary Cu, Fe, Zn, and Mn levels of 7.15, 116.28, 82.82, and 60.95 mg/kg, respectively.

Regression equation 2 revealed that dietary Cu level had a highly significant negative effect on fecal Fe excretion ($P < 0.01$), while Fe excretion increased

significantly with dietary Fe level ($P < 0.05$). The Zn×Mn interaction had a highly significant positive effect on Fe excretion ($P < 0.01$). Minimum fecal Fe excretion of 438.34 mg/kg occurred at dietary Cu, Fe, Zn, and Mn levels of 14.78, 116.81, 38.15, and 63.72 mg/kg, respectively.

Regression equation 3 indicated that dietary Cu and Fe levels both had highly significant effects on fecal Zn excretion ($P < 0.01$), with Cu showing a positive effect and Fe a negative effect. The Cu×Fe interaction had a significant negative effect on Zn excretion ($P < 0.05$), while the Zn×Mn interaction showed the opposite effect ($P < 0.05$). Minimum fecal Zn excretion of 102.60 mg/kg occurred at dietary Cu, Fe, Zn, and Mn levels of 7.69, 127.40, 40.70, and 133.26 mg/kg, respectively.

Regression equation 4 showed that fecal Mn excretion increased significantly with dietary Mn level ($P < 0.05$), while Cu×Zn, Cu×Mn, and Zn×Mn interactions had highly significant negative effects on Mn excretion ($P < 0.01$). Minimum fecal Mn excretion of 159.37 mg/kg occurred at dietary Cu, Fe, Zn, and Mn levels of 11.07, 136.97, 46.13, and 59.05 mg/kg, respectively.

Regression equation 5 demonstrated that dietary Cu and Fe levels significantly affected total fecal excretion of Cu, Fe, Zn, and Mn ($P < 0.05$). Although Zn and Mn alone had no significant effects ($P > 0.05$), their interaction significantly affected total excretion ($P < 0.05$), and interactions of Cu with Fe, Zn, and Mn all had highly significant effects on total excretion ($P < 0.01$). Minimum total fecal excretion of 218.94 mg/kg occurred at dietary Cu, Fe, Zn, and Mn levels of 7.36, 125.74, 38.23, and 62.76 mg/kg, respectively.

Discussion

3.1 Effects of Dietary Cu, Fe, Zn, and Mn Levels on Growth Performance of 22-42 Day-Old Broilers The NRC recommends 8, 80, 40, and 60 mg/kg of Cu, Fe, Zn, and Mn for broilers. Our results showed that increasing dietary trace element levels from NRC recommendations to twice the NRC recommendations did not significantly improve ADG or reduce F/G in 22-42 day-old broilers. Zhou (1995) reported that broiler growth performance did not improve with increasing dietary Cu supplementation, and that 9 mg/kg Cu in 22-42 day-old broiler diets could satisfy growth requirements, with no significant improvement even at 200 mg/kg. In our study, dietary Cu levels (8-16 mg/kg) also had no significant effect on growth performance, indicating that low Cu levels can meet broiler growth needs. Huang et al. (2008) found that adding 47.61-87.61 mg/kg Zn to broiler diets containing 22.61 mg/kg Zn did not significantly affect growth performance, consistent with our Zn recommendation range (40-80 mg/kg). When dietary Zn reached eight times the NRC recommendation, no significant effect on AA broiler body weight was observed, suggesting that high Zn cannot significantly improve growth performance and that low Zn levels can satisfy requirements. Vahl and van 't Klooster (1987) found that broiler weight gain did not change with Fe supplementation (20-60

mg/kg) in diets containing 100 mg/kg Fe, consistent with our Fe recommendation range (125–160 mg/kg). Luo et al. (1991) reported that 18–62 mg/kg Mn had no significant effects on ADG or ADFI. Tang (2004) also noted that adding 40, 80, or 160 mg/kg Fe and 60, 120, or 180 mg/kg Mn to basal diets did not significantly affect broiler growth performance. Therefore, high levels of Fe and Mn had no significant effects on growth performance, and NRC recommendations for Fe and Mn can satisfy growth requirements. Gajula et al. (2011) also indicated that NRC recommendations for Zn and Mn (40 and 60 mg/kg) could meet broiler requirements, meaning high Zn and Mn levels are unnecessary for normal growth. Wang (2007) used D-optimal design to determine that dietary Cu, Fe, Zn, and Mn levels of 13.7, 243.1, 44.4, and 16.34 mg/kg could satisfy broiler production needs, suggesting that when trace elements are balanced, levels below NRC recommendations can meet growth requirements, and high-dose supplementation cannot improve growth performance.

3.2 Effects of Dietary Cu, Fe, Zn, and Mn Levels on Fecal Excretion in 22–42 Day-Old Broilers

Our results showed significant linear relationships between dietary Fe and Mn levels and their respective fecal excretions, but no significant linear relationships for Cu and Zn. Jiang (2002) reported that apparent Fe retention rate decreased significantly with increasing dietary Fe, meaning Fe excretion increased with dietary Fe level. Yuan et al. (2008) also noted that Mn excretion increased with dietary Mn level, consistent with our findings for Fe and Mn. However, Lin and Ali (2000) added 40, 90, or 140 mg/kg Cu and 50, 150, or 250 mg/kg Zn to basal diets containing 10 mg/kg Cu and 50 mg/kg Zn, finding that Cu and Zn excretion increased with dietary levels. This differs from our results, possibly because our dietary Cu and Zn levels (Cu 8–16 mg/kg, Zn 40–80 mg/kg) were lower, with most being deposited in liver and other tissues, whereas Lin and Ali's supplementation exceeded maximum deposition capacity, causing excess Cu and Zn to be excreted.

When using minimum fecal excretion of each element as the evaluation criterion, the optimal combination ratios differed among Cu, Fe, Zn, and Mn, indicating complex interactions affecting their absorption and utilization. Excessive Zn supplementation can form metallothionein that hinders copper transport in intestinal epithelial cells, affecting Cu absorption and increasing Cu excretion. Antagonism between Mn and Fe has been consistently reported in mammals and chickens, with high Fe levels inhibiting Mn. In China, high dietary Cu is commonly used as a growth promoter, and due to trace element interactions, high Zn and Fe levels must be added to ensure normal physiological needs. Trace element excretion in livestock feces increases with dietary supplementation, causing substantial waste and serious environmental pollution. Therefore, reducing trace element supplementation while maintaining appropriate ratios is an effective measure to minimize excretion and environmental impact without compromising growth performance. However, trace element interactions are complex, and mechanisms ensuring balanced supply require further research. Wang (2007) suggested that trace element requirements determined by growth performance

are lower than those determined by other indicators (such as tissue deposition), which may satisfy optimal physiological and biochemical status but increase excretion. For short-cycle animals like broilers, whether it is necessary to waste resources and pollute the environment to achieve optimal physiological status warrants consideration. Additionally, this experiment only determined optimal combinations for different indicators, which varied among indicators. Therefore, establishing comprehensive evaluation indicators for both growth performance and metabolism is a key issue for determining trace element requirements.

Conclusions

1. Dietary trace element levels ranging from NRC recommendations to twice the NRC recommendations had no significant effects on ADG or F/G in 22-42 day-old broilers.
2. Fecal excretion of any single trace element was affected by dietary levels of other trace elements, and different optimal combinations existed for minimizing excretion of different elements.
3. Under our experimental conditions, dietary Cu, Fe, Zn, and Mn levels of 7.36, 125.74, 38.23, and 62.76 mg/kg, respectively, could satisfy broiler trace element requirements while minimizing total fecal excretion of Cu, Fe, Zn, and Mn.

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