Effects of Trace Element Supplementation Patterns on Growth Performance, Trace Element Metabolism, and Plasma Antioxidant Capacity in Broiler Chickens: Postprint

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Abstract

This study aimed to investigate the effects of different trace element supplementation patterns on growth performance, trace element metabolism, and plasma antioxidant capacity in broiler chickens. A total of 720 one-day-old Cobb 500 broiler chickens were randomly allocated into 4 groups with 10 replicates per group and 18 birds per replicate. Industry Standard group: copper, iron, zinc, and manganese were supplemented according to the Agricultural Industry Standard NY/T 33—2004. NRC Standard group: copper, iron, zinc, and manganese were supplemented according to the recommendations of NRC (1994). NRC Ratio group: the contents of copper, iron, zinc, and manganese in the basal diet were measured, and the remaining three elements were supplemented to match the excess ratio of copper (the most excessive element relative to the NRC standard). Relative Bioavailability group: assuming the biological utilization rate of trace elements in the basal diet was 30% of that of supplemental sulfates, the contents were calibrated and supplemented using the same method as the NRC Ratio group. All trace elements were added in sulfate form, and the experimental period lasted 42 days. The results showed: 1) Different trace element supplementation patterns did not cause significant differences in growth performance or mortality of broiler chickens ($P>0.05$). 2) At 21 days of age, the relative mRNA expression level of duodenal copper transporter 1 (Ctr1) in the NRC Ratio group was significantly higher than that in the other groups ($P<0.05$), and the relative mRNA expression levels of duodenal divalent metal transporter 1 (DMT1) in both the NRC Ratio group and Relative Bioavailability group were significantly higher than those in the other two groups ($P<0.05$); at 42 days of age, the relative mRNA expression levels of duodenal DMT1 in the NRC Standard group and Relative Bioavailability group were significantly

higher than those in the other two groups (P<0.05); there were no significant differences in the relative mRNA expression levels of duodenal zinc transporter 1 (ZnT1) and zinc transporter 5 (ZnT5) at 21 and 42 days of age among all groups (P>0.05). 3) At 21 days of age, the plasma total antioxidant capacity (T-AOC) in the NRC Ratio group and Relative Bioavailability group was significantly higher than that in the other two groups (P<0.05); at 42 days of age, compared with the Relative Bioavailability group, the plasma catalase (CAT) activity in the NRC Ratio group decreased significantly (P<0.05). 4) There was a significant positive correlation between trace element concentrations in feces and trace element supplementation levels in the diet (P<0.05). Therefore, considering trace element absorption efficiency and plasma antioxidant capacity in broiler chickens, supplementation based on the relative bioavailability of trace elements in the basal diet according to the NRC ratio is a more appropriate pattern, which also reduces trace element excretion in feces.

Full Text

Effects of Supplemental Patterns of Trace Minerals on Growth Performance, Trace Mineral Metabolism and Plasma Antioxidant Ability in Broilers

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Abstract

This experiment was conducted to determine the effects of different supplemental patterns of trace minerals on growth performance, trace mineral metabolism and plasma antioxidant ability in broilers. A total of 720 one-day-old Cobb 500 broilers were randomly assigned to 4 groups, each containing 10 replicates with 18 broilers per replicate. The industrial standard group received copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn) according to agriculture industrial standards NY/T 33−2004. The NRC standard group received Cu, Fe, Zn and Mn following NRC (1994) recommendations. The NRC proportion group: after measurement of mineral content in the basal diet, the highest multiple of Cu relative to the NRC standard was identified, and the remaining three minerals were supplemented to reach the same multiple. The relative bioavailability group: bioavailability of minerals in feed ingredients was assumed to be 30% relative to sulfates, and practical contents were transformed into valid content of equivalent sulfate before adding sulfates as in the NRC proportion group. Trace minerals were provided as inorganic sulfates. The experiment lasted 42 days.

The results showed: 1) Different supplemental patterns of trace minerals did not cause significant differences in growth performance or mortality rate of

broilers (P>0.05). 2) At 21 days of age, the relative mRNA expression of copper transporter 1 (Ctr1) in duodenum of broilers in the NRC proportion group was significantly higher than in other groups (P<0.05); the relative mRNA expression of divalent metal ion transporter 1 (DMT1) in duodenum of broilers in the NRC proportion group and relative bioavailability group was significantly higher than in the other two groups (P<0.05). At 42 days of age, the relative mRNA expression of DMT1 in duodenum of broilers in the NRC standard group and relative bioavailability group was significantly higher than in the other groups (P<0.05). There were no significant differences in the relative mRNA expression of zinc transporter 1 (ZnT1) and zinc transporter 5 (ZnT5) among all groups at 21 and 42 days of age (P>0.05). 3) The plasma total antioxidant capacity (T-AOC) of broilers in the NRC proportion group and relative bioavailability group was significantly higher than in the other groups at 21 days of age (P<0.05), while the plasma catalase (CAT) activity of broilers in the NRC proportion group was significantly lower than in the relative bioavailability group at 42 days of age (P<0.05). 4) A positive correlation between fecal trace mineral concentration and dietary supplemental concentration of trace minerals was observed (P<0.05). In conclusion, from the perspective of absorption efficiency and plasma antioxidant ability, the program considering the relative bioavailability of trace minerals in the basal diet is optimal, while simultaneously reducing mineral excretion.

Key words: trace mineral; growth performance; antioxidant; mineral metabolism; broilers

Introduction

Copper, iron, zinc and manganese are essential trace minerals for poultry, participating in various physiological and metabolic reactions as enzyme cofactors, involving multiple aspects including digestion and absorption, biosynthesis, and immune function maintenance [1]. However, research on trace mineral requirements has lagged behind that of macronutrients. Although NRC (1994) provides clear recommendations, to avoid potential trace mineral deficiencies during the current rapid growth phase of broilers, production practices often ignore the content of Cu, Fe, Zn and Mn in the basal diet and add excessive amounts. Excessive supplementation not only reduces the overall utilization efficiency of trace minerals but may also cause absorption antagonism among trace minerals, leading to deficiencies of certain minerals [2]. Therefore, rational and efficient supplementation of Cu, Fe, Zn and Mn is key to ensuring optimal physiological status and improving trace mineral utilization in broilers.

Studies have shown that the biological availability of amino acid chelates is higher than that of inorganic salts [3-4]. Replacing traditional inorganic salts with lower levels of amino acid chelates in diets can significantly reduce trace mineral content in feces without affecting broiler growth performance. Under

stress conditions, the utilization efficiency advantage becomes more pronounced and can significantly improve broiler immune performance [5]. However, due to high cost and utilization efficiency being greatly affected by chelation strength [6], they are not yet widely used in production. Regarding inorganic trace minerals, studies on single trace mineral requirements have proven that broiler trace mineral requirements vary with different basal diets [7], and results from single-element addition trials are limited in application because they ignore interactions among trace minerals. In recent years, research on combined supplementation of Cu, Fe, Zn and Mn in broiler diets has gradually increased. Some studies have shown that reducing Cu, Fe, Zn and Mn in broiler trace mineral premix by 80% does not affect broiler growth performance [8]. Additionally, research has begun to focus on the impact of basal diet trace minerals on synergistic supplementation, such as Zhong et al. [9] supplementing Cu, Fe, Zn and Mn to layer basal diets to gradually balance trace minerals, proving that balanced supplementation promoted deposition of Cu, Fe, Zn and Mn in layers. Due to differences in the state of trace minerals between basal diet and additionally supplemented sulfates [10], their utilization rates may differ. However, studies simultaneously considering both basal diet trace mineral content and utilization efficiency are rarely reported. This study used the supplementation ratio of Cu, Fe, Zn and Mn for broilers provided by NRC (1994) as a basis, considered basal diet trace minerals and their relative utilization efficiency, and investigated the effects of different supplementation patterns on broiler growth performance, trace mineral absorption and excretion, and plasma antioxidant ability, aiming to provide useful reference for rational trace mineral supplementation in broilers.

1.1 Experimental Animals, Diets and Experimental Design

A total of 720 one-day-old Cobb 500 broiler chicks were randomly divided into 4 groups, with 10 replicates per group and 18 chicks per replicate. Dietary nutrient levels referenced NRC (1994) broiler nutrient requirements. The composition and nutrient levels of the basal diets are shown in Table 1 . The industrial standard group (hereinafter referred to as “industrial standard group”) directly followed the recommendations of agriculture industrial standard NY/T 33—2004. The NRC standard group (hereinafter referred to as “NRC standard group”) directly followed NRC (1994) recommendations. The NRC proportion group: after measuring the content of Cu, Fe, Zn and Mn in the basal diet, the multiple of the element exceeding the NRC standard the most was identified, and the remaining three elements were supplemented to the same multiple. The relative bioavailability group: after measuring trace mineral content in the basal diet, since organic molecules in plants easily complex with metal ions [10], and taking phytic acid as an example, 0.4%~0.6% phytic acid in diets can reduce metal ion absorption rate to 30%~40% [11], while common feed ingredients contain more than 1% phytic acid [12], the biological utilization rate of trace minerals in the basal diet was assumed to be 30% of additionally supplemented sulfates. After calibrating trace mineral content in the diet, supplementation followed the

method of the NRC proportion group. All trace minerals were added as sulfates, and the experimental period lasted 42 days. The trace mineral supplementation concentrations for each group are shown in Table 2 . Broilers had free access to feed and water, and were vaccinated according to normal procedures.

1.2.1 Growth Performance

On days 21 and 42 of the experimental period, broilers were weighed by replicate after fasting for 8 hours. Feed intake was recorded to calculate average daily feed intake (ADFI), average daily gain (ADG) and feed to gain ratio (F/G) for days 1-21, days 22-42, and the entire period.

1.2.2 Plasma Antioxidant Ability

On days 21 and 42, one broiler with body weight close to the average was selected from each replicate. Five milliliters of blood was collected from the wing vein with heparin sodium as anticoagulant, immediately centrifuged at 3,000 r/min for 10 minutes, and the plasma was collected, aliquoted and stored at -20°C. Plasma total antioxidant capacity (T-AOC), catalase (CAT) activity, and total superoxide dismutase (T-SOD) activity were measured using Nanjing Jiancheng assay kits.

1.2.3 Relative mRNA Expression of Duodenal Metal Ion Transporters

Similar duodenal segments were excised from broilers, rinsed with pre-cooled sterile saline, and the mucosa from the middle segment was scraped off with a sterile glass slide and stored in liquid nitrogen for detection of relative mRNA expression of divalent metal transporter 1 (DMT1), copper transporter 1 (Ctr1), zinc transporter 1 (ZnT1) and zinc transporter 5 (ZnT5). Total RNA from mucosa was extracted using a column-based animal total RNA extraction kit (Tianenze, Beijing) following the kit instructions strictly. RNA quality and purity were immediately assessed before reverse transcription and real-time quantitative PCR. The PrimeScriptRT reagent Kit With gDNA Eraser and SYBR Premix Ex Taq™ II (Takara) were used for reverse transcription and quantitative reactions, respectively. Primers for target genes were designed using Premier 5.0 (Table 3) and synthesized by Shanghai Sangon Co., Ltd. Relative mRNA expression of target genes was calculated using the 2-ΔΔCt formula [13], where ΔΔCt = (Ct value of target gene in test group -Ct value of reference gene in test group) -(Ct value of target gene in control group -Ct value of reference gene in control group).

1.2.4 Trace Mineral Concentration in Feces

On days 18 and 38 of the experiment, feces were continuously collected for 3 days by replicate, weighed and stored at -20°C. The concentrations of Cu, Fe, Zn and Mn in absolutely dry fecal samples were determined by flame atomic absorption spectrometry.

1.3 Statistical Analysis

Mortality data were analyzed using chi-square test, while other data were analyzed using one-way ANOVA with SPSS 21.0.

2.1 Effects of Different Dietary Trace Mineral Supplemental Patterns on Growth Performance and Mortality Rate of Broilers

As shown in Table 4 , different trace mineral supplemental patterns had no significant effects on average daily feed intake, average daily gain, feed to gain ratio, or mortality rate of broilers ($P>0.05$).

2.2 Effects of Different Trace Mineral Supplemental Patterns on Relative mRNA Expression of Duodenal Metal Transporters in Broilers

As shown in Table 5 , at 21 days of age, the relative mRNA expression of Ctr1 in duodenum of broilers in the NRC proportion group was significantly higher than in other groups ($P<0.05$), while at 42 days of age, there were no significant differences in Ctr1 mRNA expression among groups ($P>0.05$). At 21 days of age, the relative mRNA expression of DMT1 in duodenum of broilers in the NRC proportion group and relative bioavailability group was significantly higher than in the industrial standard and NRC standard groups ($P<0.05$). At 42 days of age, the relative mRNA expression of DMT1 in duodenum of broilers in the NRC standard group and relative bioavailability group was significantly higher than in the other two groups ($P<0.05$). There were no significant differences in the relative mRNA expression of ZnT1 and ZnT5 in duodenum of broilers among all groups at both 21 and 42 days of age ($P>0.05$).

2.3 Effects of Different Trace Mineral Supplemental Patterns on Plasma Antioxidant Ability of Broilers

As shown in Table 6 , at 21 days of age, plasma T-AOC of broilers in the NRC proportion group and relative bioavailability group was significantly higher than in the industrial standard and NRC standard groups ($P<0.05$). At 42 days of age, plasma CAT activity of broilers in the NRC proportion group was significantly lower than in the relative bioavailability group ($P<0.05$), while other groups showed no significant differences ($P>0.05$). No significant differences in plasma T-SOD activity were observed among groups at either 21 or 42 days of age ($P>0.05$).

2.4 Regression Analysis Between Fecal Trace Mineral Concentration and Dietary Trace Mineral Supplemental Concentration

As shown in Table 7 , at both 21 and 42 days of age, fecal Cu concentration in the NRC proportion group was significantly lower than in other groups ($P<0.05$). Fecal Fe concentration in the NRC proportion group and relative bioavailability group was significantly lower than in the industrial standard and NRC standard groups ($P<0.05$). Fecal Zn concentration in the industrial standard group was significantly higher than in other groups ($P<0.05$). Fecal Mn concentration in the industrial standard and NRC proportion groups was significantly higher than in the NRC standard and relative bioavailability groups ($P<0.05$).

As shown in Table 8 , significant positive correlations were observed between concentrations of Cu, Fe, Zn and Mn in broiler feces and their corresponding dietary supplemental concentrations ($P<0.05$).

3.1 Effects of Different Trace Mineral Supplemental Patterns on Growth Performance and Mortality Rate of Broilers

The results of this experiment showed that compared with the industrial standard and NRC standard groups, the two supplementation patterns considering basal diet trace minerals and their relative bioavailability both reduced sulfate usage while ensuring no adverse effects on broiler growth performance. The lack of growth performance differences despite higher supplementation levels in groups ignoring basal diet trace minerals may be due to the imbalance of trace minerals in the basal diet. Previous studies have also demonstrated that broilers consuming purified diets without trace minerals have significantly lower requirements for Cu, Fe, Zn and Mn than those consuming commercial diets [14], because trace minerals in the diet interfere with overall balance and reduce utilization efficiency, leading to increased requirements. No differences in growth performance were observed between the NRC proportion group and relative bioavailability group, indicating that under the conditions of this experiment, whether to consider the relative bioavailability of basal diet trace minerals did not cause significant differences in growth performance, though the relative bioavailability group reduced sulfate usage during days 22-42.

3.2 Effects of Different Trace Mineral Supplemental Patterns on Relative mRNA Expression of Duodenal Metal Transporters in Broilers

DMT1 is a key protein for duodenal epithelial cell uptake of Fe and Mn and plays a role in maintaining Fe and Mn homeostasis [15-16]. This study found that at 21 days of age, DMT1 mRNA expression in duodenum of broilers in the NRC proportion group and relative bioavailability group was significantly

higher than in the industrial standard and NRC standard groups, reflecting large differences in duodenal absorption efficiency of divalent metal ions Fe and Mn among different supplementation patterns. Current research shows that Fe absorption efficiency is greatly affected by Fe nutritional status. When body Fe is excessive, hepcidin (Hepc) synthesis in the liver increases. Hepc can bind to its receptor ferroportin1 (Fpn1), causing internalization and degradation of Fpn1. Since Fpn1 is the Fe export carrier in duodenal epithelial cells and macrophages, Fpn1 degradation reduces blood Fe content, while intracellular Fe concentration in intestinal epithelial cells gradually increases [17]. At this point, the intracellular iron-responsive element/iron regulatory protein (IRE/IRP) system responds to increased intracellular Fe concentration by reducing DMT1 expression [18]. Therefore, the reduced DMT1 mRNA expression in duodenum of broilers in the industrial standard and NRC standard groups may be related to higher Fe supplementation levels in these two patterns.

At 42 days of age, DMT1 mRNA expression in duodenum of broilers in the NRC proportion group considering basal diet trace minerals was lower than in the NRC standard group, possibly due to the effect of Mn levels in the NRC proportion group diet during days 22-42. Previous studies in laying hens showed that duodenal DMT1 mRNA expression decreased with increasing dietary Mn concentration [19], indicating that DMT1 expression is co-regulated by dietary Fe and Mn, but the dominant element may be determined by supplementation level and growth stage. Studies have found that Fe in the basal diet during days 1-21 already meets broiler growth and development needs [20], while broilers have higher Mn requirements in the early stage than in the later stage. During days 1-21, broilers are in a rapid growth period with more Mn-dependent physiological and biochemical processes, so the appropriate Mn supplementation is about twice the NRC (1994) recommendation [21], while Mn requirement significantly decreases during days 22-42 [22]. These results suggest that duodenal DMT1 mRNA expression in broilers during the early stage may be mainly affected by excessive dietary Fe levels, while in the later stage, due to reduced Mn requirement, Mn levels in the NRC proportion group diet exceeded broiler requirements, leading to decreased duodenal DMT1 mRNA expression.

This experiment showed no significant differences in duodenal ZnT1 and ZnT5 mRNA expression among groups, indicating that different supplementation patterns did not cause significant differences in duodenal Zn absorption. As Zn transporters, ZnT5 and ZnT1 are responsible for Zn absorption and export in duodenal epithelial cells, respectively [23-24]. Studies have shown that increased dietary Zn levels in mice induce increased ZnT1 mRNA and protein expression, but Zn deficiency does not significantly affect ZnT1 expression [25-26]. Yu et al. [24] also found a decreasing trend in duodenal ZnT5 expression with increasing Zn ion concentration in perfusion solution through in situ ligated intestinal perfusion experiments in broilers. Therefore, Zn transporter expression levels in duodenum can reflect Zn nutritional status to some extent.

Copper transporters (Ctr) are a class of Cu ion-specific uptake proteins, with

Ctr1 having the strongest transport capacity [27]. Li et al. [28] analyzed the expression profile of solute carrier family genes in chicken small intestine from embryonic day 18 to 14 days post-hatch using whole-genome microarrays, identifying 162 genes from 41 solute carrier families in small intestine, including Ctr1 which can transport Cu. Kuo et al. [29] confirmed that duodenal Ctr1 mRNA expression levels and protein abundance in mice increase with Cu deficiency. This study found that at 21 days of age, duodenal Ctr1 mRNA expression in broilers in the NRC proportion group without Cu supplementation was significantly higher than in other groups, while at 42 days of age, no significant differences were observed among groups. This may be due to reduced Cu requirements in broilers during days 22-42, or improved Cu nutritional status from compensatory Ctr1 mRNA expression in the NRC proportion group during the early stage.

These results indicate that excessive supplementation of Cu, Fe, Zn and Mn may reduce transporter expression and affect utilization efficiency. Since some elements share the same transporter, such as Fe and Mn, imbalanced supplementation can hinder utilization of certain elements. The relative bioavailability group improved transporter expression through balanced and appropriate supplementation.

3.3 Effects of Different Trace Mineral Supplemental Patterns on Plasma Antioxidant Ability of Broilers

Cu is the catalytic active center of copper-zinc superoxide dismutase (CuZn-SOD), while Zn plays an important role in maintaining enzyme structure. Studies have shown that plasma CuZn-SOD activity decreases when broilers are fed Cu-deficient diets. Similarly, Mn superoxide dismutase (Mn-SOD) activity in liver and heart tissues is greatly affected by dietary Mn levels [30]. Furthermore, interactions among Cu, Fe, Zn and Mn affect plasma antioxidant enzyme activity. For example, high dietary Zn reduces Cu absorption, thereby affecting Cu-SOD activity [31], while Cu deficiency also hinders Fe utilization, reducing CAT activity [32]. Therefore, balanced supplementation of dietary Cu, Fe, Zn and Mn is beneficial for broiler antioxidant performance. This experiment found that plasma T-AOC in the industrial standard and NRC standard groups was significantly lower than in the NRC proportion and relative bioavailability groups at 21 days of age, indicating that high-dose supplementation actually reduced plasma antioxidant performance, possibly because direct supplementation led to imbalanced trace mineral absorption and affected utilization [9]. At 42 days of age, plasma CAT activity in the NRC proportion group was significantly lower than in the relative bioavailability group because DMT1 expression and Fe supplementation levels were relatively low in the NRC proportion group during the later stage.

3.4 Regression Analysis Between Fecal Trace Mineral Concentration and Dietary Trace Mineral Supplemental Concentration

Linear regression analysis revealed that concentrations of Cu, Fe, Zn and Mn in broiler feces increased with increasing dietary trace mineral supplementation because broiler requirements and deposition of these minerals are limited [33]. When trace minerals are continuously supplemented in excess, absorption efficiency decreases significantly, and most ingested minerals are excreted in feces, causing environmental pollution and resource waste.

Under the conditions of this experiment, the supplementation pattern considering relative bioavailability of basal diet trace minerals improved plasma antioxidant performance through balanced intestinal absorption of trace minerals and reduced trace mineral excretion in feces.

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