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# An evaluation of the effects of dietary copper sulphate level on growth performance, nutrient digestibility, organ weight, and excreta score in Ross308-Broilers

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## Abstract

Copper sulphate ($CuSO_4$) level was analyzed in this study to determine the effects on growth performance, nutrient digestibility, organ weight, and excreta score in Ross308-broilers. A total of 1,134 mixed sex, 1-d old Ross-308 chicks were randomly allocated to one of three treatment groups with 21 replication and 18 chicks / cage. For a period of 32 days, chicks were fed with the following treatment groups: CON: Basal diet, TRT1: 0.032% $CuSO_4$, TRT2: 0.047% $CuSO_4$ (Phase 1), 0.032% $CuSO_4$ (Phase 2), 0.025% $CuSO_4$ (Phase 3). The experiment was divided into three phases. Phase 1 (day 1 to 9), phase 2 (day 9 to 21), and phase 3 (day 21 to 32). During days 1 to 9, broilers receiving 0.032% or 0.047% of $CuSO_4$ in TRT1 and TRT2, respectively, had significantly higher body weight gain (BWG) ($p = 0.042$) than CON. Likewise, comparing TRT1 and TRT2 to CON during days 10 to 21, 0.032% of $CuSO_4$ significantly increased BWG ($p = 0.013$) and feed intake (FI), ($p = 0.024$) in the broiler. When compared to TRT1, the administration of 0.032% and 0.025% of $CuSO_4$ during days 22 to 32 reduced BWG and FI in CON and TRT2, respectively among the treatment groups. Throughout the experiment, the feed conversion ratio (FCR) did not change. However, the nutrient digestibility, weight of organs, and the score of excreta remained unchanged. The study found that $CuSO_4$ administration increased broiler BWG and FI without affecting FCR, nutrients digestibility, weight of organs, and excreta score.

**Keywords:** copper sulphate, excreta score, growth performance, nutrient digestibility, organ weight

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## Introduction

There is ample evidence that copper is essential for poultry and livestock (Mills et al., 1987) as well as playing an integral part in haematopoiesis, reproduction, antioxidants, cardiovascular systems, and skin pigmentation (Close, 1998; Mayer et al., 2018). For the inhalation of cells, metalloenzymes are also essential components. Livestock and poultry are negatively affected by excess copper use during breeding (Hu et al., 2017; Minervino et al., 2018). However, copper residues are also present in livestock and poultry products, which is hazardous to human health

(Cresswell et al., 1990; Zhao et al., 2016).

Various compounds containing copper have been added to poultry diets as antimicrobial agents at concentrations far exceeding the 8 ppm level recommended by the NRC (1994). Dietary minerals like sulphates, carbonates, chlorides, and oxides, which are inorganic sources of minerals, comprise a significant part of conventional animal diets. As these salts break down in the digestive tract, they are converted into free ions that are then absorbed into the body. Due to their reactive nature, free ions form complexes with other dietary molecules, making them difficult to absorb or in some cases not absorbable, which is not beneficial for animals (Close, 1998).

Despite this, copper sulphate ($CuSO_4$) has the advantages of absorbing water and aggregating as well as catalyzing unsaturated fat oxidation in feed animal products (Lu et al., 2012; Olukosi et al., 2018). It is usually added at higher doses as a dietary supplement (100 to 300 mg/kg). Low-density lipoproteins are oxidized by copper *in vitro* (Strain, 1994). As an unbound metal, copper promotes oxidation (Diplock et al., 1998). As well as it plays a fundamental role in the synthesis of superoxide dismutase, a molecule that protects living organisms from reactive oxygen species (Barman, 1969). As a result of pharmacological concentrations of copper salt, Konjufca et al. (1997) observed a reduced activity of cholesterol 7-hydroxylase (an enzyme involved in the formation of cholic acid). According to studies published by (Bakalli et al., 1995; Skrivanova et al., 2001) rabbit and broiler meat are reduced in cholesterol when dietary copper is in excess.

Therefore, we hypothesized that $CuSO_4$ may enhance broiler performance primarily through intestinal stability. The study employs a meticulously planned series of experiments to reveal the complex reactions of broilers to copper supplementation in authentic feeding environments.

The objective is to review the current understanding of growth performance, nutrient digestibility, organ weight, and excreta condition with $CuSO_4$ supplementation in broilers, aiming to provide valuable insights into optimizing broiler production practices through copper supplementation.

## Materials and Methods

According to the protocol of this study (DK-1-2128) and animal ethics guidelines approved by Dankook University's Institutional Animal Care and Use Committee, the present study was conducted.

### Source of copper sulphate

The supplemental copper (Table 1) was obtained from a commercial company name Daeho Co., Ltd. and the source was reagent-grade copper sulphate ($CuSO_4\_5H_2O$).

**Table 1.** Basal diet composition (as-fed basis) (Continued).

| Ingredient (%) | Phase 1 | | Phase 2 | | Phase 3 | |
|---|---|---|---|---|---|---|
| | CON | TRT2 | CON | TRT2 | CON | TRT2 |
| Corn | 43.378 | 43.363 | 55.058 | 55.058 | 58.558 | 58.565 |
| SBM (CP 45%) | 25.70 | 25.70 | 22.60 | 22.60 | 19.61 | 19.61 |
| Wheat bran | 10.300 | 10.300 | 0.300 | 0.300 | 0.300 | 0.300 |
| Wheat flour | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |

**Table 1.** Basal diet composition (as-fed basis).

| Ingredient (%) | Phase 1 | | Phase 2 | | Phase 3 | |
|---|---|---|---|---|---|---|
| | CON | TRT2 | CON | TRT2 | CON | TRT2 |
| RSM (CP 38%) | - | - | 2.00 | 2.00 | - | - |
| Canola | - | - | 2.00 | 2.00 | - | - |
| Corn gluten | 2.90 | 2.90 | - | - | - | - |
| Sesame meal | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| DDGS (corn) | 3.00 | 3.00 | 3.00 | 3.00 | 5.00 | 5.00 |
| Meat meal (CP 60%, low P) | 2.00 | 2.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Tallow | 1.00 | 1.00 | 1.80 | 1.80 | 3.10 | 3.10 |
| Soy oil | 0.50 | 0.50 | - | - | - | - |
| Limestone | 1.33 | 1.33 | 1.25 | 1.25 | 1.29 | 1.29 |
| MDCP | 0.77 | 0.77 | 0.19 | 0.19 | 0.35 | 0.35 |
| Salt | 0.33 | 0.33 | 0.26 | 0.26 | 0.24 | 0.24 |
| Methionine (99%, DL-form) | 0.36 | 0.36 | 0.33 | 0.33 | 0.34 | 0.34 |
| Lysine (50%) | 0.83 | 0.83 | 0.63 | 0.63 | 0.67 | 0.67 |
| Threonine (98.5%) | 0.19 | 0.19 | 0.18 | 0.18 | 0.14 | 0.14 |
| Choline (50%) | 0.13 | 0.13 | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin premix[y] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Mineral premix[z] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Phytsase | 0.05 | 0.05 | 0.07 | 0.07 | 0.07 | 0.07 |
| $CuSO_4$ | 0.032 | 0.047 | 0.032 | 0.032 | 0.032 | 0.025 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated composition (%) | | | | | | |
| Crude protein | 21.99 | | 20.49 | | 18.49 | |
| Crude fat | 4.08 | | 4.95 | | 6.08 | |
| Crude fiber | 2.44 | | 2.66 | | 2.40 | |
| Crude ash | 5.85 | | 5.27 | | 5.06 | |
| ME (kcal/kg) | 3,045 | | 3,135 | | 3,251 | |
| Ca | 0.96 | | 0.90 | | 0.89 | |
| Tp | 0.57 | | 0.50 | | 0.49 | |
| M + C | 1.05 | | 0.99 | | 0.93 | |

CON, basal diet; CP, crude protein; DDGS, distillers dried grains with solubles; MDCP, mono dicalcium phosphate; ME, metabolizable energy; Tp, total phosphorus; M + C, methionine + cysteine.

[y] Provided per kg of complete diet: 11,025 IU vitamin A; 1,103 IU vitamin D3; 44 IU vitamin E; 4.4 mg vitamin K; 8.3 mg riboflavin; 50 mg niacin; 4 mg thiamine; 29 mg d-pantothenic; 166 mg choline; 33 µg vitamin B12.

[z] Provided per kg of complete diet: 12 mg Cu (as $CuSO_4$ $5H_2O$; 8 mg Mn (as $MnO_2$); 0.28 mg I (as KI); 0.15 mg Se (as $Na_2SeO_3$ $5H_2O$).

## Animals and housing

Prior to the introduction of chickens, Dankook University's poultry experimental facility underwent a thorough cleaning and disinfection process. A total of 1,134, 1-day-old mixed Ross 308 broiler chicks were utilized in a 32-day trial with an initial mean body weight of 47.91 ± 0.89 g. A vaccination and initial weight assessment were performed when the broilers arrived. Temperatures were initially set at 33 ± 1℃ for the first week and gradually reduced to 24 ± 1℃ while maintaining 60% humidity until the trial was completed. A floor pen was used during the development of the bird equipped with slatted flooring, with each pen measuring 1 m × 1.6 m, and the stocking density was less than 25 kg/$m^2$. Feeders and nipples were placed with each of the floor pen to provide to provide birds with unrestricted access to food and water until the conclusion of the trial. The lighting system was set up to provide 18 hours of fluorescent lighting per day, followed by a 6 hour period of darkness. To uphold a sanitary environment, the broiler facility underwent regular cleaning procedures until day 32.

## Growth performance

With the aim of accomplishing the research objectives and ascertaining the average daily gain (ADG), average daily feed intake (ADFI), and the feed conversion ratio (FCR), a total of 1,134 broilers were weighed on days 1, 9, 21, and 32, with their initial body weight measured on day 1 and their final body weight measured on day 32 establishing their growth performance. The feed conversion ratio (FCR) was computed by rationalizing the feed intake (FI) with the body weight gain (BWG).

## Nutrient digestibility

The apparent total tract digestibility of dry matter (DM), nitrogen (N), and gross energy (Gas) was evaluated (Fenton and Fenton, 1979) during day 32 by adding 0.2% chromium oxide to the diet on the form of an indigestible marker (Duksan Pure Chemicals, Korea). An experimental diet containing chromium oxide was fed to the birds for a period of 25 to 32 days. Samples of excreta were collected from each pen under each replication on day 32 and stored them at -2 0℃ until they were analyzed. We defrosted and desiccated samples of excreta at 60℃ for 72 hours before finely pulverizing them to a particle size that was able to pass through a screen of 1 mm diameter in preparation for chemical analysis. An analysis of all feed and excreta samples was conducted for the determination of DM (method 930.15, AOAC, 2000). A UV absorption spectrometer was used for chromium analysis (UV-1201, Shimadzu, Japan). Nitrogen levels were determined using an N analyzer (Kjeltec 2300 Nitrogen Analyzer; Foss Tecator AB, Sweden). An oxygen bomb calorimeter was used to determine the metabolism energy of combustion (Parr 6100 Instrument Co., USA). Using the following formula, we calculated the apparent total tract digestibility:

Total tract digestibility (%) = $[1 - \{(Nf \times Cd)/(Nd \times Cf)\}] \times 100$, where Nf = excreta nutrition concentrations (% DM), Nd = diet nutrient concentrations (% DM), Cd = diet chromium concentration (% DM), and Cf = excreta chromium concentration (% DM).

## Organ weight

In order to measure organ weight, the broilers were slaughtered after being weighed. In this procedure, an individual trained in the procedure removes the breast meat, the gizzard, the bursa of Fabricius, the spleen, the liver, and the abdominal fat. To determine the relative organ weight percentage, excess moisture was removed from all samples by patting and drying.

### Excreta score

Amount of excreta per pen was observed at the beginning and on days 9, 20, 30, and 32 and scored by a professional. The stickiness of excreta was assessed on a scale of 1 to 5 (1 = normal excretion and 5 = describing watery and highly sticky excretion).

### Statistical analysis

Each cage was served as an experimental unit, and the data were analyzed using GLM (SAS, 2014). The variables were statistically examined in designed as a complete block the one-way ANOVA result, with feeding strategies as the determining variables. A Duncan Multi-range testing was conducted for the purpose of comparing the means of control and treatment groups. Since standard error of means (SEM) serves as a measure of variability in data, statistical significance is indicated by $p < 0.05$, while $p < 0.10$ represents a trend in the data.

## Results and Discussion

### Growth performance

Table 2 presents the impact of copper sulphate supplementation on growth performance. During days 1 to 9, the administration of 0.032% and 0.047% of $CuSO_4$ in TRT1 and TRT2, respectively, resulted in a significant increase in BWG ($p = 0.042$) compared to CON in broilers. Similarly, during Days 10 to 21, the administration of 0.032% of

**Table 2.** The effect of dietary copper level on growth performance in broiler.

| Item | CON | TRT1 | TRT2 | SEM | p-value |
|---|---|---|---|---|---|
| D 1 to 9 | | | | | |
| BWG (g) | 144b | 152a | 154a | 1.913 | 0.042 |
| FI (g) | 176 | 177 | 180 | 2.135 | 0.134 |
| FCR | 1.184 | 1.180 | 1.169 | 0.020 | 0.541 |
| D 10 to 21 | | | | | |
| BWG (g) | 630b | 634a | 652a | 8.822 | 0.013 |
| FI (g) | 867b | 882a | 889a | 5.789 | 0.024 |
| FCR | 1.381 | 1.372 | 1.353 | 0.021 | 0.356 |
| D 22 to 32 | | | | | |
| BWG (g) | 944b | 958a | 952b | 12.187 | 0.536 |
| FI (g) | 1,838b | 1,850a | 1,843b | 18.214 | 0.657 |
| FCR | 1.955 | 1.947 | 1.943 | 0.035 | 0.863 |
| Overall | | | | | |
| BWG (g) | 1,723 | 1,741 | 1,764 | 15.903 | 0.135 |
| FI (g) | 2,880 | 2,894 | 2,908 | 17.011 | 0.319 |
| FCR | 1.675 | 1.666 | 1.652 | 0.017 | 0.492 |

CON, basal diet; TRT1, 0.032% $CuSO_4$; TRT2, 0.047% $CuSO_4$ (phase 1), 0.032% $CuSO_4$ (phase 2), 0.025% $CuSO_4$ (phase 3); SEM, standard error of means; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.
a, b: Means in the same row with different superscripts differ ($p < 0.05$).

$CuSO_4$ led to a significant increase in BWG ($p = 0.013$) and FI ($p = 0.024$) in TRT1 and TRT2 when compared to CON in broilers. In contrast, during Days 22 to 32, the administration of 0.025% of $CuSO_4$ in TRT2 caused a reduction in BWG and FI when compared to TRT1 among the groups. FCR did not exhibit any changes during the entire experiment. In a previous study, Skrivan et al. (2000) found that the supplementation of $CuSO_4$ pentahydrate (4.3%) significantly ($p < 0.05$) improved the chickens' final body weight. By the supplementation 0.025% of $CuSO_4$ decreased BWG in CON and TRT2 than TRT1 among the TRT groups. The results of Skrivan et al. (2002) showed that 7.0% and 6.1% of $CuSO_4 \cdot 5H_2O$ significantly reduced BWG ($p < 0.05$) in chickens. It was found in the study of Forouzandeh et al. (2021) that broilers supplemented with 150 mg/kg of Cu from $Cu_2O$ had higher ($p = 0.05$) BW and ADG than broilers supplemented with 15 mg/kg of Cu from $CuSO_4$. In our experiment, 0.025% decreased FI and BWG in CON and TRT3 than TRT2 among the treatment groups where 0.032% and 0.047% of $CuSO_4$ increased FI significantly ($p < 0.05$) in TRT2 and TRT3 than CON. Unlike our investigation, Forouzandeh et al. (2021) discovered that the inclusion of $CuSO_4$ resulted in a tendency towards decreased ADFI and FCR, while growth performance, ADFI, and FCR remained unaltered at therapeutic doses (150 mg/kg). On the other hand, Luo et al. (2005) observed that the addition of 0.45% $CuSO_4$ resulted in a substantial reduction ($p < 0.05$) in average daily feed intake (ADFI) and average daily gain (ADG) in chickens. According to Miles et al. (1998), there was no significant difference in BW and FCR in birds that were fed up with 400 mg/kg Cu from $CuSO_4$ or TBCC. However, as reported by Ledoux et al. (1991) and Miles et al. (1998), the addition of 0.45% $CuSO_4$ led to a reduction in feed intake (FI) in chicks. A maximum of 35 mg/kg of copper supplementation has been permitted in poultry diets in the European Union.

Among the hypotheses by which Cu stimulates growth are regulating intestinal flora (Pang et al., 2009), enhancing neuropeptide Y expression (Li et al., 2008), and increasing dietary fat digestibility by stimulating lipase and phospholipase activity (Luo and Dove, 1996). Researchers have previously demonstrated that feeding Cu over the minimum requirements can improve the growth performance of poultry and pigs. Arias and Koutsos (2006) found that supplementing broilers' diets with 188 mg/kg copper chloride or $CuSO_4$ improved their growth compared with those fed a non-supplemented diet. Samanta et al. (2011) observed a growth improvement of 8.9% and a decrease in FCR when broilers were fed 150 mg/kg of $CuSO_4$ over 42 days. A study by Villagomez-Estrada et al. (2020) has also found that supplementation of Cu with 160 mg/kg of $CuSO_4$ or Cu hydroxychloride enhanced performance at day 42 in pigs. It is important to note that the response to Cu may vary based on the source of the supplement and the dose given. It was found by Lu et al. (2010) that the addition of 200 mg/kg of copper from tribasic copper chloride improved ADG without increasing ADFI in comparison to 200 mg/kg of Copper from $CuSO_4$ in their study of broilers. In contrast to other Cu sources, $CuSO_4$ has little impact on growth owing to several factors, such as damage to the mucosa and muscle layers in the intestinal tract (Chiou et al., 1999), increasing solubility (Pang and Applegate, 2006), accumulation of oxidants (Miles et al., 1998), decreased phytase efficiency and phosphorus retention, and toxic effects (Banks et al., 2004; Lu et al., 2010; Hamdi et al., 2018).

## Nutrient digestibility

The effect of copper sulphate supplementation on nutrient digestibility is presented in Table 3. There was no change in dry matter, nitrogen and metabolism digestion energy by the supplementation of $CuSO_4$ diet in broilers. As a result of our experiment, the broiler showed no significant difference in nutrient digestibility of dry matter, nitrogen, and metabolism energy. Likewise, Sarvestani et al. (2016) found that adding 100 mg/kg Cu nanoparticles to poultry feed had no significant impact on nutrient digestibility. A study conducted by Gonzales-Eguia et al. (2009) showed that Cu-NP performed better on piglets in terms of energy digestibility compared to $CuSO_4$. This might be due to the better bioavailability of Cu-NP and its higher penetration into the gastrointestinal tract (GIT). Since nanoparticles are small, they might diffuse faster through GIT mucus to reach the intestinal lining, then uptake through the GIT barrier to reach the blood (Singh, 2016). Moreover, Cu's low digestibility is due to antagonisms with other microminerals (Richards et al., 2010). Cu digestibility can be reduced by low pH in the stomach, causing the dissociation of inorganic salts (Underwood and Suttle, 1999). Zn and Cu are trapped in insoluble hydroxide precipitates in the small intestine when pH rises (Powell, 2000). Among these factors can be affected by Cu are feed intake, digestibility, and metabolic rate (Berntssen et al., 1999).

**Table 3.** The effect of dietary copper level on nutrient digestibility in broiler.

| Item (%) | CON | TRT1 | TRT2 | SEM | p-value |
|---|---|---|---|---|---|
| Finish | | | | | |
| Dry matter | 70.29 | 71.56 | 72.02 | 1.0247 | 0.167 |
| Nitrogen | 70.14 | 71.32 | 72.02 | 0.977 | 0.196 |
| Metabolism energy | 71.17 | 72.48 | 73.11 | 0.984 | 0.182 |

CON, basal diet; TRT1, 0.032% $CuSO_4$; TRT2, 0.047% $CuSO_4$ (phase 1), 0.032% $CuSO_4$ (phase 2), 0.025% $CuSO_4$ (phase 3); SEM, standard error of means.

## Organ weight

A comparison of the effect of copper sulphate supplementation on organ weight can be found in Table 4. There was no change in organ weight by the supplementation of $CuSO_4$ diet in broilers. The absence of a significant change in organ weight following $CuSO_4$ diet supplementation in broilers warrants further investigation. Given the limited research on $CuSO_4$ supplementation in broilers, it's possible that the mechanisms underlying its effects on organ weight remain poorly understood. Factors such as dosage, duration of supplementation, and individual bird variability could influence the outcomes. Additionally, interactions with other dietary components or environmental factors may play a role. These complexities highlight the need for more comprehensive studies to elucidate the reasons behind the observed lack of difference in organ weight and to better understand the effects of $CuSO_4$ supplementation in broilers.

**Table 4.** The effect of dietary copper level on organ weight in broiler.

| Items | CON | TRT1 | TRT2 | SEM | p-value |
|---|---|---|---|---|---|
| Relative organ weight (%) | | | | | |
| Breast muscle | 9.83 | 9.96 | 10.31 | 0.422 | 0.464 |
| Liver | 3.13 | 3.22 | 3.36 | 0.286 | 0.593 |
| Spleen | 0.10 | 0.11 | 0.12 | 0.008 | 0.218 |
| Kidney | 0.83 | 0.76 | 0.80 | 0.037 | 0.535 |
| Bursa of Fabricius | 0.16 | 0.20 | 0.21 | 0.023 | 0.181 |

CON, basal diet; TRT1, 0.032% $CuSO_4$; TRT2, 0.047% $CuSO_4$ (phase 1), 0.032% $CuSO_4$ (phase 2), 0.025% $CuSO_4$ (phase 3); SEM, standard error of means.

### Faecal score

Table 5 illustrates the effects of copper sulphate supplementation on excreta score. Supplementation of $CuSO_4$ diet to broilers did not result in a change in excreta score. It may be because of normal intestinal transit and better-quality feces elimination that there were no changes in broiler feces in our study. It would be helpful to further investigate these reasons.

**Table 5.** The effect of dietary copper level on excreta[z] score in broiler.

| Item | CON | TRT1 | TRT2 | SEM | p-value |
|---|---|---|---|---|---|
| Initial | 1.69 | 1.67 | 1.67 | 0.14 | 0.902 |
| Day 9 | 1.64 | 1.64 | 1.62 | 0.10 | 0.864 |
| Day 20 | 1.62 | 1.60 | 1.62 | 0.11 | 0.881 |
| Day 30 | 1.60 | 1.64 | 1.60 | 0.10 | 0.715 |
| Day 32 | 1.64 | 1.62 | 1.60 | 0.11 | 0.768 |

CON, basal diet; TRT1, 0.032% $CuSO_4$; TRT2, 0.047% $CuSO_4$ (phase 1), 0.032% $CuSO_4$ (phase 2), 0.025% $CuSO_4$ (phase 3); SEM, standard error of means.
[z] Excreta were scored for stickiness on a scale of 1 to 5 (1 = representing normally formed excreta and 5 = representing watery and very sticky excreta).

## Conclusion

In conclusion, the findings demonstrate that $CuSO_4$ supplementation positively influences broiler performance, as evidenced by improved body weight gain BWG and feed intake FI, while maintaining favourable outcomes for nutrient digestibility, organ weight, and excreta condition. Notably, among the three doses tested, 0.032% and 0.047% $CuSO_4$ exhibited the most significant effects on BWG and feed efficiency in broilers, suggesting optimal supplementation levels for enhancing productivity without compromising other health parameters. These results underscore the potential of $CuSO_4$ as a beneficial additive in broiler diets for optimizing growth performance.

## Conflict of Interests

No potential conflict of interest relevant to this article was reported.

## References

AOAC (Association of Official Analytical Chemists). 2000. Official methods of analysis. 16th ed. AOAC, Washington, D.C., USA.

Arias VJ, Koutsos EA. 2006. Effects of copper source and level on intestinal physiology and growth of broiler chickens. Poultry Science 85:999-1007.

Bakalli RI, Pesti GM, Ragland WL, Konjufca V. 1995. Dietary copper in excess of nutritional requirement reduces plasma and breast muscle cholesterol of chickens. Poultry Science 74:360-365.

Banks KM, Thompson KL, Jaynes P, Applegate TJ. 2004. The effects of copper on the efficacy of phytase, growth, and phosphorus retention in broiler chicks. Poultry Science 83:1335-1341.

Barman TE. 1969. Catalase and peroxidase. Enzyme Handbook. Vol. 1. pp. 232-235. Springer-Verlag, Berlin, Germany.

Berntssen MH, Lundebye AK, Maage A. 1999. Effects of elevated dietary copper concentrations on growth, feed utilisation and nutritional status of Atlantic salmon (*Salmo salar* L.) fry. Aquaculture 174:167-181.

Chiou PWS, Chen CL, Chen KL, Wu CP. 1999. Effect of high dietary copper on the morphology of gastro-intestinal tract in broiler chickens. Asian-Australasian Journal of Animal Science 12:548-553.

Close WH. 1998. The role of trace mineral proteinates in pig nutrition. Biotechnology in the Feed Industry 14:469-474.

Cresswell GC, Nair NG, Evans JC. 1990. Effect of boron and copper contaminants in poultry manure on the growth of the common mushroom, *Agaricus bisporus*. Australian Journal of Experimental Agriculture 30:707-712.

Diplock AT, Charuleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Stahl W, Vina-Ribes J. 1998. Functional food science and defence against reactive oxidative species. British Journal of Nutrition 80:S77-112.

Fenton TW, Fenton M. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Canadian Journal of Animal Science 59:631-634.

Forouzandeh A, Blavi L, Abdelli N, Melo-Duran D, Vidal A, Rodríguez M, Monteiro ANTR, Pérez JF, Darwich L, Solà-Oriol D. 2021. Effects of dicopper oxide and copper sulfate on growth performance and gut microbiota in broilers. Poultry Science 100:101224.

Gonzales-Eguia A, Fu CM, Lu FY, Lien TF. 2009. Effects of nanocopper on copper availability and nutrients digestibility, growth performance and serum traits of piglets. Livestock Science 126:122-129.

Hamdi M, Solà D, Franco R, Durosoy S, Roméo A, Pérez JF. 2018. Including copper sulphate or dicopper oxide in the diet of broiler chickens affects performance and copper content in the liver. Animal Feed Science and Technology 237:89-97.

Hu Y, Cheng H, Tao S. 2017. Environmental and human health challenges of industrial livestock and poultry farming in China and their mitigation. Environment International 107:111-130.

Konjufca VH, Pesti GM, Bakalli RI. 1997. Modulation of cholesterol levels in broiler meat by dietary garlic and copper. Poultry Science 76:1264-1271.

Ledoux DR, Henry PR, Ammerman CB, Rao PV, Miles RD. 1991. Estimation of the relative bioavailability of inorganic copper sources for chicks using tissue uptake of copper. Journal of Animal Science 69:215-222.

Li J, Yan L, Zheng X, Liu G, Zhang N, Wang Z. 2008. Effect of high dietary copper on weight gain and neuropeptide Y level in the hypothalamus of pigs. Journal of Trace Elements in Medicine and Biology 22:33-38.

Lu L, Hao S, Zhang L, Luo X. 2012. Effect of copper source on phytase stability in the premix of weanling piglets. Animal Production Science 53:142-145.

Lu L, Wang RL, Zhang ZJ, Steward FA, Luo X, Liu B. 2010. Effect of dietary supplementation with copper sulfate or tribasic copper chloride on the growth performance, liver copper concentrations of broilers fed in floor pens, and stabilities of vitamin E and phytase in feeds. Biological Trace Element Research 138:181-189.

Luo XG, Dove CR. 1996. Effect of dietary copper and fat on nutrient utilization, digestive enzyme activities, and tissue mineral levels in weanling pigs. Journal of Animal Science 74:1888-1896.

Luo XG, Ji F, Lin YX, Steward FA, Lu L, Liu B, Yu SX. 2005. Effects of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and oxidation stability of vitamin E in feed. Poultry Science 84:888-893.

Mayer AN, Berwanger E, Vieira SL, Angel CR, Kindlein L, Ebbing MA, Lopes M. 2018. Copper requirements of broiler breeder hens. Poultry Science 97:2785-2797.

Miles RD, O'keefe SF, Henry PR, Ammerman CB, Luo XG. 1998. The effect of dietary supplementation with copper sulfate or tribasic copper chloride on broiler performance, relative copper bioavailability, and dietary prooxidant activity. Poultry Science 77:416-425.

Mills CF, Davis GK. 1987. Molybdenum. In Trace elements in human and animal nutrition. Edited by Mertz W. pp. 429-463. Academic Press, New York, USA.

Minervino AH, López-Alonso M, Barrêto Júnior RA, Rodrigues FA, Araújo CA, Sousa RS, Mori CS, Miranda M, Oliveira FL, Antonelli AC, et al. 2018. Dietary zinc supplementation to prevent chronic copper poisoning in sheep. Animals 8:227.

NRC (National Research Council). 1994. Nutrient requirements of poultry. 9th rev. ed. National Academy Press, Washington, D.C., USA.

Olukosi OA, van Kuijk S, Han Y. 2018. Copper and zinc sources and levels of zinc inclusion influence growth performance, tissue trace mineral content, and carcass yield of broiler chickens. Poultry Science 97:3891-3898.

Pang Y, Applegate TJ. 2006. Effects of copper source and concentration on *in vitro* phytate phosphorus hydrolysis by phytase. Journal of Agricultural and Food Chemistry 54:1792-1796.

Pang Y, Patterson JA, Applegate TJ. 2009. The influence of copper concentration and source on ileal microbiota. Poultry Science 88:586-592.

Powell SR. 2000. The antioxidant properties of zinc. The Journal of nutrition 130:1447-1454.

Richards JD, Zhao J, Harrell RJ, Atwell CA, Dibner JJ. 2010. Trace mineral nutrition in poultry and swine. Asian-Australasian Journal of Animal Sciences 23:1527-1534.

Samanta B, Biswas A, Ghosh PR. 2011. Effects of dietary copper supplementation on production performance and plasma biochemical parameters in broiler chickens. British Poultry Science 52:573-577.

Sarvestani SS, Resvani M, Zamiri MJ, Shekarforoush S, Atashi H, Mosleh N. 2016. The effect of nanocopper and mannan oligosaccharide supplementation on nutrient digestibility and performance in broiler chickens. Journal of Veterinary Research 71:153-161.

SAS (Statistical Analysis System). 2014. SAS user's guide. SAS Institute Inc., Cary, NC, USA.

Singh P. 2016. Use of nano feed additives in livestock feeding. International Journal of Livestock Research 6:1-4.

Skrivan M, Sevcikova S, Tumova E, Skrivanova V, Marounek M. 2002. Effect of copper sulphate supplementation on performance of broiler chickens, cholesterol content and fatty acid profile of meat. Czech Journal of Animal Science 47:275-280.

Skrivan M, Skrivanova V, Marounek M, Tumova E, Wolf J. 2000. Influence of dietary fat source and copper supplementation on broiler performance, fatty acid profile of meat and depot fat, and on cholesterol content in meat. British Poultry Science 41:608-614.

Skrivanova V, Skrivan M, Marounek M, Baran M. 2001. Effect of feeding supplemental copper on performance, fatty acid profile and on cholesterol contents and oxidative stability of meat of rabbits. Archives of Animal Nutrition 54:329-339.

Strain JJ. 1994. Newer aspects of micronutrients in chronic disease: Copper. Proceedings of the Nutrition Society 53:583-598.

Underwood EJ, Suttle NF. 1999. The mineral nutrition of livestock. 3rd edition. p. 614. CABI Publishing, Wallingford, UK.

Villagomez-Estrada S, Perez JF, Darwich L, Vidal A, van Kuijk S, Melo-Duran D, Sola-Oriol D. 2020. Effects of copper and zinc sources and inclusion levels of copper on weanling pig performance and intestinal microbiota. Journal of Animal Science 98:1-15.

Zhao Y, Wang D, Yang S. 2016. Effect of organic and conventional rearing system on the mineral content of pork. Meat Science 118:103-107.