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Response of ROSS 308 Broiler Chickens (*Gallus Domesticus*) to Dietary Supplementation with Inorganic Copper

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ABSTRACT

A dose-response experiment was conducted to investigate the effects of dietary supplementation with Copper (II) Sulphate (CuSO4) at 0, 62.5, 125, 187.5 and 250mg/kg on the growth performance, and carcass and meat quality of broilers (Ross 308). Forty-five 2-week old Ross broiler chicks with an initial average weight of 490±0.6g/bird were randomly assigned to 4 dietary treatments with three replicates of three chicks per treatment. Diets were fed over the grower day (14-35) and finisher day (35-49) phases. Average daily water intake (ADWI), feed intake (ADFI), water to feed ratios (WFR), average daily gain (ADG), body weight (BW), and feed conversion ratio (FCR) were measured from 14 to 49 days of age. The mean ADWI and WFR were significantly higher in birds fed a diet containing 250mg/kg CuSO4, while ADFI, ADG and BW were significantly higher in birds fed diets containing 125 mg/kg CuSO4 compared to the rest of the treatments ($P<0.05$). FCR, carcass and viscera weights, meat pH and colour were similar among the experimental diets ($P > 0.05$). In conclusion, supplementation with 125mg/kg CuSO4 was more efficacious than the rest of the treatments in promoting growth performance.

Abbreviations:

ADWI: Average daily water intake
ADFI: Average feed intake
WFR: Water to feed ratios
ADG: Average daily gain
BW: Body weight
FCR: Feed conversion ratio

INTRODUCTION

Copper plays a vital role in the growth of broilers, having significant influences on the maintenance of production performance, regulation of enzymatic activity, bone growth, glucose metabolism, hemoglobin synthesis, cardiovascular structural integrity, and other physiological functions (Feng *et al*., 2020).

The recommended nutritional requirement of copper in broiler diets is in the range from 8 mg/kg (NRC, 1994) to 15 mg/kg (Aviagen, 2014). However, higher doses are often administered in the production process to obtain better economic benefits (Leeson, 2009). Supplementation of trace minerals with a large safety margin in broiler chicken diets has resulted in broiler diets with several fold increases in some trace minerals as compared with NRC recommendations (Guclu *et al*., 2008), which has led to a high level of mineral excretion into the environment (Dozier *et al*., 2002; Skrivan *et al*., 2005). With the application of poultry manure, these elements accumulate in the soil in some regions, and crop yields have been reduced because of high copper levels (Karimi *et al*., 2011). Furthermore, some of the elements leach into the groundwater and potentially cause problems for humans (Bao *et al*., 2007). Today, the optimum addition of dietary copper is still controversial. For example, Arias and Koutsos (2006) showed that with increasing copper level (100–300 mg/kg), the performance of broilers was improved whereas Pang and Applegate (2007) did not observe any significant effects on bird's growth performance when copper was supplemented at levels excess to the requirements (up to 250 mg/kg). Karimi *et al* (2011), observed that feeding 250 mg/kg of $CuSO_4$ during the starter period had negative consequences on bird performance, and moderate levels of $CuSO_4$ (125 mg/kg) had beneficial effects on bird performance at market age. Against this background, the level of copper supplementation needs to be urgently reassessed.

The objective of this study was to compare the effects of graded levels of dietary $CuSO_4$ supplementation on feed intake, water intake, feed conversion ratio, water to feed ratios, body weight gains, carcass and internal organ weights, breast meat pH and colour of broiler chickens. It was hypothesized that incremental dietary $CuSO_4$ supplementation increases growth performance, carcass and meat quality of broiler chickens.

MATERIALS AND METHODS

Site Description

The study was carried out at a farm in a Highveld area of Zimbabwe at an altitude of 1600m above sea level at 18° 22' 54.066'' S. The farm is located in natural farming region 2b where average annual rainfall ranges between 600 and 800mm per year. Highest temperatures are experienced in October averaging to 28^0C, lowest temperature being attained in June and averaging around 5^0C.The minimum, maximum and average temperature during the experimental period are summarized in Table 1. The average ambient temperature was 23.5°C.

Table 1: Average minimum and maximum temperature from 1 week to 5 weeks of age during the experiment.

Week	Temperature (ºC)		
	Minimum	Maximum	Mean
1	14	34	24
2	15	32	23.5
3	20	31	25.5
4	19	28	23.5
5	19	27	23

Experimental design and layout

The experiment was a completely randomized design with graded levels of $CuSO_4$ in the feed. Treatments were 0, 62.5, 125, 187.5 and 250mg/kg $CuSO_4$ respectively. Each treatment was replicated 3 times. The basal diet (0mg/kg) had no additional $CuSO_4$ and was used as a control.

Birds, treatments and management

A total of 45-day old Ross 308 broiler chickens were used in the experiment. The chicks were weighed prior to placement and housed in a deep litter system. The birds were fed a Broiler starter meal from day old to 14 days. At day 14 of age, the chicks were randomly assigned to five experimental diets, each diet being replicated 3 times. Supplementation with $CuSO_4$ was done at day 15 and continued until day 49. All birds received *ad libitum* access to assigned diets and water, and the formulated diets met the requirements of growing broiler birds (NRC, 1994). Other factors were held constant except the amount of copper. Environmental conditions were monitored and heat was adjusted using 250-watt infrared lamps and ventilation by opening or closing curtains.

Formulation of experimental diets

Commercial starter (21 % CP) diet was fed with no restriction from day 1 to 14. Grower diet (19.5% CP) was fed to the birds from day 15 to day 28 and birds switched to the finisher diet (18% CP) from day 29 to day 49. Experimental diets were formulated using broiler concentrate (41% crude protein), maize meal (9% crude protein) using the Pearson square method (Table 3.3). Graded levels of $CuSO_4$ were added to the diets followed by thorough mixing using a rotary drum mixer.

Table 2: Chemical composition (label values) of broiler starter, grower and finisher used in the experiment.

Dietary composition	Starter crumbles %	Grower pellets %	Finisher pellets %
Crude protein	21	19.5	18
Moisture	12	12	12
Crude fibre	3	3.7	3.7
Fat	4	4.5	5
Calcium	0.9	0.87	0.85
Phosphorus	0.42	0.42	0.4

According to Profeeds, Zimbabwe

Data collection

Zootechnical evaluation

The daily feed intake (ADFI g/bird) was determined for birds in each treatment group. This was done by measuring the difference between the feed supplied every morning and the residuals on the following day. Body weight changes were determined by measuring body weight for each bird on a weekly basis from week zero of the experiment using a digital electronic scale (Jadever JPS-1050, Micro Precision Calibration Inc, USA). Feed conversion ratio was calculated as the amount of feed consumed per unit of live weight gain. Average daily water intake was determined by measuring the difference between water supplied in founts and water remaining after a 24-hour period. Evaporation losses were corrected for by using a 1-liter fount similar in capacity and design to other drinking founts. The evaporation fount was placed at the center of the poultry house. Water to feed ratio was calculated as the proportion of water to feed consumed daily during the experimental period.

Carcass, organ weights and meat quality characteristics

At day 49 of age, after being deprived of feed overnight, 2 birds per replicate (6 birds from each treatment group) were randomly selected and slaughtered by exsanguination to determine carcass weight, quality and organ weights (liver, heart, gizzard and intestines). Slaughtered birds were left to bleed for four minutes followed by hot water scalding and plucking of feathers manually. Birds were eviscerated and the dressed carcass weight, liver, heart, gizzard, intestine weights measured using an electronic digital scale.

Samples of breast meat were taken from the birds for colour and pH determination. Samples were chilled for 24 hours after slaughter to facilitate optimum rigor mortis. A precision calorimeter 110 was used to measure breast meat colour coordinates (lightness, redness, yellowness, hue and chroma angle). The colour spectrum for breast meat lightness (L*) values were classified as follows: lighter than normal (light, L* > 53), normal (48 < L* < 53), and darker than normal (dark, L* < 46) (Qiao *et al.,* 2001). A hanna pH meter calibrated with buffers at pH 4 and pH 7 was used for breast meat pH analysis.

Statistical analysis

Data was analyzed using the General Linear Model procedures of SAS (2010) for a completely randomized design.

The model used was:

$$Y_{ij} = \mu + t_i + e_{ij}$$

Where:

Y_{ij} = Response variable (ADWI, ADFI), WFR, ADG), FBW, FCR, meat colour and pH)
μ = overall mean
t_i =effect of the i^{th} treatment
e_{ij} = error
Least square means were compared using the PDIFF procedure of SAS (2010). Statistical significance was considered at the 5% level of probability.

RESULTS

The effects of $CuSO_4$ supplementation on performance parameters of the birds are presented in Table 3. The mean water intake was significantly higher in birds fed a diet containing 250mg/kg $CuSO_4$ compared to other birds (P<0.05). The water intake of birds fed a diet containing 250mg/kg $CuSO_4$ was 10, 8, 8 and 9% higher than birds fed 0, 62.5, 125 and 187.5mg/kg $CuSO_4$, respectively. The highest feed intake was observed in birds fed a diet containing 125mg/kg $CuSO_4$, while the water to feed ratio tended to be higher for the birds fed on diets containing 250mg/kg $CuSO_4$ as compared to the other birds (P<0.05).The water to feed ratio of birds on the 250mg/kg $CuSO_4$ diet was 8%, 7%, 12%, 8% higher than those of birds on 0, 62.5, 125 and 187.5mg/kg $CuSO_4$ diets respectively. Copper II Sulphate supplementation significantly affected average daily gain (P<0.05). The mean average daily gain of birds that received 125mg/kg $CuSO_4$ was 13%, 6%, 11%, 14% higher than the mean of birds that received 0, 62.5, 187.5 and 250mg/kg $CuSO_4$ diets respectively. Feed conversion ratios, final live weights, carcass and organ weights (Table 4) were similar among the diets (P> 0.05). Although no significant differences were observed, birds that received the 125mg/kg $CuSO_4$ diet had relatively higher final, dressed carcass and organ weights compared to the other birds (P > 0.05). The final live-weight of birds that received the control diet (0mg/kg $CuSO_4$) were 9% lower than for birds fed the 125mg/kg $CuSO_4$ diet, while dressed carcass weight were 14%, 9%, 0.03% and 12% higher for birds that received the 125mg/kg $CuSO_4$ diet compared to other birds. Supplementation of the broiler diet with $CuSO_4$ did not significantly affect breast meat pH and colour (Table 5: P>0.05). Breast meat pH was however slightly high in birds that received the 62.5mg/kg $CuSO_4$ diet compared to birds fed on the rest of the diets.

Table 3: Growth performance parameters of broilers fed with Copper II Sulphate from week 2 to week 7 of age.

Performance Characteristics	Treatments					R^2	P- Value
	0mg/kg $CuSO_4$	62.5mg/kg $CuSO_4$	125mg/kg $CuSO_4$	187.5mg/kg $CuSO_4$	250mg/kg $CuSO_4$		
Water intake (ml/bird/day)	242.12^b±4.278	247.10^b±4.278	248.09^b ±4.332	245.22^b ±4.354	270.11^a±4.302	0.5	0.0001
Feed intake (g/bird/day)	162.85^b±2.055	164.66^b ±2.055	176.13^a±2.055	161.85^b ±2.055	168.57^{ab} ±2.057	0.7	0.0001
Water/feed ratio (ml/g/bird/day)	1.582^b±0.044	1.601^b ±0.044	1.519^b±0.045	1.587^b ±0.046	1.738^a ± 0.045	0.12	0.015
Daily gain (g/bird/day)	71.88^b ±1.82	77.28^{ab} ±1.82	82.853^a ±1.82	73.663^b ±1.82	71.146^b ±1.82	0.79	0.01
Live weight (g/bird)	3073.66^{ab} ±64.87	3210.55^{ab} ±64.87	3385.22^a ±64.87	3096.71^{ab} ±64.87	3037.55^b±64.87	0.71	0.06
Feed conversion ratio (g/g)	2.4189^a ± 0.11	2.113^a ± 0.11	1.96^a ± 0.11	2.33^a ±0.11	2.473^a ± 0.11	0.66	0.10

Means within a row followed by a different superscript are significantly different (P>0.05). [1]±SE = standard error. [2]R^2= Coefficient of determination.

Table 4: Effects of graded levels of Copper II Sulphate on carcass and viscera weights of broiler chickens.

Carcass Characteristics	Treatments					S. E	R^2	P- Value
	0mg/kg $CuSO_4$	62.5mg/kg $CuSO_4$	125mg/kg $CuSO_4$	187.5mg/kg $CuSO_4$	250mg/kg $CuSO_4$			
Dressed weight (g)	2031.66^a	2140.33^a	2363.83^a	2356.16^a	2067.66^a	±96.17	0.63	0.14
Liver (g)	41.50^a	38.33^a	45.33^a	38.33^a	40.50^a	±2.33	0.52	0.30
Heart (g)	13.66^a	10.50^a	10.66^a	12.33^a	12.33^a	±0.72	0.63	0.13
GIT (g)	74.83^a	72.00^a	77^a	75^a	75^a	±1.92	0.42	0.49
Gizzard (g)	47.50^a	45.00^a	49.83^a	47.00^a	46.33^a	±2.23	0.26	0.81

Means within a same row followed by different superscript are significantly different (P<0.05).[1]SE = Standard Error. [2]R^2= Coefficient of determination.

Table 5: Effects of graded levels of Copper II Sulphate supplementation on meat pH and colour of broilers.

Meat quality characteristics	Treatments					S. E	R^2	P-Value
	0mg/kg $CuSO_4$	62.5mg/kg $CuSO_4$	125mg/kg $CuSO_4$	187.5mg/kg $CuSO_4$	250mg/kg $CuSO_4$			
pH	6.59[a]	6.62[a]	6.59[a]	6.51[a]	6.51[a]	0.03	0.65	0.11
Lightness	57.42[a]	59.66[a]	60.87[a]	55.16[a]	56.61[a]	3.29	0.35	0.63
Redness	0.40[a]	2.34[a]	0.53[a]	0.49[a]	1.42[a]	0.512	0.68	0.08
Yellowness	1.90[a]	6.30[a]	3.5[a]	1.9[a]	4.4[a]	1.49	0.52	0.31
Chroma	2.67[a]	6.75[a]	3.75[a]	3.16[a]	4.75[a]	1.46	0.51	0.32
Hue angle	76.89[a]	66.24[a]	111.36[a]	101.90[a]	72.89[a]	20.46	0.41	0.53

Means within a row followed by a different superscript are significantly different ($p<0.05$). [1]SE= Standard Error. [2]R^2= Coefficient of determination.

DISCUSSION

Water intake, water:feed ratio, feed intake

The observation that increasing $CuSO_4$ concentration in feed resulted in increased water intake compared to the control diet (0mg/kg $CuSO_4$) is consistent with previous findings (Deyhim and Teeter *et al*., 1995). The increase in water intake was attributed to a high metabolic heat production. When metabolic heat production exceeds the capacity of passive heat dissipation, the birds activate their physiological and behavioral defense mechanisms for heat stress (Dai and Bessei, 2008). The defensive mechanisms comprise the decrease in blood flow to the uterus, panting, reduction of feed consumption and increase in water consumption (May and Lott, 1992). The immediate increase in water intake helps birds to increase the dissipation of heat from respiratory surfaces, and the decrease in feed intake reduces the contribution of metabolic heat to the total heat load. In contrast, Miles *et al* (1998), noted that $CuSO_4$ supplementation did not show significant effect on water intake. The high water: feed ratio observed in birds fed a diet containing 250mg/kg $CuSO_4$ could be attributed to the higher water intake of birds (Persia *et al*., 2004).

The observation that feed intake increased as Copper II Sulphate increased until it reached a plateau at the 125mg/kg $CuSO_4$ level of supplementation then declined in birds fed diets containing 187.5 and 250mg/kg $CuSO_4$ suggests that the 125mg/kg $CuSO_4$ level of supplementation was optimal. Similar results were reported by Feng et al (2020). On the contrary, Luo *et al.,* (2005) reported that copper had no effect on feed intake.

FCR is an important determinant of profitability for broiler producers. Because the feed constitutes 70–80% of the cost of raising broiler chickens, changes in the feed-conversion ratio can have a major impact on the profitability of an operation (Aggrey et al., 2010). In agreement with the current study, Hamdi *et al.,* (2015) reported that feed conversion ratio was not affected by addition of $CuSO_4$ to the diet. The lack of response of broiler chicken to $CuSO_4$ supplementation suggests that the feed conversion efficiency of broilers was influenced by other nutritional or environmental factors. Further studies are needed to support this hypothesis.

Daily gain and final live weight

Average daily gain and final live weight reached a peak in birds fed a diet containing 125mg/kg $CuSO_4$ whilst higher levels (187.5 and 250mg/kg of $CuSO_4$) retarded growth performance. The increase in average daily gain and final live weight at 125mg/kg $CuSO_4$ might be might be a consequence of the significant reduction in the total number of pathogenic organisms in the gut (Pang and Applegate, 2007; Samanta *et al*, 2011). The reason for the growth-depressing effects of a higher doses of $CuSO_4$ (250 mg/kg) in the present study is not clear and is in disagreement with studies reporting growth-stimulating effects from supplementing broiler diets with an excessive amount of $CuSO_4$ (Bortoluzzi *et al*., 2019). Banks et al., (2004) reported that increasing concentrations of $CuSO_4$ (0, 62.5, 125, 250, or 375 mg/kg) had growth depressing effects on broiler chicks. Reduced growth rate is used as a proxy indicator of mineral toxicity to evaluate the toxic effects of certain levels of specific minerals. Persia *et al*., (2004), reported that 500 and 800 mg of supplemental $CuSO_4$ depressed body weight gain and final live-weight. In another study, they suggested levels of 642 and 781 mg/kg $CuSO_4$ as a toxic break point for chick body weight gain and final body weight gain, respectively. Luo *et al*. (2005) also reported that feeding 450 mg/kg of $CuSO_4$ resulted in decreased broiler average daily gain and feed intake. Because the copper level used in the present study was less than the toxic copper levels reported in other studies (Persia *et al*., 2004; Banks *et al*., 2004; Luo *et al*., 2005), further research is needed to clarify the nature of the depressive effects of the higher level of $CuSO_4$ (>125mg/kg) exhibited in the present study.

Internal viscera and Meat quality

Dressed weight was not affected by different copper levels fed to broilers in this study. Our results are partially consistent with work conducted by Liu *et al*.

(2012) in terms of carcass weight. In contrast, Chowdhury *et al* (2003) demonstrated the positive influence of copper supplementation on carcass yields. No significant differences were observed in liver, gastrointestinal and gizzard weights between treatments irrespective of copper supplementation level. The results obtained in the present study are consistent with findings of Attia *et al* (2012) who reported no significant differences between internal organs due to copper supplementation. However, Liu *et al* (2012) found a significant reduction in the weight of liver due to increasing copper levels in layer diets. Several factors may affect the final results of the trials, such as copper sulphate source, management practices applied, or environmental conditions under which experiments were conducted.

The relationship between poultry meat color and pH has been well documented, but the relative influence on poultry meat quality is not as well established. Barbut (1993) suggested that apparent pale color in turkey meat is associated with lower pH and is similar to the pale soft exudative condition in pork meat. Fletcher (1999a) reported that extremes of light and dark poultry breast fillets showed corresponding differences in muscle pH, and Allen *et al.* (1998) reported on differences in color extremes on functional properties similar to those for PSE- and dark, firm, and dry-like conditions in red meat. In the present study, no significant differences in pH were detected between diets. However, birds fed a diet containing 62.5mg/kg $CuSO_4$ had higher pH values than birds fed diets containing 187.5mg/kg and 250mg/kg $CuSO_4$, respectively. Meat color is one of the proxy indicators of meat quality, and copper is responsible for erythropoiesis, hemoglobin, myoglobin formation, so the potential to alter meat color. Supplementation with graded levels of $CuSO_4$ in broiler diets did not significantly affect meat color coordinates. Our results are partially consistent with those reported by Yang *et al* (2011) who also found no effect on the meat redness a* and yellowness b* values, but differ on lightness L* values which they reported an increase in the lightness L* value of the breast meat. There is a paucity of information on the effects of $CuSO_4$ on broiler carcass and meat quality, and further experiments are needed to elucidate the effect of $CuSO_4$ on meat quality (Zhang *et al*., 2009).

CONCLUSION

Results of the present study indicate that dietary supplementation with 125mg/kg $CuSO_4$ produced beneficial changes in feed intake, daily gain and final live-weight while the converse was true for doses exceeding 125mg/kg $CuSO_4$. Doses exceeding 125mg/kg $CuSO_4$ increased water intake, and reduced the average daily feed intake, daily gain and final live-weight. Supplementation with $CuSO_4$ had no effect on meat quality characteristics and internal organs of broilers. Small-scale poultry producers can boost food and nutrition security in developing countries by using 125mg/kg $CuSO_4$ as a supplement in broiler enterprises. Future research should focus on the effect dietary $CuSO_4$ supplementation on the sensory attributes of meat.

Competing interests

The authors declare that they have no conflict of interests.

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Ethics declarations

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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