Research Article

Comparative Evaluation of Hydroxy Copper vs. Copper Sulfate on Growth, Meat Quality, and Serum Biochemistry of Commercial Broilers

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Abstract | This study evaluated two different forms of copper (hydroxy copper and copper sulfate) on the growth, meat quality, and blood biochemistry of broiler chickens. To this end, 240-day old broiler chicks (Ross 308) were randomly divided into four dietary treatments having six replicates of 10 birds each according to completely randomized design. The dietary treatments were: a control group with no added copper (T1), copper sulfate at 8 mg/kg (T2), hydroxy copper at 8 mg/kg (T3), and hydroxy copper at 16 mg/kg (T4). Data were collected regarding growth performance, carcass traits, meat quality, serum chemistry, and copper retention in the liver and bones. The findings revealed that birds fed hydroxy copper (especially at the higher 16 mg/kg dose (T4)) performed significantly better in terms of weight gain, feed efficiency, and meat quality traits. In addition, these birds had higher levels of triglycerides, glucose, and total protein in their blood, but their liver enzymes stayed within normal limits. Notably, copper accumulation in the bone tissue was highest in the group receiving the 16 mg/kg hydroxy copper. In conclusion, supplementing broiler diets with hydroxy copper at 16 mg/kg is more beneficial than using copper sulfate; it promotes growth, meat quality, and improves copper absorption, without any adverse effect on birds' metabolism.

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Introduction

Poultry farming stands as one of the fastest-growing and most vibrant sectors within Pakistan's agricultural industry, playing a critical role in the country's food supply and economic development. As highlighted in the Pakistan Economic Survey (2023), the poultry sector contributes around 1.55% to the national GDP and over 6% to the agricultural value-added, underscoring its importance not just for food production, but also for livelihoods and employment across the country. With consumer demand rising for safe, high-quality chicken meat, there is increasing pressure to refine feeding strategies and improve nutrient management in broiler production systems.

Among the key nutrients in poultry diets, trace minerals like copper are especially important due to their role in supporting growth, immune function, and meat quality. Copper participates in various physiological processes, such as iron metabolism, collagen synthesis, antioxidant defense, and maintaining a robust immune system (Leeson and Summers, 2001). Traditionally, poultry producers have believed on copper sulfate ($CuSO_4$) as a dietary supplement due to its affordability and antimicrobial benefits. However, this form of copper is characterized by low bioavailability with an increase in excretion rates and potential environmental impact (Boa and Choct, 2009). In addition, when copper sulfate levels are too high, they can interfere with absorption of needed minerals (e.g., calcium and iron) and may actually cause toxicity if not used carefully and properly in poultry (Miroshnikova *et al*., 2015).

In poultry nutrition trace minerals are crucial for enzymatic activity, metabolism and health of animals. One such trace mineral, copper (Cu), has been well recognized for their positive effects on growth, feed efficiency, and carcass quality of broilers and turkeys. As a cofactor for many metalloenzymes, copper is involved in the metabolism of iron, the formation of collagen and antioxidant defense systems, and it has been proposed to be a copper-dependent factor that deranges in the serum of patients with T2DM and obesity (Harms and Buresh, 1987). These roles highlight both the nutritional and functional importance of copper in poultry diets.

Recently, hydroxy copper has gained attention as a better-performing replacement for the traditional product – copper sulfate. Its unique molecular structure allows it to maintain its stability in feed and increase absorption from the gastrointestinal tract (Swiątkiewicz *et al*., 2014). This copper source is known for being able to increase growth rates, feed efficiency and mineral retention, while decreasing nutrient interactions and environmental contamination (Ferreira Júnior *et al*. 2022).

Copper sulfate ($CuSO_4$) has been the most used source in commercial feed additives, because of its low cost and easy accessibility. Nevertheless, efficiency of the protocol has been questioned by some studies showing performance outcomes variability. For instance, feeding broiler chickens at different doses levels of copper sulphate enhanced growth rate and carcass characteristics but the responses differed based on the amount and the duration of feeding (Hassan *et al*. 2020). Likewise, Harms and Buresh (1987) observed that moderate dietary Cu levels enhanced feed efficiency and BW gain in turkeys, whereas extreme supplementation resulted in deleterious effects. These findings highlight the importance of identifying optimal inclusion levels that maximize benefits without impairing metabolism.

The stability and bioavailability have been a major concern for the use of copper and recent investigations have developed non inorganic sources: chelated copper and hydroxy complexes. Makarski *et al*. (2014) evaluated copper sulfate and chelated copper in turkey hens at 50 mg/kg and observed no significant changes in hematological and biochemical parameters. Nonetheless, altered triglyceride levels and enhanced copper accumulation in tissues were detected, indicating different metabolic handling based on the form of copper. The fact that copper tissue levels remain within the safe range of human consumption offers promising prospects for the use of alternative copper sources in poultry husbandry.

Collectively, these studies show that dietary copper can exert a beneficial effect on growth performance, nutrient retention, and carcass quality in poultry, but the degree of the response depends greatly on the dietary concentration and the source of copper. Although moderate inclusions are beneficial to growth and feed efficiency, too much supplementation of alpha-lipoic acid might not improve production or change lipid metabolism, with or without production advantages. The chemical semblance of the copper (sulfate, chelated and hydroxy), can modify how copper is absorbed, metabolized, and excreted, and therefore its efficiency and environmental sustainability (Harms and Buresh, 1987; Makarski *et al*., 2014; Hassan *et al*., 2020).

Even though more than fifty years of research have been dedicated to this, the most effective form and level of copper inclusion for digestion and growth remains controversial for modern day production of poultry. Differences in genetic, dietary, and management factors could explain some of the differences. Therefore, further comparative studies are needed to fully understand the benefits of adding copper to diets in order to increase broiler and turkey performance while decreasing the environmental impact (Makarski *et al.*, 2014; Hassan *et al.* 2020).

Notwithstanding these possible benefits, there are incomplete and un-biased side-by-side comparisons between hydroxy copper verses copper sulfate under practical broiler production conditions. In addition, both sources of copper were monitored for their effects on meat quality, blood biochemical components and tissue mineral retention which are important to animal health as well as the product quality offered to consumers.

This study was undertaken to compare the effects of hydroxy copper and copper sulfate (provided at equivalent dietary levels) on growth performance, carcass yield, meat quality, serum biochemistry, and copper retention in broilers. The overarching goal is to determine whether hydroxy copper can serve as a more effective and environmentally sustainable option for commercial poultry nutrition. The 8 mg/kg level was selected to match the recommended dietary requirement for broilers and allow a direct comparison of copper sources at equivalent elemental copper. The 16 mg/kg level was included only for hydroxy copper due to its higher bioavailability and chemical stability, which reduces the risk of toxicity at higher inclusion levels. Copper sulfate was not evaluated at 16 mg/kg to avoid potential pro-oxidative effects, mineral antagonism, and environmental excretion associated with higher levels of inorganic copper. This limitation has now been clearly acknowledged in the Discussion section

Novelty and rationale of the study

Although copper source comparisons in broiler nutrition have been previously reported, the novelty of this study lies in its integrated evaluation of hydroxy copper versus copper sulfate under practical production conditions. Unlike earlier studies focusing mainly on growth performance, this research simultaneously assesses growth performance, comprehensive meat quality traits, serum biochemistry, tissue copper retention, and economic viability within a single experimental framework. In addition, the study evaluates two inclusion levels of hydroxy copper (8 and 16 mg/kg) against an equivalent level of copper sulfate, allowing assessment of dose-dependent responses that are insufficiently explored in existing literature. The inclusion of economic analysis and local production conditions using Ross 308 broilers further enhances the practical and regional relevance of the findings. Overall, this work advances knowledge by providing a holistic and application-oriented evaluation of copper supplementation strategies in broiler production.

Materials and Methods

Experimental design and bird's management

This study was carried out at the Experimental Broiler House, Block A, University of Veterinary and Animal Sciences (UVAS), Ravi Campus, Pattoki, Pakistan. All bird handling procedures followed institutional ethical guidelines, as approved by the UVAS Ethical Review Committee (Approval No. DR/176). The trial spanned 35 days and was divided into three distinct feeding phases: starter (days 0–10), grower (days 11–24), and finisher (days 25–35).

A total of 240-day-old Ross 308 broiler chicks were randomly distributed across four dietary treatment groups using a completely randomized design. Each group included six replicates, with ten chicks housed per replicate. The dietary treatments were consisted of **T1:** Control group supplemented a basal diet without added copper; **T2:** Basal diet supplemented with 8 mg/kg copper from copper sulfate ($CuSO_4$); **T3:** Basal diet supplemented with 8 mg/kg copper from hydroxy copper; **T4:** Basal diet supplemented with 16 mg/kg copper from hydroxy copper.

Birds were kept under a controlled environment and managed using standard commercial husbandry practices. Feed and clean drinking water were made available at all times. Diets were formulated according to Ross 308 nutritional guidelines (2025), with adjustments made for each growth stage. Details regarding the diet composition and nutrient profiles are provided in Table 1.

The inclusion level of 8 mg/kg elemental copper was selected to reflect the recommended dietary requirement for broilers, as reported by NRC (1994), and to allow a direct comparison between copper sulfate and hydroxy copper at equivalent copper concentrations. An additional higher level of hydroxy copper (16 mg/kg) was included to evaluate potential dose-dependent responses, as hydroxy copper is characterized by greater bioavailability and chemical stability compared to inorganic copper sources. Previous studies have reported improved performance and mineral utilization with organic or hydroxy copper sources at levels above the minimum requirement without adverse physiological effects (Świątkiewicz *et al*., 2014; Ferreira Júnior *et al*., 2022).

Table 1: *Ingredients and nutrient composition of feeds offered*

Formulation	Starter (1-14 d)	Grower (15-21 d)	Finisher (22-35 d)
Ingredients	%	%	%
Corn grain	55	56	63
Rice Polishing	5	5	3
Molasses	4	4	4
Poultry fat	1.5	1.5	1.5
Soybean meal	8	8	8
Rapeseed meal	9.5	7	5
Corn Gluten 30 %	9	7	5
Fish Meal	5.5	5.5	4.8
Poultry hydroxy copper Meal	6	5	5
L-Lysine HCl	0.1	0.05	0
DL-Methionine	0.07	0.05	0.05
Limestone	1.15	0.8	0.55
Micro Mineral	0.05	0.05	0.05
Vitamin Premix	0.05	0.05	0.05
Total	100	100	100
Nutrient composition (%)			
Crude Protein	23	21.5	19.5
ME (Kcal/Kg)	2975	3050	3100
Crude Fiber	3.66	3.44	3.06
Ether Extract	5.08	4.94	4.63
Calcium	0.95	0.75	0.65
Phosphorus (Av)	0.5	0.42	0.36
Lysine (dig)	1.32	1.18	1.08
Methionine (dig)	0.55	0.51	0.48
Threonine (dig)	0.88	0.79	0.72
Arginine(dig)	1.4	1.27	1.17
Tryptophan(dig)	0.21	0.19	0.17

Traits evaluated

Growth performance

Throughout the study, body weight and feed intake were measured weekly. Based on these measurements, body weight gain (BWG), feed conversion ratio (FCR), feed efficiency (FE), and the European Production Efficiency Factor (EPEF) were calculated by the method adopted by Akeem *et al*. (2021). Mortality was checked daily to account for performance data accuracy.

Body weight (BW, g): It was recorded at day 1; after that, weekly body weights were recorded.

Body weight gain (BWG, g) = Final BW -Initial BW
Feed intake (FI) = Total feed offered – Feed refused

$$\text{Feed conversion ratio (FCR)} = \frac{\text{FI (g)}}{\text{BWG (g)}}$$

$$\text{Feed Efficiency (FE)} = \frac{\text{WG (g)}}{\text{FI (g)}}$$

$$\text{European production index (EPI)} = \frac{\text{BWG (kg)} \times \text{Livability (\%)}}{\text{FCR} \times \text{Age (d)}} \times 100$$

Carcass traits

On day 35, two birds from each replicate were randomly selected and slaughtered to assess carcass traits. The relative weights of carcass components (including breast, thighs, drumsticks, wings, liver, heart, gizzard, spleen, and bursa) were expressed as percentages of pre-slaughter body weight.

Meat quality assessment

Breast muscle samples were used for meat quality analysis. Muscle pH was measured at both 2- and 24-hours postmortem using a digital pH meter. Meat color parameters (lightness (L*), redness (a*), and yellowness (b*)) were determined with a Minolta® CR-410 colorimeter. Drip loss and cooking loss were evaluated following the method described by Honikel (1998). All meat quality analyses were performed at the Meat Technology Laboratory, Department of Meat Science and Technology, UVAS, Lahore.

Meat pH: The pH of the breast was measured by a pH meter at three places on the breast. the pH was recorded 2 hours and 24 hours after slaughtering (Albrecht *et al*., 2019).

Meat Color: For meat color, the breast samples' measurements were taken at 2 hours and 24 hours post slaughtering. for this purpose, the breast samples were placed at a chilling temperature (8-10°C) packed in the tray. the color was measured using Minolta® CR-410 colori meter. the color measurements were including Lightness (L*), Redness (a*), and yellowness(b*) (Albrecht *et al*., 2019).

Drip Loss (%) and cooking Loss (%): The breast samples from each slaughtered bird were collected for Measurements of cooking loss and drip loss. Drip loss % and cooking loss % were calculated in the Meat Technology Lab at the Department of Meat Science and Technology, UVAS, Lahore.

The samples were weighed and put in polyethylene bags for the purpose of measuring drip loss. the bags are going to be vacuum-packed, sealed, and kept at 4°C. Samples were taken out of the bags and reweighed after being stored for 24 hours. The Honikel procedure were used to calculate the percentage of drip loss (Albrecht *et al*., 2019).

$$\text{Drip loss \%} = \frac{\text{Wa}-\text{Wb}}{\text{wa}} \times 100$$

Where, Wa = Muscle weight before storage (g) and Wb = Muscle weight after storage (g).

The meat sample were weighed and placed in plastic bags in order to assess the amount of cooking loss. the bags were placed in a pre-heated water bath set at 80 degrees. the bags should be allowed to cool and gently blotted dry with paper towels without being squeezed after the internal temperature reaches 78 °C. after being taken out of the polyethylene bags, the muscle samples were weighed again. the following formula by Honikel (1998) were used to determine the proportion of cooking losses.

$$\text{Cooking loss \%} = \frac{\text{Wx}-\text{Wy}}{\text{wx}} \times 100$$

Where, Wx = Muscle weight before cooking (g) Wy = muscle weight after cooking (g).

Serum biochemistry

On day 35, two birds per replicate were randomly selected for blood collection via the wing vein using EDTA-treated tubes. Samples were centrifuged at 1500 rpm for 10 minutes at 4°C, and the serum was stored at −20°C for analysis. Commercial diagnostic kits were used to measure serum levels of glucose, cholesterol, triglycerides, total protein, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). These analyses were conducted at the Quality Operations Lab, UVAS, Lahore, Pakistan.

Mineral retention

To evaluate copper retention, liver and tibia bone samples were collected from two birds per replicate at the end of the experiment. Copper concentrations in the tissues were determined using atomic absorption spectrophotometry, following established procedures for mineral analysis.

Statistical analysis

Collected data were first checked for normality and homogeneity of variance; normal distribution and homogeneity of variance of the data were confirmed via Kolmogorov–Smirnov test and Levene test, respectively. Subsequently, data were analyzed through the one-way ANOVA technique using PROC GLM in SAS software (version 9.1). For the comparison of significant treatment means, Duncan's Multiple Range test was applied. The experimental unit was the replicate pen, as birds were housed and managed collectively within each replicate. Therefore, data were analyzed using replicate mean values, while individual bird measurements within a replicate were averaged prior to statistical analysis.

Treatment means were separated using Duncan's multiple range test when a significant F-test was observed. Statistical significance was declared at $P < 0.05$, and results showing a tendency were discussed at $0.05 \leq P < 0.10$. The data were presented as least square means ± standard errors. The following mathematical model was applied:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where,

Y_{ij} = observation of dependent variable record on i^{th} treatment group

μ = population mean

τ_i = effect of i^{th} treatment (i = 1, 2, 3,4)

ε_{ij} = residual effect of j^{th} observation on i^{th} treatment, NID ~ 0, σ^2

Results and Discussion

Growth performance

Commercial broilers fed different copper sources revealed differences among the treatment groups (Table 2). Chickens supplementing hydroxy copper at 16 mg/kg (T4) achieved the highest average daily gain, recording 2093.17 ± 40.70 g, along with the most favorable feed conversion ratio of 1.52 ± 0.02.

Feed efficiency was also superior in this group at 0.66 ± 0.01. In contrast, birds in the control group (T1) demonstrated the poorest performance, with the lowest ADG (1895.31 ± 28.42 g) and the highest FCR (1.76 ± 0.03). Statistical analysis confirmed significant effects of both copper source and level on ADG (p = 0.0128), FCR (p = 0.006), and FE (p = 0.004). Additionally, the highest European Production Efficiency Factor was recorded in the T4 group (347.89 ± 2.12), highlighting its superior productivity.

Growth performance parameters were calculated using the following standardized equations:

Feed Conversion Ratio (FCR) FCR = Total feed intake (g) / Total body weight gain (g)
Feed Efficiency (FE) FE = Total body weight gain (g) / Total feed intake (g)
European Production Efficiency Factor (EPEF) EPEF = [Live body weight (kg) × Livability (%) × 100] / [Age (days) × FCR]

Terminology was standardized throughout the manuscript, and the term European Production Efficiency Factor (EPEF) is used consistently in place of EPI.

Carcass traits

As detailed in Table 3, broilers fed hydroxy copper (T3 and T4) showed marked improvements in carcass traits. These groups had significantly higher pre-slaughter weights, carcass weights, and yields of prime cuts such as breast, thighs, and drumsticks (P < 0.05). Among them, the T3 group achieved the highest overall carcass yield at 71.97 ± 3.79%. Birds

Table 2: *Growth performance of commercial broiler among different treatment groups*

Traits	Control	Copper Sulfate (8mg/kg Diet)	Hydroxy Copper (8mg/kg Diet)	Hydroxy Copper (16mg/kg Diet)	p-value
Total Feed Intake (g/bird)	3324.80^{ab} ± 38.5	3419.83^{a} ± 82.26	3222.32^{b} ± 49.99	3183.12^{b} ± 43.87	0.032
Weigh Gain (g/bird)	1895.31^{b} ± 28.42	2053.77^{ab} ± 110.51	2087.66^{ab} ± 39.34	2093.17^{a} ± 40.7	0.012
Cum. FCR	1.76^{a} ± 0.03	1.68^{ab} ± 0.08	1.55^{bc} ± 0.04	1.52^{c} ± 0.02	0.006
Cum. FE	0.57^{c} ± 0.01	0.60^{bc} ± 0.03	0.65^{ab} ± 0.02	0.66^{a} ± 0.01	0.004
Cum. Liv %	88.89^{a} ± 2.78	63.89^{b} ± 2.78	91.67^{a} ± 2.15	90.28^{a} ± 3.34	<0.0001
EPEF	228.31^{b} ± 2.14	203.20^{b} ± 3.11	340.83^{a} ± 2.77	347.89^{a} ± 2.12	0.0001

[abcd]Superscripts on different means within row differ significantly at $p \le 0.05$. Cum = cumulative; FCR = feed conversion ratio; FE = feed efficiency; EPEF = European production efficiency factor; Liv = livability.

Table 3: *Carcass traits of commercial broiler among different treatment groups*

Traits	Control	Copper Sulfate (8mg/kg Diet)	Hydroxy copper (8mg/kg Diet)	Hydroxy copper (16mg/kg Diet)	p-value
Pre-slaughter weight (g)	2185.78^{b} ± 5.78	2115.60^{c} ± 4.80	2178.43^{b} ± 2.11	2245.02^{a} ± 5.00	<0.0001
Carcass weight (g)	1216.00^{b} ± 96.00	1209.00^{b} ± 39.00	1568.00^{a} ± 84.00	1531.00^{a} ± 43.00	0.038
Carcass yield (%)	55.62^{b} ± 4.25	57.14^{b} ± 1.71	71.97^{a} ± 3.79	68.20^{ab} ± 2.07	0.050
Breast yield (%)	21.45 ± 1.18	24.16 ± 1.61	27.50 ± 1.17	24.94 ± 2.97	0.296
Wings (%)	5.08 ± 0.15	3.78 ± 0.94	5.14 ± 0.36	5.88 ± 0.19	0.168
Leg (%)	20.99 ± 2.46	22.26 ± 1.51	25.61 ± 0.07	24.72 ± 1.26	0.279
Thigh (%)	6.86 ± 0.99	7.14 ± 0.69	7.35 ± 0.74	7.49 ± 0.82	0.949
Drumstick (%)	4.07^{c} ± 0.22	4.16^{bc} ± 0.18	5.42^{a} ± 0.27	4.90^{ab} ± 0.08	0.024
Heart (%)	0.48^{a} ± 0.02	0.28^{b} ± 0.05	0.48^{a} ± 0.02	0.45^{a} ± 0.05	0.050
Liver (%)	1.67^{ab} ± 0.06	1.89^{a} ± 0.14	1.95^{a} ± 0.02	1.38^{b} ± 0.04	0.023
Gizzard (%)	2.04^{a} ± 0.06	1.47^{c} ± 0.04	2.02^{a} ± 0.04	1.69^{b} ± 0.00	0.002
Spleen (%)	0.09 ± 0.00	0.12 ± 0.02	0.09 ± 0.00	0.09 ± 0.00	0.383
Bursa (%)	0.11 ± 0.02	0.09 ± 0.00	0.11 ± 0.02	0.13 ± 0.00	0.483

[abcd]Superscripts on different means within row differ significantly at $p \le 0.05$; Carcass traits were evaluated on d 35 and presented as percent of pre-slaughter body weight.

in the T4 group also exhibited greater liver and heart weights, suggesting enhanced organ development and better nutrient utilization. These findings point to the positive influence of hydroxy copper on both carcass quantity and quality.

Table 4: *Meat quality traits of commercial broiler among different treatment groups at day 35*

Traits	Control	Copper sulfate (8 mg/kg diet)	Hydroxy copper (8 mg/kg diet)	Hydroxy copper (16 mg/kg diet)	p-value
Initial pH	6.03[a] ± 0.04	6.07[a] ± 0.04	6.05[a] ± 0.04	5.95[b] ± 0.04	0.040
Final pH	5.11[b] ± 0.04	5.09[b] ± 0.04	5.24[a] ± 0.04	5.21[a] ± 0.04	0.030
Drip loss (%)	3.90[b] ± 0.05	4.40[a] ± 0.05	4.50[a] ± 0.05	3.80[b] ± 0.05	0.050
Cooking loss (%)	29.05[a] ± 0.20	22.61[b] ± 0.20	31.58[a] ± 0.20	28.97[a] ± 0.20	0.010
L* (Lightness)	57.10[b] ± 0.05	55.85[b] ± 0.05	62.66[a] ± 0.06	60.50[a] ± 0.04	0.010
a* (Redness)	14.87[a] ± 0.04	13.56[a] ± 0.03	10.76[b] ± 0.02	10.74[b] ± 0.04	0.010
b* (Yellowness)	12.10[b] ± 0.04	11.81[b] ± 0.04	12.65[a] ± 0.01	12.66[a] ± 0.02	0.040

[abcd]Superscripts on different means within row differ significantly at $p \leq 0.05$

Meat quality

Meat quality parameters varied notably across treatments (Table 4). The ultimate muscle pH measured 24 hours postmortem was significantly higher in birds from T3 (5.24 ± 0.04) and T4 (5.21 ± 0.04), indicating improved water-holding capacity. Hydroxy copper also influenced meat color, producing lighter (higher L*) and less reddish (lower a*) meat, which are desirable traits for consumer acceptance. The lowest drip loss was observed in the T4 group (3.8 ± 0.05%), reflecting better moisture retention. Although cooking loss was lowest in T2 (22.61 ± 0.2%), the overall meat quality—particularly visual appeal and freshness—was enhanced in broilers fed hydroxy copper.

Serum biochemistry

Analysis of blood samples (Table 5) revealed significant differences in serum metabolites across the treatment groups ($P < 0.01$). Birds in the T4 group had the highest levels of glucose (169.80 ± 7.83 mg/dL), triglycerides (159.63 ± 6.57 mg/dL), and total protein (7.70 ± 0.20 g/dL), indicating a higher metabolic rate and improved energy status. While ALT levels were slightly elevated in T4, AST values across all groups remained within normal physiological ranges, suggesting no adverse liver impact. These results imply enhanced metabolic function and nutrient utilization with hydroxy copper supplementation. In the present study, broilers supplemented with hydroxy copper, particularly at 16 mg/kg, exhibited higher serum concentrations of glucose, triglycerides, and total protein compared with the control and copper sulfate groups. These changes should be interpreted cautiously, as elevations in circulating metabolites do not necessarily indicate improved physiological status but may reflect alterations in energy metabolism, nutrient utilization, or hepatic activity. Increased serum glucose and triglyceride concentrations may suggest enhanced dietary energy availability and lipid metabolism rather than pathological dysregulation, as previously reported in broilers receiving bioavailable copper sources (El-Ghalid *et al*., 2019). Similarly, higher total protein levels may be associated with improved protein synthesis and nutrient assimilation; however, excessive elevations could also indicate metabolic stress if accompanied by abnormal liver enzyme activity.

Table 5: *Serum chemistry of commercial broiler among different treatment groups at day 35*

Traits	Control	Copper sulfate (8 mg/kg diet)	Hydroxy copper (8 mg/kg diet)	Hydroxy copper (16 mg/kg diet)	p-value
Glucose (mg/dL)	131.19[c] ± 0.78	159.63[ab] ± 9.16	169.80[a] ± 7.83	142.42[b] ± 7.19	0.001
Cholesterol (mg/dL)	140.63[d] ± 6.32	179.88[bc] ± 8.51	174.51[c] ± 7.67	197.91[ab] ± 8.97	< 0.001
Total protein (g/dL)	6.49[b] ± 0.12	7.66[a] ± 0.28	7.35[a] ± 0.19	7.70[a] ± 0.20	0.001
Triglycerides (mg/dL)	104.88[d] ± 2.18	109.31[d] ± 13.70	148.76[bc] ± 9.23	159.63[b] ± 6.57	< 0.001
ALT (U/L)	6.00[b] ± 0.84	14.75[ab] ± 0.68	14.16 [ab] ± 0.73	16.96 [a] ± 0.71	0.002
AST (U/L)	217.50[a] ± 5.06	269.38[a] ± 6.52	225.10[b] ± 9.83	225.73[b] ± 10.32	0.001

[abcd] Superscripts on different means within row differ significantly at $p \leq 0.05$

In the current study, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities remained within established physiological reference ranges for broiler chickens, indicating no evidence of hepatic damage or toxicity. Reported normal reference values for broilers range from approximately 5–20 U/L for ALT and 180–350 U/L for AST, depending on age, strain, and analytical method (Kaneko *et al*., 2008). The enzyme values observed across all treatments in this study fell within these limits, supporting the conclusion that hydroxy copper supplementation, even at the higher inclusion level, did not adversely affect liver function.

Collectively, these findings suggest that the observed changes in serum metabolites likely reflect adaptive metabolic responses to improved copper bioavailability rather than pathological alterations, although long-term studies would be valuable to further confirm metabolic safety.

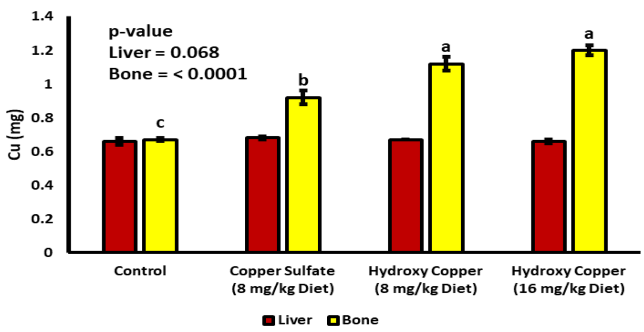

Figure 1: *Copper retention in liver and bone of commercial broiler among different treatment groups at day 35 of age*

Mineral retention

Copper accumulation in liver and bone is presented in Figure 1. Birds supplemented with hydroxy copper – particularly in T3 (1.12 ± 0.04 mg) and T4 (1.20 ± 0.03 mg) – had significantly higher bone copper content compared to the control group (0.67 ± 0.01 mg) ($p < 0.0001$). However, no significant differences were observed in liver copper levels among the treatments. These findings indicate improved copper bioavailability and retention in skeletal tissue when hydroxy copper is used instead of copper sulfate. In the present study, dietary supplementation with different copper sources and inclusion levels did not result in significant differences in liver copper concentrations among treatments, despite clear increases in copper retention observed in bone tissue. This finding is consistent with the tight homeostatic regulation of copper metabolism in poultry. The liver serves as the primary organ for copper storage and regulation, and excess absorbed copper is rapidly bound to metallothioneins or excreted via bile to maintain hepatic copper concentrations within a narrow physiological range (Kim *et al*., 2015). As a result, liver copper levels often remain stable across dietary treatments unless copper is supplied at pharmacological or toxic levels.

The absence of significant differences in liver copper concentration in the current study suggests that both copper sulfate and hydroxy copper were efficiently regulated and did not exceed the birds' hepatic storage capacity. In contrast, the higher copper retention observed in bone tissue in birds supplemented with hydroxy copper indicates improved bioavailability and utilization of this source for structural mineral deposition rather than excessive hepatic accumulation. Similar patterns of stable liver copper levels with increased skeletal retention have been reported in broilers fed organic or hydroxy trace mineral sources (Ferreira Júnior *et al*., 2022; Dos Santos *et al*., 2021). These findings support the conclusion that hydroxy copper enhances copper utilization efficiency while maintaining safe systemic regulation. Copper concentrations in liver and bone tissues were expressed as mg/kg dry matter (DM) and are reported consistently throughout the manuscript and tables. Tissue samples were dried prior to analysis, and copper content was determined using atomic

Table 6: *Economic evaluation of broilers fed different copper sources*

Treatment	Inclusion Level (mg/kg)	Cost of Cu Source (PKR/kg)	Feed Conversion Ratio (FCR)	Feed Cost Index (Relative)	Economic Viability	p-value
Control (Basal diet, no Cu)	0	–	1.76^{a}	100	Lowest efficiency	–
Copper Sulfate	32	720	1.68^{ab}	96	Slightly economical	0.006
Hydroxy Copper	13.79	1000	1.55^{bc}	88	More economical	0.006
Hydroxy Copper	27.58	1000	1.52^{c}	86	Most economical	0.006

[abcd]*Superscripts within the FCR column show significant differences ($p \leq 0.05$). Feed Cost Index is expressed relative to control (lower is better).*

absorption spectrophotometry following standard mineral analysis procedures.

Economic evaluation

Economic evaluation is presented in Table 6. Economic analysis indicated that the T4 treatment group was the most profitable, whereas use of hydroxy copper ingredient in the poultry diet resulted in somewhat more feed cost. This is reflected by the increased weight gain and improved feed efficiency for this group, which is identified as having higher profits. However, the smallest economic return and performance came from T1 (control). The outcome of our study can conclude that addition of 16 mg hydroxy Cu/kg could be better among the tested levels for broiler chickens and economically feasible as well to the commercial poultry industry. An economic analysis was performed to evaluate the cost-effectiveness of dietary copper supplementation. Feed cost calculations were based on prevailing local market prices of feed ingredients during the experimental period, expressed as cost per kilogram of feed. The market price of live broilers was calculated using the average farm-gate price (PKR/kg live weight) at the time of the study.

The cost of copper supplementation was included in the analysis, with copper sulfate and hydroxy copper priced according to their respective commercial market values. Total feed cost per bird was calculated by multiplying cumulative feed intake by feed cost per kilogram for each treatment.

Net profit per bird was calculated using the following formula:
Net profit (PKR/bird) = Total revenue from live weight – Total feed cost – Cost of copper supplementation
Economic efficiency was compared among treatments based on net profit and feed cost per kilogram of live weight gain.

The present study was conducted to compare the influence on growth, carcass quality, meat traits and some blood biochemical parameters of hydroxy copper (HC) and copper sulfate (CS), also tissue mineral retention in broiler chickens. Copper sulfate was assumed to be the least digestible as based on its solubility and bioavailability, that hydroxy copper and in particular at 16mg/kg would certainly do better than it. There was a significant improvement in the growth performance and carcass yield of the birds supplemented hydroxy copper as compared to the overall performance of both copper sulphate and control group which actually confirmed our assumption that compared with copper sulfate dietary supplementation of hydroxy copper is more efficacious on poultry production.

The inclusion of hydroxy copper improved average daily weight and consequently feed conversion ratios, and feed efficiency. These results are consistent with previous studies from El-Ghalid *et al.* (2019) and Skřivan *et al.* (2002). While there are limits to how much organic or more bioavailable sources of copper can mimic the effects from inorganic $CuSO_4$, generally these forms provide better performance compared with $CuSO_4$. The most likely explanation might be its chemical stability and greater gastrointestinal absorption and subsequent higher true digestibility that increases nutrient retention efficiency, especially in the presence of other minerals. Contrastingly, the pro-oxidative effects of copper sulfate at higher levels have been demonstrated to compromise performance, as suggested by Boa and Choct (2009).

In addition, in broilers, copper supplementation has been shown to have positive effect on growth rate and carcass quality, which might be due to the antimicrobial and nutrient metabolism modulation properties of copper (Hassan *et al.*, 2020). Occasionally, even higher levels of copper failed to elicit an additional response, suggesting that nutritional copper requirements can be met at lower levels.

The slaughter weights and post-slaughter characteristics that were improved show the carcass benefits to feeding hydroxy copper. These results agree with previous studies done by Ibrahim *et al.* (2022) and Ferreira Júnior *et al.* (2022), both showed similar benefits of hydroxy or methionine supplements; Increased breast muscle deposition and greater organ development could be due to improved protein metabolism with hydroxy copper in diets and better energy utilization. The heavier liver and gizzard weights observed with hydroxy copper groups also suggest improvements in digestive health and metabolic function.

Makarski *et al.* (2014) also observed that adding copper at a level of 50 mg/L in chelated or sulfate form to the drinking water for turkeys did not result in improvement of hematological or biochemical parameters; rather, they found suppressed level of triglycerides in the blood. Although there were higher copper accumulations in the liver and pectoral muscle, the levels were not hazardous to human health suggesting that product safety was maintained even in the absence of any functional benefit.

The final pH of meat quality was better in the birds treated with hydroxy coppers, which also resulted in lower drip loss and better color of the product, also important for freshness and water-holding capacity. These components are elementary for the shelf life and the consumer-friendly aspect of the product. It is in agreement with the previous research by El-Ghalid *et al*. (2019). that also experienced some improvements while using it as an organic copper source. Similar to our findings, the enhancement was more significant in the hydroxy copper groups, even though copper sulfate also created some benefit. This fact confirms the importance of the copper source in the muscle physiology of the chicken post slaughtering.

Serum biochemical profiles provided further evidence of hydroxy copper's metabolic advantages. Higher levels of glucose, triglycerides, and total protein in the T4 group suggest enhanced energy metabolism and nutrient assimilation. These results support findings from Kumar and Thakur (2007), who observed that increased copper bio-availability boosts lipid metabolism and protein turnover. Importantly, liver enzyme levels (ALT and AST) remained within normal physiological limits across all treatments, indicating that hydroxy copper did not induce liver stress, even at the higher dose.

Tissue mineral analysis showed a clear increase in bone copper content in birds fed hydroxy copper, while liver copper levels remained stable. This supports earlier studies by Ferreira Júnior *et al*. (2022) and Kim *et al*. (2015), who found that organic copper sources promote greater mineral retention in skeletal tissue without causing excessive liver accumulation. The data suggests that hydroxy copper is efficiently absorbed and regulated within the body, allowing for safe use at both 8 mg/kg and 16 mg/kg levels.

From an economic perspective, hydroxy copper supplementation at 16 mg/kg resulted in the highest net return, despite a modest increase in feed cost. The improved growth performance, better feed efficiency, and enhanced carcass yield contributed to greater profitability, echoing findings from Dos Santos *et al*. (2021), who reported favorable cost-benefit ratios for hydroxy mineral use in broiler production.

It is worth noting that while some earlier studies, such as Akeem *et al*. (2021), found minimal differences between copper sources, the present results strongly support the use of hydroxy copper over copper sulfate. Variations in findings across studies may be due to differences in trial length, bird genetics, feed formulations, or copper source quality and bioavailability.

Conclusions and Recommendations

It was concluded that supplementing broiler diets with hydroxy copper at 16 mg/kg is more beneficial than using copper sulfate. It promotes better growth, meat quality, and this study comply with field standards and available on demand.

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Novelty Statement

This study pioneers the comparative evaluation of hydroxy copper (CuOHCl) and copper sulfate (CuSO4) on growth performance, meat quality, and serum biochemistry of commercial broilers, aiming to elucidate the superior copper source for enhanced poultry production and meat quality.

Authors' Contribution

Nasrat Ullah Rahimi: conducted this study as part of his M.Phil.
Sohail Ahmad: Research work under the supervision, helped in the statistical analysis and formatting of the manuscript, helped in reviewing the manuscript.
Saeed Ahmed, Ehsaan Ullah Khan: Research work under the supervision, Helped in reviewing the manuscript.
Jamshid Ahmad and Sameen Sargand: Helped in data
Ghulam Qadir: Helped in proof reading, Sameen Sargand helped in data collection
All authors read and approve the final manuscript.

Generative AI or AI assisted technology statement

No generative AI or AI assisted technology were used in the preparation of this manuscript.

Conflict of interest

The authors have no conflict of interest.

References

Akeem, A., O. Adeyemi and F.A. Igbasan. 2021. Effects of dietary copper supplementation on growth performance, nutrient digestibility and mineral retention in broilers. Trop Anim Health Prod., 53: 412. https://doi.org/10.1007/s11250-021-02864-0

Albrecht A., U. Herbert and D. Miskel. 2019. Effect of methionine supplementation in chicken feed on the quality and shelf life of fresh poultry meat. Poultr. Sci., 96: 2853–2861. https://doi.org/10.3382/ps/pex071, 2-s2.0-85028374048.

Boa, A.M and M. Choct. 2009. Trace minerals in poultry nutrition: review. World's Poult. Sci. J., 65(3): 515–528

Dos, S.T.S., RFM. Donzele and F.M. Oliveira. 2021. Effect of hydroxychloride trace minerals on performance, carcass yield, and meat quality in broilers. Anim Feed Sci. Technol., 278: 114975. https://doi.org/10.1016/j.anifeedsci.2021.114975

El-Ghalid, O.A., M.A. El-Sherbiny and M.E. Abd El-Hack. 2019. Comparative effects of different copper sources on growth performance, meat quality, and blood profile in broilers. Anim. Nutr. Feed Technol., 19(3): 497–507

Ferreira, J.A.A., J.B. Lopes and L.F. Oliveira. 2022. Hydroxy copper supplementation improves bone strength and mineral retention in broilers. Poult. Sci., 101(4): 101688. https://doi.org/10.1016/j.psj.2021.101688

Harms, R.H. and R.E. Buresh. 1987. Influence of three levels of copper on the performance of turkey poults. Poult. Sci., 66(6): 1060–1063. https://doi.org/10.3382/ps.0661060

Hassan, A.M., N.M. Abdel-Azeem and S.A.A. El-Latif. 2020. Growth response and carcass quality of broiler chickens fed diets supplemented with different levels and sources of copper. Egypt Poult. Sci. J., 40(2): 507–520. https://doi.org/10.21608/epsj.2020.94131

Ibrahim, D., A.H. El-Far and M.A.A. Latif. 2022. Influence of nano and organic trace minerals on broiler carcass characteristics. Front. Vet. Sci., 9: 845297. https://doi.org/10.3389/fvets.2022.845297

Kaneko, J., J. Harvey and M. Bruss. 2007. Clinical biochemistry of domestic animals. 6th edition, Academic press Pub, Elsevier Inc., p. 928.

Kim, J.H., S.H. Seo and J.S. Yoo. 2015. Effects of dietary copper sources on growth, tissue mineral concentration, and immune responses in broiler chickens. Asian-Aust. J. Ani. Sci., 28(11): 1605–1610. https://doi.org/10.5713/ajas.15.0127

Kumar, S. and S.S. Thakur. 2007. Effect of dietary copper supplementation on performance, nutrient utilization, and immune response in broiler chicks. Animal Feed Science and Technology.

Leeson, S. and J.D. Summers. 2001. Scott's Nutrition of the Chicken. 4th ed. University Books, Guelph, Canada

Makarski, B., M. Bednarczyk and M. Kwiecień. 2014. Impact of copper (Cu) at the dose of 50 mg administered in sulfate and chelate form on selected haematological, biochemical, and tissue parameters in turkey hens. Ann. Ani. Sci., 14(3): 599–610. https://doi.org/10.2478/aoas-2014-0003

Miroshnikov, S., E. Yausheva, E. Sizova and E. Miroshnikova. 2015. Comparative assessment of effect of copper nano- and microparticles in chicken. Orient J Chem. 31(4):2327–2336.

NRC (National Research Council). 1994. Nutrient requirements of poultry. 9th Edition, National Academy Press, Washington DC.

Pakistan Economic Survey 2023. https://finance.gov.pk/survey/chapter_24/2_agriculture.pdf

Pakistan Economic Survey 2024.https://finance.gov.pk/survey/chapter_24/2_agriculture.pdf

Skřivan, M., G. Dlouhá and O. Mašata. 2002. Effect of dietary copper supplementation on performance of broiler chickens. Czech J. Ani. Sci., 47(8): 376–382.

Swiatkiewicz, S., M. Swiatkiewicz, A. Arczewska-Wlosek and D. Jozefiak. 2015. Chitosan and its oligosaccharide derivatives (Chitooligosaccharides) as feed supplements in poultry and swine nutrition. J. Anim. Physio. Anim. Nutr., 99 (1): 1-12.