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Effects of different copper sources on performance, carcass characteristics and intestinal histomorphology in broiler chickens

Navar Ahmad Kazem[1], Mohsen Daneshyar[1]*, Abbas Nikoo[2], Gholamreza Najafi[3], Seyyed Meysam Abtahi Froushani[4]

[1] Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran; [2] Department of Organic Chemistry, Faculty of Chemistry, Urmia University, Urmia, Iran; [3] Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran; [4] Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

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Abstract

This study aimed to evaluate the effects of different copper sources experimental copper sulfate (CS), copper methionine (CM), copper carbohydrate, copper dihydrogen phosphate (CP), and nano copper (CN) on growth performance, carcass characteristics and intestinal histomorphology in broiler chickens. A total number of 300-day-old broilers were randomly assigned to five dietary treatments with six replicates each. During the finisher and the whole experimental periods, CM- and CN-fed birds had the highest and lowest body weight gain, respectively compared to the birds in other treatments. Consumption of the CM diet increased the feed intake compared to the different treatments. Feed conversion ratio was increased by consumption of CN compared to CM during the finisher and the whole experimental periods. In the duodenum, villus height (VH) was significantly increased by CN as compared to control. In the jejunum, consumption of both the CM and CC diets caused the lowest VH, whereas dietary CN inclusion resulted in the highest VH. The VH/crypt depth (CD) ratio was at the lowest and the highest levels by CM and CN, respectively. In the ileum, villus width was numerically decreased by CP. The CS-fed birds had the highest CD between the treatments. The VH/CD ratio was higher in CP and CN-fed birds compared to CS ones. Moreover, the goblet cell number was decreased by dietary CP compared to CM. In conclusion, broiler performance was increased by both CM and CP sources of copper, with methionine-based copper being suggested as a component of chicken diets.

Introduction

Because trace elements take part in various biochemical events required for appropriate growth and maintenance, their inclusion in poultry diets is crucial.[1] Copper (Cu), an essential trace element with numerous physiological activities in animals and poultry, is one of the significant trace minerals. Moreover, Cu plays a role in skeletal mineralization, erythropoiesis, leukopoiesis, connective tissue synthesis, myelin formation, melanin pigment synthesis, catecholamine metabolism, thermal regulation, cholesterol metabolism, immune function, cardiac function and glucose metabolic regulation.[2-4]

Additionally, it promotes the immune system and guards against oxidative stress.[5] In the poultry sector, there is increasing interest in substituting organic trace elements for inorganic ones. Increased mineral availability, improved performance and less environmental contamination are the goals of this substitution.[6] Furthermore, it has been shown that adding organic components to the diet shields the metals from dietary antagonists such phytate, non-starch polysaccharides, and crude fiber, as well as from interactions between minerals and their detrimental effects on vitamins.[7] The most popular source of Cu for poultry is copper sulfate (CS). It dissolves readily in acidic and watery solutions. Copper methionine (CM) is another Cu source that is being exploited and taken into consideration in the poultry industry.[8]

The intestinal flora may be affected differentially by CS due to its varying relative bioavailability and solubility. The effects of CM on broiler chickens have been the subject of numerous reports. It has been found that, in the presence or absence of zinc or iron, rats utilized more organic Cu from Cu-proteinate or Cu-lysine than from inorganic CS.[9]

There was no significant difference in performance between broilers fed on high levels of inorganic mineral chelates and those fed on low levels of organic chelates.[6]

***Correspondence:**

Mohsen Daneshyar. PhD
Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran
E-mail: m.daneshyar@urmia.ac.ir

When compared to inorganic minerals, using organic trace minerals allows supplementing rates to be reduced by at least 33.00% when 15.00 ppm CS is replaced with 10.00 ppm Cu proteinate, all without affecting broiler performance.[10] Dietary supplementation of organic Cu (50.00, 100, 150, and 200 mg kg^{-1} CM) did not significantly affect growth performance, however, it did increase the excretion of Cu in the excreta due to greater Cu inclusion and the buildup of Cu and fat in the liver.[11] Feeding dietary Cu concentrations up to 200 mg Cu kg^{-1} diet enhanced growth and feed conversion efficiency regardless of different sources.[12] The experiment included three sources of Cs, Cu-chloride and Cu-propionate as well as four Cu concentrations of 8.00, 100, 150 and 200 mg kg^{-1}. Inorganic Cu (Cu proteinate) has shown positive benefits on feed conversion, bone mineralization and reduced Cu requirements (8.84 *versus* 9.87 mg).[13]

No reports are available on the new sources of Cu and their effects on broiler chickens, despite several published papers comparing the efficacy of organic sources of Cu, like CM, to inorganic forms (CS). Compared to the other organic Cu sources (amino acid-based ones), the copper carbohydrate (CC), a new organic Cu source based on processed carbohydrates, is less expensive. In addition to Cu, the copper dihydrogen phosphate (CP) is a novel chemical molecule that also contains phosphorus. There is no information on the inclusion of these latter Cu sources in animal diets and they are novel.

There are some published reports on nano copper (CN). Supplementing broiler chicks with 10.00, 15.00 and 20.00 mg kg^{-1} of CN enhanced their performance, carcass yield, immunity, lipid profile, antioxidants, blood minerals, digestive enzymes and cecal microbiota.[14] It has been found that adding up to 12.00 mg of CN *per* bird—a maximum of 7.00% more than the National Research Council (NRC) to the meals of chickens improved their immunological and antioxidant defenses.[15] In the other study, adding CN to the standard dietary CS supplement could improve the antioxidant status of chicken liver and breast meat.[16] However, the CN level should not exceed 25.00% of the Cu content that the NRC recommends for broiler chickens. The safest level is 7.00% of the Cu content that the NRC recommends. Supplementation of green CN (8.00 mg kg^{-1}, Nano-copper oxide) improved carcass features as well as liver and kidney functions under climate change difficulties.[17] In another study, broiler hens treated with 5.00, 10.00, and 15.00 mg kg^{-1} of CN showed a linear improvement in growth performance, muscle histological features, bone strength and serum metabolites.[18]

This experiment goal was to find out how the growth performance, carcass yield, internal organs and intestinal histomorphology of broiler chickens were affected by five distinct Cu sources: CS, CM, CC, CP and CN as the sole source of the mineral premix.

Materials and Methods

Birds, treatments, and management. This study used a completely randomized design with five treatments and six repeats (10 chickens *per* repetition) on 300-day-old broiler chicks (Ross 308). Care and management of chicks were performed according to commercial guidelines and were approved by Urmia University Animal Ethic Committee (IR-UU-AEC-3/22). After being placed at 32.00 ˚C for 1 to 3 days, the temperature was progressively lowered by 3.00 ˚C *per* week until it reached 22.00 ˚C. Until the end of study period, the temperature was maintained at 22.00 ˚C. Additionally, during the trial, birds had unrestricted access to food and water, and the illumination program was run with 23 hr light and 1 hour dark. After being weighed, the birds were divided into five treatments, each consisting of six repetitions of 10 birds. The experimental treatments included the control treatment (containing 16.00 mg of CS), the second treatment (containing 16.00 mg of CM), the third treatment (containing 16.00 mg of CC), the fourth treatment (containing 16.00 mg of CP) and the fifth treatment (containing 16.00 mg of CN from the oxide source). During the starter (1 to 10 days old), grower (11 to 24 days old) and finisher (25 to 42 days old) phases, three experimental meals were ingested. The experimental meals were formulated in accordance with Ross 308 suggestions (Ross 308 nutrients requirements, 2014, Table 1).

Performance. During the starter, grower, finisher and entire experimental periods, body weight growth (BWG) and feed intake (FI) were measured and the feed conversion ratio (FCR) was computed for each period.

Carcass and internal organs. Six chickens from each treatment and one from each repetition were chosen at random and killed on day 42 of the experiment. The internal organs, such as the heart, liver, bursa, spleen, pancreas, duodenum, jejunum, ileum, and cecum as well as the carcass, breast and thighs, were weighed.

Intestine histomorphology. Segments of the duodenum, jejunum, and ileum were used to obtain intestinal samples from one bird each repeat. The beginning, middle and end of the small intestine where the three areas from which intestinal samples were taken. Following 48 hr of fixation in 10.00% formaldehyde phosphate buffer, intestinal tissues were processed and paraffin-embedded. A microtome (MicroTec, Walldorf, Germany) was used to cut each paraffin block into 5.00 µm slices, which were then put on a glass slide. Sections were then stained with Hematoxylin and Eosin and examined under a light microscope (Olympus, Tokyo, Japan) to determine the thicknesses of the mucosa and muscle layers as well as the villus height (VH), crypt depth (CD) and villus width (VW). The CD was measured from the base of the villus to the sub-mucosa and VH was measured from the tip of the villus to the villus-crypt junction.

Table 1. Components and chemical compositions of the diets used in the starter, growth and finisher periods.

Diet ingredients (%)	Starter (day 1 - 10)	Grower (day 11 - 24)	Finisher (day 25 - 42)
Corn	53.77	53.98	60.84
Soybean meal 42.00%	39.69	39.21	33.32
Soybean oil	2.31	3.17	2.52
Dicalcium phosphate	1.87	1.45	1.15
Calcium carbonate	1.08	0.81	0.77
L-lysine hydrochloride	0.32	0.16	0.19
DL-methionine	0.25	0.19	0.18
L-Threonine	0.19	0.11	0.10
Sodium bicarbonate	0.13	0.14	0.14
Salt	0.28	0.28	0.28
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
Nutrients			
AMEn (kcal kg^{-1})	2900	2960	3010
Crude protein (%)	21.26	20.86	18.92
Calcium (%)	0.92	0.73	0.63
Available phosphor (%)	0.49	0.41	0.35
Sodium (%)	0.17	0.17	0.17
Chloride	0.20	0.20	0.20
Digestible methionine (%)	0.56	0.49	0.47
Digestible methionine + cysteine (%)	0.97	0.90	0.84
Digestible lysine (%)	1.28	1.14	1.05
Digestible threonine (%)	0.85	0.77	0.70
Digestible arginine (%)	1.36	1.35	1.20
Digestible valine (%)	0.99	0.99	0.90
DCAB (mEq kg^{-1})	259.93	255.96	232.22

AMEn: Apparent metabolizable energy corrected for nitrogen; DCAB: Dietary cation-anion balance.

[1] Provides the following *per* kg of diet: 10,000 IU retinol, 4,500 IU chole-calciferol, 65.00 mg Dl-α-tocopherol, 3.00 mg menadione, 2.50 mg thiamine, 6.50 mg riboflavin, 3.20 mg pyridoxine, 17.00 μg cyanocobalamin, 1.90 mg folic acid, 180 μg biotin, 18.00 mg calcium pantotenate and 60.00 mg nicotinic acid.

[2] Provides the following *per* kg of diet: 120 mg manganese sulfate, 110 mg zinc oxide, 1.25 mg potassium iodate, 20.00 mg ferrous sulfate, 16.00 mg copper sulfate, 0.30 mg sodium selenite and 150 mg ethoxyquin. The same Cu content from separate sources was used for different treatment diets.

At each villus base, the VW was computed. The distance between the muscular layer and the mucosal epithelium was used to assess the thickness of the mucosa, and the inner circular and outer longitudinal layers of smooth muscle cells were used to identify the muscular layer. The following formulas were used to determine the villus surface area (VSA) and apparent absorptive surface area (AASA).[19]

$$VSA = 2\pi \times \tfrac{1}{2}VW \times VH$$

$$AASA = (3.10 \times VW + 3.20 \times VH) \times 1.00 - (20 \times VH)$$

Copper sources. The CM, CP, CC and CN have Cu concentrations of 17.65, 39.83, 13.59 and 79.88, respectively. The Iranian company (Danesh Pajohan Novin Khorak Co., Urmia, Iran) supplied all of these supplies. An X-ray fluorescence analyzer (Magix PRO-PW 2440; Philips, Amsterdam, The Netherlands) was used to measure the Cu content.

Statistical analysis. The SAS Software (version 9.1; SAS Institute, Cary, USA) and the GLM technique were used to statistically evaluate all of the data in a completely random design. The Tukey test was used to compare the means at the 5.00% probability level.

Results

Broiler Performance. The BWG of broiler chicks during the grower period was unaffected by dietary treatments ($p > 0.05$, Table 2). Birds fed CM and CN during the starter period exhibited lower BWG than the other birds ($p < 0.05$). When compared to the birds in other treatments, the CM and CN-fed birds exhibited the highest and the lowest BWG during the finisher and the whole experimental periods, respectively ($p < 0.05$). Dietary treatments had an impact on FI during the starter period and CM diet consumption raised FI relative to the other treatments (Table 2). Furthermore, when compared to the control birds, the consumption of the CM and CHP diet exhibited the lowest FI ($p < 0.05$). Throughout the grower, finisher and the whole experimental periods, no discernible differences among the FI treatments were seen ($p > 0.05$). During the starter and grower periods, there were no impacts of the Cu source on FCR ($p > 0.05$, Table 2). Consuming more CN than CM during the finisher and the whole experimental periods resulted in a higher FCR ($p < 0.05$). For FCR, there were no appreciable differences between the other treatments.

Carcass and internal organs. There were no discernible impacts of the various Cu sources on carcass, breast, thigh or abdominal fat weights ($p > 0.05$, Table 3). Furthermore, all internal organ proportionate weights were unchanged, with the exception of the duodenum and gizzard ($p > 0.05$, Table 3). Consuming CP increased the weight of the gizzard, while consuming CN increased the weight of the duodenum ($p < 0.05$).

Intestine histomorphology. Table 4 shows the impact of dietary treatments on intestinal histomorphology. When compared to the control, CN considerably raised the VH, VSA and AASA in the duodenum ($p < 0.05$). Regarding VH, there were no appreciable variations among the other treatments ($p > 0.05$). Additionally, broilers fed on CM and CN had a deeper CD than the control group ($p < 0.05$). Regarding CD, no discernible variations between the other treatments were found ($p > 0.05$). The ingestion of various treatments did not alter the VW or the VH to CD ($p > 0.05$). CP ingestion resulted in a numerical decrease in GCs ($p = 0.06$). In the jejunum, CN produced the maximum VH, VSA and ASAA while ingestion of CC and CM produced the lowest VH ($p < 0.05$). Different Cu sources had no effect on VW ($p > 0.05$). Consuming CC significantly reduced the depth of the crypt ($p < 0.05$). In terms of VH to CD ratio, CM and CN had the lowest and the greatest values, respectively ($p < 0.05$). Similar to the duodenum, CP intake reduced the number of GCs ($p < 0.05$). There was no discernible impact of dietary treatments on VH, VSA and ASAA in the ileum ($p > 0.05$). The CP quantitatively reduced VW ($p < 0.05$). Among the treatments, the birds fed on a control diet had the deepest crypt ($p < 0.05$). The VH/CD ratio was greater in birds given CP and CN than in birds fed CS ($p < 0.05$). Additionally, there were fewer GCs in CP than in CM ($p < 0.05$).

Table 2. The effects of different sources of copper on performance of broiler chickens during different experimental periods

Treatments	Starter (1 - 10 days)	Grower (11 - 24 days)	Finisher (25 - 42 days)	Total (1 - 42 days)
Body weight gain (g per day)				
CS	19.90[a]	54.58	78.59[ab]	51.02[ab]
CM	18.89[b]	56.85	83.64[a]	53.13[a]
CP	19.52[ab]	57.26	77.63[ab]	51.47[ab]
CC	19.95[a]	55.58	73.34[ab]	49.59[bc]
CN	18.82[b]	57.21	67.22[b]	47.75[c]
SEM	0.13	0.64	1.66	0.50
***p*-value**	0.001	0.63	0.008	0.002
Feed intake (g per day)				
CS	23.86[ab]	86.92	148.86	86.59
CM	22.50[c]	86.37	152.71	87.19
CP	23.16[bc]	87.37	148.86	86.46
CC	24.32[a]	87.96	149.09	87.13
CN	23.84[ab]	88.01	147.31	86.39
SEM	0.17	0.30	0.85	0.30
***p*-value**	0.001	0.38	0.34	0.85
FCR (g per day)				
CS	1.20	1.60	1.91[ab]	1.70[ab]
CM	1.19	1.52	1.83[b]	1.64[b]
CP	1.18	1.53	1.93[ab]	1.68[b]
CC	1.22	1.59	2.03[ab]	1.76[ab]
CN	1.27	1.54	2.20[a]	1.81[a]
SEM	0.01	0.02	0.04	0.02
***p*-value**	0.10	0.68	0.02	0.005

CS: Copper sulfate (Control diet); CM: Copper-methionine; CP: Copper dihydrogen phosphate; CC: Copper carbohydrate; CN: Nano copper; SEM: Standard error of means.
[abc] Different superscripts denotes significant difference in the same column ($p < 0.05$).

Table 3. The effects of different sources of copper on internal organ weights (g) of broiler chickens at day 42 of age.

Treatments	Heart	Gizzard	Liver	Pancreas	Bursa	Spleen	Duodenum	Jejunum	Ileum
CS	0.58	1.60[ab]	2.07	0.24	0.11	0.10	0.54[b]	1.59	1.38
CM	0.43	1.57[bc]	2.00	0.21	0.16	0.11	0.62[ab]	1.46	1.31
CP	0.50	1.73[a]	2.04	0.25	0.17	0.10	0.61[ab]	1.50	1.43
CC	0.47	1.52[bc]	1.90	0.23	0.16	0.10	0.56[ab]	1.35	1.43
CN	0.47	1.52[b]	1.92	0.25	0.21	0.11	0.61[a]	1.72	1.29
SEM	0.02	0.04	0.04	0.007	0.01	0.005	0.02	0.07	0.08
***p*-value**	0.96	0.005	0.32	0.46	0.56	0.80	0.045	0.69	0.42

CS: Copper sulfate (Control diet); CM: Copper-methionine; CP: Copper dihydrogen phosphate; CC: Copper carbohydrate; CN: Nano copper; SEM: Standard error of means.
[abc] Different superscripts denotes significant difference in the same column ($p < 0.05$).

Table 4. The effects of different sources of copper on duodenum, jejunum and ileum morphology of broiler chickens at day 42 of age.

Treatments	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Villus height/crypt depth	Goblet cells (number *per* mm²)	VSA (mm²)	AASA (µm²)
Duodenum							
CS	1,074.30[b]	168.06	186.21[b]	5.80	3,815.20	0.57[b]	1,810.2[b]
CM	1,088.57[ab]	163.53	205.37[a]	5.29	3,870.60	0.56[b]	1,813.2[b]
CP	1,304.59[ab]	169.91	200.42[ab]	6.50	2,711.60	0.69[ab]	2,092.2[ab]
CC	1,112.50[ab]	172.79	185.52[b]	6.01	3,238.80	0.60[ab]	1,870.7[ab]
CN	1,350.94[a]	175.44	207.29[a]	6.55	3,239.00	0.74[a]	2,165.0[a]
SEM	34.63	2.62	2.65	0.17	149.14	0.02	42.94
***p*-value**	0.01	0.71	0.004	0.09	0.06	0.01	0.008
Jejunum							
CS	1,047.22[ab]	163.27	186.58[ab]	5.62[ab]	3,903.80	0.54[ab]	1,762.8[ab]
CM	1,004.08[b]	164.36	205.25[a]	4.90[b]	4,190.00	0.51[b]	1,714.4[b]
CP	1,189.01[ab]	158.16	192.27[a]	6.18[ab]	2,944.20	0.59[ab]	1,917.1[ab]
CC	926.51[b]	173.49	169.21[b]	5.59[ab]	3,343.80	0.50[b]	1,648.7[b]
CN	1,314.86[a]	164.36	202.09[a]	6.56[a]	3,742.00	0.68[a]	2,088.2[a]
SEM	39.07	2.61	3.45	0.19	155.23	0.02	44.84
***p*-value**	0.007	0.47	0.0004	0.05	0.07	0.02	0.008
Ileum							
CS	772.22	208.20	111.01[a]	7.04[b]	4,391.40[ab]	0.51	1,572.08
CM	731.22	200.90	88.59[b]	8.32[ab]	4,644.20[a]	0.46	1,496.44
CP	842.01	189.17	91.63[ab]	9.21[a]	3,155.20[b]	0.50	1,596.84
CC	713.03	208.09	85.50[b]	8.39[ab]	3,796.80[ab]	0.47	1,500.73
CN	772.22	200.14	85.82[b]	9.19[a]	4,272.50[ab]	0.49	1,565.33
SEM	19.35	2.46	2.78	0.26	168.01	0.01	22.75
***p*-value**	0.22	0.06	0.005	0.02	0.02	0.71	0.56

VSA: Villus surface area; AASA: Apparent absorptive surface area; CS: Copper sulfate (Control diet); CM: Copper-methionine; CP: Copper dihydrogen phosphate; CC: Copper carbohydrate; CN: Nano copper; SEM: Standard error of means.

[abc] Denotes significant difference in the same column with different superscripts differ significantly ($p < 0.05$).

Discussion

The findings demonstrated that birds' performance responses to Cu sources was different. In comparison with the other sources, CC inclusion produced superior BWG and FI during the first phase (starting period), but this effect vanished during the grower, finisher and entire trial periods. The CM incorporation during the finisher and entire periods allowed for the detection of the increased performance (BWG and FCR). According to certain reports, freshly hatched chicks are very capable of absorbing and digesting large quantities of carbohydrates.[20] However, due to insufficient pancreatic and peptic proteases in young birds, which impede protein digestion, this ability does not exist for other nutrients such as proteins. Therefore, the superior performance of CC-fed birds in the current experiment during the first 10 days of life may be linked to their better use of carbohydrate-based Cu (CC) in comparison with the other sources. However, the enhanced capacity of the broilers to use amino acid-based Cu (CM) at older ages (25 to 42 days of age) may be linked to the superior performance of CM-fed birds throughout the last stages of production (finisher and entire period). It has been indicated that following week one, protein digestion begins to rise.[21] Despite the lack of published data regarding broiler consumption of CC, the superior performance of birds fed on CC during the starting period might be due to the higher and faster availability of carbs compared to other nutrients (fats and proteins) during the early stages of life.

The enhanced performance of broilers fed on CM is in line with the findings of earlier studies. For example, supplementing broilers with chelated Cu and at super nutritious levels has been shown to boost their performance.[22] Additionally, it has been noted that adding 100 ppm of Cu in the form of Met-Cu or Met-CuZn to broiler feed enhances performance,[23] and boosts egg production.[24] Additionally, FI, dry matter availability, and weight gain were all enhanced by dietary supplementation of 100 ppm Cu in chelates.[24]

Results of studies using additional Cu chelates have also been published. It has been found that broiler and pig performance was improved by supplementing with 100 - 125 ppm Cu of CM.[22] Additionally, it was noted that broiler performance was enhanced by supplementing 100 ppm of Cu as CM or copper zinc (CuZn) methionine.[23] It has been stated that Cu growth-promoting effects were caused by its antimicrobial properties.[25] According to the experiment findings, Cu has a major impact on the growth regulatory system in a variety of ways.[26] Therefore, the antibacterial properties of this substance and its increased availability may be linked to the improved performance of broilers fed on CM in recent experiments. In our experiment, CM did not significantly alter intestinal shape, despite the fact that the bacterial population was not examined in the recent investigation. Naturally, the jejunum CD was lower than the control, indicating better ileal health, which might have contributed to the CM-fed birds increased performance. Dietary inclusion of organic Cu sources (CM and Cu-glycine, or Cu-glycine) perform better in broiler chickens.[27]

Contrary to our findings, other research has found no impact of organic forms of Cu on broiler chicken performance. For instance, no impact of 50.00, 100, 150, and 200 mg kg^{-1} of CM have been reported on broiler chicken growth performance.[11]

In the current experiment, CN also discovered a lower BWG and a higher FCR. Previous research has reported improved performance due to the introduction of CN. Furthermore, the improved intestinal histomorphology of the CN-fed birds in our experiment did not match the performance outcomes. In contrast, adding CN to drinking water enhanced the performance of broilers.[28] They linked these benefits to increased nanoparticle levels and better absorption and bioavailability of CN in the gastrointestinal tract. Because of their small size, nanoparticles may diffuse through the GIT mucus more quickly, reaching the intestinal lining cells. They may then be absorbed through the GIT barrier and into the bloodstream.[29] It has been shown that CN have a higher bioavailability than CS.[30]

Even though there is no any solid evidence supporting increased gizzard weight due to CP consumption, it appears that the gizzard may be able to absorb proton ions through amphoteric effects of produced dihydrogen phosphateions. In addition to boosting gizzard muscular tone, structural components like hydroxyl (OH) also increase grinding activity and digesta retention time in the gizzard and proventriculus which enhances nutrition utilization.[20]

The higher VH and VH/CD ratio of CN-fed birds is correlated with their increased duodenal weight. We discovered that adding CN to the diet improved intestinal shape and might had led to increased absorption of Cu, which led to conditions like Cu poisoning that just impaired performance without causing any additional adverse effects.

In contrast, CM-fed birds exhibited improved performance throughout the whole study period, with higher CD and lower VH, VSA and ASAA in the duodenum and jejunum. This might have related to the various absorption locations and solubility of Cu sources.[31] The duodenum and upper jejunum are said to be the primary sites for Cu absorption from mineral sources.[32,33] Lower CD and VH/CD ratios might have been seen because more Cu from the CM source might have reached to the ileum, the last section of the intestine, where it was absorbed. For both CP and CN sources, the same scenario could apply.

The way the crypts and villi are shaped will show how the intestines work, how digestive enzymes are secreted and how nutrients are absorbed. The form of intestinal villi is associated with intestinal growth rate and function. A longer intestinal villus increases the surface area available for absorbing nutrients. Intestinal villus shortening has thus been linked to poor gut health in broilers. Since Cu directly promotes villus regeneration in birds and indirectly influences intestinal architecture by influencing the gut microbiota population, broiler performance and intestinal physiology can be significantly impacted by dietary Cu sources.[8]

In an experiment, the maximum VH and VW were generated by CN treatment (CS + 15 mg kg^{-1}). Additionally, the CS + 15 mg kg^{-1} fed birds showed the highest VH/CD ratio in the ileum and duodenum.[34] Villous epithelial cell renewal is significantly aided by the stem cells in the crypt.[35] Regeneration and mitosis are associated with the VH/CD ratio.[36] These outcomes could be explained by Cu capacity to actively affect enzyme function, improving energy digestibility and metabolic pathways.[29]

All intestinal segments include GCs, which secrete mucin glycoprotein and mucus.[37] Research has indicated that nutrition has an impact on mucin diversity, activity and population of GCs.[38] The increased anti-bacterial properties of CM in with other sources may be the cause of the higher number of GCs in all three intestinal segments in the CM-fed birds in the current experiment. The greater numbers of GCs in the ileum in our study might have been induced by the maximum bacterial burden and the bactericidal effects.[39] The lower GC counts in the ileum of CP birds might have been caused by their improved intestinal health, particularly in the ileum (higher VH/CD ratio). Since this work was the first to demonstrate the impact of CP on intestinal histomorphology, further potential regulation mechanisms by CP inclusion could be examined in relation to GC syntheses.

In conclusion, recent experiment results indicated that broiler performance was increased by both CM and CP sources of Cu, with methionine-based Cu being suggested as a component of chicken diets. Even though dietary CN showed improved intestinal histomorphology at the end of the experiment, performance was found to have declined. Therefore, when adding CN to diets, levels below the broiler requirements demand might be more beneficial.

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Conflict of interest

There is not conflict of interest with any person or institute/organization regarding this manuscript.

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