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Copper Nanoparticles Improve Growth Performance and Modulate Biochemical and Lipid Profiles in Broilers Exposed to Chronic Heat Stress

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THIS experiment was designed to evaluate the impact of dietary supplementation with copper oxide nanoparticles (CuO_NPs) on growth, serum biochemical and lipid profiles, and relative immune organs weights of Ross 308 and Cobb 500 broilers exposed to chronic heat stress (HS). Chicks were allotted in three treatments with three replicates per each treatment. Chicks in the 1st treatment group fed 100 % of its recommended Cu needs in the form of CuO while, 2nd & 3rd groups received 100 % and 50% of their Cu needs as CuO_NPs, respectively. At 21 days old, each group was split into two, normal (housed at 24 °C) and HS (exposed to 33±2°C for 5 h/day for 2 weeks). CuO_NPs supplementation to Cobb and Ross broilers significantly modified their growth. Furthermore, it altered their serum globulin concentration while non-significant improvement in liver function indicators was recorded. In addition, dietary inclusion of CuO_NPs for the two studied strains significantly modified their serum ALP levels and significantly modified their abdominal fat content and serum lipid profile (cholesterol, triglycerides, HDL and LDL). In conclusion, dietary supplementation of CuO_NPs to Cobb and Ross birds reared under HS conditions can effectively improve growth performance and biochemical profile, reduce serum cholesterol content, and restored the relative weight of the bursa, thymus, and spleen.

Keywords: Copper Oxide Nanoparticles (CuO_NPs), Growth performance, biochemical profile, Broilers

Introduction

The trace minerals including copper (Cu) are essential in poultry feed. They are crucial for oxygen utilization, growth, energy metabolism, and overwhelming the oxidative stress [1,2]. Cu has an important role in free radical elimination and protect cells from lipid peroxidation [3]. It is regarded as a crucial part of the enzyme copper-zinc-superoxide dismutase (Cu-ZN-SOD) enzyme [4,5]. Under normal housing temperature, the addition of CU to chicken feed significantly enhanced the antioxidant activity [6,7]. In addition, it decreases blood cholesterol, triglycerides, along

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with elevates the levels of HDL and total cholesterol in meat [8,9]. Further, Cu supplementation to broiler diet improves the actions of proteases, amylase and lipase enzymes in the small intestinal leading to improving the intestinal mucosal morphology and as a result higher daily gain is obtained [10]. Despite of the importance of Cu to poultry and due to its low bioavailability, Cu slats usually supplemented in higher levels which may cause toxicity and environmental pollution. Therefore, Cu-NPs are recommended. However, Cu-NPs many induce toxic effects that vary depending on the animal species, delivery, concentration, and size of Cu-NP. These factors impact Cu-NPs toxicity by facilitating particle absorption and translocation in the animal's body. Additionally, it has been observed that exposure to copper nanoparticles may cause toxicity, and induce negative impacts on bird's immune system [11].

At the commercial level, most of the Cu supplementation in bird feeds as inorganic salts which have low availability and associated with excess Cu excretion in the litter [2,12]. So, searching for new alternatives replacing these inorganic salts, and improve Cu utilization and bioavailability attracted the attention of nutritionists. One of these alternatives is the Cu-nanoparticles (Cu_NPs) which is an auspicious recent trend for better chicken performance. Under normal rearing temperature, Cu_NPs supplementation to animal diet increases its intestinal absorption, serum and tissue concentration, consequently improved the animal health [13]. Moreover, an enhancement of chicken's embryo development and the postnatal performance have been proved following in ova administration of Cu-NPs [14]. In addition, copper nanoparticles injection increases RBCs count, haemoglobin level, serum protein and copper nanoparticles level [15].

Commercial broilers are highly sensitive to the deleterious impacts of heat stress (HS) because of its higher metabolic rate and the ineffective thermoregulation under HS [16-18]. Therefore, HS impairs broiler performance [19], lowers feed consumption, egg production and quality and reduces bird's immune response [20,21]. Therefore, several methods have been applied to decrease these effects including nutritional manipulation through supplementation of variable feed additives such as Nano minerals [21-23]. However, previous literatures did not consider dietary CuO_NPs addition effects on the blood biochemical profile of broilers during chronic HS. To evaluate the effects of CuO_NPs addition to broilers' diets on growth performance, serum biochemical changes, and the relative weights of the immunological organs in Cobb 500 and Ross 308 broilers subjected to HS, this feeding trial was conducted.

Material and Methods

Bird source

Bird management was accomplished depending on the procedures approved by the animal care and ethics committee at the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt. This study has been done on Ross 308 and Cobb 500 obtained from Abd Elsalam Hegazy group, Cairo, Egypt as an example to the broiler strains. A total of 288-day old mixed sex chicks (144 chicks for each strain) were used. Broiler chicks with non-significantly different body weights (average 48.7 ± 0.72 g) of the two commercial strains were randomly kept in isolated and environmentally controlled rooms (15×3×3 m^2) at the experimental poultry farmhouse, Kafrelsheikh University, Egypt, with average housing density ≈ 8 birds/m^2.

Experimental design and bird's management

Birds of each strain were randomly allocated into 3 treatments with 3 replicates for each group with 16 birds per replicate (n= 48 bird/group). The chicks were brooded in distinct floor pens (1 m × 1.5 m). On 21st day of age, each replicate was randomly split into two groups of 8 birds each. One group transferred to HS room while the other group was kept in normal temperature room. HS was cyclic chronic HS lasted for 14 days, 5h per day (10: 15pm) at 33 ± 2°C, then kept at (24°C) for the rest of the day (Table 1) [16,24]. Birds' partitioning was done randomly in which one-half of the chicks' number (72 chicks of Ross 308 and 72 chicks of Cobb 500) was housed in one room under normal housing temperature and the other half (72 chicks/ strain) was housed in another room for HS treatment. To safeguard isolate the experimental rooms from the external environment and outside heating, the roof of the experimental rooms was sealed. To ensure data accuracy, three checks/ day were made on the room's temperature and relative humidity using both digital and manual thermometer and hygrometer above the level of the bird's head.

Bird's feeding and diet formulation

Birds consumed basal diet (BD) which was prepared (Table 2) based on [25] of poultry nutrient requirement. Cu was included in the diet depending on the recommended nutrients requirements guide (commercial catalog) of each broiler strain [26,27]. In the current study, copper was added at different rates and forms, 100 % of its normal level was supplemented as CuO (Sigma- Aldrich®) (control group). While the 2nd and 3rd groups, Cu was included at 100 % and 50% of its recommended level as CuO_NPs, respectively. CuO_NPs were made at the Nano-chemistry Laboratory, Faculty of

Science, Kafrelsheikh University, Egypt, based on sol-gel technology using the wet-chemical method. Then the particles were well characterized [2]. Throughout the study, birds had free access (*ad libitum)* of food and water access and housed on a deep litter system. On 1st day of the experiment, birds in all experimental groups were brooded at 34 °C then was decreased gradually, till reached 22–25 °C at 21 days old. To maintain a stable room temperature, automatic heaters with digital thermometers specific for ambient temperature were used. Over the course of the feeding trial, relative humidity remained nearly constant at an average of 70%. The birds in all groups were reared under the same management procedures such as floor space, light requirements, and duration along with the access to food and water.

TABLE 1. Experimental design

Bird strain	Normal (N) (24±2°C)	Heat stress (HS) (33±2°C)	Copper treatment
Cobb 500 (C)	CN1	CH1	100% CuO (1st group, control)
	CN2	CH2	100% CuO-NPs (2nd group)
	CN3	CH3	50% CuO-NP (3rd group)
Ross 308 (R)	RN1	RH1	100% CuO (1st group, control)
	RN2	RH2	100% CuO-NPs (2nd group)
	RN3	RH3	50% CuO-NP (3rd group)

CN1, CN2 and CN3 represent Cobb birds reared under normal temperature and supplemented with Cu as 100 % of recommended requirements as CuO, 100% as CuO-NPs and 50 % as CuO-NPs, respectively. CH1, CH2 and CH3: The same as the first 3 groups of Cobb birds but reared under HS conditions. RN1, RN2 and RN3 donate Ross birds reared under normal temperature and supplemented with Cu as 100 % of recommended requirements as CuO, 100% as CuO-NPs and 50 % as CuO-NPs, respectively. RH1, RH2 and RH3: The same as the first 3 groups of Ross birds but reared under HS conditions.

TABLE 2. Ingredients and calculated composition of the used basal diet

Ingredients (%)	Starter	Grower	Finisher
Yellow corn	56.15	62.26	66.36
Corn Gluten Meal	6.00	3.70	2.00
Soybean meal (47%)	31.7	27.61	25.21
Soybean Oil	2.30	2.50	2.50
DCP[1]	1.50	1.50	1.50
Limestone [2]	1.40	1.40	1.40
Mineral premix [3]	0.15	0.15	0.15
Vitamin premix [4]	0.10	0.10	0.10
Common Salt	0.30	0.30	0.30
DL- methionine [5]	0.20	0.22	0.22
Lysine HCl [6]	0.15	0.21	0.21
Choline chloride	0.05	0.05	0.05
Calculated Composition [7] (%)			
Crude protein	23.0	20.13	18.26
Calcium	0.92	0.91	0.90
Available Phosphorus	0.47	0.46	0.46
Lysine	1.21	1.15	1.08
Methionine	0.59	0.56	0.52
ME (Kcal/kg)	3058.46	3108.86	3132.7
Calorie/protein ratio [8]	123.97	154.43	171.56

[1]DCP=Dicalcium phosphate (contain 18% Phosphorus and 21% Calcium).[2]Limestone (contain 35 % calcium).[3]Mineral premix: formulated and composed of (each 1.5 kg) 110000 mg Mn (Mn oxide), 105000 mg Zn (Zn oxide), 15500 mg Cu (copper oxide), 30000 mg Fe (Fe sulphate), 1125 mg I (sodium iodide), 325 mg Se (sodium selenite) and 100 mg Co (cobalt sulphate) and calcium carbonate as a carrier. [4]Vitamin premix produced by Nutristar international Co. and composed (per 1 kg) of vitamin A 12000000 IU, vitamin D3 2500000 IU, vitamin E 10000 mg, vitamin K3 1000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B1210 mg, niacin 30000mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10000 mg, antioxidant- ethoxiquin 250 mg with calcium carbonate upto 1 kg as carrier. [5]DL-Methionine (Produced by Evonic Co. and contain 99 % methionine).[6]Lysine = lysine hydrochloride (contains 98 % Lysine).[7] calculated composition according to NRC (1994). [8] Calorie/protein ratio = ME Kcal /CP % .

Growth Performance measurements

Up until the fifth week of the experiment, feed intake (FI) and body weight (BW) were measured weekly. Additionally, weekly body gain (WG) and feed conversion ratio (FCR) were computed according to [28].

Analytical Measurements

At the end of 5th week of age, blood samples (3 ml) were collected from six randomly selected birds (2 birds / replicate) by wing vein puncture. Blood samples were centrifuged at 3000 rpm for 10 minutes then the serum samples were kept in the freezer at -20 °C until used for analysis. The following biochemical parameters were analysed: total protein (biuret method), albumin (bromocresol green method), and globulin (calculated as the difference between the total protein and albumin) while the total cholesterol, and triglycerides (Trinder enzymatic method) were determined in individual samples in an automatic analyzer (Chemwell®, Megazyme, Wicklow, Ireland) using commercial kits (Labtest Diagnóstica S.A, Lagoa Santa, Brazil). The automatic analyzer was previously calibrated with caliber 1 and measured with the control serum Qualitrol 1H, both manufactured by Labtest Diagnóstica®. After that the birds were slaughtered and the abdominal fat content and relative immune organs weights (bursa of Fabricius, spleen and thymus) were recorded.

Statistical analysis

Results were statistically analyzed using GraphPad Prism 6 software (GraphPrism Software, La Jolla, California, USA). Before analysing, data was tested for normality distribution by analysis of the residuals. The data were analyzed by Two-way ANOVA based on this model: $Y_{ij}=\mu + T_i + S_j + (TS)_{ij} + e_{ij}$, where Y_{ij} denotes the assessed variables, μ is the total average, T_i represents the different treatment's effect (HS and copper treatments), S_j is for the effect broiler strain, $(TS)_{ij}$ is the interaction between T and S, and e_{ij} is the random error. Statistical significances was defined at P values < 0.05 depending on the Tukey's multiple comparisons. Results were stated as mean ± SEM (Standard error of means).

Results

Growth Performance

CuO-NPs significantly (P<0.05) modified Cobb and Ross birds' BW, WG, and FCR (Table 3 and Fig. 1) for HS & Cu treatments as well as broiler's strain, and for treatment-strain interaction). Supplementing CuO_NPs to Cobb birds which housed under normal temperature did not change BW and WG (P>0.05). Interestingly, when added at 50% to heat-stressed birds significantly (P < 0.05) improved the reduction of BW and WG resulted from HS. Regarding Ross raised under both types of conditions, CuO_NPs supplementation non-significantly (P>0.05) increased BW. Moreover, at the normal temperature, CuO_NPs caused significant and non-significant increases of WG when supplemented at 50 % (P<0.05) and 100% (P>0.05), respectively. The influence of CuO_NPs on WG was significantly higher in Ross (RH2) than Cobb (CH2) (P<0.05). Regarding feed intake (FI), CuO-NPs supplementation non-significantly reduced FI of Cobb and Ross birds raised under normal housing temperature and Cobb exposed to HS conditions. While under HS, CuO_NPs supplementation to Ross birds caused a non-significant increase of FI. The changes in WG and FI due to CuO_NPs dietary supplementation were associated with changes in FCR, the 100 % supplementation of CuO_NPs caused significant (P<0.05) improvement of FCR in Cobb birds (CN2 and CH2). On the other hand, 50 % supplementation (RN3 and RH3) as CuO_NPs distinctly improved the FCR.

Serum Biochemical Parameters

Supplementing CuO-NPs did not modify the total protein level in serum (P>0.05 for all factors) (**Table 4**). Following HS**,** Cobb birds received 100 % of Cu level as CuO_NPs (CH2) showed non-significantly (P>0.05) reduced serum albumin concentration. Besides, using CuO_NPs to Cobb and Ross birds significantly altered their serum globulin concentration (P<0.001 for treatment, P>0.05 for strain). For Cobb, 50 % of Cu supplied as CuO_NPs significantly (P<0.05) improved the serum globulin levels under HS conditions

(CH3) compared with its effect under normal temperature (CN3). While in Ross strain under HS, the 50 % supplementation as CuO_NPs (RH3) had a better effect on the serum globulin levels compared to the 100 % supplementation level as CuO_NPs (RH2) (P<0.05). Also, under HS, Ross received 50 % of Cu as CuO-NPs (RH3) showed slight elevations in globulin level in serum than Cobb (CH3).

TABLE 3. Growth performance of commercial broilers supplemented with CuO-NPs under normal and heat stress conditions.

Groups	Initial Wt. (g)	Final Wt. (g)	Total body gain (g)	Total FI (g)	Average FCR
CN1	48.08 ± 0.88 [aA]	1642.38 ± 39.19 [aA]	1594.11± 11.47 [aA]	2654.47	1.71 ± 0.071 [aB]
CN2	47.9 ± 0.65 [aA]	1680.38 ± 41.84 [aA]	1632.48± 13.85 [aA]	2604.59	1.58 ± 0.083 [abA]
CN3	47.8 ± 0.66 [aA]	1567.08 ± 31.18 [aA]	1519.28± 16.039 [aA]	2548.00	1.68 ± 0.048 [abA]
CH1	49.08 ± 0.48 [aA]	1474.02 ± 43.77 [bA]	1424.94 ± 20.85 [bA]	2548.89	1.71 ± 0.078 [aA]
CH2	47.5 ± 0.53 [aA]	1423.63 ± 20.0 0[bA]	1376.13± 16.25 [bB]	2112.34	1.52 ± 0.025 [bA]
CH3	47.2 ± 0.71 [aA]	1525.21 ± 33.91 [abA]	1478.01± 13.54 [aA]	2441.79	1.62 ± 0.055 [abA]
RN1	49.5± 0.86 [aA]	1563.04 ± 28.11 [aA]	1513.54± 12.22 [bA]	2820.48	1.91 ± 0.079 [aA]
RN2	50.08 ± 0.67 [aA]	1585.25 ± 36.66 [aA]	1535.17± 14.66 [abA]	2798.56	1.79 ± 0.055 [abB]
RN3	48.01 ± 0.75 [aA]	1616.54± 48.96 [aA]	1568.53± 18.37 [aA]	2714.12	1.64 ± 0.057 [bA]
RH1	50.02 ± 0.92 [aA]	1493.81 ± 33.80 [bA]	1443.79± 11.42 [bA]	2430.27	1.72 ± 0.083 [bA]
RH2	49.1 ± 0.69 [aA]	1529.25 ± 29.46 [abA]	1480.15± 10.48 [bA]	2498.30	1.67 0.034 [bA]
RH3	47.6 ± 0.54 [aA]	1512.86 ± 53.21 [abA]	1465.26± 15.20 [bA]	2489.98	1.53 ± 0.040 [cA]

The data are presented as Means ± SEM. Different lowercase letters represent the statistical differences (P<0.05) between different treatments within the same strain. While the uppercase ones for the statistical significance (P<0.05) between Ross and Cobb chicken. CN1, CN2 and CN3: Cobb birds reared under normal temperature and supplemented with Cu as 100 % of recommended requirements as CuO, 100% as CuO-NPs and 50 % as CuO-NPs, respectively. CH1, CH2 and CH3: The same as the first 3 groups of Cobb birds but reared under HS conditions. RN1, RN2 and RN3: Ross birds reared under normal temperature and supplemented with Cu as 100 % of recommended requirements as CuO, 100% as CuO-NPs and 50 % as CuO-NPs, respectively. RH1, RH2 and RH3: The same as the first 3 groups of Ross birds but reared under HS conditions. This experiment included 3 replicates with 16 birds in each (n= 48 bird/group).
Absence of significant letters donates non-significant differences.

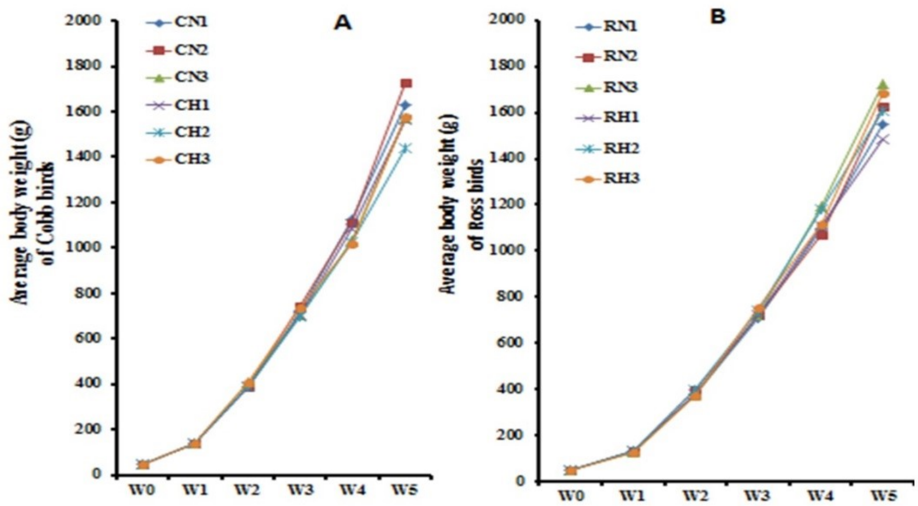

Fig 1. Effect of CuO-NPs supplementation on average body weight of normally and following HS in Cobb (A) and Ross birds (B). N1, N2 and N3 represents relative weight of organ of birds kept at normal temperature and supplemented with their Cu requirement as 100 % of CuO, 100 % of CuO-NPs and 50 % of CuO-NPs respectively, while H1, H2 and H3 for those birds exposed to chronic HS and supplemented with Cu as 100 % of CuO, 100 % of CuO-NPs and 50 % of CuO-NPs, respectively.

TABLE 4. Biochemical parameters of commercial broilers supplemented with CuO-NPs under normal and heat stress conditions.

Groups	Total protein gm/dl	Albumin gm/dl	Globulin[1] gm/dl	GOT[2] U/L	GPT[3] U/L	Glucose mg/dl	ALP[4] U/L
CN1	2.95 ± 0.12	1.62 ± 0.09	1.33 ± 0.04 [a]	141.66 ± 25.09	5.67 ± 1.45	253.00 ± 16.92 [a]	4592.33 ± 124.07 [a]
CN2	2.85 ± 0.07	1.69 ± 0.06	1.16 ±0.07 [b]	161.00 ± 25.10	6.00 ± 0.58	235.67 ± 13.57 [a]	4949.66 ± 13.32 [aA]
CN3	2.91 ± 0.11	1.71 ± 0.04	1.19 ±0.14 [b]	211.00 ± 13.00	6.67 ± 0.33	213.67 ± 29.56 [b]	5131.66 ± 42.78 [aA]
CH1	3.16 ± 0.11	1.72 ± 0.04	1.37 ± 0.07 [a]	209.50 ± 0.86	6.00 ± 1.15	225.33 ± 4.18 [a]	4341.33± 223.98 [aA]
CH2	2.93 ± 0.09	1.51 ± 0.06	1.34 ±0.06 [a]	180.00 ± 7.02	8.67± 3.71	213.00± 10.44 [b]	3377.66 ± 67.03 [bB]
CH3	2.95 ± 0.06	1.73 ± 0.01	1.55 ± 0.21 [a]	188.00 ± 2.88	4.67 ± 1.02	239.00 ± 3.00 [a]	4379.66 ± 122.45 [a]
RN1	3.16 ± 0.11	1.78 ± 0.03	1.37 ± 0.14 [c]	192.66 ± 7.35	4.67 ± 0.33	173.67± 12.20 [b]	4802.00± 45.50 [a]
RN2	2.93 ± 0.09	1.72 ± 0.04	1.21 ± 0.05 [c]	188.66 ± 29.38	5.33 ± 0.33	212.00 ± 10.41 [a]	3898.00 ± 100.53 [bB]
RN3	2.95 ± 0.06	1.70 ± 0.04	1.25 ±0.04 [c]	171.66 ± 13.38	6.00 ± 0.57	221.33 ± 10.73 [a]	2376.00 ± 100.68 [cB]
RH1	3.09 ± 0.09	1.64 ± 0.10	1.63 ± 0.06 [b]	185.00 ± 10.39	6.33 ± 1.85	217.33 ± 7.62 [a]	2817.00 ± 471.00 [dB]
RH2	2.85 ±0.10	1.72 ± 0.04	1.44 ±0.16 [b]	183.33 ± 3.84	6.67 ± 1.20	232.00 ± 4.73 [a]	4476.33 ± 97.79 [aA]
RH3	3.28 ± 0.23	1.61 ± 0.02	1.86 ± 0.11 [a]	170.66 ± 3.18	4.33 ± 0.33	238.00± 8.33 [a]	3616.00± 111.77 [b]

The data are presented as Means ± SEM. Different lowercase letters represent the statistical differences (P<0.05) between different treatments within the same strain. While the uppercase ones for the statistical significance (P<0.05) between Ross and Cobb chicken. Absence of significant letters donates non-significant differences. [1] Globulin: total protein – albumin, [2] GOT: Glutamic oxaloacetic transaminase, [3] GPT: Glutamic pyruvic transaminase,[4] ALP: alkaline phosphatase. This experiment included 3 replicates with 16 birds in each (n= 48 bird/group).

Regarding liver function enzymes, supplementation of CuO_NPs to Cobb birds diet reared under normal temperature (CN2 and CN3) non-significantly (P>0.05) increased the serum GOT levels, while non significantly reduced it under HS (CH2 and CH3) when compared with their control groups received copper as CuO (CN1 and CH1). The same response of reducing serum GOT levels was also observed in Ross birds received copper as CuO_NPs either reared within normal temperature or exposed to HS. Birds in CN3 and RN3 groups (P > 0.05) showed slight increases in serum GPT levels, while minor reduction of serum GPT level was reported in case of heat stress (CH3 and RH3) when compared to the other experimental groups. Glucose level in serum was considerably decreased (P<0.05) in Cobb birds in CN3 group and those received 100 % of Cu as CuO_NPs and subjected to HS. While in Ross birds, glucose level was increased with CuO_NPs with the highest concentration was obtained at 50 %. Moreover, HS when CuO was used as Cu source significantly modulated the blood glucose level in Ross birds.

Dietary inclusion of CuO-NPs in diet of both strains significantly modified their serum ALP levels (P<0.0001 for all factors and their interaction). Cobb chicken raised on 100 % of Cu as CuO_NPs (CH2) and exposed to HS showed significant (P<0.0001) decrease of serum ALP levels compared with its effect in normal temperature (CN2) or those received 50 % of Cu as CuO-NPs (CH3) under HS. In Ross strain, the 50 % CuO_NPs supplement, under normal temperature (RN3), significantly decreased the serum ALP levels compared with the 100 % CuO_NPs supplementation (RN2), 100% CuO (RN1) and the 50 % CuO_NPs supplement during HS (RH3) (P<0.05). Heat- stressed Ross birds which fed 100% of its Cu as CuO (RH1) had low serum ALP levels compared with same treatment when the rearing temperature is normal (RN1) (P<0.0001) and the 100 % CuO_NPs supplement (RH2) in heat stressed birds (P<0.0001). However, Cobb strain fed on 50 % of Cu as CuO_NPs under normal temperature (CN3) and those received 100 % of Cu as CuO under HS (CH1) showed significant elevation (P<0.0001) in serum ALP levels compared to Ross strain with the previous treatment (RN3& RH1). While under HS, Cobb birds supplemented with 100 % as CuO_NPs (CH2) showed lower serum ALP levels compared to Ross strain with the same treatment (RH2) (P<0.05).

Concerning serum lipid profiles described in Table 5, supplementing CuO-NPs to Cobb birds significantly (P<0.05) modified their serum cholesterol concentrations. For Cobb birds reared in normal temperature or exposed to HS, the 50% supplementation of Cu as CuO_NPs (CN3 and CH3) considerably diminished blood cholesterol concentrations (P<0.05). Besides, HS plus CuO_NPs supplementation in Cobb diet significantly (P<0.05 for treatment) modulated serum high density lipoprotein (HDL) without strain differences. Blood HDL level was markedly increased in Cobb birds raised when rearing temperature is normal and received 100 and 50 % of its Cu as CuO_NPs (CN2 and CN3, respectively) (P<0.05), while reduced in Cobb birds received the same Cu levels but reared under HS (CH2 and CH3) (P < 0.05) compared with their control groups (CN1 and CH1). For Cobb reared in normal housing temperature and received 50% as CuO_NPs (CN3) showed reduced serum triglycerides levels compared to those receiving 100 % of the same supplement (CN2), 100 % as CuO (CN1) or those receiving the same level of Cu but reared under HS (CH3) (P<0.05, respectively). Alternatively, Ross housed in normal temperature and fed CuO (RN1) displayed decreased serum TG levels compared with those received CuO_NPs (50% and 100%) (RN2 and RN3) (P<0.05).

Cobb birds exposed to HS and supplied with 50 % as CuO_NPs (CH3) showed reduced abdominal fat content (P<0.05) compared with the same group reared under normal temperature (CN3) or those raised on CuO (CN1). On the other hand, Ross birds reared under HS and received 100 % as CuO-NPs showed the highest abdominal fat content (P<0.05) compared to other experimental groups of the same strain reared under both conditions.

TABLE 5. Lipid profiles and abdominal fat content of commercial broilers supplemented with CuO-NPs under normal and heat stress conditions.

Groups	**Cholesterol** mg/dl	**Triglycerides** mg/dl	**HDL**[1] mg/dl	**LDL**[2] mg/dl	**Abdominal Fat Content** (g)
CN1	132.66 ± 4.25 [b]	191.33 ± 12.99 [a]	60.06 ± 4.12 [c]	34.33 ± 1.70	19.00 ± 2.67 [a]
CN2	155.00 ± 1.73 [a]	226.00 ± 18.47 [a]	81.23 ± 4.90 [a]	28.56 ± 3.01	11.30 ± 1.02 [b]
CN3	140.33 ± 5.81 [b]	156.33 ± 17.93 [b]	77.63 ± 5.26 [a]	31.43 ± 5.19	15.90 ± 2.17 [a]
CH1	157.66 ± 4.84 [a]	180.33 ± 4.41 [a]	94.16 ± 2.80 [a]	27.43 ± 5.37	12.00 ± 1.00 [b]
CH2	139.00 ± 5.68 [b]	178.66 ± 6.93 [a]	76.00 ± 5.59 [b]	26.26 ± 1.94	11.20 ± 0.26 [Bb]
CH3	132.33 ± 3.18 [b]	163.00 ± 4.04 [a]	72.50 ± 4.31 [b]	27.20 ± 5.35	14.90 ± 0.69 [b]
RN1	146.33 ± 5.84 [b]	142.66 ± 11.86 [b]	88.90 ± 3.53	28.90 ± 3.40	14.20 ± 1.50 [b]
RN2	158.66 ± 12.71 [a]	206.66 ± 20.69 [a]	78.00 ± 7.73	39.26 ± 8.30	15.50 ± 0.99 [b]
RN3	152.66 ± 2.33 [a]	182.66 ± 29.49 [a]	81.00 ± 1.53	35.13 ± 8.12	10.20 ± 0.53 [b]
RH1	159.66 ± 12.66 [a]	168.66 ± 8.57 [a]	83.83 ± 2.49	48.76 ± 6.86	16.70 ± 1.22 [b]
RH2	154.00 ± 7.93 [a]	167.00 ± 10.81 [a]	78.56 ± 4.12	35.36 ± 2.35	21.70 ± 1.38 [Aa]
RH3	155.33 ± 10.17 [a]	188.00 ± 7.50 [a]	79.83 ± 7.88	42.36 ± 2.27	16.72 ± 2.01 [b]

The data are presented as Means ± SEM. Different lowercase letters represent the statistical differences (P<0.05) between different treatments within the same strain. While the uppercase ones for the statistical significance (P<0.05) between Ross and Cobb chicken. Absence of significant letters donates non-significant differences. [1] HDL: high density lipoprotein, [2] LDL: low density lipoprotein. This experiment included 3 replicates with 16 birds in each (n= 48 bird/group).

The Relative Immune Organs Weights

Generally, different forms of supplemented Cu and HS similarly altered the relative weights of the immune organs Ross and Cobb broilers (P<0.05 for treatments and P>0.05 for broiler strain) (Fig.2). For the bursa relative weights (Fig.2.A), in Ross strain, supplementing CuO_NP at 100% of Cu's needs (N2) significantly (P<0.05) increased the relative weight of bursa in normal housed chicken than those supplemented with CuO (N1). Another distinct increase was recorded for Ross chicken supplemented with its Cu need as 50% CuO_NPs and exposed to HS (H3) (P<0.05). For Cobb chicken, a non-significant variation of bursa relative weight was found among the different treated chicken.

Likewise, thymus relative weights (Fig.2B) were modified with different Cu treatments without strain differences (P<0.05). Hence, CuO_NPs supplementation at 100% of recommended Cu's level markedly increased thymus relative weight of normally housed Cobb chicken (N2) compared to those reared on CuO (N1) containing diet (P<0.05). In contrast, HS caused a slight decrease of thymus weight which was restored with CuO_NPs supplementation. Spleen relative weight was also, measured following CuO_NPs and HS treatments (Fig.2C). A significant change (P<0.05) in its weight was reported in the two broiler strains. Following HS, Cobb chicken received CuO_NPs supplementation (H2 and H3) caused marked reduction of spleen weight (P<0.05). On the other hand, this reduction in spleen weight reported in case of Cobb birds, was improved in case of Ross chicken received 50% of its Cu need as CuO_NPs (P<0.001).

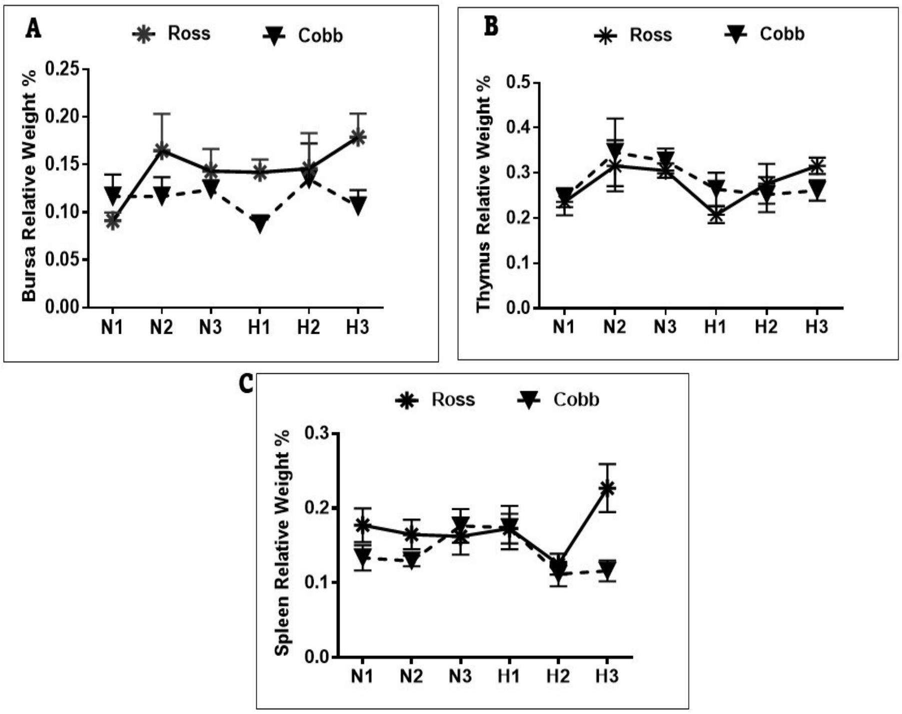

Fig. 2 . Effect of CuO-NPs supplementation on relative weight of immune organs normally and following HS in Ross and Cobb birds. N1, N2 and N3 represents relative weight of organ of birds kept at normal temperature and supplemented with their Cu requirement as 100 % of CuO, 100 % of CuO-NPs and 50 % of CuO-NPs respectively, while H1, H2 and H3 for those birds exposed to chronic HS and supplemented with Cu as 100 % of CuO, 100 % of CuO-NPs and 50 % of CuO-NPs, respectively. A represents bursa relative weight, B represents thymus relative weight and C represents spleen relative weight. The Results are expressed as mean ± SEM.

Discussion

Copper is an important trace element which efficiently maintains animal hemostasis including hematopoiesis, growth performance, and regular metabolic functions [29]. Cu is included in poultry diets as inorganic salts such as CuO [30]. These inorganic forms of Cu usually show poor bioavailability resulted from antagonism with other ingredients in diets affecting their absorption as well as increasing their excretion to the environment [31,32]. With the development of nanotechnology and emerging of nanomaterials with higher bioavailability and bioactive function, copper nanoparticles has gradually replaced other Cu sources and became more widely used in animal diets [2,24,33,34]. Heat stress (HS) is considered one of the dangerous and common stressors threatened poultry sector all over the world [35,36]. Cu has a several biological activities which has been documented in several studies [37,38], so investigating the bioactivity Cu nanoparticles is attracting and as a mean of relieving the deleterious consequences of HS.

In the present feeding trail, chronic HS for two weeks significantly decreased bird's body weight and gain in the two studied broiler strains under this experimental condition. This effect following HS exposure might be because of lower feed efficiency utilization and raised body temperature [39]. Additionally, HS has a destructive effects on the intestinal villi structure and intestinal enzyme activity which consequently interfere with the absorption and utilization of nutrients [40]. In the present trial, CuO_NPs supplementation was found to improve the growth parameters of birds especially under HS conditions. This finding agreed with [41] findings which reported that adding Cu at 50 % of its requirements as CuO_NPs improved the birds growth under heat stress condition. Moreover, [42] showed that copper nanoparticles dietary supplementation at 25% of the standard level of CuSO4 (7.5 mg/kg diet) could replace it and even enhance body weight gain of broilers, although the FCR was not significantly different among groups. While in contrast, [43] stated that Cu sources in diet (Cu Sul.

vs Cu-NP) did not alter turkey's growth performance.

Dietary copper shortage could induced several metabolic disturbance including hypercholesterolemia, elevated blood pressure, and glucose intolerance in ducks [44] and laying hens [45]. The 100 % CuO-NPs (CH2) under HS non-significantly lowered albumin content while the 50 % supplementation significantly increased the serum globulin concentration. Consistently with our results, [46] documented a non-significant decline in the albumin level in the Cu-NP treated birds as well as, Fausto*, et al.* [47] who found that Cu addition obviously increased the total protein and gamma globulin concentrations in lambs experimentally challenged with *Haemonchus contortus*. The present results also showed that, Cu-NPs supplementation to Cobb birds at 50 % under normal temperature or 100 % of requirements under HS conditions reduced the serum glucose level which could be due to increasing oxidation of glucose. However, it was increased in Ross birds. This differences in broiler's strains response might be due to the variable genetic structure of the birds. Similarly, [43] reported reduced glucose and albumin levels in plasma of turkey received Cu-NP compared with those supplemented with Cu sulphate. Additionally, [34] found that dietary supplementation with Cu-NPs caused a significant reduction in glucose level of Red sea bream more than in the CuSO4 supplemented group.

Serum SGPT, SGOT, and ALP are the most reliable indicators of hepatic damage as they increased in the circulation after their release from the damaged hepatic cells. [48]. Scott, Vadalasetty Krishna, Chwalibog and Sawosz [31] reported a reduced activity of these enzymes in the Cu-NPs supplemented birds in comparison with their reference group which received Cu sulphate. In our trial, adding CuO_NPs at 50 % of birds required needs did not modify their serum GOT and GPT enzyme activities. Moreover, El Basuini, El-Hais, Dawood, Abou-Zeid, EL-Damrawy, Khalafalla, Koshio, Ishikawa and Dossou [34] observed that dietary CU levels didn't change the metabolic activity of (GOT and GPT), thus could reflected the absence of Cu level toxicity in red sea bream. Supplementation 50 % CuO-NPs to Cobb birds (CH3) increased the serum ALP levels ($P < 0.05$) which is involved in maintaining homeostasis and energy generation in animal body, while reduced it in Ross birds. Our results noted that in Cobb birds under HS, the 100 % CuO-NPs treatment (CH2) significantly lowered the serum ALP levels. As, CuO-NPs supplementation alleviated the hepatic degenerative changes induced by HS compared to birds supplemented with CuO [41].

Several studies have been described the effect of Cu on lipid metabolism in poultry. Copper supplementation decreased the serum total cholesterol concentrations [43,46,49,50] which agreed with our results (the 50% of the needed Cu levels). By reducing the reduced form of glutathione (GSH) and raising the oxidized form, Cu indirectly controls the production of cholesterol [51]. GSH induced 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA) production, which aids in the organic synthesis of cholesterol. However, [52,53] found that plasma cholesterol of pigs and steers respectively were not influenced by supplemental Cu. Copper exposure increased lipogenesis and fatty acid uptake [54] as its effect on the hepatic lipid metabolism was dose and time dependent in vitro [55].

Cobb chicken exposed to HS +CuO-NPs supplementation by 100 and 50% (H2 and H3) caused marked reduction of the spleen weight ($P<0.05$). As, chronic HS lowered antibody production in broiler chicks due to activation of hypothalamic-pituitary-adrenal axis [56] and causes greater amounts of corticosterone to be released which induce reduction in the lymphoid organs' weights [57]. While a significant increase was recorded for Ross chicken supplemented with its 50% CuO-NPs and exposed to HS (H3) ($P<0.05$). This agreed with Ognik*, et al.* [58] findings which reported that an increase of level of Cu (just 7% over the recommended) in chickens feed was associated with negative results, including deterioration in RBCs parameters and stimulation of the immune system (an increase in IL-6, IgA, IgM and IgY). Despite of the foremost results obtained in this study, there were limitations such as lack of molecular approach, and examining the effect of dietary supplementation of CuO-NPs on egg production, and meat, and egg qualities. Therefore, these limitations are recommended for future investigations.

Conclusion

Dietary supplementation of CuO_NPs to Cobb and Ross birds raised under HS environment can ameliorate the negative effects of HS, improve growth performance, reduce serum cholesterol levels, and restore biochemical profile and the relative weights of bursa, thymus, and spleen. Further future studies on the effect of CuO_NPs dietary supplementation on histopathological parameters are recommended to evaluate the appropriate doses to avoid toxicity.

Authors' Contributions

Abeer A. K. Kirrella and Rasha A. Al-Wakeel collected data, performed analysis and helped in writing manuscript. Karima El-Naggar formulated diet and took part in data collection and manuscript writing. Radi A Mohamed and Safaa E. Abdo helped in the experiment, bird's management and writing manuscript. Ibrahim El-Mehaseeb prepared CuO-NPs. Seham El-Kassas designed the experiment, collected, and analyzed data, performed statistical analysis, and wrote the manuscript. Mohammed Abu El-Magd revised the manuscript. All authors approved the submitted final version of the manuscript.

Disclosure statement

The authors declare that there is no conflict of interests regarding the publication of this article.

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تحسين جزيئات النحاس النانوية أداء النمو وتعديل خصائص الكيمياء الحيوية والدهون في الدجاج التسمين تحت ظروف الإجهاد الحرارى المزمن

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تم تصميم تجربة التغذية هذه لتقييم تأثير مكمل جزيئات أكسيد النحاس النانوية (CuO_NPs) على النظام الغذائي على أداء النمو - والكيمياء الحيوية للسيرم والدهون في السيرم - وأوزان أعضاء المناعة النسبية لـ Ross 308 و Cobb 500 التي تتعرض للإجهاد الحراري المزمن (HS). تم تقسيم الكتاكيت إلى ثلاث معاملات بكل معاملة ثلاث مكررات. غذت الكتاكيت في المجموعة العلاجية الأولى ١٠٠٪ من احتياجاتها من النحاس الموصى بها في شكل CuO بينما تلقت المجموعتان الثانية والثالثة ١٠٠٪ و ٥٠٪ من احتياجاتها من النحاس على شكل CuO_NPs - على التوالي. في عمر ٢١ يومًا - تم تقسيم كل مجموعة إلى مجموعتين ؛ مجموعة تتعرض لدرجات الحرارة الطبيعية (٢٤ درجة مئوية) وأخرى تتعرض للإجهاد الحرارى (٣٣ ± ٢ درجة مئوية لمدة ٥ ساعات / يوم لمدة أسبوعين). أظهرت النتائج أن المكملات الغذائية لـ CuO_NPs إلى دجاج التسمين كوب وروس عدل بشكل كبير BW و WG و FCR. علاوة على ذلك - فقد غيرت من تركيز الجلوبيولين في الدم بينما تم تسجيل تحسن غير ملحوظ في مؤشرات وظائف الكبد. بالإضافة إلى ذلك - أدى التضمين الغذائي لـ CuO_NPs للسلالتين المدروستين إلى تعديل مستويات ALP في سيرم الدم بشكل كبير وتعديل محتوى الدهون في البطن ودهون سيرم الدم بشكل كبير (الكوليسترول - Triglycerides- HDL و LDL). في الختام - يمكن للمكملات الغذائية من CuO_NPs لطيور Cobb و Ross التي تمت تربيتها تحت ظروف الإجهاد الحرارى أن تحسن أداء النمو بشكل فعال - الكيميائي الحيوية للدم وتقليل محتوى الكوليسترول في الدم واستعادة الوزن النسبي للبرساوالغدة الثيموسية والطحال.

الكلمات الدالة: جزيئات أكسيد النحاس النانوية (CuO_NPs) ؛ أداء النمو؛ بيوكيمياء الدم ودجاج التسمين.