The impact of dietary supplementation with copper nanoparticle, nitrate, and copper sulphate on productive performance, hematological indicators and antioxidant reactivity in broiler chicks

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Abstract

The current study examined the effects of dietary copper (Cu) supplementation on productive performance, hematological indicators, immunoglobins, and antioxidant capabilities in broilers. A number of 420, one-day old, broiler chicks (Ross 308) were split up into four groups: the control group (n= 42 chicks) and three experimental groups (n= 126 chicks/ each), based on copper forms copper nitrate (Cu (NO3)2), copper nano-particles (Cu- NPs), and copper sulphate (CuSO4). Depending on copper levels (75, 100, and 125 mg/kg), each main group was split into three subgroups (n= 42 chicks per each level), chicks of each subgroup were divided into three replicates (n= 14 chicks/ each). At the age of 5 th weeks, the selected productive performance traits like body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and mortality rate (MR%) improved considerably ($P<0.05$) in chicks treated with Cu-NPs at a level of 125 mg/kg diet. also, the interaction among them detected the same trend. Plasma levels of total protein (T.P) and globulin (GL) were increased; plasma levels of total cholesterol (T.Ch), triglycerides (T.G), low density lipoproteins (LDL), aspartate amino transferase (AST), alanine amino transferase (ALT) activity, creatinine (Cr) and uric acid (U.A) decreased in Cu-NPs treatment, while high density lipoproteins (HDL) increased in Cu (NO3)2. Serum immunoglobulins (IgM and IgG) were increased by Cu-NPs, 125 mg/kg and the interaction between them. Feeding diet supplemented with Cu-

NPs at a level of 125 mg/kg significantly($P<0.05$) improved plasma total antioxidant (TAOC), glutathione peroxidase (GPx), superoxide dismutase (SOD) and decreased malondialdehyde (MDA) than control and other treatments. As the antibacterial properties results found that all Cu treatments with all additional dose's levels inhibited bacterial proliferation. It could be concluded supplemental Cu-NPS at 125 mg/kg improved broiler growth performance, feed utilization, plasma biochemical markers, immunoglobulins, antioxidant activity, and antibacterial properties.
Keywords: *Copper; Nano-Particles; Immunoglobulins; Antioxidants; Anti-Bacterial.*

INTRODUCTION

Nutrition in birds depends on the basic food elements (protein, carbohydrate and fats) and additives from both synthetic and natural origins, which are regularly added to diets to meet the dietary requirements of animals (Świątkiewicz et al., 2014). The metabolic activities of animals depend on the availability of micro metals (MM) in their diet (KO et al., 2010). Micro metals and /or trace minerals (TM) like (Zinc, Manganese, Copper, Iron, and Selenium) are crucial for maintaining the health and production of all livestock animals, including broiler chickens. In reality, they function as catalysts or components in a number of enzyme systems and are found in numerous proteins and organic compounds that are implicated in immune protection mechanisms, hormonal secretion routes, and intermediate metabolism (Dieck et al., 2003). Therefore, they affect the broiler bird's growth, skeletal development, rate of feathering, enzyme functions, and feed consumption rates (Nollet et al., 2007). To supply levels of minerals that either avoid clinical shortages, enable attainment of potential growth based on genetics, or both, typically the trace metals are supplied as dissolved salts such as carbonates, oxides, and sulfates. (Bao et al., 2007).

In the production of poultry, copper is frequently used to stimulate growth (Scott et al., 2016). A crucial trace element called copper (Cu) is needed for several biological activities, including the formation of hemoglobin, mitochondrial respiration, and enzyme active reactions (Kim et al., 2008). Additionally, it is employed to effectively boost the health and growth of chickens (Richards et al., 2010). There is a significant focus on administering copper to poultry as a substitute for antibiotics that can have comparable benefits on chicken productivity (Ibrahim et al., 2022). In reality, large amounts of copper are added to feed combinations to encourage development; nevertheless, increasing the amount of copper in an animal's diet might cause low digestion and absorption in chickens, which would increase the amount of copper discharged in faeces and cause environmental contamination (Karimi et al., 2011).

In the past decade, inorganic copper ($CuSO_4$) has been the most extensively employed supplement in animal diets. (Chowdhury et al., 2004). The primary source of copper in the diets of chickens and other animals is copper sulphate ($CuSO_4$); nevertheless, the artificial salt has low bioavailability as a result of components that might block absorption. Therefore, adding inorganic minerals to animal feed puts the ecosystem in danger since increased mineral outflow taints soil and water (Scott et al., 2018). Nanoscale minerals are now used commercially as a result of nanotechnology, which has also been used as a tool to study various animal sciences (Peters et al., 2016). According to several writers, Cu salts are less bioavailable than organic and nanosized Cu (Creech et al., 2004, Gonzales-Eguia et al., 2009, Scott et al., 2016).

Numerous studies have recently identified copper nanoparticles (Cu-NPs) as viable growth stimulants and substitutes for antibacterial compounds (Scott et al., 2018). Better immunological response, performance, feed efficiency, and a greater meat production in broiler chicks have been seen after in ovo feeding nano-Cu (Josuha et al., 2016). By adding nano-Cu to their diets, turkey chickens' thigh muscles' aminopeptidases were substantially more active (Jóźwik et al., 2018). Additionally, the concentration of serum Cu was linearly enhanced by adding nanosized-Cu to consumped water (Ognik et al., 2016). The femoral bone characteristics of chicken were boosted by nano-Cu, reducing the likelihood of fracture. Additionally, proliferating cell nuclear antigen was improved (Scott et al., 2017). Previously, referred mentioned that the chicken feed sector uses copper as a growth enhancer. Comparative research between Cu-NPs and CuSO4 on the performance of chickens' growth found that Cu-NPs may be more effective than CuSO4 at enhancing the birds' growth performance (Al-Bairuty et al., 2016, Mroczek-Sosnowska et al., 2015a). So, the main objectives of the study were to determine the impacts of different dietary forms of copper with varying levels on productive performance, hematological indicators, immunoglobulins and antioxidant reactivities in broilers.

Materials and methods

Location and Ethics Approval for the Experiment

The Poultry Research Farm, part of Benha University's Faculty of Agriculture in Egypt, served as the site of the study. The Institutional Animal Care and Use Committee (IACUC) at Benha University gave its approval to all study plans from May 21 through June 26, 2021.

Birds management and Experimental Design:

All birds were housed in experimental pens with regulated environmental and sanitary conditions in the same geographic region. All pens were in one location, where electric lighting was used continuously for the first five days, then during the next 30 days, lighting applied 23 hrs. and one hour of darkness were used. The air was heated to 33°C and had a relative humidity of 63% three days prior to the chicks being put in the pens. For the first week of the experiment, the temperature was set at 33°C, and then it dropped by 2-3°C each following week to a final degree of 20–22°C. The chicks were given ad libitum meals made specifically for each stage of their development, that is, starter (days 1–21) and grower (days 22–35), both with unrestricted water availability (Table,1). A diet of crumble was used for starting period while, pellets was used for growing period. There was a 35-day rearing period in total.

Table.1 The experimental basal meals' components and quantitative analyses.

Basal diet Components (%)		Starter	Gower
*per kilogram	Yellow Corn	58.00	63.00
of diet, provided that:	Soybean (44% CP)	28.20	24.90
Vitamin A: 13,000 IU;	Gluten meet (60% CP)	7.00	6.0
Vitamin D: 1,300 IU;	Dicalcium phosphate	1.80	1.20
Vitamin E: 65	Limestone	1.30	1.30
IU; Menadione:	Soybean oil	2.00	2.00
3.4 mg;	Na Cl	0.30	0.30
Pantothenic	L-Lysine	0.20	0.20
Acid: 37 mg; Vitamin B2:	DL-Methionine	0.20	0.10
6.6 mg; Folic Acid: 3.7 mg;	Vit. And premix *	1	1
Niacin: 39 mg;	Total	100	100
Thiamine: 1.0	Calculated composition		
mg; Vitamin B6: 4.3 mg;	ME (kcal/kg) **	3012.26	3068.97
Biotin: 0.23 mg; Vitamin	Crude protein (%)	21.99	20.28
B12: 0.075 mg;	Calcium (%)	0.98	0.84

Choline chloride dosage of 43 mg 170 mg zinc, 140 mg iron, 34 mg manganese, 0.29 mg iodine, and 0.29 mg selenium make up this dose. **energy that is metabolizable.	Avaible phosphor (%)	0.40	0.39
	Methionine (%)	0.59	0.47
	Methionine + cysteine (%)	0.95	0.80
	Lysine (%)	1.14	1.06

A number of 420 birds broiler chicks (Ross 308) were identified into four groups: the first group (42 chicks/ control) and three experimental groups of 126 chicks each, based on copper sources organic copper nitrate (Cu (NO3)2), copper nanoparticles (Cu– NPs) sigma-Alderic,99% purity, NPs<75 nm, and in organic copper sulphate (CuSO4). Each of the experimental groups were divided into three subgroups according to copper levels (75, 100, and 125 mg/kg for each copper form n= 42 / level), divided into three replicates (n= 14 chicks/replicate). The chicks in the control also got a mineral and vitamin premix without copper along with a balanced feed combination that met the nutritional needs of Ross 308 chickens.

Measurements, data, and laboratory testing

Growth performance and feed utilization:

Birds in each experimental group were individually weighed weekly for determine (BW) and (BWG), Feed intake (FI) was also recorded twice in the same week to determine ratio of feed conversion efficiency (FCR) (g feed: g gain). The rate of mortality was noted as it happed, to be computed after the experiment had ended as live birds were subtracted from the original birds.

Blood parameters:

After the experiment had ended (35 days later), blood samples (n = 3 per replica) were drawn from the wing vein of chicks and kept into hygienic, heparinized tubes and other tubes with a serum separator for analysis of serum immunoglobulins. Blood samples were centrifuged at 3500 rpm for 10 minutes at 4 °C. Plasma was stored in a deep freezer at around -20°C until it was time for chemical analysis. Plasma protein fractions (total protein (T.P.), albumin (AL), globulin (GL), and albumin/globulin ratio (A/G ratio)); total lipid profile (total cholesterol(T.Ch.), triglycerides(T.G.), low-density lipoproteins (LDL), and high-density lipoproteins (HDL)); liver enzymes (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) and kidney function tests (creatinine (Cr) and uric acid (U.A)) were measured using a colorimetric approach using commercial kits.

Immunological analysis

Serum immunoglobulins (IgG and IgM) were calculated using the analysis technique presented by (El Basuini et al., 2016) using (Alpha Diagnostic international) kits in an ELISA reader in line with the instructions given by the manufacturer.

Antioxidant capacities:

The total anti-oxidant (T-AOC) content of plasma was analyzed using a commercial kit (Randox, UK) based on technique used by Miller et al., (1993). Blood glutathione peroxidase activity (GPx, EC 1.11.1.9) was tested with readily accessible GPx kits (Randox, Crumlin, UK) following the kit's manufacturer's instructions in accordance with the methodology described by (Paglia and Valentine 1967) . According to Jo and Ahn (1998), the amount of malondialdehyde (MDA) in plasma was assessed using a modified fluorometric technique. Evaluated the superoxide dismutase (EC 1.1.5.1; SOD) activity in erythrocyte lysates using kits from

Randox Laboratories Ltd. (Crumlin, UK), following the instructions provided by (Woolliams et al., 1983).

Bacteriological analysis of meat:

Sample preparation in accordance with (Hunt et al., 2001), for evaluating aerobic bacteria by using conventional techniques (Brown and Smith 2014). Salmonella was found using the Xylose Lysine Deoxycholate Agar (XLD) medium, were red colonies with a black core were noted as positive for Salmonella. The number of Escherichia coli (E. coli) spices per game was detected using differential counts of green color (DOWNES and ITO 2001). (DOWNES and ITO 2001).

Statistical analysis

Data were examined using the General Linear Models (GLM) approach of two-way ANOVA (SAS 2004). The interaction between the components of the main impact was evaluated using a factorial design (3×3), (dietary copper source and its levels). When there were substantial changes between treatments, the Duncan's multiple range test was used to examine such differences (Duncan 1955). The following liner model states:

$X_{ijk}=\mu+\alpha_i+\beta_j+(\alpha\beta)_{ij}+e_{ijk}$

Whereas: Xijk is the observation of ijkth broiler chicks; μ= the overall mean; αi= the impact of ith Cu sources (Cu (NO3)2, (Cu - NPs), and (CuSO4); βj= the impact of jth varying levels of Cu (75, 100 and 125 mg/kg diet); (αβ)ij = permanent impact of the interaction between the Cu sources and their levels; eijk = the experimental error, accordingly mean = zero and variance =

Results:

Growth performance and feed utilization

Table.2 Growth performance, feed utilization, and mortality rate are expressed as least-square means and standard error (in relation to copper forms and levels

Sources	Levels (mg/kg diet)	BW (g) at 1 day	BW (g) at 35 day	BWG (g) during 1-35 day	FI during 1-35 day	FCR during 1-35 day	MR. % during 1 -35 day
Cu forms (F)	**Cu (NO3)2**	44.2±0.34	2042.7±19.8^{ab}	1998.5±19.9^{ab}	3184.9±11.1	1.59±0.02	2.20±0.44
	Cu – NPs	43.9±0.35	2082.5±20.2^{a}	2038.5±20.3^{a}	3177.7±11.1	1.56±0.02	2.20±0.44
	CuSO4	43.6±0.35	1999.8±20.2^{b}	1956.1±20.4^{b}	3189.8±11.1	1.62±0.02	3.30±0.44
Cu levels (L)	**0**	43.43±0.64	1915.5±35.4^{b}	1872.1±35.4^{b}	3375.1±11.9^{a}	1.85±0.01^{a}	6.60±0.53^{a}
	75	43.72±0.35	1956.6±19.4^{b}	1912.9±19.4^{b}	3218.0±8.4^{b}	1.67±0.01^{b}	3.30±0.30^{b}
	100	44.16±0.35	2062.6±19.4^{a}	2018.4±19.4^{a}	3181.3±8.4^{c}	1.57±0.01^{c}	3.30±0.30^{b}
	125	43.98±0.35	2104.3±19.4^{a}	2060.3±19.1^{a}	3153.1±8.4^{d}	1.53±0.01^{d}	1.10±0.30^{c}
Control	**0**	43.43±0.65	1915.5±34.7^{d}	1819.4±34.7^{d}	3375.1±11.3^{a}	1.85±0.006^{a}	6.60±0.05^{a}
Cu (NO3)2	**75**	44.11±0.60	2007.0±32.5^{bc}	1962.9±32.1^{bc}	3228.0±13.8^{b}	1.64±0.008^{c}	3.30±0.05^{b}
	100	44.03±0.64	2060.3±32.5^{b}	2016.3±32.6^{b}	3193.2±13.8^{bcde}	1.58±0.008^{d}	3.30±0.05^{b}
	125	44.47±0.60	2062.0±32.5^{b}	2017.5±32.6^{b}	3133.5±13.8^{f}	1.55±0.008^{e}	0.00±0.05^{c}
Cu – NPs	**75**	44.01±0.61	1987.5±32.5^{bc}	1943.5±32.6^{bc}	3211.2±13.8^{bc}	1.66±0.008^{c}	3.30±0.05^{b}
	100	44.0±0.61	2085.6±32.5^{ab}	2041.6±34.2^{ab}	3153.2±13.8^{ef}	1.54±0.008^{e}	3.30±0.05$_{b}$
	125	43.92±0.61	2174.5±32.5^{a}	2130.6±32.6^{a}	3168.7±13.8^{cdef}	1.49±0.008^{cf}	0.00±0.05^{c}
CuSO4	**75**	42.93±0.61	1915.5±32.5^{cd}	1872.1±34.7^{cd}	3214.7±13.8^{b}	1.72±0.008^{b}	3.30±0.05^{b}
	100	44.44±0.61	2044.0±32.5^{b}	1999.6±32.6^{b}	3197.5±13.8^{bcd}	1.59±0.008^{d}	3.30±0.05^{b}
	125	43.56±0.61	2076.4±32.5^{b}	2032.8±32.6^{b}	3157.2±13.8^{def}	1.56±0.008^{e}	3.30±0.05^{b}

Two-way ANOVA							
F	-	P=0.5704	P=0.0001	P=0.0001	P=0.7466	P=0.1618	P= 0.1581
L	-	P=0.7126	P=0.0001	P=0.0001	P=0.0001	P=0.0001	P=<.0001
F x L	-	p= 0.301	P=0.0002	P=0.0001	P=0.0001	P=0.0001	p= <.0001

A significant difference ($P<0.05$) is indicated by superscript letters in the columns.

Abbreviations: "BW (g) = body weight/ g; BWG (g)= body weight gain; FI= feed intake/bird/ day; FCR= feed conversion ratio g feed: g gain and MR. (%) = relative embryonic mortality".

Table. 2 shows the effects of dietary three forms of copper and their varied levels supplementation, either alone or in an interaction between them, on the starting BW, final BW, BWG, FI, FCR, and MR.% of treated broiler chicks. At the 5th week of the experiment, the BW and BWG were increased in the Cu-NPs groups in comparison to the Cu (NO3)2 and CuSO4 groups ($P < 0.0001$). The Cu-forms variant of the experiment had no discernible effects on FI, FCR, or MR%. According to varying levels of Cu supplementation, it was found that adding 125 mg Cu/kg diet increased BW (s) and BWG, decreased FI and MR.%, and gave the best FCR when compared to the control, 75, and 100 mg Cu/kg diet groups ($P < 0.001$). Additionally, the interaction between the Cu- NPs and the level of 125 mg/kg showed the same tendency as the experimental results.

Blood parameters

Table.3 displays the impacts of dietary supplementation with the three different copper forms at various levels on the plasma concentrations of total protein, albumin, globulin, A/G ratio, total cholesterol, triglycerides, LDL, HDL, AST, ALT, creatinine, and uric acid in broiler chicks. Cu-NPs supplementation had a substantial impact ($P<0.01$) on plasma levels of T.P., GL, T.Ch., TG, LDL, HDL, AST, ALT, Cr, and U.A. compared to other treated forms. Broiler chicks fed a diet with 125 mg/kg recorded significant ($P<0.01$) variations in the plasma T.P., AL, GL, T.Ch., TG, LDL, AST, ALT, Cr, and U.A.

Table.3 Plasm indicators are expressed as least- square means and standard error ($\bar{X} \pm S.E$) in relation to copper forms and levels

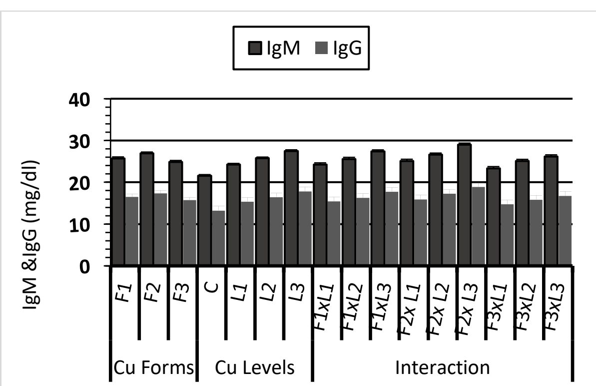
independent variables		Plasma protein fraction (g /dl)				Lipid profile				Liver function		Kidney function	
		T. P	AL	GL	A/G	T. Ch	T. G	LDL	HDL	AST	ALT	Cr	U. A
Cu forms (F)	Cu (NO3)$_2$	7.15^{b}	4.26	2.89^{ab}	1.51	146.8^{a}	101.7^{b}	44.0^{a}	82.4^{b}	26.40^{b}	16.40^{b}	0.79^{a}	18.0^{ab}
	Cu – NPs	7.44^{a}	4.33	3.11^{a}	1.40	136.5^{b}	91.50^{c}	36.3^{b}	81.9^{b}	23.82^{c}	13.82^{c}	0.69^{b}	17.12^{b}
	CuSO4	6.88^{c}	4.20	2.68^{b}	1.59	154.6^{a}	109.5^{a}	37.4^{ab}	95.3^{a}	27.41^{a}	17.41^{a}	0.84^{a}	18.61^{a}
MSE±	-	±0.09	±0.08	±0.08	±0.06	±3.31	±2.46	±1.3	±2.0	±0.34	±0.16	±0.02	±0.32
Cu levels (L)	0	6.12^{c}	3.84^{c}	2.28^{c}	1.72	182.3^{a}	122.6^{a}	42.0^{a}	115.8^{a}	32.20^{a}	19.88^{a}	1.01^{a}	20.14^{a}
	75	6.80^{b}	4.11^{ab}	2.70^{b}	1.57	156.6^{b}	108.0^{b}	52.6^{a}	82.4^{c}	27.05^{b}	17.49^{b}	0.85^{b}	18.56^{b}
	100	7.20^{a}	4.28^{a}	2.92^{ab}	1.48	144.3^{c}	100.5^{bc}	42.4^{b}	81.9^{c}	25.85^{bc}	16.48^{b}	0.77^{bc}	17.88^{bc}
	125	7.46^{a}	4.40^{a}	3.06^{a}	1.46	137.1^{c}	94.14^{c}	23.0^{ab}	95.3^{b}	24.74^{c}	15.30^{c}	0.69^{c}	17.28^{c}
MSE±	-	±0.08	±0.07	±0.09	± 0.07	±3.12	±2.53	±1.2	±2.0	±0.33	±0.02	±0.02	±0.33
Control	0	6.12^{g}	3.84^{c}	2.28^{d}	1.72	182.3^{a}	122.6^{a}	42.0^{a}	115.8^{a}	32.20^{a}	19.88^{a}	1.01^{a}	20.14^{a}
Cu (NO3)$_2$	75	6.76^{ef}	4.22^{abc}	2.54^{cd}	1.74	156.0^{bc}	108.2^{bc}	42.6^{ab}	91.8^{b}	27.50^{b}	17.86^{c}	0.88^{abc}	18.58^{abc}
	100	7.24^{bc}	4.22^{abc}	3.02^{ab}	1.40	145.1^{cde}	101.1^{cd}	47.7^{a}	77.2^{c}	26.52^{cd}	16.92^{de}	0.78^{cd}	18.00^{bcd}
	125	7.46^{ab}	4.34^{ab}	3.12^{a}	1.75	139.3^{de}	95.7^{de}	41.8^{ab}	78.4^{c}	25.20^{de}	15.38^{gh}	0.71^{de}	17.42^{bcd}
	75	7.12^{cd}	4.10^{abc}	3.02^{ab}	1.36	151.0^{bcd}	101.8^{cd}	41.4^{ab}	89.2^{b}	24.70^{fg}	15.84^{gf}	0.77^{cd}	17.94^{bcd}

Cu – NPs	**100**	7.50^{a}	4.40^{ab}	3.10^{ab}	1.44	134.0^{ef}	90.14^{ef}	36.4^{bc}	79.6^{c}	23.88^{gh}	15.0^{h}	0.70^{de}	17.06^{cd}
	125	7.72	4.50^{a}	3.22^{a}	1.42	124.7^{f}	82.5^{f}	31.2^{c}	77.0^{c}	22.90^{h}	14.06^{i}	0.61^{e}	16.36^{d}
CuSO4	**75**	6.54^{f}	4.02^{bc}	2.54^{cd}	1.62	162.9^{b}	114.1^{ab}	57.5^{ab}	97.6^{b}	28.96^{b}	18.78^{b}	0.92^{ab}	19.18^{ab}
	100	6.88^{de}	4.22^{abc}	2.66^{bcd}	1.60	153.7^{bc}	110.2^{bc}	58.7^{abc}	95.2^{b}	27.16^{cd}	17.54^{cd}	0.83^{bcd}	18.58^{abc}
	125	7.22^{bc}	4.36^{ab}	2.86^{abc}	1.56	147.3^{cde}	104.2^{cd}	33.3^{bc}	93.2^{b}	26.12^{de}	16.48^{df}	0.77^{cd}	18.08^{bcd}
MSE±	**-**	±0.10	±0.14	±0.03	±0.12	±0.14	±0.10	±2.6	±2.8	±0.42	±0.23	±0.04	±0.55
Two-way ANOVA													
F	**-**	0.005	0.527	0.006	0.178	0.001	0.001	0.018	0.001	0.001	0.001	0.004	0.008
L	**-**	0.001	0.003	0.004	0.283	0.001	0.001	0.004	0.283	0.007	0.001	0.001	0.007
F x L	**-**	0.01	0.076	0.002	0.299	0.001	0.001	0.06	0.001	0.001	0.001	0.001	0.001

A significant difference (P<0.05) is indicated by superscript letters in the columns.

Whereas, increased plasma levels of T.P., AL, and GL and decreased plasma T.Ch., TG, LDL, AST, ALT, Cr, and U.A. concentrations were observed compared with the zero and other levels of Cu applied. Statistically (P <0.001), significant variations of blood biochemical markers occurred in all the interactions applied in comparison with the control. The increased plasma T.P. and GL and the decreased concentrations of plasma T.Ch., TG, LDL, AST, ALT, Cr, and U.A. were observed in the interaction between Cu-NPs and the level of 125 mg/kg in comparison with the control and different interactions applied. While the higher LDL value was detected in the control group.

Figure 1: Serum immunoglobulins (IgM and IgG) are expressed as least-square means and standard error (X̅±S.E) in relation to copper forms and levels.



Immunological analysis

Figure.1 displays impacts different forms of Cu supplementation affect serum immunoglobulins. The effects of Cu (NO3)2, Cu-NPs, and CuSO4 on serum immunoglobulins did not result in any significant changes (P < 0.05). Additionally, the results showed that broiler chicks fed a diet enriched with Cu-NPs had higher serum levels of IgM (26.90 mg/dl) and IgG (17.35 mg/dl). At the end of the experimental period, the group that received Cu at the level of 125 mg/kg diet had a significantly higher (P < 0.001) levels of IgM (27.50 mg/dl) and IgG (17.79 mg/dl) in the chicken serum as compared to the control group and other applied levels of Cu. The concentration of serum IgM and IgG were statistically greater (P < 0.001) in all experimental groups than in the group that was fed basal diet at the time of estimation. In comparison to the other interactions, the serum IgM (28.98 mg/dl) and IgG (18.90 mg/dl) concentrations were higher in the broilers of the Cu-NPs with a level of 125 mg/kg.

Antioxidant capacities

The Cu- NPs treatment increased plasma TAOC, GPX and SOD activity and decreased plasma MAD as compared to Cu (NO3)2 and CuSO4 (Table 4). As compared to the control chicks and other Cu addition levels, birds in the 125 mg Cu/kg diet had a statistically significant increase (P < 0.001) in plasma TAOC, GPX, and SOD activity and a decrease in MAD. A statistical interaction between Cu forms and their varying application levels revealed a linear increase in antioxidant status. Results obtained showed that the interaction between Cu-NPs and its level of 125 mg/kg recorded significantly (P < 0.001) higher plasma TAOC, GPX, and SOD activities and a lower MDA value compared with other interactions detected in the study. Results obtained showed that the interaction between Cu-NPs and its level of 125 mg/kg recorded significantly (P < 0.001) higher plasma TAOC, GPX, and SOD activities and a lower value of MDA compared with other interactions detected in the study.

Table.4 Plasma Total antioxidants, glutathione peroxidase, super oxidases dismutase and malondialdehyde are expressed as least- square means and standard error ($\bar{X} \pm S.E$) .

Items	Levels (mg/kg diet)	TAOC (Mm/ L)	GPX (U/ ml)	SOD (U/ ml)	MDA (nmol/ mL)
	Cu (NO3)$_2$	0.83±0.03$_{b}$	289.9±5.2$_{ab}$	4.80±0.17	3.78±0.15$_{ab}$
Cu forms (F)	**Cu –NPs**	0.93±0.03^{a}	301.2±5.2^{a}	5.08±0.17	3.44±0.15^{b}
	CuSO4	0.78±0.03^{b}	281.3±5.2^{b}	4.60±0.17	4.02±0.15^{a}
	0	0.63±0.04^{d}	251.6±8.0^{c}	3.84±0.29^{c}	4.86±0.25^{a}
Cu levels (L)	**75**	0.74±0.02^{c}	276.1±4.6^{b}	4.52±0.16^{b}	4.16±0.14^{b}
	100	0.83±0.02^{b}	290.0±4.6^{b}	4.80±0.16^{ab}	3.77±0.14^{bc}
	125	0.97±0.02^{a}	306.3±4.6^{a}	5.16±0.16^{a}	3.32±0.14^{c}
Control	**0**	0.63±0.03^{f}	251.6±7.6^{e}	3.84±0.29^{c}	4.86±0.29^{a}
	75	0.73±0.03^{de}	276.0±7.6^{cd}	4.56±0.29^{bc}	4.18±0.29^{abc}
	100	0.82±0.03^{cd}	288.4±7.6^{bcd}	4.76±0.29^{abc}	3.82±0.29^{bcd}
Cu (NO3)$_2$	**125**	0.95±0.03^{b}	305.4±7.6^{ab}	5.10±0.29^{ab}	3.36±0.29^{de}
	75	0.79±0.03^{cd}	284.0±7.6^{bcd}	4.68±0.29^{abc}	3.92±0.29^{bcd}
	100	0.92±0.03^{b}	299.0±7.6^{abc}	5.02±0.29^{ab}	3.52±0.29^{cde}
Cu –NPs	**125**	1.07±0.03^{a}	320.6±7.6^{a}	5.54±0.29^{a}	2.90±0.29^{e}
	75	0.69±0.03^{ef}	268.4±7.6^{de}	28.96±0.29^{b}	4.40±0.29^{ab}
	100	0.76±0.03^{de}	282.6±7.6^{bcd}	4.32±0.29^{bc}	3.98±0.29^{bcd}
CuSO4	**125**	0.88±0.03^{bc}	293.0±7.6^{bcd}	4.86±0.29^{ab}	3.70±0.29^{bcd}
Two-way ANOVA					
F	-	P=0.0037	P=0.036	P=0.172	P=0.042
L	-	P=0.0001	P=0.0001	P=0.0016	P=0.0001
F x L	-	p= 0.0001	P=0.0001	P=0.027	P=0.0001

Superscript letters in columns refer to a significant difference (P<0 .05).

Abbreviations: TAOC = Total antioxidants; GPX = glutathione peroxidase; SOD = super oxidases dismutase and MAD = malondialdehyde

Bacteriological analysis of meat:

Bacteriological examination (APC, coliform count bacteria, E. coli and salmonella) in fresh meat of broiler was presented in (Table,5). In general, dietary supplementation with copper in the broiler diet reduces the coliform count of bacteria and inhibits E. coli and salmonella proliferation in meat. The greatest number of ABC and the lowest value of coliform bacteria were recorded in the chicks fed a basic diet, which including 100 and 75 mg Cu/kg, respectively. Cu-NPs interacted with a level of 75 mg/kg in a significantly (P 0.001) increased ABC bacterial number and decreased coliform count bacteria when compared to other interactions. All experimental treatments of Cu applied in this study recorded negative counts of E. coli and Salmonella proliferation as compared with the Cu (NO3)2 group.

Table.5 The influence of different forms and varying levels of Cu on meat bacterial counts in broiler chickens are expressed as least- square means and standard error ($\bar{X} \pm S.E$) .

Items	Levels (mg/kg diet)	ABC (cfu/g)	Coliform C. (cfu/g)	E. coli	Salmonellae
Cu forms (F)	**Cu (NO3)2**	$4.96\times10^4\pm0.51$	$3.33\times10^2\pm0.40$	+ve	-ve
	Cu –NPs	$6.18\times10^4\pm0.51$	$3.10\times10^2\pm0.40$	-ve	-ve
	CuSO4	$5.25\times10^4\pm0.51$	$3.33\times10^2\pm0.40$	-ve	-ve
Cu levels (L)	**0**	$2.20\times10^4\pm0.91^{b}$	$6.33\times10^2\pm0.60^{a}$	-ve	-ve
	75	$5.26\times10^4\pm0.53^{a}$	$2.55\times10^2\pm0.34^{c}$	-ve	-ve
	100	$5.57\times10^4\pm0.53^{a}$	$3.22\times10^2\pm0.34^{bc}$	-ve	-ve
	125	$5.56\times10^4\pm0.53^{a}$	$3.99\times10^2\pm0.34^{b}$	-ve	-ve
Control	**0**	$2.20\times10^4\pm0.57^{e}$	$6.33\times10^2\pm0.54^{a}$	-ve	-ve
Cu (NO3)2	**75**	$3.53\times10^4\pm0.57^{de}$	$2.66\times10^2\pm0.54^{bcd}$	-ve	-ve
	100	$4.80\times10^4\pm0.57^{bcd}$	$4.0\times10^2\pm0.54^{bc}$	-ve	-ve
	125	$6.56\times10^4\pm0.57^{ab}$	$3.33\times10^2\pm0.54^{bcd}$	-ve	-ve
Cu –NPs	**75**	$8.13\times10^4\pm0.57^{a}$	$1.66\times10^2\pm0.54^{d}$	-ve	-ve
	100	$5.63\times10^4\pm0.57^{bc}$	$3.33\times10^2\pm0.54^{bcd}$	-ve	-ve
	125	$4.80\times10^4\pm0.57^{bcd}$	$4.33\times10^2\pm0.54^{b}$	-ve	-ve
CuSO4	**75**	$4.13\times10^4\pm0.57^{cd}$	$3.33\times10^2\pm0.54^{bcd}$	-ve	-ve
	100	$6.30\times10^4\pm0.57^{b}$	$2.33\times10^2\pm0.54^{cd}$	-ve	-ve
	125	$5.33\times10^4\pm0.57^{bcd}$	$4.33\times10^2\pm0.54^{b}$	-ve	-ve
Two-way ANOVA					
F	**-**	P=0.0331	P=0.9045	P=0.172	P=0.042
L	**-**	P=0.0205	P=0.0001	P=0.0060	P=0.0003
F x L	**-**	p= 0.0001	P=0.0007	P=0.0613	P=0.0086

Superscript letters in columns refer to a significant difference (P<0 .05).

Discussion

The current study investigated how broilers' performance responded to various Cu forms and varying levels of dietary supplements. The findings suggested that adding Cu-NPs to the diet might enhance growth performance. The BW(s), BWG, FI, FCR, and MR.% in chicks receiving basal diets plus Cu-NPs improved, especially with enhancement with 125 mg/kg of Cu- nanoparticles (Cu- NPs). These outcomes were consistent with how copper affected broiler chicks reported by (Wang et al., 2011), however, the appropriate level differed.. Various ideal amount of Cu might be caused by the variations in experimental animals. Due to their distinct properties, Cu-NPs may boost the activity of particular growth factors more effectively than other Cu forms. This might contribute to enhancing growth performance. Kim et al., (2011) demonstrated that both Cu- NPs enhanced broiler growth performance. Due to Cu-NPs can be more beneficial than bulk Cu at lower dosages due to its compact size, which can improve GIT absorption (Civardi et al., 2015). Cu-NPS combines with both organic and inorganic substances in the animal body more efficiently because of its larger surface area (Zaboli et al., 2013). Cu-NPs have the benefit of preventing mineral dissociation from other nutrients, which reduces conflict (Scott et al., 2018). Additionally,(Mroczek-Sosnowska et al., 2015a, Al-Bairuty et al., 2016) indicating that Cu-NP is more effective than CuSO4 at promoting animal growth and performance. Animal growth, development, and health may be impacted if Cu-NP absorption in the GIT is increased. Cu-NPs, used as a feed enhancer for birds and fish, have been shown to enhance feed utilization and growth compared with CuSO4 (Mroczek - Sosnowska et al., 2015b, El Basuini et al., 2016). The enhancement was credited to Cu-NP's bioavailability when compared to CuSO4 salts. According to the study of Arias and Koutsos (2006) indicated that Cu NP's impact can be attributed to its antibacterial qualities and/or the enhanced fat and energy digestion (Gonzales-Eguia et al., 2009, Scott et al., 2016). According to studies done on several animal species, immunological dysfunction caused by copper deficiency manifests itself as a greater incidence of illnesses and a higher death rate(Jarosz et al., 2018, Jarosz et al., 2021, Skřivan et al., 2002). Jarosz et al., (2018) and Stabel et al., (1993) recognized that maintaining homeostasis, even when exposed to internal and external stimuli, is necessary for the organism to operate well. This involves interactions between a large number of immune-competent cells, or the effective body's immune activity. This is why enriching feeds with compounds derived from copper is crucial in intensive poultry production since modern methods of rearing these animals seek to produce the maximum potential slaughter bodyweight in a short amount of time. The present research attempted to determine the influence of Cu-forms and their varying levels on selected parameters of growth performance, feed utilization, and mortality rate in broilers. The feed additive Cu-NPs at 125 mg/kg produced the most benefits, which most obviously improved BW(s), BWG, FI, FCR, and MR% in the treated birds.

Blood parameters

Blood biochemical markers have an importance role in predicting the overall health, metabolic performance and physiological response to stress in broiler chickens. The blood tests in the present study are regarded as being within the usual range of a healthy birds. Plasma levels T. P .and GL were higher while, T.Ch., TG, LDL, HDL, AST, ALT, Cr, and U.A declined in Cu- NPs treatment. And numerical improvement was recorded on plasma constituents in birds fed with Cu (NO3)2 like HDL. Important indicators of protein synthesis include serum TP, AL concentrations, and the amount of urea nitrogen (U.N) in the serum (Wang et al., 2011). The low concentration of U.N in the serum may indicate that protein synthesis has increased (Fukawa et al., 1982). In the current investigation, it was discovered that CU-NPs, particularly when combined with a 125 mg/kg diet, significantly ($P < 0.01$) raised plasma levels of T.P., AL, and kidney function by secreting lowering concentrations of Cr and U.A in blood plasma. The results may be attributed to Cu-NPs may be a factor in improving plasma protein synthesis, which would improve growth performance, in the line with reported by (Wang et al., 2011). The reduction in total cholesterol, triglyceride, and LDL were observed as results of supplementation with Cu-NPs, showing that the nanoparticles of Cu are more effective in reducing T.Ch., TG and LDL than CuSO4 and Cu (NO3)2 when fed to broiler chicks. The results obtained in the line with reported by (Jegede et al., 2011, Jegede et al., 2012) revealed that when given to broilers, the proteinate form of copper reduced cholesterol levels more than the sulphate form. This discovery may be the cause due to Cu influences cholesterol production by lowering hepatic glutathione levels and altering the ratio of oxidised glutathione, which reduces the activity of 3-hydroxyl-3-methylglutaryl CoA (HMG-CoA) reductase (Jegede et al., 2011, Mondal et al., 2007, Jegede et al., 2012, Kim et al., 1992). CANLI and Canli (2017) showed that oral treatment of 5 mg/kg of oxide Cu-NPs dramatically reduced their blood sugar, total cholesterol, and triglyceride levels compared to the control. This may be linked to hyperglycemia brought on by gluconeogenesis mediated by glucocorticoid. In contrast Gonzales-Eguia et al., (2009) discovered that Cu-NPs supplementation had no effect on the blood concentrations of cholesterol. may be to blame for these gains the greater absorption and antibacterial activity of Cu-NPs than CuSO4. The research revealed that taking supplements with Cu-NPs and Cu (NO3)2 decreased LDL and increased HDL as compared to CuSO4. There are two regulators—LDL cholesterol and a non-sterol product made from mevalonate—that are necessary for the total regulation of cholesterol production since they both affect HMG-CoA reductase activity (Goldstein and Brown 1990, Jegede et al., 2012). It may be concluded that the non-sterol product was likely controlled by Cu-NPs supplementation to lower plasma cholesterol levels. The addition of copper nanoparticles, it supposedly has a higher bioavailability than CuSO4 (Scott et al., 2018, Scott et al., 2017) may have improved the number of hepatic binding sites, which helped the liver use cholesterol more effectively (Jegede et al., 2011).

The metabolic vector, which displays the concentrations of total protein, AST activity, ALT activity, GGT activity, and urea level, indicates the direction of the organism's biochemical activities (Sizova et al., 2020). Considering that AST is a sign of mitochondrial function in the organism's cells (Sookoian and Pirola 2015) we can presume that nanoform accelerates the Krebs cycle's use of unbound amino acids to produce energy. The results obtained agree with those reported by (Attia et al., 2012) who reported that dietary supplementation Cu at 150 mg/kg diet increasing activity of liver enzymes AST and ALT. Makarski and Zadura (2006) revealed that copper supplementation increased ALT and AST activities in male turkeys. It seems to be working on the combining of the key amino acids required for protein synthesis (El-Hady and Mohamed 2019).

Immunological analysis

In order to explore the effects of various Cu forms at different levels on the immune system, the quantities of immunoglobulins in serum were assessed in present study. It was discovered that birds treated with Cu-NPs at a dosage of 125 mg/kg produced greater immunoglobulin levels (IgM and IgG). Results in the line of (Wang et al., 2011) who demonstrated that supplemented proteinate Cu to broiler diet significantly increase serum immunoglobulins (IgA, IgG and IgM). Future uses of Cu-NP in chicken feed and treatment are encouraged by their ability to modulate the immune system of poultry (Sharif et al., 2021). Feeding birds dietary consists of copper sources has an impact on lymphatic organs and enhancing their development and growth like bursa of Fabricius gland (Yang et al., 2009). This is supported by (Arshami et al., 2010) in research on broilers, showed that copper increased the amount of immunoglobulins in the blood. Birds' particular immune systems are strengthened by an increase in immunoglobulin content, stimulating complement components, and defence against infections (Jarosz et al., 2018). However, research has also shown that the reason Cu-NP did not activate the immune system was because antigen-presenting cells did not detect it (Scott et al., 2016). As ACPs' absorption of nanoparticles influences immune responses (Bao and Choct 2009). Additionally, Cu-NP may be poisonous and have negative effects on the immune system ((Bao and Choct 2009, Scott et al., 2016). According to reports as well, the levels of IgM, IgA, and nuclear factor-kappa B were not affected by Cu-NP(Pineda et al., 2013).

Plasma antioxidant capacities:

The results showed that antioxidant status (TAOC, GPX, SOD, and MAD) was better in Cu-NPs chickens compared to other forms in the study. That is true for some antioxidants that have copper as an essential component, such as superoxide dismutase (Sharif et al., 2021). Copper has biological effects because of its activity in the active site of metalloenzymes (Scott et al., 2018). Numerous metalloenzymes, including cytochrome oxidase, superoxide dismutase (SOD), lysyl oxidase, dopamine hydroxylase, and tyrosinase, include copper Makarski and Zadura (2006). Wu et al., (2015) shown how Cu influences the control of SOD activity. The SOD enzyme facilitates the disproportionation of two superoxide radicals into hydrogen peroxide and oxygen, hence assisting in the removal of ROS-induced damage (Lin et al., 2008). Nano-Cu is one of several elements that perform a defensive function by preventing oxidative damage (Sizova et al., 2020). The addition of copper in nano form can improve poultry's antioxidant status (Sharif et al., 2021). According to the dose given, broiler chickens have varying antioxidant statuses. For instance, adding 12 mg of nano-Cu per chicken over a six-week period to their food may enhance the antioxidant and immunological defenses of chickens (Zhao et al., 2014). Ognik et al., (2018) suggested that SOD activity, in addition to MAD activity, was found to increase as the level of Cu-NPs in the broiler diet increased from 0.5 to 1.5 mg/kg BW/d. The three distinct concentrations of Cu oxide nanoparticles (30, 60, and 90 mg/kg feed) in the broiler were examined in the research of (Nassiri and Ahmadi 2015) it showed that birds given nanoparticles at 60 and 90 mg/kg feed had significantly higher levels of SOD and glutathione peroxidase. It could be hypotheses that Cu-NPs has the capacity to improve the antioxidant enzymes in birds and so improving their antioxidant state in the present study in agreement with the detection by (Sharif et al., 2021) .

Bacteriological analysis of meat:

As the antibacterial properties were assessed, it was found that all Cu treatments with all additional dose's levels decreased and/or inhibited bacterial proliferation. These may be due to the Cu antibacterial properties (Scott et al., 2018). Cu-antibacterial nano particles efficacy is dependent on the nanoparticle's physical properties (Rakhmetova et al., 2010). In addition to Cu-NPs has antibacterial qualities, it has been employed as a sanitizer for a long time (Sánchez-Sanhueza et al., 2016). The results obtained in the same trend with Duffy et al., (2018) reported that in chicken, Cu-NPs has demonstrated effective antibacterial action against Salmonella and Campylobacter additionally, against Escherichia coli (DeAlba-Montero et al., 2017). It is significant to mention that Cu-physical NP's properties are what give them their antibacterial properties (Rakhmetova et al., 2010). After administering Cu-NPs, an increase in reactive oxygen species (ROS) strengthens the bactericidal activity of nanoparticles Chang et al., (2012), based on the temperature, pH, and nanostructure concentration (Pramanik et al., 2017). Cu-NPs damaged the cell walls of the microorganisms, halted DNA polymerase, and prevented protein synthesis, all of which prevented the germs from growing (Huh and Kwon 2011). Cu-NPs has the wildly effective bactericidal effects due to its larger surface area compared to other sources of Cu (Sharif et al., 2021).

Conclusion

Broiler performance, feed utilization, plasma biochemical indicators, immunoglobulins, antioxidant activity, and antibacterial capabilities all increased with the addition of copper nanoparticles. Furthermore, supplemented of 125 mg/kg to broiler diets diet enhanced studded traits than different level applied. These findings imply that dietary inclusion of Cu-NPs at a rate of 125 mg/kg might substitute for the supplementation of Cu $(NO_3)_2$ and $CuSO_4$ in broiler diets.

Data availability statement

The data sets are available upon reasonable request.

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Author contributions

Mahmoud. El-Attrouny, Ibrahim Abdul El-Kareem and Hamada Okasha prepared the original text, participated in the experimental design, conducted out the experimental, and performed statistics. Gafaar. El-Gendi, Mahmoud. El-Attrouny and Hamada Okasha helped with both the statistics and the experimental deigning. Gafaar. El-Gendi and Nihad Ali interpret and discuss the results of the findings, and write a final essay.

Declaration of interest

The authors attest that they don't have any conflicts of priorities.

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