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PRODUCTIVE AND IMMUNOLOGICAL RESPONSES OF BROILER CHICKS TO SUPPLEMENTATION OF DIFFERENT COPPER FORMS

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ABSTRACT

Two hundred and ten unsexed broiler chicks (Arbor Acres) at 7 day of age were used to study the effect of dietary supplementation of different Copper (Cu) forms *i.e*. inorganic (copper sulfate, $CuSO_4$), organic (copper methionine Cu_2-Met) or nano inorganic copper particles (Cu-NP) on the productive performance, hematological and biochemical constituents of blood and immune response of broiler chicks. They were randomly divided into seven dietary treatments with five replicate cages per treatment, six chicks for each. The first group was fed the basal diet without any supplementation (control); while the 2nd and 3rd groups were fed the same diet supplemented with 50 and 100 ppm of inorganic Cu (copper sulfate, $CuSO_4$), respectively. The 4th and 5th groups were fed the basal diet supplemented with 50 and 100 ppm of organic Cu (copper methionine Cu_2-Met), respectively. The 6th and 7th groups were fed the basal diet supplemented with 50 and 100 ppb of nano inorganic copper (Cu-NP), respectively. Results showed that chicks fed the basal diet supplemented with different forms of Cu had significantly better LBW, BWG, FCR, economic efficiency and production index. Both organic and nano Cu-fed groups showed significantly better productive performance traits compared with the inorganic Cu – fed groups. Supplementation of different Cu forms decreased serum levels of total lipids, triglycerides, cholesterol, low density lipoprotein (LDL), whereas RBCs count, hemoglobin, PCV, glucose, globulin, thyroid hormones (T3-T4), immunoglobulin (IgM, IgG), and antioxidant enzymes activity (TAC–GPX- GSH-SOD**)** were significantly increased compared with the control treatment. Additionally, Cu supplementation increased lactobacillus sp. while decreased total bacterial count (*Salmonella*, *E. coli* and *Proteus)* compared with the control group. In conclusion, Cu supplementation improved the growth performance, immune response and physiological status of broiler chickens. Broilers fed organic and nano Cu-supplemented diets had better growth performance and immune response than those fed inorganic copper.

Keywords: Copper, Broilers, Performance, Blood parameters, Immune response.

INTRODUCTION

The beneficial effect of copper (Cu) on the development and functioning aspects of birds is very well documented in the literature **(Collins et al 2010; Hatoriand Lutsenko, 2016; Ognik et al 2016 and Jarosz et al 2018)**.This element has an effective impact in conditioning and modulating many important metabolic processes in the living body *i.e*. it participates in binding of iron to haem in haemoglobin, synthesis of red blood cells, responsible for the structure of connective tissue and ensuring proper collagen cross-linking **(Collins et al 2010)**. In addition, as a co-factor of many enzymes, Cu determines the correct course of certain metabolic pathways via enhancing energy metabolism in the respiratory- mitochondrial chains, synthesis and degradation of neurotransmitters, or redox signaling in angiogenesis **(Hatori and Lutsenko, 2016)**.

Davis and Mertz (1987) studied the essentiality and bioavailability of different Cu sources along with its safe levels for use by the commercial feed industry in poultry and livestock diets. Since, Cu from various sources has often been included to diets at different levels as an antimicrobial agent. Copper is a component of various antioxidant enzymes (*i.e*. cytochrome oxidase, superoxide dismutase), metal enzymes involved in energy and ferric metabolism (Klasing, 1998). Since, it is an important trace element for many physiological and biochemical processes. In poultry, copper deficiency results in growth retardation, abnormal bone formation and remodeling, hypochromic anemia and therefore it is considered as an essential element that has been used worldwide as a growth promoter in poultry and pigs. Indeed, several reports **(Aboul-Ela et al 2000; Skrivanova et al 2001**) indicated that Cu supplementation to poultry diets could improve growth performance and immunity. Recently, the tremendous nanotechnology development and its applications are ever more varied and specific, with a high potential for improving poultry and animal production**.** In this concern, **Mroczek-Sosnowska et al (2015)** declared that either Nano Cu or copper sulfate (CuSO4) are effective for stimulating the development of blood vessels and enhancing muscle growth during late stage of embryogenesis by increasing myofibrils size (hypertrophy) over their number (hyperplasia). This effect was confirmed by **Velleman (2007)** who found that the number of muscle fibers is established mainly in the prenatal period. (**Febré, et al 2016**) have reported that different Cu forms could be used as promising alternatives to prevent harmful bacteria and to promote growth performance. They added that both $CuSO_4$ and Cu nanoparticles (Cu-NP) were effective as antibacterial and/ or growth promoters. Moreover, Cu-NP was found to exhibit a variety of effects on animal performance depending on the source, particle size, dose growth period and animal species.

Little information about the immune-physiological effects of different Cu forms, especially nanoparticles, in birds is available in the literature. Therefore, the main objective of this investigation was to evaluate the influence of different forms of cupper i.e., inorganic (copper sulfate, $CuSO_4$), organic (copper methionine Cu_2-Met) and nano copper on the productive performance, blood profile and immunity of broilers.

MATERIALS AND METHODS

The present experiment was carried out at the Animal and Poultry Research Centre (El-Bostan Farm) belonging to Faculty of Agriculture, Damanhour University, during the period from April to June 2019. The study aimed at elucidating the beneficial effect(s) of supplementing broiler chicks diet with copper different levels and forms *i.e*. inorganic (copper sulfate, $CuSO_4$), organic (copper-methionine) or nano inorganic copper (Cu-NP) on productive performance, blood plasma constituents, complete blood cell count (CBC) and immune – related parameters and responses.

Birds and experimental design

Total of 210 one day old broiler chicks (Arbor Acres) of both sexes, purchased from a local hatchery were used in this study. Upon arrival all chicks were brooded at 33^{o}C for the first seven days of age. From the second week of age, they were randomly distributed into seven treatment groups of 30 chicks, each of five replicates, six birds per replicate. The first group was fed the basal diet without any supplementation (control), while the 2nd and 3rd groups were fed the same diet supplemented with 50 and 100 ppm inorganic Cu (copper sulfate, $CuSO_4$), while diets of the 4th and 5th groups were supplied with 50 and 100 ppm organic Cu (copper methionine Cu-Met), and then the 6th and 7th groups have been fed diets with 50 and 100 ppb nano inorganic copper (Cu-NP), respectively. Birds of all treatments were reared in similar hygienic and managerial conditions. Feed and fresh water were offered for *ad libitum*. The basal diet was formulated according to the strain management guide as listed in **Table 1.**

Housing and management

Chicks were housed in breeding pens in semi-closed system house. They exposed to a standard light program that recommended for broiler chicks production under commercial conditions, where a 23 h light was supplied during the first week of age followed by 20 h light from the second week until the end of the fattening period. The brooding temperature (indoor) was 33C^{o} in the first week, then declined gradually to reach 30-27 and 24-21 C^{o} during the second week and from 3 to 6 week of age, respectively.

Table 1. Ingredients and calculated analysis of the basal 'diets

(%)	Starter phase (8- 21 days)	Growing (22 to 35 days)
Yellow Corn	57.60	61.00
Soybean Meal (48%)	29.50	26.00
Corn gluten meal (60%)	5.20	6.00
Soy oil	1.10	2.70
Full fat soya bean	2.00	0.00
Mono calcium Phosphate	1.50	1.65
Lime stone	1.90	1.50
Choline Chloride	0.10	0.10
Sodium Bicarbonate	0.20	0.20
Salt (NaCl)	0.20	0.20
DL –methionine	0.10	0.10
L-lysine	0.30	0.25
Broiler Premix *	0.30	0.30
Total %	**100**	**100**
Calculated analysis (DM basis)		
CP %	22.9	21.4
ME (kcal/kg) **	3042	3147
Ether extract	4.10	4.40
Calcium	1.05	0.90
Av. Phosphorus	0.51	0.43
Methionine	0.50	0.46
Lysine	1.40	1.23
Methionine + cysteine	0.98	0.89

* Each kg premix contains: vit. A (12 I.U.), vit. D3 (5 I.U.), vit. E (75 I.U.), vit. K menadione (2 mg), vit. B1 (2 mg), vit. B2 (6 mg), vit. B6 (4 mg), vit. B12 (0.016 mg), Pantathenic acid (13 mg), Nicotinic acid (55 mg), Folic acid (2 mg), Biotin (0.2 mg), Copper (16 mg), Iodine (1.25 mg), Iron (40 mg), Manganese (120 mg), Zinc (100 mg), Selenium (0.3 mg). ** ME=Metabolizable Energy

Data collection

Performance parameters including individual live body weight (LBW, g), gain of weight (BWG, g), and feed consumption (FI, g) were recorded weekly throughout the trial period (7-35 d of age). For each replicate within treatment groups, feed / gain ratio (FCR) was calculated according to the formula:

FCR = FI (g) / BWG (g).

Economic efficiency of experimental diets was estimated **(Zeweil, 1996)** as the ratio between income and total feed cost during the experimental growth period. The price of the fed diets and Cu supplements was calculated according to the local market price at the same time of the experiment in 2019 by the Egyptian pound (L.E.). Economic efficiency (%) = (Net revenue/Total feed cost)*100.

Net revenue = Total revenue - Total feed cost. European production efficiency index (EPEI, production index) was calculated to Hubbard broiler management manual as follows.

$$\mathbf{EPEI} = \frac{\mathbf{BW\ (kg)}\,x\,\mathbf{SR}}{\mathbf{PP}\,x\,\mathbf{FCR}}\,x\,\mathbf{100}$$

Where:

BW = Body weight (kg), SR = Survival rate (100% - mortality), PP = Production period (days), FCR = Feed conversion ratio (kg feed / kg gain)

Apparent digestibility of dry matter, crude protein, fiber, ash and ether extract, were determined by using five birds per treatment that were housed individually in metabolic cages using the procedures reported by **Abou-Raya and Galal (1971)** with aid of total collection method. Nitrogen, ether extract, crude fiber and ash content of the excreta as well as those of feed were determined according to **AOAC (2004)**.

At slaughtering time, blood samples were withdrawn from six (randomly chosen) chicks / treatment group in two sterilized test tubes. The first one was heparinized tubes for the determination of the hematological parameters. The second non-heparinized tubes were allowed to coagulate at room temperature for 30 min and then centrifuged at 4000 rpm/min for 10 min then sera were decanted and stored at – 20 C^{o} until the biochemical analyses were done by using available commercial kits. Blood glucose concentration (mg/dl) was determined according to **Trinder (1969)**, total protein **(Henry et al 1974)**, albumin **(Doumas, 1971)**, globulin **(Coles, 1974)** and its fractions (α ; β and γ-globulins) as described by **Bossuyt et al (2003)**. In addition, further assays was done in terms of serum creatinine and uric acid **(Bartles et al 1972)**, total cholesterol **(Stein, 1986)**, triglycerides **(Fossati and Prencipe, 1982)**, HDL **(Lopez-Virella, 1977)**, while LDL was calculated by the formula of **Friedewald et al (1972)**. Transaminases (ALT and AST) and alkaline phosphatase (ALP) activities were also measured according to **Reitman and Frankle (1957) and Bauer (1982)**, respectively.

The heparinized blood samples were used to determine number of red blood cells, total white blood cells count and differential types. Besides, packed cell volume (%), Hemoglobin concentration and CBC indices (MCH and MCHC) were determined with the following equations:

Mean Corpuscular Hemoglobin (MCH, Pg) = Hb x 10/ Red blood cell

Mean Corpuscular Hemoglobin Concentration (MCHC) (g/dl) = Hb x100/ Packed cell volume

Total antioxidant capacity was determined according to **Koracevic et al (2001)**, Superoxide dismutase activity **(Misra and Fridovich, 1972)**, Glutathione peroxidase activity **(Paglia and Valentine, 1967)** and Glutathione activity **(Ellman, 1959)**. Phagocytic activity and index was determined according to **Kawahara et al (1991)**. Phagocytic activity (PA) = Percentage of phagocytic cells containing yeast cells.

Phagocytic index (PI) = Number of yeast cell phagocytized/ Number of phagocytic cells.

Serum immunoglobulins (IgY, IgM and IgA) were measured by using commercial ELISA kits (Kamiya Biomed. Comp., USA) as reported by **Bianchi et al (1995)**. All measurements were conducted according to the manufacturer's instructions. Lymphocyte transformation test was determined following the method described by **Balhaa et al (1985)**. Serum bactericidal activity against *Aeromonas hydrophila* strain was determined according to **Rainger and Rowley (1993)**. Serum lysozyme activity was measured with the turbidimetric method described by **Engstad et al (1992)** and the results are expressed as one unit of lysozyme activity that defined as a reduction in absorbance at 0.001/min. Lysozyme activity = (A0 - A) / A.

Bacterial count

The microbial load of the digestive system was evaluated through measuring total bacterial count and also counting some pathogenic bacteria harboring the intestine such as salmonella, E.coli and proteus spp. according to methods described by **ICMSF (1980)**.

Statistical analysis

Data were subjected to the one way ANOVA procedure using Statistical Analysis System (SAS), 2002, with the following model:

$$Yij = \mu + Ti + eij$$

Where Y is the dependent variable; μ the general mean; T the fixed effect of i^{th} treatment and e the random error.

The difference among means was determined using Duncan's new multiple range est (Duncan, 1955) at $P < 0.05$.

RESULTS AND DISCUSSION

Productive performance traits

Data of growth performance, economical efficiency and production index are shown in **Table (2).** Results revealed that supplementation of different forms and levels of Cu has resulted in a significant ($p \leq 0.05$) increase in body weight (BW) at 35 d of age and body weight gain (BWG) from 7-35 d, when compared with the control group. However, the best results were achieved by Cu-Meth and Cu-NP addition than the other inorganic or non –supplemented control group. Moreover, feed conversion ratio (FCR) of broiler chicks that fed the experimental diets supplied with different forms and levels of Cu displayed better values when comparison with the control group, with significant differences being obtained for broilers fed Cu-Meth and Cu-Np – supplemented diets. They had significantly ($p \leq 0.05$) better economic efficiency and production index compared with the control group, but the best values were recorded for groups that fed on organic and nano Cu. These observations are in the line with those of **Luo et al (2005), Paik, (2001), Surai (2016), El-kazaz and Hafez (2020) and El-Ghalid et al (2019)** who found that all sources and levels of Cu supplementation created significant increases in body weight at 35 d of age when compared with the non- supplemented group, with significant differences shown by those fed organic copper.

The significant increases in LBW and BWG that observed in the present study could be attributed to the role of copper in skeletal muscles building via modulating synthesis and release of some anabolic hormones, enhancing nitrogen retention and / or muscle fibers hypertrophy. This holds true as **Arias and Koutsos (2006)** revealed that Cu works to increase muscle mass in the body through clear mechanisms as it regulates the balance of nitrogen in the body and increase the availability of amino acids for absorption in the intestines (**Scott et al 2018)** which is essential in the process of building protein inside the cells and the result of increasing the amount of muscle in the body will positively reflected on body weight as it has an important role in the oxidation of lysine. These findings confirm and support our results. Moreover, **Zhou et al (1994)** cited the mechanism(s) by which copper can exert its effect on growth. Such mechanisms are: 1) its antibacterial effect which undoubtedly change the microflora populations in the digestive tract; 2) induce mitogenesis activity; 3) stimulate growth hormone synthesis release from adenohypophysis

Table 2. Effect of different copper forms on productive performance of broiler chicks

Parameter		LBW 7 d (g)	LBW 35 d (g)	BWG 7-35d (g)	FI 7-35d (g)	FCR 7-35d (g)	Economic efficiency	Production index
Control	**0 ppm**	**201**	**1780^{c}**	**1580^{c}**	**3132**	**1.98^{a}**	**0.880^{c}**	**140^{c}**
CuSO4	50 ppm	202	1890^{b}	1690^{b}	3141	1.85^{b}	1.24^{b}	155^{b}
	100 ppm	200	1900^{b}	1700^{b}	3009	1.8^{b}	1.31^{b}	159^{b}
Cu-Met	50 ppm	201	2200^{a}	1980^{a}	3198	1.70^{c}	1.73^{a}	180^{a}
	100 ppm	202	2290^{a}	2020^{a}	3190	1.60^{c}	1.80^{a}	180^{a}
Nano-Cu	50 ppb	203	2400^{a}	1990^{a}	3181	1.60^{c}	1.96^{a}	170^{a}
	100 ppb	200	2410^{a}	2300^{a}	3200	1.60^{c}	1.90^{a}	169^{a}
SEM		1.447	4.975	45.82	39.11	0.064	9.98	11.9
P-Value		0.987	0.004	0.007	0.0801	0.0001	0.001	0.002

^{a,b,c} different letters in the same column are significantly different at $p \leq 0.05$.SEM=Standard error of means.

and increasing GH-gene expression (**LaBella et al 1973**); 4) increased neuropeptide Y formation and release (**Tsou et al 1977**); 5) modulation of regulatory peptides action (**Eipper and Mains 1988**) and 6) as an element in the human growth factor (lamin) specific for wound healing **(Parkart, 1987**).

Apparent digestibility of nutrients (ADN)

Table 3 demonstrates the influence of different forms of copper on the AND of chicks. It is clear that different forms of Cu had significantly affected the coefficient of digestibility of crude protein, ether extract and dry matter. Thus, the basal diet supplemented with each of Cu-organic, Cu-inorganic and Cu nano forms significantly increased their digestibility compared to the control. However, there were no significant effects of different Cu sources on crude fiber and Apparent Ash retention digestibility. Our findings are in accordance with those reported by **Wu et al (2014 and 2018).**

The blood hematological criteria

The blood hematological criteria of 35 d old chicks that fed the basal diet supplied with different forms and levels of Cu are shown in **Table 4.**

Different forms of Cu supplementation improved RBC's, Hb, PCV, MCV and MCH nut not MCHC concentration of chicks compared with the control group. Data concerning the effects of Cu sources on the total WBCs and its differential counts of chicks at 35 day of age are shown in **Table 5**. The different sources of Cu had improved the WBC's and Lymphocytes (L, %) only. However, the differences between treatments in the percentages of Monocytes, Basophils, Eosinophils, Heterophils (H) and H/L ratio were not significant.

These findings are in agreement with those of **Makaraski and Zdura (2006)** found that the higher number of RBCs recorded with dietary Cu-supplemented may be due to Cu involvement in hemoglobin synthesis. They concluded that supplementing turkey's diet with Cu lysine chelate has a significant effect on the level of hematological indices. Moreover, addition of both antibiotics and Cu products resulted in reduced levels of RBC and HCT paralleled to RBC and HCT in the control group. The MCV value was decrease in the Cu-supplemented groups paralleled with that in control group **(Kim et al 2011).**

The observed changes in blood hematology in response to Cu-supplementation could be explained by the relationship between copper and iron metabolism. It is well known that Cu is not actually a constituent of hemoglobin, but it is a main element in certain specific blood proteins, mainly ceruloplasmin, which is responsible with the release of iron from the cells into the plasma. In this concern, **Fox (2003)** reported that copper deficiency retarded the ability of animals to absorb iron, mobilize it from the tissues to be utilized in hemoglobin formation. Moreover, Cu has a significant and direct impact in the process of RBCs formation, as it interferes in the rate of and amount of iron absorbed into the body and its incorporation to hemoglobin. Also, Cu is an important element of other blood proteins, *i.e.* erythrocuprein which present in the erythrocytes, where

Table 3. Effect of different cupper forms on the apparent digestibility of the nutrients of broiler chicks

Treatment	Control	CuSO4		Cu-Meth		Nano – Cu		SEM	P-Value
Parameter	**0**	**50**	**100**	**50**	**100**	**50**	**100**		
Crude protein	69.11^{c}	73.3^{b}	75.9^{b}	77.0^{a}	80.1^{a}	81.9^{a}	82.9^{a}	1.99	0.001
Ether extract	70.1^{b}	66.9^{b}	82.3^{a}	85.6^{a}	83.3^{a}	83.3^{a}	82.6^{a}	11.9	0.0090
Crude fiber	17.1	19.6	18.63	19.5	20.8	19.62	19.5	1.19	0.081
Apparent Ash retention,%	33.2	39.7	39.0	38.8	37.8	37. 9	37.8	8.34	0.156
Dry matter	64.9^{c}	70.1^{b}	71.9^{b}	75.5^{a}	74.9^{a}	77.9^{a}	77.5^{a}	10.72	0.001

^{a,b,c} different letters in the same row are significantly different at $p \leq 0.05$.SEM=Standard error of means

Table 4. Effect of different cupper forms on hematological parameters of broiler chicks

Treatment	Control	CuSO4		Cu-Met		Nano – Cu		SEM	P-Value
Parameter	**0**	**50**	**100**	**50**	**100**	**50**	**100**		
RBC's (10^6/mm^3)	1.56^{b}	1.76^{ab}	1.90^{a}	1.86^{a}	1.96^{a}	1.86^{a}	1.86^{a}	0.0534	0.0006
Hb (g/100ml)	9.66^{c}	11.9^{b}	10.3^{b}	15.9^{a}	14.66^{a}	14.6^{a}	16.9^{a}	0.7968	0.0115
PCV %	33.6^{b}	37.3^{b}	39.9^{ab}	42.6^{a}	41.6^{a}	41.6^{a}	43.9^{a}	0.9920	0.0013
MCVum^3	215^{b}	212^{b}	210^{b}	229^{a}	212^{b}	223^{a}	236^{a}	11.9	0.001
MCH (pg)	61.9^{b}	67.6^{b}	54.2^{b}	85.5^{a}	74.8^{a}	80.1^{a}	90.9^{a}	13.9	0.006
MCHC (g/dl)	28.75	31.9	25.8	37.3	35.2	35.0	38.5	9.99	0.093

^{a,b,c} different letters in the same row are significantly different at $p \leq 0.05$.SEM=Standard error of means

Table 5. Effect of different cupper forms on the differential count of leukocytes

Treatment	Control	CuSO4		Cu-Met		Nano – Cu		SEM	P-Value
Parameter	**0**	**50**	**100**	**50**	**100**	**50**	**100**		
WBC's (10^3/cmm^3)	20.66^{c}	24.33^{b}	25.33^{b}	26.66^{ab}	29.9^{a}	29.6^{a}	30.9^{a}	0.7126	0.004
Lymphocytes (%)	35.3^{c}	41.3^{b}	42.6^{b}	41.1^{b}	41.6^{b}	45.4^{a}	41.9^{b}	0.6666	0.001
Monocytes (%)	11.9	10.4	10.6	16.3	17.3	13.3	10.6	0.3563	0.441
Eosinophils, (%)	12.8	14.2	13.3	11.4	13.6	10.1	13.3	0.6546	0.540
Heterophils, (%)	39.6	33.9	32.8	30.8	26.9	30.9	33.9	0.765	0.334
Basophils, (%)	0.33	0.33	0.70	0.33	0.61	0.30	0.33	0.6546	0.138
H/L ratio	1.12	0.82	0.76	0.75	0.70	0.75	0.80	0.987	0.099

^{a,b,c} Means in the same row with different letters differ significantly ($p \leq 0.05$) SEM=Standard error of the mean.

it plays a role in oxygen metabolism. This assumption confirm the findings of **Winnica (2008)** who reported that the higher number of RBCs recorded with Cu-supplements to diet is due to the participation of Cu in the process of hemoglobin synthesis. Also, studies by **Makaraski and Zdura (2006)** declared that enriching turkey's diet with Copper- lysine chelate has significant effects on the level of hematological measurements of blood which support our results.

Biochemical constituents of blood

Protein profile

Total serum protein of chicks fed Cu – supplemented diets is illustrated in **Table 6**. Different forms of Cu supplementation significantly increased total protein, total globulin and γ –Globulin concentrations than the control group. Organic and nano Cu resulted in the highest total protein globulin concentrations and γ –Globulin of chicks. However the effect of different sources of Cu supplementation on serum albumin, α –globulin and β– globulin of chicks was not significant. These findings are in agreement with those of **El-Ghalid et al (2019), Chowdhury et al (2004), Kim et al (2011) and Reham, (2018)** through clear mechanisms as the copper regulates the balance of nitrogen in the body and increase the availability of amino acids for absorption in the intestines **(Pastorelli et al 2010)** which is essential in the process of building protein inside the cells and the result of increasing the amount of muscle in the body will positively reflected on body weight as it has an important role in the oxidation of lysine. This result was consistent with what was mentioned by a number of researchers (**Scott et al 2018).**

Blood glucose and thyroid hormones

The blood glucose and thyroid hormones of chicks fed the basal diet supplemented with different sources of Cu are shown in **Table 7**. Different sources of Cu supplementation significantly increased serum level of thyroxin (T_4), triiodothyronine (T_3) and glucose than the control group. Organic and nano Cu supplementation resulted in a significant increase in blood glucose, T3 and T4 than the control group and Cu inorganic. These findings are in agreement with those of **El-Ghalid et al (2019), Chowdhury et al (2004), Kim et al (2011) and Reham, (2018).**

Lipid profile

Data concerning the effects of Cu forms on the lipid profile of chicks are presented in **Table 8**. It appears that serum concentrations of total lipids, cholesterol, triglycerides and low-density lipoprotein (LDL) were significantly reduced by copper inclusion in the diets. Furthermore, addition of organic and nano organic sources of Cu to the feed had a significantly lower total lipids and LDL in blood plasma compared with the Cu inorganic and control group. The hypolipedimic effect of CuNP was more obvious than the other forms in this respect, especially when serum cholesterol is taken as the criteria of response. However, high density lipoprotein level (HDL) was not affected in all treatments. Our findings are nearly similar to those observed by **Ognik et al (2018), El-Ghalid et al (2019), Chowdhury et al (2004), Kim et al (2011) and Reham (2018)**. In this respect, **Sevcikova et al (2003)** observed that cholesterol concentration was declined by 24.9% in Cu- glycine chelate fed group compared to the control one. Also, **Aksu et al (2010)** observed a drop in cholesterol and LDL fraction levels with a raise in HDL level in the plasma of chickens consumed rations containing different organic forms of Zinc, Copper and Manganese. **Mondal et al (2007)** fed broiler chickens on diets contained 200 or 400 mg /kg from organic and inorganic copper salts, they declared that the first level (200mg / Kg) has a lowering effect on serum cholesterol, while the high inclusion level (400 mg/kg) resulted in higher level of HDL. This was not observed by **Konjifca et al (1997)** that a supplementation of Cu in an organic form leads to a higher content of cholesterol and HDL fraction. The hypo-cholesterolemic - mechanism of copper sulfate was firstly established in rats. Since**, Kim et al (1992)** showed that liver copper results in reducing hepatic reduced glutathione concentration which inhibits the enzymatic activity of HMG-COA-reductase, the rate-limiting step in the synthesis of mevalonate, and finally reduced cholesterol biosynthesis. **Bakalli et al (1995)** verified that such mechanism is also operative in the chicken where they observed that a high dietary copper level (250 mg/kg) fed to broiler chicks decreased blood reduced glutathione concentration and subsequently reduced plasma total cholesterol. Similarly, the observed increase in HDL level with levels of 50 and 100mg/kg diet is in accordance with the findings by **Pearce et al (1983)** and **Lien et al (2004)** who found dietary supplemental copper to reduce serum VLDL-cholesterol and increase HDL-cholesterol of laying hens. Similar results were also reported by **Bakalli et al (1995)** and **Hamdi et al (2018)** in broiler chickens**,** and in Japanese quail **(Reham, 2018)** The observed increase in serum HDL-cholesterol might be attributed to the high dissociation rate of cholesterol to be esterified by transformation of the long chain fatty acid pattern. It is also suggested that the triglycerides-lowering effect of Cu supplementation be mediated via its lowering impact on fatty acid synthetase (FAS) enzyme activity which in turn resulted in a reduction in the synthesis of fatty acid from Acetyl COA and finally decreasing triglycerides biosynthesis. This mechanism of Cu to reduce plasma triglycerides level was evidenced by the results of **Qureshi et al (1983) and Konjufca et al (1997)** who illustrate that FAS activity was significantly suppressed by copper feeding.

Table 6. Effect of different cupper forms on blood protein fraction of broiler chicks

Treatment	Control	CuSO4		Cu-Met		Nano – Cu		SEM	P-Value
Parameter	0	50	100	50	100	50	100		
Total protein (g/dl)	4.96^{c}	5.43^{b}	5.83^{b}	6.42^{a}	6.11^{a}	6.34^{a}	6.64^{a}	0.218	0.0026
Albumin (g/dl)	2.73	2.33	2.61	1.96	2.00	2.01	2.23	0.130	0.1091
Globulin (g/dl)	2.23^{c}	3.20^{b}	3.23^{b}	4.46^{a}	4.11^{a}	4.33^{a}	4.41^{a}	0.271	0.0025
α –globulin(mg/dl)	0.531	0.730	0.800	0.632	0.501	0.800	0.900	0.081	0.13
β– globulin (mg/dl)	0.742	0.461	0.660	0.700	0.611	0.716	0.613	0.051	0.492
γ –Globulin (mg/dl)	0.961^{c}	1.99^{b}	1.76^{b}	2.93^{a}	2.99^{a}	2.89^{a}	2.33^{a}	0.302	0.0056

^{a,b,c} Means in the same row with different letters differ significantly (p≤ 0.05) SEM=Standard error of the mean

Table 7. Effect of different cupper forms on blood glucose and thyroid hormones concentrations of broiler chicks

Treatment	Control	CuSO4 mg/kg		Cu-Met mg/kg		Nano – cu		SEM	P-Value
Parameter	**0**	**50**	**100**	**50**	**100**	**50**	**100**		
Glucose (mg/dl)	166^{c}	179^{b}	177^{b}	191^{a}	189^{a}	181^{a}	187^{a}	1.70	0.004
T3 (ng / ml)	2.05^{c}	2.13^{b}	2.22^{b}	2.41^{a}	2.33^{a}	2.49^{a}	2.35^{a}	0.021	0.005
T4 (ng / ml)	11.1^{b}	12.2^{b}	13.2^{b}	16.1^{a}	17.2^{a}	15.4^{a}	17.4^{a}	0.029	0.008

^{a,b,c} Means in the same row with different letters differ significantly (p≤ 0.05) SEM=Standard error of the mean

Table 8. Effect of different cupper forms supplementation on lipids profile of broiler chicks

Treatment	Control	CuSO4		Cu-Met		Nano – cu		SEM	P-Value
Parameter	**0**	**50**	**100**	**50**	**100**	**50**	**100**		
Total Lipids (mg/dl)	730^{a}	620^{b}	600^{b}	520^{c}	520^{c}	540^{c}	510^{c}	1.99	0.001
Cholesterol (mg/dl)	216^{a}	202^{b}	207^{b}	212^{b}	207^{b}	188^{c}	192^{c}	2.63	0.039
Triglycerides(mg/dl)	188^{a}	171^{b}	175^{b}	175^{b}	174.3^{b}	175^{b}	174^{b}	1.16	0.001
HDL (mg/dl)	48.1	48.7	50.6	49.3	53.3	54.7	52.6	1.36	0.113
LDL (mg/dl)	130.0^{a}	119.0^{b}	121.0^{b}	127.0^{b}	119.0^{b}	98.3^{c}	104^{c}	2.76	0.002

^{a,b,c} Means in the same row with different letters differ significantly (p≤ 0.05) SEM=Standard error of the mean

Antioxidative defense indicators

The role of copper supplements in boosting antioxidants in blood serum of treated broiler chicks is shown in **Table 9**.

Chicks fed basal diet with different forms of Cu showed significant increases in the total antioxidants capacity (TAC), GSH activity, GSH and SOD in comparison with those fed the control diet. In addition, dietary organic and Cu-NP inclusion significantly increased TAC and SOD activity than the control and inorganic Cu- fed groups, respectively. These findings are in agreement with those of **Ferrari and Cagliero (1993)**; **Sevcikova et al (2003)**; **Chowdhury et al (2004)**, **Mondal et al (2007)**, **Reham (2018)** and **El-Ghalid et al (2019)**. Also, **Bakalli et al (1995) and Kaneko et al (1997)** sustained the second probability where they stated that copper is well known as a strong oxidizer, being readily associated with free SH-groups, may affect the bioavailability of glutathione because the Cu-SH bond cannot easily dissociate. On the other hand, copper in the form of methionine or glycine decreased the level of MDA, which is considered as a reliable indicator for oxidative stress, because of its involvement in the antioxidant defense system leading to potential damage of living cells. **(Kim et al 2011).** Moreover, **Jarosz et al (2018)** indicated that SOD activity increased with feeding broiler chickens on diets rich in copper sulfate. It is well-known that copper has the ability to regulate SOD activity in the tissues of growing animals **(Surai 2016).** Meanwhile, the excess of dietary copper might be the main reason of high SOD activity in quails fed Cu-supplemented diets **(Dameron and Harris 1987; Oztürk and Tarhan, 2001).** As a component of the antioxidant enzyme superoxide dismutase, copper has an active role in the body's antioxidant defence against the effects of free radicals, thereby limiting lipid oxidation reactions **(Ognik et al 2018).**

Immune response indices

The immunity indicators of chicks from different Cu treatments are listed in **Table 10**. Results revealed that Cu supplemented groups had a significant effect on **LA**, **BA**, **LTT**, **PI**, **PA**, **IgA**, **IgM** and **IgG** compared with the control group. Furthermore, additions of organic and nano organic sources of Cu to the feed increased significantly the levels of **PI**, **PA**, **IgA** and **IgG** than that from Cu inorganic and control group. On the other hand, there was insignificant influence of the different sources of Cu on **LA**, **BA**, **LTT** and **IgG**. This might support the results of **Ferrari and Cagliero (1993)**; **Kim et al (2011**; **Reham (2018) and El-Ghalid et al (2019),** they found a positive impact of amino acid chelates on immune system functions, improving the health state and vitality of chickens.

Bacterial count

The bacterial count of chicks fed diet supplemented with different forms of Cu is shown in **Table 11**. Chicken fed basal diet supplemented with different sources of Cu had significantly increased lactobacillus sp. while different sources of Cu had significantly lower total bacterial count, Salmonella, E. coli and Proteus chicks. Our results showed significant reduction in the number of pathogenic bacteria, indicating the role of Cu as a good element to relieve bacterial disorders in the digestive tract of broiler chickens. It is well known that the beneficial bacterial community in the digestive tract could enhance and maintain the structural and functional integrity of the epithelial lining mucosa, and affecting the immune system, as well as preventing the development of intestinal disease in chickens (**Chambers and Gang, 2011).** In addition**, El-Ghalid et al (2019 and Chowdhury et al (2004)** showed that copper has a significant impact on the regulation of growth rate in broiler chickens, pathogenic bacteria in the jejunum small intestine were prevented by incremented with Cu supported by our results.

Table 9. Effect of different cupper forms on antioxidants enzymes of broiler chicks

Treatment	Control	CuSO4		Cu-Met		Nano – cu		SEM	P-Value
Parameter	0	50	100	50	100	50	100		
TAC (Mmol/dl)	411^{c}	425.3^{b}	417^{b}	443^{a}	433a	444^{a}	438a	1.8257	0.0013
GPX (U/dl)	0.213^{b}	0.452^{a}	0.432^{a}	0.441^{a}	0.343^{a}	0.413^{a}	0.451^{a}	0.0152	0.0001
GSH (U/dl)	955^{b}	991^{a}	972^{a}	970^{a}	994^{a}	968^{a}	981^{a}	5.9441	0.0239
SOD (U/dl)	222^{c}	241^{b}	243^{b}	256^{a}	258^{a}	255^{a}	258^{a}	2.4168	0.0001

^{a,b,c} Means within the same row with different letters are significantly different (p≤ 0.05).SEM=Standard error of the mean.

Table 10. Effect of supplemental different cupper forms on blood plasma immune indices of broiler chicks

Treatment	Control	CuSO4 mg/kg		Cu-Met mg/kg		Nano – cu		SEM	P-Value
Parameter	0	50	100	50	100	50	100		
LA (IU %)	0.111^{b}	0.176^{a}	0.181^{a}	0.183^{a}	0.171^{a}	0.171^{a}	0.173^{a}	0.010	0.009
BA (%)	34.1^{b}	39.6^{a}	38.3^{a}	39.6^{a}	36.6^{a}	38.6^{a}	37.3^{a}	0.967	0.005
LTT(%)	20.6^{b}	27.6^{a}	25.3^{a}	27.6^{a}	27.7^{a}	26.9^{a}	27.6^{a}	0.854	0.007
PI (%)	1.23^{c}	1.45^{b}	1.58^{b}	1.98^{a}	2.09^{a}	1.99^{a}	1.96^{a}	0.103	0.008
PA (%)	16.9^{c}	17.9^{b}	19.3^{ab}	21.3a	22.6a	21.3a	22.9^{a}	0.666	0.002
IgA(mg/dl)	61.2^{c}	70.3^{b}	71.6^{b}	80.9^{a}	79.6^{a}	79.6^{a}	79.1^{a}	1.4853	0.0172
IgM(mg/dl)	226^{b}	225^{b}	300^{a}	325^{a}	299^{a}	294^{b}	290^{a}	13.8	0.002
IgG(mg/dl)	917.6^{c}	946^{b}	949^{b}	983^{a}	996^{a}	988^{a}	998^{a}	6.97	0.001

^{a,b,c} Means within the same row with different letters are significantly different (p≤ 0.05).SEM=Standard error of the mean.

Table 11. Effect of of different cupper forms on bacterial count of broiler chicks

Treatment	Control	CuSO4 mg/kg		Cu-Met mg/kg		Nano – cu		SEM	P-Value
Parameter	0	50	100	50	100	50	100		
TBC (10^3)	3.27^{a}	1.95^{b}	2.28^{b}	1.97^{b}	2.07^{b}	2.17^{b}	2.09^{b}	0.088	0.002
Lactobacillus x 10^3	1.97^{c}	2.51^{b}	2.91^{b}	4.12^{a}	3.66^{a}	4.82^{a}	4.53^{a}	0.998	0.001
Salmonella x 10^2	1.01^{a}	0.808^{b}	0.729^{b}	0.679^{c}	0.629^{c}	0.651^{c}	0.620^{c}	0.018	0.002
E. coli x 10^3	1.19^{a}	0.903^{b}	0.901^{b}	0.856^{c}	0.774^{c}	0.723^{c}	0.792^{c}	0.022	0.001
Proteus x 10^2	0.779^{a}	0.615^{b}	0.601^{b}	0.458^{c}	0.429^{c}	0.428^{c}	0.379^{c}	0.044	0.001

^{a,b,c} Means within the same row with different letters are significantly different (p≤ 0.05).SEM=Standard error of the mean.

CONCLUSION

The results can be summarized that the addition of different forms of Cu inorganic, organic and nano copper to broiler chicken diets improved performance trait. Chickens fed diet with organic and nano copper showed better growth performance than other groups. The use of copper increases hemoglobin synthesis by higher number of RBCs recorded with Cu-supplemented chickens' diet. Different forms of Cu exert positive impacts on immune profile, antioxidant status and enhanced lipid profile. An increase in protein content may indicate faster biosynthesis of tissue protein, or a slowing down of protein metabolism. Our results showed significant

reduction in the number of pathogenic bacteria (*Salmonella, E. coli* and *Proteus*), indicating the role of Cu as a good element to relieve bacterial disorders in the digestive tract of broiler chickens. Further research is necessary to elucidate the effect of organic copper on the immune response and cellular reaction of the digestive system epithelial lining, which helps improve nutrient absorption and disease resistance in chickens.

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الإستجابة الإنتاجية والمناعية لإضافة صور مختلفة من النحاس لبداري التسمين [60]

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أجريت هذه الدراسة في وحدة بحوث الدواجن بمزرعه البستان، قسم الانتاج الحيواني والداجني، كلية الزراعة – جامعة دمنهور خلال الفترة من ابريل حتي يونيه 2019. وكان الهدف منها تقييم التأثيرات الناتجة عن إضافة صور مختلفة من عنصر النحاس : غير عضوي (كبريتات النحاس) عضوي (نحاس– ميثيونين). وكذلك الصورة النانومترية للنحاس (نانو نحاس) علي الصفات الانتاجيه والفسيولوجيه والاستجابة المناعية لكتاكيت اللحم. ولتحقيق هذا الهدف تم توزيع عدد 210 من كتاكيت اللحم عشوائيا بداية من عمر أسبوع إلى سبعه معاملات بكل معاملة 30 طائر في خمسة مكررات بكل منها 5 كتاكيت علي النحو التالي: المجموعة الاولى هى الضابطة (الكنترول) وكانت بدون إضافات؛ والمعاملتين الثانيه والثالثة تغذت على العليقه الأساسيه مع اضافه النحاس في الصورة غير عضوية (كبريتات النحاس) بمستويات 50–100 جزء في المليون بينما المعاملتين الرابعه والخامسة تم اضافة النحاس في الصورة العضوية (نحاس مرتبط مع الميثيونين) بمستويات 50–100 جزء في المليون بينما المعاملتين السادسة والسابعة تم اضافة النحاس في صورة النانو بمستويات 50–100 جزء في البليون. أظهرت النتائج حدوث زيادة معنوية في وزن الجسم الحي ومعدل الزيادة في وزن الجسم مع تحسن في الكفاءة الغذائية والكفاءة الاقتصادية ووزن الذبيحة في المجموعات التي غذيت علي صور النحاس المختلفة بالمقارنة بمجموعة المقارنة. وكان افضل المعاملات تلك التي غذيت علي النحاس العضوي والنانو نحاس مقارنة بباقي المعاملات. وأظهرت النتائج أيضا حدوث زيادة معنوية في مستوي بروتينات الدم والجلوبيولينات المناعية في المجموعات المضاف لها النحاس بصوره المختلفة مقارنة بمجموعة المقارنة. بينما كان هناك انخفاض معنوي في مستوي الدهون الكلية في الدم والكوليسترول وكذلك انخفاض في مستوي LDL في المجموعات المغذاة علي صور النحاس المختلفة. لوحظ أيضا وجود زيادة في مستوي جلوكوز الدم وفي تركيز هرمونات الغدة الدرقية وأيضا تحسن في مستوي انزيمات الأكسدة المختلفة في سيرم الدم في المجموعات المغذاة علي صور النحاس المختلفة. من ناحية أخرى كان لهذه الإضافات تأثير معنوي علي زيادة العدد الكلي لكرات الدم البيضاء، نسبة كرات الدم البيضاء الليمفاوية، مع زيادة جلوبيولينات الدم (ألفا وجاما). وبالمثل تحسنت الحالة الضد– تأكسدية للطيورمن واقع زيادة مستوى انزيم (SOD) والجلوتاثيون (GSH) والجلوتاثيون بيروكسيديز والقدرة الكية المضادة للأكسدة والنشاط البلعمى ودليل النشاط البلعمى ومعامل تحويل الخلايا الليمفاوية ونشاط مقاومة البكتريا والنشاط الليسوسومى كما حدثت زيادة معنوية في مستوي الجلوبيولينات المناعية (IgA – IgM)– IgG. كما أدت جميع الإضافات إلى حدوث انخفاض في أعداد البكتريا الممرضة في الامعاء في المجموعات المغذاه علي صور النحاس. وكان أفضل المعاملات تلك المغذاه علي النحاس العضوي والنانو نحاس مقارنة ببقية المعاملات.