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Research Article

Effects of Copper (Sulfate, Acetate and Nano) *in ovo* injection on Hatching Traits and some Physiological Parameters of Newly-hatched Broiler Chicks

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Abstract: This study was conducted to investigate the effect of different sources of copper (sulfate, acetate and nano) *in ovo* injection at 10 days of embryogenesis period on hatching traits and some physiological parameters of newly-hatched broiler chicks. A total number of 462 fertile eggs were used (with an average weight of 66.24±0.23g in 7 groups, each group containing 66 fertile eggs in three replicate. The experimental design was as follows the group 1 as control, while the groups 2, 3 and 4 injected with 0.1ml deionized water containing 8µg/egg of Cu sulfate, Cu acetate and nano Cu, respectively, and the groups 5, 6 and 7 injected with 0.1ml deionized water containing 16µg/egg of Cu sulfate, Cu acetate and nano Cu, respectively. After hatching eight one-day chicks around the average of each group were used in this study and the results as follows, different sources of Cu *in ovo* injection at 10 days of incubation by two levels (8 and 16µg/egg) did not affect hatching traits except elevation chick weight and chick weight %, did not affect yolk sac %, relative weight of organs (liver, gizzard, heart and bursa) of newly hatched chicks, did not affect hematological parameters and some plasma constituents, levels of 16µg/egg Cu led to increasing plasma Alb compared to the levels of 8µg/egg Cu and control, Cu sources at 8 or 16µg/egg decreased the values of AST enzyme.

From the results of this study, it can be concluded that using Cu sources *in ovo* injection at 10 days of incubation up to the level of 16µg/egg, did not cause harmful effect but caused best results in chick weight and chick weight %.

Keywords: Ovo injection, Cu sulfate, Cu acetate, Nano Cu, Hatching traits, Blood parameters.

1. Introduction

The period of broiler chick embryogenesis is critical for growth and development because it represents a greater proportion of its total life span [1]. It has been suggested that the nutrients stored in the egg might not be sufficient for modern chicken embryonic development and postnatal growth [2]. Also, Naber [3] and Angel [4] reported that the high consumption of minerals was from 11-17 days of embryogenesis. At 17 days of incubation, the majority of minerals were consumed, therefore, the embryo may suffer from a deficiency of some minerals during the last days of incubation. Therefore, it began to supply embryos with various biologic and supplements that improve intestinal growth of the fetus and newly hatched chicks, it also improves hatchability, growth of body and reduces chick mortality after hatching [5,6,7,8,9]. Uni and Ferket [10] reported that the degree of response to *in ovo* feeding may depend on genetic, breeder hen age, egg size, incubation conditions and time of injection. Also, Ferket *et al.*, [11] and Chen *et al.*, [12] confirmed that the amnion is an effective site for injecting the nutrients, which represent an external feed that digested and absorbed in the intestine of embryo before piping**.** The weight of newly-hatched chicks is a major indicator of marketing weight in chickens as observed by [13]. Blood biochemical indices are important indicators for general health and physiological state of broiler chickens [14].

Copper (Cu) is necessary for hemoglobin formation and it is known to play a vital role in many enzyme systems; for example, it is a component of cytochrome oxidase and superoxide dismutase [15,16,17,18]. Mroczek-Sosnowska *et al.*, [19] demonstrated that both forms of Cu (Nano Cu and $CuSO_4$) stimulate the development of blood vessels at the molecular and systemic level, resulting in increasing

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of hyperplasia over hypertrophy to the end of embryogenesis. Febré *et al*., [20] studied Cu acetate and nanoparticles (Cu-NP) as a promising alternative to antibacterial reagents and promoter of growth and noted that the animal performance by the effects of Cu-NP depends on the size, shape, dose and animal species.

Little information on the effect of different Cu sources (sulfate, acetate and nano) *in ovo* injection at 10 days of embryogenesis periods on hatching traits and yolk sac %, relative weight of organs, hematological parameters and some plasma constituents of newly-hatched broiler chicks, therefore, the current study was conducted to fill this gap.

2. Materials and Methods

The present study was conducted in two stages, the 1st stage at hatching plant in El Takamoly Poultry Project, EL-Azab and the 2nd stage at Poultry Production Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt,

2.1 Preparation and characterization of Cu nanoparticles

Preparation and characterization processes of copper oxide nanoparticles were conducted at Nanotechnology and Advanced Materials Central Lab (NAMCL), Agricultural Research Center, Egypt. It prepared by chemical reduction method using Copper(II) sulfate pentahydrate as precursor salt and starch as capping agent according to [21]. The X-Ray diffraction (XRD) pattern of copper oxide (CuO) nanoparticles is shown in Fig. (1). The particle size were investigated by high-resolution transmission electron microscopy (HRTEM) and the size ranged from 59.2–88.3nm with nearly spherical shaped particles. Dynamic light scattering (DLS) analysis was used to measure hydrodynamic diameter of CuO nanoparticles and the size was 32.52nm (Fig. 2) and Fig. (3) shown the final form of nano Cu.

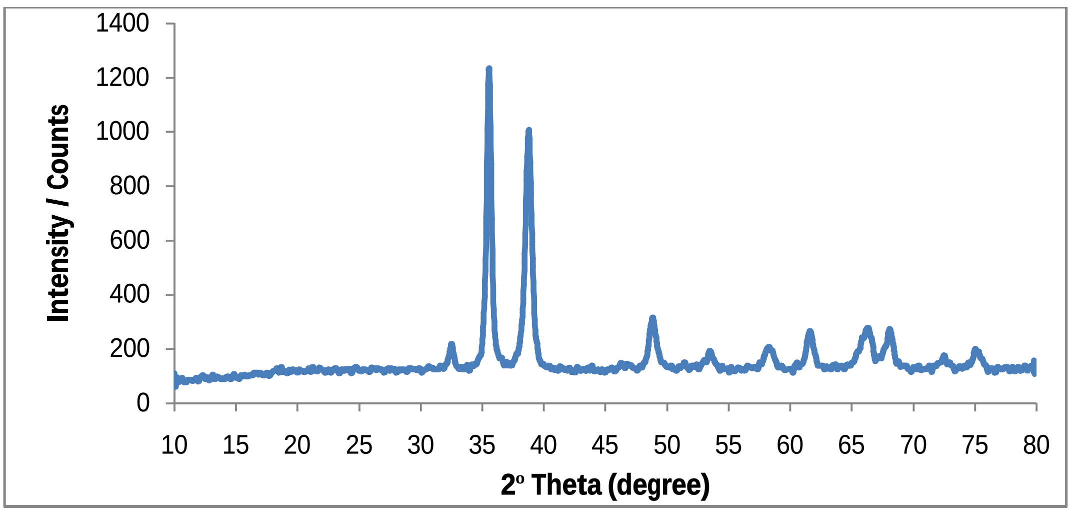

Fig. 1: X-ray diffraction (XRD) patterns of copper oxide nanoparticles synthesized by chemical reduction method.

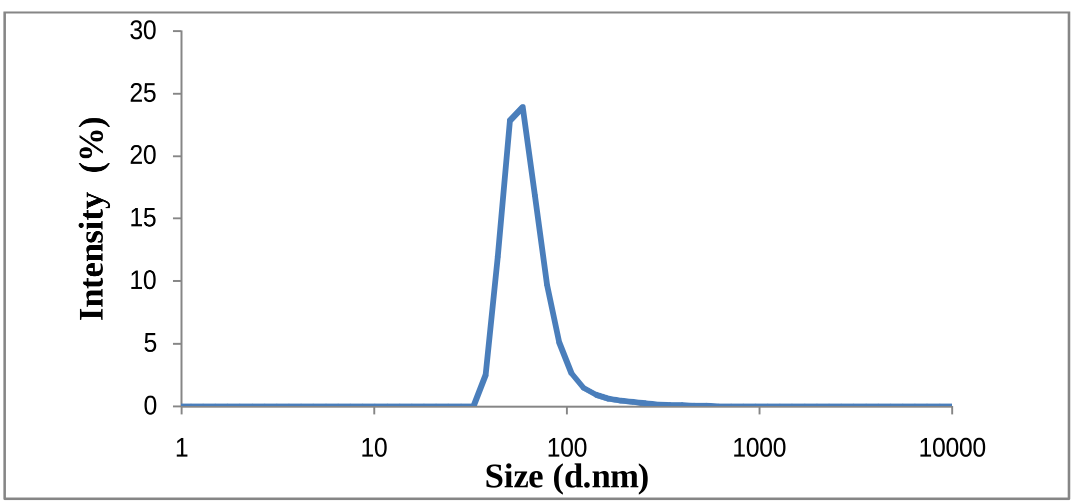

Fig. 2: Particle size distribution of CuO nanoparticles.

Fig. 3: Cu-Cu_2O nanoparticles synthesized through chemical reduction method.

2.2 Experimental design

A total of 560 eggs obtained from a commercial broiler flock (Avian 48) at 38 weeks of age were stored 2 days at a temperature of 17ºC and humidity of 70-80%, the eggs were marked, weighed individually and initially divided into similar 7 groups until beginning injection experiments. Eggs fumigated and incubated for 21 days under standard conditions (99-100ºF, 60-70% relative humidity and turned each one-hour for 18 days in an automatic electrical incubator and on the 19th day of incubation the eggs were transferred to the hatchery with a temperature of 97-98ºF and 80-90% relative humidity until hatching). After candling, 462 total number fertile eggs were used (with an average weight of 66.24±0.23gm) in experimental injection at 10 days of incubation, in 7 groups, each group containing 66 fertile eggs in three replicates. As follow the group 1 as control (without injection), while the groups 2, 3 and 4 injected with 0.1ml deionized water containing 8µg/egg of Cu sulfate, Cu acetate and nano Cu, respectively, and the groups 5, 6 and 7 injected with 0.1ml deionized water containing 16µg/egg of Cu sulfate, Cu acetate and nano Cu, respectively.

2.3 *In ovo* injection procedure

In ovo injection were carried out on the 10th day of incubation into amnion, before injection the broad end of each egg was sterilized with ethyl alcohol and a small pinpoint hole was made in the broad end to injection. The treatment solutions were injected into the eggs using automatic siring by a 24G hypodermic needle (25mm long) according to [22], and the pinpoint hole was sealed with sterile paraffin wax. The *in ovo* injection of each treatment was completed within 10 – 15 minutes also the eggs of the control remained outside the setter for the same time.

2.4 Hatching and one-day post hatch traits

After hatching, the hatched chicks from all groups were counted and weighed individually and the eggs did not hatch of each treatment were recorded and checked to calculate hatching %, chick weight % and embryonic mortality by the following formulas.

$$\text{Hatchability \%} = \frac{\text{Number of hatched chicks}}{\text{Number of fertile eggs}} \times 100 \qquad (1)$$

$$\text{Chick weight \%} = \frac{\text{Chick weight}}{\text{Hatched egg weight}} \times 100 \qquad (2)$$

The different types of embryonic mortality (mid and late) were calculated as percent of fertile eggs.

Eight one-day chicks around the average of each group were taken and weighted individually before slaughter and the blood samples were collected in two tubes the first tube containing EDTA to determine hematological parameters and second tube containing heparin as coagulant, after that centrifuged at 3,000 rpm for 10 minutes to separate plasma, and stored in sterile tubes at -20°C until the time of determination plasma constituent and T_3 hormone. Plasma constituent and T_3 hormone were calorimetrically determined by commercial kits purchased from BioVision Company.

The yolk sac and the different organs (liver, gizzard, heart and bursa) were weighed to calculate the relative weights.

2.5 Statistical analysis

The data obtained in this study was statistically analyzed general linear models procedure (GLM) of SPSS for Windows [23]. Significant differences among treatment means were tested by Duncan multiple range test [24].

3. Results and Discussion

Results in Table (1) shown that, Cu sources (Cu sulfate, Cu acetate and nano Cu) or Cu levels (8 or 16µg/egg) *in ovo* injection in amnion at 10 days of incubation did not affect significantly ($P \geq 0.05$) on each of hatchability %, mid and late embryonic mortality and dead % after hatching but chick weight and chick weight % increased significantly compared to the control.

In ovo injection with 8 or 16µg/egg of each Cu sulfate, Cu acetate and nano Cu at 10 days of incubation led to insignificant effect on hatchability %, mid and late embryonic mortality and dead % after hatching, however, significant differences were found in each of chick weight and chick weight % and the control recorded the lowest value as illustrated in Table (1). Our results are in agreement with that obtained by [25] of hatchability %, death before or after piping and dead chicks when broilers fertile eggs injected with 8µg/egg at 14 days of age, but the same authors noted no variation in hatch weight between treated group and the control. Mroczek-Sosnowska *et al.*, [19] reported that both Cu Nano and $CuSO_4$ improved chicken development by stimulation of blood vessel development, increasing hyperplasia over hypertrophy during the embryogenesis and the number of muscle fibres is established mainly in the prenatal period [26]. Moreover, *in ovo* administration of 50mg/kg Cu-NP resulted in O_2 consumption, elevation at 16 and 19 days of embryonic development, but the two levels of $CuSO_4$ (50 or 100mg/kg) led to higher O_2 on days 10 and 13 and subsequently improved embryonic development as noted by [27] this because Cu is important element for hemoglobin formation and for essential enzymes (cytochrome oxidase) that essential in cellular energy generation [28]. Miroshnikov *et al*., [29] suggested that intramuscular injections of copper nanoparticles stimulate growth and metabolic changes quickly of chicken and the Cu preparations promoted elevating the arginine content in chicken liver. It is noteworthy that arginine is the prime factor for maximizing growth potential in young animals [30,31]. As well as copper acetate, containing acetate (Short-Chain Fatty Acid) that is most commonly used in the form acetyl coenzyme A, that is important for lipid, carbohydrate and protein metabolism and energy production. Also, acetate lowers intestinal pH and increased minerals solubility and absorption [32].

No significant effects were found from Cu sources *in ovo* injection at 10 days of incubation on percentage of yolk sac, liver, gizzard, heart or bursa of broiler chicks at one day of age as observed in Table (2). Also, the relative organ weights were used as a measure of hatching development by [27] and observed that organ weights (liver and heart) relative to the yolk-free body weight were not affected in the injected groups by 50mg/kg Cu-NP and $CuSO_4$.

Results in Table (3) shown that no significant differences have existed in hematological parameters of newly hatched broiler chicks that produced from treated eggs at 10 days of incubation by two levels (8 or 16µg/egg) of Cu sulfate, acetate and nano as observed in Table (3).

No variation ($P \geq 0.05$) were found in plasma T_3 hormone of one day broiler chicks produced from treated eggs at 10 days of incubation by different sources of Cu with 8 and 16µg/egg compared with control, but a tendency to increase the level of T_3 hormone by the effect of Cu sources, levels and their interactions (Table 4). Thyroid hormones are important in maintaining normal growth and development of the chick embryo, and a positive correlation was observed between thyroid hormones and chick embryonic weight as found by [33]. Thyroid hormones are needed in controlling metabolic rate, and the T_3 concentration in blood is positively correlated with oxygen consumption in broilers [34].

Table (1): Effects of different sources of copper *in ovo* injection at 10 days of incubation on hatching traits of broilers fertile eggs.

Treatments		Egg weight (g)	Hatchability %	Embryonic mortality %		Dead %	Chicks weight (g)	Chicks Weight %
				Mid	Late			
Cu sources								
Control		65.59	87.69	0.00	12.31	4.62	40.16 [b]	61.48 [b]
Cu sulfate		66.58	83.33	1.52	15.15	0.76	42.55 [a]	64.06 [a]
Cu Acetate		66.34	83.97	1.52	14.50	2.29	42.07 [a]	63.65 [a]
Nano Cu		66.13	86.36	1.52	12.12	0.76	42.05 [a]	63.76 [a]
SEM		0.46	3.45	1.10	3.33	1.26	0.37	0.44
***P*-value**		0.55	0.81	0,80	0.88	0.18	0.000	0.001
Cu levels								
0.0µg/egg		65.59	87.69	0.00	12.31	4.62	40.16 [b]	61.48 [b]
8µg/egg		66.10	85.28	2.03	12.69	0.51	42.38 [a]	64.35 [a]
16µg/egg		66.58	83.84	1.01	15.15	2.02	42.06 [a]	63.34 [a]
SEM		0.42	3.81	1.01	3.06	1.16	0.33	0.40
***P*-value**		0.29	0.75	0.41	0.73	0.08	0.000	0.000
Interaction (Source x Level)								
Control		65.59	87.69	0.00	12.31	4.62	40.16 [b]	61.48 [b]
Cu sulfate	8µg/egg	66.48	83.33	1.52	15.15	1.52	42.55 [a]	64.20 [a]
Cu Acetate	8µg/egg	66.16	83.08	1.54	15.38	.00	42.33 [a]	64.30 [a]
Nano Cu	8µg/egg	65.68	89.39	3.03	7.58	.00	42.28 [a]	64.54 [a]
Cu sulfate	16µg/egg	66.68	83.33	1.52	15.15	.00	42.55 [a]	63.92 [a]
Cu Acetate	16µg/egg	66.49	84.85	1.52	13.64	4.55	41.86 [a]	63.11 [ab]
Nano Cu	16µg/egg	66.57	83.33	.00	16.67	1.52	41.81 [a]	63.00 [ab]
SEM		0.60	4.23	1.41	4.26	1.61	0.48	0.57
***P*-value**		0.76	0.92	0.77	0.80	0.137	0.003	0.001

Table (2): Effects of different sources of copper *in ovo* injection at 10 days of incubation on Yolk sac % and relative organs weight of newly hatched broiler chicks.

Treatments		body weight (g)	Yolk sac %	Liver %	Gizzard %	Heart %	Bursa %
Cu Sources							
Control		39.80	6.93	2.81	7.07	0.855	0.153
Cu sulfate		41.49	6.68	2.94	7.90	0.831	0.125
Cu Acetate		41.59	5.94	3.00	7.48	0.812	0.133
Nano Cu		41.25	6.70	2.85	7.67	0.777	0.142
SEM		0.70	0.65	0.10	0.32	0.04	0.02
***P*-value**		0.372	0.747	0.608	0.380	0.457	0.796
Cu Levels							
0.0µg/egg		39.80	6.93	2.81	7.07	0.855	0.153
8µg/egg		41.25	6.15	2.95	7.74	0.803	0.139
16µg/egg		41.60	6.77	2.90	7.64	0.806	0.129
SEM		0.63	0.59	0.09	0.29	0.03	0.02
***P*-value**		0.197	0.583	0.584	0.329	0.572	0.667
Interaction (Source x Level)							
Control		39.80	6.93	2.81	7.07	0.855	0.153
Cu sulfate	**8µg/egg**	40.96	6.31	2.83	8.00	0.797	0.142
Cu Acetate	**8µg/egg**	41.70	5.66	3.14	7.60	0.823	0.135
Nano Cu	**8µg/egg**	41.20	6.35	2.97	7.62	0.796	0.139
Cu sulfate	**16µg/egg**	42.03	7.05	3.05	7.79	0.865	0.105
Cu Acetate	**16µg/egg**	41.50	6.17	2.88	7.38	0.803	0.131
Nano Cu	**16µg/egg**	41.31	7.05	2.76	7.73	0.759	0.146
SEM		0.95	0.88	0.15	0.43	0.06	0.03
***P*-value**		0.721	0.914	0.398	0.783	0.680	0.919

Table (3): Effects of different sources of copper *in ovo* injection at 10 days of incubation on hematological parameters of newly hatched broiler chicks.

Treatments		RBC ($10^6/mm^3$)	Hb (%)	HCT (%)	MCV (µm)	MCH (Pg)	MCHC (%)
Cu Sources							
Control		2.36	11.82	31.60	135.48	50.38	37.20
Cu sulfate		2.38	12.25	33.225	138.72	51.31	37.08
Cu acetate		2.28	11.70	31.64	139.12	51.28	36.85
Nano Cu		2.14	10.93	30.06	135.25	49.39	36.51
SEM		0.09	0.57	1.39	2.11	0.97	0.46
***P*-value**		0.246	0.384	0.417	0.405	0.390	0.715
Cu Levels							
0.0µg/egg		2.36	11.82	31.60	135.48	50.38	37.20
8µg/egg		2.23	11.39	31.15	136.830	50.04	36.56
16µg/egg		2.30	11.78	31.95	138.376	50.61	37.01
SEM		0.09	0.54	1.31	1.96	0.89	0.41
***P*-value**		0.614	0.793	0.876	0.603	0.562	0.522
Interaction (Source x Level)							
Control		2.36	11.82	31.60	135.48	50.38	37.20
Cu sulfate	**8µg/egg**	2.42	12.12	32.60	135.02	50.05	37.09
Cu acetate	**8µg/egg**	2.27	11.57	31.37	139.56	51.17	36.66
Nano Cu	**8µg/egg**	2.00	10.47	29.50	135.89	48.87	35.93
Cu sulfate	**16µg/egg**	2.35	12.37	33.85	142.41	52.56	37.07
Cu acetate	**16µg/egg**	2.29	11.80	31.86	138.76	51.37	37.00
Nano Cu	**16µg/egg**	2.26	11.30	30.52	134.75	49.81	36.97
SEM		0.12	0.78	1.91	2.72	1.28	0.61
***P*-value**		0.358	0.730	0.798	0.394	0.550	0.809

Plasma TP differed ($P \leq 0.05$) by the effect of Cu sources, but not by Cu levels or by the interaction between Cu sources and Cu levels. The levels of 16µg/egg Cu led to increasing plasma Alb compared to the levels of 8µg/egg Cu and control. Also, significant variations were observed in plasma Alb concentration among the treatment groups. Whereas Glo or Alb/Glo ratio did not affect by Cu sources, Cu levels or by the interaction between them, and the tendency to increase the level of Alb/Glo ratio by Cu sources (sulfate and nano) compared to acetate or control and by levels of 8 and 16µg/egg Cu compared to the control as illustrated in Table (4). And this may cause improving in chick weight as found in Table (1) because plasma Alb has a nutritive role, acts as a transport carrier for various biomolecules (fatty acids and trace elements) also, it maintains of osmotic pressure and fluid distribution between blood and tissues [14]. On the contrary, [27] noted that no variation in Alb of treated groups by Cu-NP and $CuSO_4$ compared to the control.

Results in Table (5) indicated that the concentrations of plasma cholesterol, Ca and P of treated groups by Cu sources (sulfate, acetate and nano) with two levels (8 or 16µg/egg) not differed significantly compared to the control group. But the AST values of one-day broiler chicks decreased significantly by Cu sources *in ovo* injection at levels 8 and 16µg/egg related to the control. Confirmed to our results [27], no changes in blood cholesterol of one-day chicks produced from Cu-NP and $CuSO_4$ *in ovo* injection compared to the control group but the same authors found no differences in AST values.

Table (4): Effects of different sources of copper *in ovo* injection at 10 days of incubation on, plasma T_3 hormone, proteins, and Alb/Glo ratio of newly hatched broiler chicks.

Treatments		T_3 (ng/ml)	TP (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Cu Sources						
Control		2.20	4.50^{ab}	2.17	2.33	1.01
Cu sulfate		3.25	3.68^{b}	2.26	1.42	1.96
Cu Acetate		3.25	4.81^{a}	2.17	2.63	1.12
Nano Cu		3.09	4.00^{ab}	2.26	1.74	1.72
SEM		0.38	0.30	0.09	0.29	0.32
***P*-value**		0.304	0.045	0.75	0.018	0.116
Cu Levels						
0.0µg/egg		2.20	4.50	2.17^{b}	2.33	1.01
8µg/egg		3.13	3.85	2.09^{b}	1.76	1.47
16µg/egg		3.25	4.31	2.38^{a}	1.93	1.83
SEM		0.34	0.29	0.06	0.29	0.30
***P*-value**		0.163	0.261	0.002	0.468	0.200
Interaction (Source x Level)						
Control		2.20	4.50	2.17^{bc}	2.33	1.01
Cu sulfate	8µg/egg	3.00	3.33	2.04^{c}	1.29	1.81
Cu acetate	8µg/egg	3.75	4.58	2.07^{bc}	2.51	1.07
Nano Cu	8µg/egg	2.80	3.89	2.17^{bc}	1.72	1.38
Cu sulfate	16µg/egg	3.50	4.03	2.49^{a}	1.54	2.12
Cu acetate	16µg/egg	2.75	5.03	2.27^{abc}	2.76	1.17
Nano Cu	16µg/egg	3.33	4.10	2.34^{ab}	1.76	2.00
SEM		0.48	0.39	0.09	0.39	0.42
***P*-value**		0.368	0.115	0.013	0.125	0.312

Table (5): Effects of different sources of copper *in ovo* injection at 10 days of incubation on plasma Cholesterol, AST, Calcium and Phosphorous of newly hatched broiler chicks.

Treatments		Cholesterol (mg/dl)	AST (U/L)	Calcium (mg/dl)	Phosphorous (mg/dl)
Cu Sources					
Control		384.40	55.03^{a}	10.67	5.85
Cu sulfate		356.20	36.08^{b}	11.30	5.68
Cu acetate		398.75	42.70^{b}	11.07	6.04
Nano Cu		407.81	38.91^{b}	10.87	5.98
SEM		26.36	3.17	0.66	0.26
***P*-value**		0.438	0.004	0.922	0.732
Cu Levels					
0.0µg/egg		384.40	55.03^{a}	10.67	5.85
8µg/egg		392.15	34.97^{b}	11.59	5.69
16µg/egg		383.75	41.75^{b}	10.55	6.07
SEM		24.77	2.76	0.58	0.23
***P*-value**		0.95	0.000	0.243	0.382
Interaction (Source x Level)					
Control		384.40	55.03^{a}	10.67	5.85
Cu sulfate	8µg/egg	365.75	35.78^{bc}	11.56	5.34
Cu acetate	8µg/egg	370.50	35.51^{bc}	12..02	5.37
Nano Cu	8µg/egg	430.60	32.80^{c}	11.29	6.22
Cu sulfate	16µg/egg	349.83	36.27^{bc}	10.98	5.91
Cu acetate	16µg/egg	427.00	46.59^{ab}	10.12	6.71
Nano Cu	16µg/egg	388.83	44.00^{abc}	10.45	5.78
SEM		34.61	4.75	0.83	0.30
***P*-value**		0.582	0.003	0.731	0.073

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