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Effects of dietary copper sources on haematological, serum biochemical and hormonal profiles of laying hens

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ADDITIONAL KEYWORDS

Copper.
Blood.
Glucose.
Diets.
Hormones.
Feed.

SUMMARY

A total of 224 pullets of 20 weeks of age were used to study the effects of two varying levels of dietary inorganic copper sources on the haematological, serum biochemical and hormonal performances of laying hens. They were assigned to two groups of experimental diets which were copper-free. Each group consists of four treatments. A group contains copper sulphate (CuSO4) supplement at 0, 100, 200 and 300 mg/ kg diets while the other group contains copper Oxide (CuO) at the same inclusion rates. The pullets were randomly allotted to the eight (8) treatment diets and replicated seven (7) times with four (4) pullets per replicate in a 2 x 4 factorial arrangement. The experiment lasted for a period of sixteen weeks. At the end of the experiment, 3 birds/ replicate were sacrificed; blood samples were collected for haematological, serum biochemical and hormonal studies. It was observed that copper oxide and copper sulphate significantly (P<0.05) enhanced the various performances of the laying hens in the parameters studied. However, CuSO4 at 100 and 200mg inclusion levels were observed to confer lower values in packed cell volume, haemoglobin and red blood cell count as compared with the control. Also, it was observed that blood viscosity significantly (P<0.05) increased as copper supplements increase in the diets. Furthermore, the result showed that there was significant difference (P<0.05) between treatments only for uric acid. However, significant differences (P<0.05) were observed at levels of inclusion for uric acid, glucose and cholesterol.

Effetti delle fonti dietetiche di rame su ematologica, siero biochimiche e ormonali profili delle galline ovaiole

PAROLE CHIAVE AGGIUNTIVE

Rame.
Sangue.
Glicemia.
Diete.
Ormoni.
Alimentazione.

SOMMARIO

Un totale di 224 vacche di 20 settimane di età è stato utilizzato per studiare gli effetti di due diversi livelli di fonti di rame inorganiche dietetiche sulle prestazioni ematologiche, seriche e biochimiche e ormonali delle galline ovaiole. Sono stati assegnati a due gruppi di diete sperimentali che erano prive di rame. Ogni gruppo è costituito da quattro trattamenti. Un gruppo contiene il supplemento di rame di solfato (CuSO4) a 0, 100, 200 e 300 mg / kg di diete mentre l'altro gruppo contiene ossido di rame (CuO) con gli stessi tassi di inclusione. Le razze sono state assegnate casualmente alle otto diete di trattamento (8) e ripetite sette (7) volte con quattro (4) gregge per replicare in una disposizione fattoriale 2 x 4. L'esperimento è durato per un periodo di sedici settimane. Alla fine dell'esperimento sono stati sacrificati 3 uccelli / repliche; Sono stati raccolti campioni di sangue per studi ematologici, sierici biochimici e ormonali. È stato osservato che l'ossido di rame e il solfato di rame significativamente (p <0,05) hanno migliorato le varie prestazioni delle galline ovaiole nei parametri studiati. Tuttavia, sono stati osservati livelli di inclusione di CuSO4 a 100 e 200 mg per conferire valori inferiori nel volume compresso della cellula, nell'emoglobina e nel conteggio dei globuli rossi rispetto al controllo. Inoltre, è stato osservato che la viscosità del sangue significativamente aumentata (p <0,05) aumentando i supplementi di rame nelle diete. Inoltre, il risultato ha mostrato una differenza significativa (P <0,05) tra i trattamenti solo per l'acido urico. Tuttavia, differenze significative (P <0,05) sono state osservate ai livelli di inclusione per l'acido urico, il glucosio e il colesterolo.

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INTRODUCTION

Copper is a vital constituent of important biological processes in animals. It is a constituent of pro-enzyme; which explains why dietary copper is essential because copper restriction in the diet can alter activity of copper pro-enzyme and impact normal physiology (Prohaska 2011, Prohaska and Gybnia, 2004), it is involved in electron transfer as constituent of copper metalloprotein, pigmentation (Xu et al., 2013) and

oxidation resistance (Wang et al., 2013), it also alters lipid metabolism (Saran-Netto et al., 2014) and it is an important cofactor for many copper proenzymes in which copper is bound to specific amino acid residues in active site (Prohaska and Gybnia, 2004). Also, other importance of copper which include: utilization of dietary iron, prevention of anaemia, assistance in blood coagulation, crosslinking of connective tissues, defense against oxidative damage and synthesis of hormone; myelination of brain and spinal cord as well as reproduction has been stressed.

Table I. Composition of the Experimental Diets (kg/MT) (Composizione delle diete sperimentali (kg / MT).

	Diets with CuSO4 inclusion				Diets with CuO inclusion			
INGREDIENTS	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Maize	460	460	460	460	460	460	460	460
Soya Meal	240	240	240	240	240	240	240	240
Corn Bran	120	120	120	120	120	120	120	120
Wheat Offal	40	40	40	40	40	40	40	40
Rice Bran	20	20	20	20	20	20	20	20
Oyster shell	90	90	90	90	90	90	90	90
Di-Calcium Phosphate	19	19	19	19	19	19	19	19
Lysine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Methionine	3	3	3	3	3	3	3	3
Mineral + Vitamin Premix*	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Salt	3	2.9	2.8	2.7	3	2.9	2.8	2.7
CuSO4	0	0.1	0.2	0.3	0	0	0	0
CuO	0	0	0	0	0	0.1	0.2	0.3
Total	1000	1000	1000	1000	1000	1000	1000	1000
CALCULATED VALUES								
Crude Protein (%)	17.06	17.06	17.06	17.06	17.06	17.06	17.06	17.06
ME (Kcal/kg)	2668.72	2668.72	2668.72	2668.72	2668.72	2668.72	2668.72	2668.72
Ca (%)	3.61	3.61	3.61	3.61	3.61	3.61	3.61	3.61
Total Phosphorus (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Crude Fibre (%)	4	4	4	4	4	4	4	4
Crude Fat (%)	4.51	4.51	4.51	4.51	4.51	4.51	4.51	4.51
Lysine	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12
Methionine	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Chlorine (%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Potassium (%)	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Sodium (%)	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Copper (mg/kg)	5.563	6.543	7.523	8.503	5.563	6.543	7.523	8.503
DEB (m Eq/kg)	198.21	198.21	198.21	198.21	198.21	198.21	198.21	198.21
ANALYSED VALUES								
Crude Protein (%)	17.49	17.49	17.49	17.49	17.49	17.49	17.49	17.49
ME (Kcal/kg)	2740.48	2740.48	2740.48	2740.48	2740.48	2740.48	2740.48	2740.48
Crude Fibre (%)	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Crude Fat (%)	2.26	2.26	2.26	2.26	2.26	2.26	2.26	2.26
Crude Ash (%)	11.02	11.02	11.02	11.02	11.02	11.02	11.02	11.02
Moisture (%)	11.97	11.97	11.97	11.97	11.97	11.97	11.97	11.97
NFE (%)	53.76	53.76	53.76	53.76	53.76	53.76	53.76	53.76

NFE = Nitrogen Free Extract; ME = Metabolizable Energy; MT = Metric Tonne; DEB: Dietary Electrolyte Balance
*Composition of Mineral + Vitamin premix: 2.5 kg of premix contains: Vit. A (10000000 iu), Vit. D3 (2500000 iu), Vit. E (12000 iu) Vit. B1 (2000 mg), Niacin (15000 mg), Vit. B6 (1500 mg), Vit. B12 (10 mg), Vit. K3 (2000 mg), Biotin (20mg) Folic Acid (600 mg), Panthothenic Acid (7000 mg), Chlorine Chloride (150000 mg), Manganese (80000 mg) Iron (40000 mg), Cobalt (500 mg), Zinc (60000 mg), Selenium (150 mg), Iodine (1000 mg), Magnesium (100 mg), Ethoxyquine (500 g), BHT (700 g), Copper (0.00mg)

Copper has been used extensively in poultry diet. The advantage of dietary copper in maximizing production performance of birds (Lien et al., 2004), increase in egg and meat production through proper nutrition have been explored among other beneficial properties (Pesti and Bakalli, 1998). Abaza et al. (2009) reported that additional copper sources have become available and the potential for commercial use as feed supplement has expanded.

Copper sulphate is a naturally-occurring inorganic salt. Copper sulphate has antibacterial and antifungal properties (Chen et al., 2014). Jegede et al. (2015) reported that birds fed diets containing 50 mg/kg $CuSO_4$ concentration laid the highest number of eggs during the early and mid laying period. They also reported significant ($P<0.05$) increase in yolk and blood cholesterol and triglycerides measure in birds fed $CuSO_4$ at 100 and 150 mg/kg inclusion rate. It has equally been stressed that dietary supplementation of broiler chicken with $CuSO_4$ up to 200 mg/kg did not have any adverse effect on production performance of broiler chicken (Vasanth, et al., 2015). Scott et al. (2017) observed that in in-ovo injection of $CuSO_4$ to chicken embryo, there was an improved final body weight, average daily gain and feed conversion ratio in relation to the control group. It has been reported that dietary $CuSO_4$ is more effective than CuO (Zia-Ur-Rahman *et al*, 2001).

We therefore examined the effects of dietary Copper Sulphate ($CuSO_4$) and Copper Oxide (CuO) supplements on laying birds being the most reared poultry commercially.

MATERIALS AND METHOD

The experiment was carried out at the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria. It was conducted in accordance to the research ethics and guidelines of the Animal Production and Health Department of the institution (FUTA/APH/15/4750). Eight (8) isocaloric and isonitogenous experimental diets **(Table I)** were formulated in a 2 × 4 factorial arrangement such that they have varying inclusion levels of the two sources of copper ($CuSO_4$, CuO). Each was supplemented at four levels of inclusion (0, 100, 200 and 300 mg/kg) **(Table I)**. The formulated diets met the nutrient requirements of laying hens according to NRC recommendations (NRC, 1994). The proximate analyses of the diet samples were carried out according to AOAC (AOAC, 1995). The metabolizable energy (ME) of feed samples was calculated using a prediction equation as follows: ME = (37xCP + 81.8xEE + 35.5xNFE) (Pauzenga, 1985).

Two hundred and twenty-four (224) Bovan Nera pullets of 16 weeks old were sourced from a reputable farm and were kept on deep litter until 20 weeks of age for stabilization before being transferred to the cage. Feed was given according to body weight and age twice daily in line with the Bovan Nera management manual and drinking water was also provided *ad libitum*. All required managerial practices such as strict bio-security measures were ensured and also as at when due, appropriate vaccines and prophylactic treatments were also be administered. The birds were housed in an open-sided building in a thoroughly cleaned, washed and disinfected three tier cage system of 32 x 38 x 42 cm dimension. Four (4) birds were conveniently housed in a unit. They remained on commercial grower mash until they attained 20% hen day production (24 weeks of age) when the experimental diets were introduced. At 24 weeks of age, the birds were then divided into 8 groups of twenty-eight (28) birds and randomly assigned to the eight (8) treatment diets in a 2 x 4 factorial experiment. Each treatment was replicated seven (7) times with 4 birds per replicate. The experimental birds were weighed before the commencement of the experiment. Throughout the sixteen weeks of the experiment, the birds were fed twice daily (morning and afternoon), water was also given *ad-libitum.* For each treatment, eggs were collected and weighed on a daily basis.

Data were also collected for parameters such as haematological and serum biochemicals indices, as well as plasma hormones. At the end of the feeding trial, three birds per replicate (i.e. 21 birds per treatment) were randomly selected for blood collection to determine the serum and blood biochemical properties. Samples of blood were taken between 2 pm and 4 pm from the jugular vein, using sterile and heparinized syringes on the last day of the experiment. Blood samples (5ml) were collected into labeled bottles, one set containing Ethylene diaminetetraacetic acid (EDTA) while the other without EDTA for serum biochemistry. After collection, tubes containing blood for serum analysis were kept at room temperature for two hours and then transferred to a refrigerator and kept overnight at 4°C. It was thereafter centrifuged at 3,000 rpm/15 min. Serum was then harvested and stored at -20°C until further analyses for serum biochemicals indices. The blood samples in the EDTA bottles were used for haematological analyses. Packed Cell Volume, Red Blood Cell, Haemoglobin, White Blood Cell, Total Protein, Globulin and Albumin were determined as described by Tietz (1995). The serum creatinine and urea nitrogen were estimated by deproteinisation and Urease-Berthelot colorimetric methods respectively, using a commercial kit (Randox Laboratories Ltd., U.K.). Also the free cholesterol was determined by nonane extraction and enzymatic colorimetric methods respectively using commercial test kits (Quimica Clinica Applicada, S.A.), while the serum enzymes Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were obtained using the Randox Laboratories Ltd, UK test kits.

The cholesterol levels of the eggs were evaluated using laboratory procedures for determining egg cholesterol as described by Idowu et al. (2004). Ten eggs per treatment were randomly selected for analyses weekly starting from the 10th week of the experiment. The selected eggs were weighed, hard cooked by immersion in boiling water for eight (8) minutes. The yolks were individually removed and individually weighed and oven-dried at 70°C, pooled and blended. Cholesterol determination was done using a commercial test kit for cholesterol analysis (Sigma diagnostic cholesterol reagent procedure No 352'Sigma Chemical

Table II. Haematology of laying hens fed diets supplemented with two sources of copper (Ematologia delle galline ovaiole alimentati con diete integrate con due fonti di rame)

Treatment	Level of Copper (mg)	ESR	Packed Cell Volume (%)	Red Blood Cell	Haemoglobin (g/dl)	Lymphocyte (%)	Heterophils	Monocytes ($x10^3/mm^3$)	Basophils (%)	Eosino-phil ($x10^3/mm^3$)	MCHC (%)	MCV (μ^3)	MCH (pg)
CuO	0	8.00 ± 3.06	27.33 ±3.33	26.97 ±3.27	9.10 ±1.10	62.33±0.67	20.67±1.45	11.67±1.20	3.33±0.03	2.00±0.00	33.30 ±0.03	101.36 ±0.99	33.75 ±0.33
CuO	100	8.33 ± 0.33	23.33 ±0.33	22.60 ±0.82	7.80 ±0.10	63.00±0.58	19.00±1.53	12.67±1.45	3.33±0.33	2.00±0.00	33.43 ±0.05	103.41 ±2.28	34.57 ±0.81
CuO	200	7.33 ± 2.67	24.67 ±1.76	22.73 ±2.98	8.20 ±0.59	63.00±0.58	19.33±1.67	12.67±1.20	3.33±0.33	1.67±0.33	33.24 ±0.05	110.60 ±8.66	36.76 ±2.84
CuO	300	8.33 ± 0.33	23.00 ±1.15	21.03 ±2.06	7.67 ±0.38	63.33±0.88	17.33±1.76	14.33±0.88	3.00±0.00	2.00±0.00	33.34 ±0.08	115.58 ±9.19	37.20 ±3.07
$CuSO_4$	0	9.67 ± 1.20	21.67 ±0.67	17.47 ±1.87	7.23 ±0.23	62.00±0.58	19.67±1.45	12.67±1.45	3.67±0.33	2.00±0.00	33.38 ±0.05	125.92 ±8.77	42.03 ±2.87
$CuSO_4$	100	5.00 ± 2.89	16.67 ±8.41	16.23 ±8.19	5.57 ±2.81	41.33±20.67	14.67±7.33	7.67±3.84	2.00±1.00	1.00±0.58	22.27± 11.14	68.45 ±34.22	22.87 ±11.43
$CuSO_4$	200	7.33 ± 3.71	14.00 ±7.02	10.20 ±5.17	4.67 ±2.34	42.33±21.17	13.00±6.66	8.00±4.16	2.00±1.00	1.33±0.67	22.23± 11.11	91.96 ±46.16	30.67± 15.40
$CuSO_4$	300	8.00 ± 0.00	24.00 ±0.58	22.93 ±1.08	8.00 ±0.17	62.00±0.58	20.67±1.33	12.33±0.88	3.33±0.33	1.67±0.33	33.34 ±0.08	104.89 ±2.61	34.97 ±0.95
Mean separation													
Level of Copper													
0		8.83 ± 1.51	24.50 ±1.98	22.22 ±2.71	8.17 ±0.65	62.17±0.40	20.17±0.95	12.17±0.87	3.50±0.22	2.00±0.00	33.34 ±0.03	113.64 ±6.76	37.89 ±2.26
100		6.67 ± 1.50	20.00 ±4.05	19.42±3.95	6.68 ±1.35	52.17±10.44	16.83±3.49	10.17±2.15	2.67±0.56	1.50±0.34	27.85 ±5.57	85.93 ±17.22	28.72 ±5.76
200		7.33 ± 2.04	19.33 ±4.02	16.47±3.87	6.43 ±1.34	52.67±10.54	16.17±3.38	10.33±2.20	2.67±0.56	1.50±0.34	27.73 ±5.55	101.28±21.41	33.72 ±7.14
300		8.17 ± 0.17	23.50 ±0.62	21.98±1.33	7.83 ±0.20	62.67±0.56	19.00±1.24	13.33±0.71	3.17±0.17	1.83±0.17	33.34 ±0.05	108.24 ±4.52	36.09 ±1.52
Treatment													
CuO		8.00 ± 0.88	24.58 ±0.99	23.33±1.29[a]	8.19 ±0.33	62.92±0.31	19.08±0.77	12.83±0.59	3.25±0.13	1.92±0.08	33.33 ±0.03	106.73 ±3.05	35.57 ±1.01
$CuSO_4$		7.50 ± 1.15	19.08 ±2.63	16.71±2.52[b]	6.37 ±0.88	51.92±7.00	17.00±2.36	10.17±1.45	2.75±0.39	1.50±0.23	27.80 ±3.75	97.81 ±13.91	32.63 ±4.64
Statistical significance													
Treatment		0.757	0.078	0.031	0.079	0.157	0.443	0.114	0.229	0.115	0.179	0.557	0.563
Level		0.785	0.532	0.453	0.532	0.619	0.691	0.459	0.400	0.418	0.587	0.594	0.601
Treatment* Level		0.734	0.573	0.331	0.576	0.607	0.602	0.531	0.266	0.568	0.585	0.559	0.556

[ab]Means with different superscripts within column differ significantly (P<0.05)

Table III. Serum biochemical profiles of laying hens fed diets supplemented with two sources of copper (profili biochimici sierici di galline ovaiole alimentate diete integrate con due fonti di rame)

Treatment	Level of Copper (mg)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin / Globulin ratio	ALT (U/L)	AST (U/L)	Uric Acid (mg/dl)	Creatinine (mg/dl)	Glucose (mg/dl)	Cholesterol (mg/dl)
CuO	0	6.20 ± 0.15	3.03 ± 0.12	3.17±0.13	0.96±0.06	7.6 ± 0.06	167.33 ±1.76	9.97 ± 0.20	1.03±0.03	161.93±8.27	140.87±9.90
CuO	100	5.85 ± 0.18	3.12 ± 0.04	2.73±0.19	1.16±0.09	7.37 ± 0.09	172.33 ±1.20	10.97 ± 0.15	1.03±0.03	151.27±2.18	138.17±5.77
CuO	200	5.83 ± 0.15	3.23 ± 0.17	2.60±0.10	1.25±0.10	7.83 ± 0.04	167.93 ±5.10	7.57 ± 1.32	0.97±0.03	142.63±2.78	118.93±3.27
CuO	300	5.70 ± 0.17	2.60 ± 0.23	3.10±0.21	0.85±0.11	7.33 ± 0.19	169.60 ±6.07	6.13 ± 0.72	0.97±0.07	151.97±3.19	110.40±1.96
$CuSO_4$	0	5.98 ± 0.12	2.95 ± 0.13	3.03±0.24	0.99±0.11	7.77 ± 0.09	170.27 ±2.15	10.20 ± 0.69	0.93±0.03	195.20±15.11	151.77±5.46
$CuSO_4$	100	5.93 ± 0.18	3.20 ± 0.15	2.73±0.33	1.23±0.22	7.77 ± 0.20	173.67 ±1.20	10.97 ± 0.15	1.03±0.03	154.60±1.29	127.17±2.46
$CuSO_4$	200	5.73 ± 0.24	2.70 ± 0.10	3.03±0.28	0.91±0.10	8.61 ± 1.21	175.47±17.57	9.40 ± 1.55	0.97±0.03	148.00±3.75	120.20±3.03
$CuSO_4$	300	6.03 ± 0.07	2.92 ± 0.15	3.12±0.11	0.94±0.08	9.18 ± 1.11	199.13 ±12.81	9.73 ± 1.12	0.97±0.09	142.87±7.66	110.30±2.32
Mean separation											
Level of Copper											
0		6.09 ± 0.10	2.99±0.08	3.10±0.13	0.98±0.06	7.69 ± 0.06	168.80 ±1.40	10.08 ±0.33^{ab}	0.98±0.03	178.57 ±10.71^{a}	146.32±5.61^{a}
100		5.89 ± 0.11	3.16±0.07	2.73±0.17	1.19±0.11	7.57 ± 0.13	173.00 ±0.82	10.97 ±0.09^{a}	1.03±0.02	152.93 ±1.36^{b}	132.67±3.73^{b}
200		5.78 ± 0.13	2.97±0.15	2.82±0.17	1.08±0.10	8.22 ± 0.57	171.70 ±8.35	8.48±1.00^{bc}	0.97±0.02	145.32 ±2.41^{b}	119.57±2.01^{c}
300		5.87 ± 0.11	2.76±0.14	3.11±0.11	0.90±0.07	8.26 ± 0.65	184.37 ±9.15	7.93±1.00^{c}	0.97±0.05	147.42 ±4.23^{b}	110.35±1.36^{c}
Treatment											
CuO		5.90 ± 0.09	3.00 ± 0.10	2.90± 0.10	1.06 ± 0.06	7. 53 ± 0.08	169.30 ±1.85	8.66±0.66^{b}	1.00±0.02	151.95±2.90	127.09±4.64
$CuSO_4$		5.92 ± 0.08	2.94 ± 0.08	2.98 ±0.12	1.02± 0.07	8.33 ± 0.40	179.63 ±5.80	10.08±0.47^{a}	0.98±0.03	160.17±7.25	127.36±4.86
Statistical significance											
Treatment		0.831	0.605	0.609	0.651	0.074	0.096	0.041	0.477	0.117	0.940
Level		0.313	0.090	0.223	0.122	0.546	0.289	0.014	0.490	0.0007	<0.0001
Treatment* Level		0.384	0.059	0.589	0.268	0.514	0.325	0.204	0.669	0.050	0.220

^{abc}Means with different superscripts within column differ significantly (P<0.05)

Co., St Louis, MO, USA). All sample extracts were analyzed in triplicates. Cholesterol concentrations were determined from the absorbance read at 500 nm using a spectrophotometer (Idowu et al., 2004). Quantification of Luteinizing hormone (LH), progesterone, oestrogen, testosterone and follicle stimulating hormone (FSH) were performed by radioimmunoassay procedure as described by Williams and Harvey (1986).

Data collected were subjected to 2 factors in 2 by 4 factorial procedures of SAS (2008). Where the analysis of variance indicated significant treatment effect, the means were compared using Duncan's Multiple Range Test of the same software. The procedure was reviewed and approved by the Federal University of Technology Akure Animal Care and Use Committee.

RESULTS AND DISCUSSION

Haematology

The results of the haematology **(Table II)** between the birds on 200 mg and 300 mg/kg diet CuO supplementation showed significant difference ($P < 0.05$) in the Erythrocyte Sedimentation Rate (ESR) Lymphocyte, Monocytes, Eosinophil, Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) while the birds in the control group had the lowest value for MCH, MCV, Monocytes and Lymphocyte. However, the birds on control diet had the highest values for packed cell volume (PCV), Red blood cell (RBC) and Heamoglobin (Hb). Furthermore, there was significant decrease in the values for PCV, RBC and Hb in birds on 300mg copper oxide diet inclusion level.

It was observed from the result that there was significant difference ($P<0.05$) between the treatments for RBC. However, there was no significant difference ($P>0.05$) among the levels and the interactions. Contrary to the report of Agunbiade and Babatunde (1995) that suggested that in laying hens, as concentration of dietary copper increased in ration, the concentration of haemoglobin also increased, it was observed in this study that increased concentration of copper in the diets of the hens did not consistently increase the concentration of haemoglobin. From the result, it was observed that there was relative increase in the concentration of the packed cell volume (PCV) and haemoglobin (Hb) from 200mg to 300mg inclusion level. This result agreed with the report of Zia-Ur-Rahman *et al* (2001) that also discovered an increased packed cell volume and haemoglobin in broiler chicken fed an increased level of dietary $CuSO_4$ but differed totally from the findings of Samanta et al. (2011) who reported that dietary $CuSO_4$ inclusion above 150mg in broilers reduced the haemoglobin and packed cell volume concentration but agreed that no significant difference ($P>0.05$) was observed in the concentrations of heterophils, monocytes, lymphocytes and basophils and eosinophils. It was observed that the erythrocyte (red blood cell) count reduced as copper concentration increased though not linearly Samanta et al. (2011). The results also showed that dietary copper in the excess of 200 mg/kg was capable of reducing Hb concentration in blood. This also agreed with the finding of Samanta et al. (2011) that postulated that excess copper above 250 mg/kg significantly reduced Hb concentration. Swenson and Reece (1996) equally reported that excess of dietary copper results in an accumulation of copper in the liver with decreased blood Hb concentration and packed cell volumes. Ozcelik et al. (2002) reported that similar effects in Wister albino rats fed $CuSO_4$ above 250 mg/kg.

Serum biochemistry

It was observed that the birds in the control group had the highest values for total protein, globulin, creatinine, glucose and cholesterol for CuO. The lowest values for these parameters for the birds on CuO diets were recorded at 300, 200, 300, 200 and 300mg inclusion levels respectively as shown in **Table III**. Furthermore, the total cholesterol for birds on both treatments decreased linearly as the concentration of copper increased. Also the total protein of birds on copper oxide treatment showed a linear decrease as concentration increased. However, in $CuSO_4$, though the same pattern was observed in the birds for total protein, the value obtained at 300mg level of inclusion increased over and above those at 100 and 200mg levels of inclusion and also the control to deviate from the observed trend in birds on copper oxide. It was observed in the birds placed on copper sulphate supplementary diet, that the 300mg group had the highest values for total protein, globulin, alanine trasaminase (ALT) and aspertate transaminase (AST) whereas the lowest values for these parameters were observed in birds in 200mg, 100mg, 100mg and control groups respectively. This agreed with the findings of Zia-ur-Rahman et al. (2001) which reported that supplement of Cu at a dose of 300 mg/kg significantly increased the ALT and AST enzyme system. The observations of Chiou et al. (1997) and Chen et al. (1997) are also in agreement with this finding. They reported an increase in enzymes level as a result of very high doses of dietary $CuSO_4$. They concluded that higher level of Cu accumulation might have damaged the liver to increase the presence of these enzymes. It was also observed that birds on 100mg copper sulphate diet had the highest value for albumin, albumin/ globulin ratio, uric acid and creatine but the lowest values for these parameters albumin, albumin/globulin ratio and uric acid were observed in birds fed copper sulphate diet at 200mg inclusion level. For creatinine, the lowest value was observed in birds on control diet which was 0.93mg/dl.

From the result, it was observed that there was significant difference ($P<0.05$) between treatments only for uric acid. However, significant differences ($P<0.05$) were observed at levels of inclusion for uric acid, glucose and cholesterol. It was also observed that there was a consistent reduction in the values of total cholesterol and serum total protein in birds as the level of inclusion of dietary CuO increases. This supports the reports of Lien et al. (2004), Abaza et al. (2009); Elsayed and Wakwak (2010) and Samata et al. (2011) that reported significant reductions in plasma total cholesterol and triglyceride in the chickens fed increasing level of dietary copper sulphate. The result obtained for total cholesterol in birds placed on $CuSO_4$ diet also is in support of Abaza et al. (2009) but the result for serum total protein deviated from the findings of Abaza et al.

Table IV. Hormone levels of laying hens fed diets supplemented with two sources of copper (i livelli ormonali delle galline ovaiole alimentate diete integrate con due fonti di rame).

Treatment	Level of Copper (mg)	Luteinizing Hormone	Progesterone	Estrogen	Testosterone	Follicle Stimulating Hormone
CuO	0	3.64 ± 0.02	1.52 ± 0.01	457.49 ± 2.62	20.58± 0.41	4.52 ± 0.04
CuO	100	3.66 ± 0.05	1.52 ± 0.00	459.10 ± 1.50	21.08 ± 0.84	4.57 ± 0.01
CuO	200	3.64 ± 0.04	1.52 ± 0.01	459.38 ± 3.05	21.35 ± 0.68	4.59 ± 0.01
CuO	300	3.65 ± 0.02	1.52 ± 0.01	456.39 ± 1.64	20.59 ± 0.40	4.54 ± 0.05
$CuSO_4$	0	3.60 ± 0.01	1.56 ± 0.03	460.13 ± 0.58	22.13 ± 0.32	4.59 ± 0.03
$CuSO_4$	100	3.67 ± 0.04	1.53 ± 0.01	457.98 ± 3.26	20.73 ± 0.57	4.59 ± 0.02
$CuSO_4$	200	3.60 ± 0.01	1.52 ± 0.01	457.69 ± 2.27	21.57 ± 0.50	4.58 ± 0.01
$CuSO_4$	300	3.64 ± 0.01	1.54 ± 0.01	461.13 ± 1.60	21.58 ± 0.41	4.62 ± 0.01
Mean separation						
Level of Copper						
0		3.62 ± 0.01	1.54 ± 0.02	458.81 ± 1.34	21.36 ± 0.42	4.55 ± 0.03
100		3.67 ± 0.03	1.52 ± 0.01	458.54 ± 1.62	20.90 ± 0.46	4.58 ± 0.01
200		3.62 ± 0.02	1.52 ± 0.01	458.54 ± 1.74	21.46 ± 0.38	4.59 ± 0.01
300		3.64 ± 0.01	1.53 ± 0.01	458.76 ± 1.47	21.09 ± 0.34	4.58 ± 0.03
Treatment						
CuO		3.65 ± 0.02	1.52 ± 0.00	458.09 ± 1.05	20.90 ± 0.28	4.56 ± 0.02
$CuSO_4$		3.63 ± 0.01	1.54 ± 0.01	459.09 ± 1.02	21.50 ± 0.25	4.60 ± 0.01
Statistical significance						
Treatment		0.4476	0.0687	0.4786	0.1363	0.0532
Level		0.3925	0.4863	0.9989	0.7283	0.6114
Treatment* Level		0.8057	0.3909	0.4404	0.3414	0.3476

(2009) and Wu et al. (2014) because the value obtained at 300mg inclusion level was higher than the values obtained for the control and other inclusion levels. The result obtained that showed decrease in values of blood sugar, total serum protein and total cholesterol as level of dietary copper increased supports the report of Zia-ur-Rahman et al. (2001) that dietary copper in the feed of broiler increased the total serum protein and cholesterol. They further postulated that a decrease in the plasma glucose concentration in Cu-supplemented birds might be due to decreased feed intake, increased packed cell volume, and haemoglobin. Paik et al. (1999) opined that high level of Cu reduced glutathion (GSH) that reduced stimulation of HMG-CoA reductase activity to reduce cholesterol synthesis.

Hormonal changes

From the hormonal profile results, there was no statistical difference between and among the treatments and also for the interaction. Progesterone production has been attributed to continuous flow of luteinizing hormone (Cooper et al., 1986). This implies that as the there is a direct relationship between amount of luteinizing hormone and progesterone. The result obtained in this study is in agreement with this as the values obtained for the progesterone are fairly in proportion with the values obtained for luteinizing hormone for both treatments at the different levels. The results obtained for copper sulphate in perfectly in support of Kuoppala (2015) as there was an increase in value of testosterone as the concentration of copper sulphate increased and also in birds fed copper oxide except for a deviation at 300mg inclusion level. Copper forms complex with gonadotropin-releasing hormone. This complex (Cu-GnRH) was capable of releasing FSH (Cooper et al., 1986). Also according to Michaluk and Kochman (2007), reported that copper complexes with GnRH are more effective than native GnRH in the release of LH and FSH from the anterior pituitary *in vivo*. This accounts for the trend observed in the results obtained for both copper oxide and copper sulphate which is in agreement with the above postulations. This also explains why the values observed in birds fed supplemented copper diets for follicle stimulating hormone are higher than the values for the control of the respective treatment. Pharmacological levels of Cu (>250mg/kg diet) was reported to cause changes in 17 ß-estradiol and enzymes involved in lipid and amino acid metabolism in matured hens (Pesti and Bakalli, 1998). This explains the changes observed in the trend of the values seen in the result. However, since estrogen is a steroid hormone and copper reduces cholesterol level of serum, it was expected that as the concentration of copper in diet increased, the estrogen level should decrease. The values obtained in birds fed copper sulphate followed the trend that is as copper concentration increased, value of estrogen decreased but there was a deviation from this trend at 300mg inclusion level. However, for copper oxide, the value of estrogen was only lower than that of the control at

Table V. Blood Viscosity of laying hens fed diets supplemented with two sources of copper (Viscosità del sangue delle galline ovaiole alimentate diete integrate con due fonti di rame)

Treatment	Level of Copper (mg)	Whole Blood	Blood Serum	Blood Plasma
CuO	0	1.91 ± 0.01	1.27 ± 0.79	1.02 ± 0.98
CuO	100	1.94 ± 0.01	1.32 ± 0.01	1.04 ± 0.01
CuO	200	1.94 ±0.01	1.33 ± 0.01	1.05 ± 0.00
CuO	300	1.95 ±0.01	1.35 ± 0.01	1.06 ± 0.01
$CuSO_4$	0	1.91 ±0.01	1.28 ± 0.00	1.02 ± 0.01
$CuSO_4$	100	1.94 ± 0.01	1.33 ± 0.01	1.06 ± 0.01
$CuSO_4$	200	1.97 ± 0.01	1.35 ± 0.01	1.07 ± 0.01
$CuSO_4$	300	1.96 ± 0.01	1.36 ± 0.01	1.07 ± 0.01
Mean separation				
Level of Copper				
0		1.91 ± 0.00[b]	1.27 ± 0.00[c]	1.02 ± 0.00[b]
100		1.94 ± 0.01[a]	1.33 ± 0.01[b]	1.05 ± 0.01[a]
200		1.96 ± 0.01[a]	1.34 ± 0.01[b]	1.06 ± 0.00[a]
300		1.96 ±0.01[a]	1.36 ± 0.01[a]	1.07 ± 0.01[a]
Treatment				
CuO		1.94 ± 0.01	1.32 ± 0.01[b]	1.04 ± 0.01
$CuSO_4$		1.95 ± 0.01	1.33 ± 0.01[a]	1.06 ± 0.01
Statistical significance				
Treatment		0.139	0.022	0.054
Level		0.0001	<0.0001	0.0003
Treatment* Level		0.263	0.801	0.842

[abc]Means with different superscripts within column differ significantly (P<0.05)

300mg inclusion but the values obtained at 200mg and 200mg inclusion levels were higher than the values obtained for the control.

Blood viscosity

The blood viscosity of laying hens fed diets supplemented with the two sources of copper is shown in **Table V**. It was observed from the result that the birds fed copper oxide diet at 300mg inclusion level consistently had the highest values for whole blood, blood serum and blood plasma. The lowest values for the three parameters were observed in the birds on control diet. From the result, it was observed that the values of viscosity for whole blood, blood serum and blood plasma in birds fed supplementary copper sulphate diet were higher than those of copper oxide at all the inclusion levels except at 100mg inclusion level for both treatments where the values were the same. It was also observed that the levels of copper inclusion had significant difference (P<0.05) for all the three blood viscosity parameters; blood serum, whole blood and blood plasma. However, for treatment, significant difference (P<0.05) was only observed in blood serum. There was no significant difference (P>0.05) in the interaction. Contrary to the report of Sushil and Darry (1988) that as the dietary copper increased, the blood viscosity reduced in rats, the result of this study showed that blood viscosity generally increased as the concentration of dietary copper increased for both dietary copper oxide and copper sulphate. This implied that copper diets enhance blood viscosity.

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