

## CAN COPPER AMINO ACID CHELATES REDUCE THE PHYSIOLOGICAL STRAIN OF GROWING RABBITS UNDER SUMMER CONDITIONS?

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**Abstract:** This study was conducted at the Poultry Research Centre, Faculty of Agriculture, Alexandria University, Egypt, during the summer season (July and August months) 2018. For five weeks, until 63 d of age, a total number of 140 weaned V line rabbits were randomly distributed into seven groups of 20 rabbits each. Rabbits of groups 2 and 3 were fed basal diet plus copper sulphate (100 and 200 mg *Cu-Sul*/kg, respectively). Groups 4 and 5 were fed basal diet plus copper methionine (100 and 200 mg *Cu-Meth*/kg, respectively) and groups 6 and 7 were fed basal diet plus copper glycine (100 and 200 mg *Cu-Gly*/kg, respectively), while the first group was fed basal diet only as control. Rabbits fed diet supplemented with higher level (200 mg/kg) of *Cu-Meth* or *Cu-Gly* chelates had significantly higher body weight and daily body weight gain ( $P<0.05$ ) compared to those fed diets supplemented with both levels of *Cu-Sul*. Rabbits fed diets supplemented with *Cu-Meth* or *Cu-Gly* chelates had significantly increased ( $P<0.05$ ) red blood cells, lymphocyte, high-density lipoprotein and Cu values in blood. Within the studied Cu sources, serum total lipids values of rabbits were significantly lower than in the control group. However, serum total antioxidant capacity, glutathione peroxidase and superoxide dismutase were higher by both organic chelates studied than other groups. In general, copper supplementation improves productive performance and physiological status under summer conditions.

**Key Words:** rabbit, heat stress, copper chelates, performance, antioxidants.

### INTRODUCTION

Rabbits are highly sensitive to heat stress because they are unable to sweat and rely on vasomotor control and panting to dissipate excess body heat (Zeferino *et al.*, 2013). When rabbits are exposed to elevated ambient temperatures, imbalances are induced in their body, adversely affecting their growth and reproductive traits (Okab *et al.*, 2008). One of the most important factors that help rabbits deal with environmental and psychological stressors, including heat, cold and unhealthy practices, is nutrition (McWilliams, 2001). There are several conflicting results as to whether dietary copper supplementation improves performance in rabbits, particularly those under stress due to poor husbandry and underlying disease challenges (Amy, 2010). When chelated amino acids are used in the preparation of copper amino acid chelates, methionine is the most common factor. For example, copper methionine chelates, with a relative bioavailability of 88 to 147% in poultry, pigs, sheep or cattle (Miles *et al.*, 2003).

Some authors have suggested that the mineral components of organic syntheses (chelates) can be better absorbed than those in mineral combinations (Liotta *et al.*, 2009), including minimising management of minerals and, consequently, the cost of breeding. Another example is copper glycine chelate; enterocytes improve copper absorption in laying hens and minimise the release of the element into the environment (Kaya *et al.*, 2018).

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<https://doi.org/10.4995/wrs.2022.13848>

**Cite as:** Goodb F., Soliman F., Elghalid O., Abd El-hady A.M. 2022. Can copper amino acid chelates reduce the physiological strain of growing rabbits under summer conditions? *World Rabbit Sci.*, 30: 277-286. <https://doi.org/10.4995/wrs.2022.13848>

This aim of this research was to evaluate the ability of different levels of copper substances from different sources in feed to alleviate physiological strain on growing rabbits raised under summer conditions.

## MATERIAL AND METHODS

This study was conducted at the Poultry Research Centre, Poultry Production Department, Faculty of Agriculture (El-Shatby), Alexandria University, Egypt, during the summer season (July and August months) 2018.

### Preparation of Cu chelates

Three sources of supplemental Cu were used in the current study, where: Copper sulphate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 98% ( $\text{Cu-SO}_4$ ; contains 25% Cu) working as inorganic form, copper methionine chelate (*Cu-Meth*; contains 47.35% Cu) and copper glycine chelate (*Cu-Gly*; contains 47.35% Cu) as organic form. *Cu-Meth* and *Cu-Gly* chelates were prepared in the laboratory by reacting D-methionine or Glycine amino acids and copper sulphate at a molar ratio 1:1 for a reaction to occur to which 50% NaOH solution was gradually added to increase pH up to 7 for maximum precipitation. Precipitate was separated, dried in an oven at 50°C for 2 d and then made into a powder, which was tested for Cu by analysis. When the precipitates were dissolved in water, the amounts of ionic form of Cu ( $\text{Cu}^{2+}$ ) were quantified by copper electrode. A half of dissolved *Cu-Meth* or *Cu-Gly* were found to be disassembled into an ionic form and the remaining other half of dissolved *Cu-Meth* or *Cu-Gly* were in a stable form, which were considered to be chelated (Lim and Paik, 2006).

**Table 1:** Ingredient (g/kg) and chemical composition (g/kg dry matter) of the basal diet.

Ingredients	Basal diet
Ground barley	135
Yellow corn	80
Wheat bran	240
Soybean meal 44%	180
Clover straw	34
Alfalfa hay	280
Molasses	27
Limestone	10
Di-calcium phosphate	6
Sodium chloride	4
DL-methionine	1
Premix <sup>1</sup>	3
Chemical composition	
Crude protein	178.9
Crude fat	37.8
Crude fibre	138.8
DE (kcal/kg) <sup>2</sup>	2530

<sup>1</sup>Each 1 kg of rabbit's premix contained: vitamin A 12 000 000 IU; vitamin D3 2 000 000 IU; vitamin E 10 000 mg; vitamin K3 2000 mg; vitamin B1 1000 mg; vitamin B2 5000 mg; vitamin B6 1500 mg; vitamin B12 10 mg; Biotin 50 mg; Choline Chloride 250 000 mg; Pantothenic acid 10 000 mg; Nicotinic acid 30 000 mg; Folic acid 1000 mg; Iron 30 000 mg; Copper 10 000 mg; Manganese 60 000 mg; Iodine 1000 mg; Selenium 100 mg; Cobalt 100 mg; Zinc 50 000 mg and Antioxidant, 1000 mg to 3000 g.

<sup>2</sup>DE: Digestible energy was calculated according to Schneider and Flatt (1975).

### Animals and design

A total of 140 weaned V line rabbits unsexed at an average age of four weeks were used in this study and initial body weight range between 547.4 to 553.5 g each were randomly distributed into seven groups of 20 rabbits. Each group was subdivided into 5 replicates of 4 rabbits each per cage. The experiment lasted five weeks after weaning to 63 d of age. Rabbits of group 1 (T1) were fed basal diet without any supplementation as a control, while rabbits of groups 2 (T2) and 3 (T3) were fed basal diet plus copper sulphate (100 and 200 mg *Cu-Sul*/kg, respectively), and groups 4 (T4) and 5 (T5) were fed basal diet plus copper methionine (100 and 200 mg *Cu-Meth*/kg, respectively) and groups 6 (T6) and 7 (T7) were fed basal diet plus copper glycine (100 and 200 mg *Cu-Gly*/kg, respectively). Basal diet in Table 1 was formulated to cover the nutrient requirements of growing rabbits recommended according to (NRC, 1994) and the chemical composition of the basal diet was analysed based on (AOAC, 1995). The dietary treatments were a control diet with 18.43 mg Cu/kg diet (from raw materials and premix), and six experimental treatments where basal diet was supplemented with 100 or 200 mg/kg *Cu-Sul* (with 43.4 and 68.4 mg Cu/kg diet), *Cu-Meth* (with 65.8 and 113.1 mg Cu/kg diet) or *Cu-Gly* (with 65.8 and 113.1 mg Cu/kg diet). The determination of Cu concentration was done in various ingredients of basal diet and the concentrations were as follows: ground barley 1.12, yellow corn 0.20, wheat bran 3.35, soybean meal 2.30, clover straw 0.12, alfalfa hay 1.34

and premix 10 mg/kg with a total concentration 18.43 mg/kg diet by atomic absorption spectrometry at the Central Laboratory of Faculty of Agriculture, Alexandria university, Egypt.

### Managements and observations

Rabbits were housed in galvanised wire cage batteries with standard dimensions of 50×45×40 cm and an elevation of 110 cm from the floor, enough for 4 rabbits per cage in a well-ventilated open system rabbitry with feeders, automatic water system and kept under the same management, hygienic and environmental conditions in summer conditions. The means of ambient temperature and relative humidity were recorded daily and registered from July to August using an automatic hydro thermograph-computerised system (Data Logger-Log 100/10, Germany) located in the farm. Their maximum daily value at 12 pm was used to estimate the daily mean of each parameter. Overall means of temperature, relative humidity and temperature-humidity index are shown in Table 2. The temperature-humidity index (THI) equation (LPHSI, 1990) modified by Marai *et al.* (2001) was used in calculation in the present study, as follows:

$$THI = db\text{ }^{\circ}\text{C} - \{(0.31 - 0.31(RH)) \times (db\text{ }^{\circ}\text{C} - 14.4)\}$$

Where: db °C=dry bulb temperature, RH=relative humidity percentage/100. The overall mean values of ambient temperature, relative humidity and THI were 30.12°C, 82.40% and 29.27, respectively. The mean values of THI acquired in this study (29.27) were classified as severe heat stress (28.9 to <30.0 category) throughout the experimental period.

### Data collected

During the five-week trial period, individual body weight (BW) and feed conversion ratio (FCR) were recorded weekly, while daily body weight gain (DBWG) and daily feed consumption (DFC) was calculated daily. The dead rabbits were recorded throughout the experimental period for each treatment, and then the survival rate was calculated as the ratio of the number of living rabbits at the end of the experiment to the number of rabbits at the beginning of the study.

About 3 mL of blood samples were collected between 8.00-9.00 h a.m. at 63 d of age from the marginal ear vein into vacutainer tubes with or without K3-EDTA (1 mg/mL); coagulated blood samples were centrifuged at 4000 rpm for 15 min and the clear serum was separated and stored in a deep freezer at -20°C until blood biochemical analysis. Non-coagulated blood was divided into two parts. The first part was centrifuged and the clear plasma was separated and stored in the same way as before, where the second part of each sample was used to evaluate the haematological parameters including red blood cell count (RBCs), percentage of packed corpuscular volume (PCV), white blood cells (WBCs) and the differential counts of lymphocytes (L), neutrophils (N) and the ratio between them (N/L) according to Feldman *et al.* (2000). Haemoglobin (Hb) concentration was measured according to Provan *et al.* (2004).

Serum total protein and albumin (g/100 mL) were measured by spectrophotometer using special kits delivered from sentinel CH Milano, Italy (Beckman DU-530, Germany). With the difference between total protein and albumin the blood globulin level (g/100 mL) was calculated, as fibrinogen usually contains a small fraction. Serum total lipids, triglyceride, total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were calculated using

**Table 2:** Overall means of temperature °C (Temp), relative humidity % (RH) and temperature-humidity index (THI) during the experimental period of growing rabbits fed diets supplemented with different sources of copper under summer conditions.

Month		Temp (°C)	RH (%)	THI
July	First week	31.4	84	30.56
	Second week	30.6	83	29.75
	Third week	30.2	86	29.51
	Forth week	29.3	80	28.38
August	First week	29.1	79	28.14
Overall means		30.12	82.40	29.27

Temp °C: temperature; RH%: relative humidity; THI: temperature-humidity index.

colorimetric method by commercial kits obtained from Reactivos GPL, Barcelona, Spain. The transaminase enzyme activities of serum aspartate amino transferase (AST) and alanine amino transferase (ALT) were determined by calorimetric method. Serum creatinine, calcium (Ca), inorganic phosphorus (IP) and copper (Cu) were assayed by a colorimetric method using commercial kits from Sclavo Diagnostics Company (Kite Italia S.P.A.). Serum iron (Fe), malondialdehyde (MDA), total antioxidant capacity (TAC), glutathione peroxidase and superoxide dismutase (SOD) level were assayed by a colorimetric method using commercial kits of Bio Systems S.A, Barcelona (Spain).

**Data analysis**

The experiment was set in a completely randomised design. Data were examined by analysis of variance using the general liner model procedure in SPSS® statistical software (SPSS, 2016). Differences among means were determined using Duncan’s test (Duncan, 1955).

The statistical model used was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where  $Y_{ij}$  is observation of the statistical measured,  $\mu$  is overall mean,  $T_i$  is effect of treatment,  $e_{ij}$  is the experimental random error.

Survival rate was analysed using a chi-square procedure.

**RESULTS**

**Productive performance traits**

The effect of productive performance traits of growing rabbits fed diets supplemented with different substances of copper (Cu) under summer condition, are presented in Table 3. Within the studied Cu sources, the rabbits fed diet supplemented with *Cu-Meth* or *Cu-Gly* chelates had, in general, higher BW compared with those fed *Cu-Sul* in their diet. However, rabbits fed diet supplemented with higher level (200 mg/kg) of *Cu-Meth* or *Cu-Gly* chelates presented significant ( $P < 0.05$ ) superiority (1868 and 1853 g, respectively) over those fed diet supplemented with both levels of *Cu-Sul*. These results reflect that the two organic Cu studied, especially at the higher level (200 mg/kg), promoted the BW of growing rabbits more than inorganic *Cu-Sul*. Daily body weight gain (DBWG) of rabbits fed diet supplemented with 100 or 200 mg/kg levels of *Cu-Sul* had insignificant differences with those fed the lower level of *Cu-Gly* (36.00, 36.25 and 36.81 g/d, respectively). This result revealed that the two organic Cu studied, especially at the higher level (200 mg/kg), supported the DBWG of growing rabbits more than inorganic *Cu-Sul*. The rabbit DFC during the whole experimental period for all studied treatments ranged between 103.8 and 108.9 g/d, with insignificant ( $P > 0.05$ ) differences among them.

**Table 3:** Means±standard error of some productive performance traits from 28 to 63 d of age of growing rabbits fed diets supplemented with different sources of copper under summer condition.

Traits	Treatments							SEM	P-value
	T1	T2	T3	T4	T5	T6	T7		
BW at 63 d (g)	1729 <sup>a</sup>	1815 <sup>b</sup>	1829 <sup>b</sup>	1849 <sup>bc</sup>	1868 <sup>c</sup>	1837 <sup>b</sup>	1853 <sup>c</sup>	22	0.002
DBWG (g/d)	33.65 <sup>a</sup>	36.00 <sup>b</sup>	36.26 <sup>b</sup>	37.21 <sup>c</sup>	37.61 <sup>c</sup>	36.81 <sup>bc</sup>	37.12 <sup>c</sup>	0.63	0.022
DFC (g/d)	106.7	107.0	108.3	106.9	108.9	103.8	107.0	1.1	0.127
FCR	3.17 <sup>b</sup>	2.98 <sup>ab</sup>	2.99 <sup>ab</sup>	2.88 <sup>a</sup>	2.90 <sup>a</sup>	2.83 <sup>a</sup>	2.88 <sup>a</sup>	0.06	0.029
Survival (%)	85.0	90.0	90.0	95.0	90.0	95.0	95.0	-	0.902

<sup>a,b,c</sup>Different letters in the same row indicate significant differences ( $P < 0.05$ ), according to Duncan test.

BW: body weight; DBWG: daily body weight gain; DFC: daily feed consumption; FCR: feed conversion ratio; SEM: Standard error mean. T1: fed basal diet without any supplementation (control), T2: basal diet+copper sulphate 100 ppm (100 mg/kg), T3: basal diet+copper sulphate 200 ppm (200 mg/kg), T4: basal diet+copper methionine 100 ppm (100 mg/kg), T5: basal diet+copper methionine 200 ppm (200 mg/kg), T6: basal diet+copper glycine 100 ppm (100 mg/kg), T7: basal diet+copper glycine 200 ppm (200 mg/kg).

These results indicated that the studied supplemented Cu, whether organic or inorganic and regardless of addition level, had no impact on DFC of growing rabbits under summer condition. Within the Cu sources studied, the rabbits fed diet supplemented with organic *Cu-Meth* or *Cu-Gly* chelates presented insignificant better FCR (ranged between 2.83 and 2.90) compared with those fed inorganic *Cu-Sul* in their diet (ranged between 2.98 and 2.99). However, rabbits fed diet supplemented with both levels of *Cu-Meth* or *Cu-Gly* chelates had significantly ( $P<0.05$ ) improved FCR values compared to those of the control group. Throughout the experimental period, no significant differences were observed in the survival rate between the different treatments.

### Blood haematological parameters

In the present results, there was a significant increase ( $P<0.05$ ) in the RBC values obtained with the organic Cu source and an insignificant effect ( $P>0.05$ ) with the inorganic *Cu-Sul* source compared to the control value. There was also an insignificant ( $P>0.05$ ) increase in Hb and PCV values in Table 4. Within the studied Cu sources, the rabbits fed diet supplemented with *Cu-Meth* or *Cu-Gly* chelates had significantly higher lymphocyte (L) values (ranged between 38.75 and 45.00%) compared with the corresponding control value (35.50%). The neutrophils (N) and N/L ratio decreased with all studied Cu sources. However, the 200 mg/kg level of *Cu-Meth* treatment had a significantly lower N/L ratio (1.12) compared with that of the control group (1.65).

### Blood biochemical parameters

Within the studied Cu sources, the total protein values of different sources of Cu significantly exceeded ( $P<0.05$ ) (ranged between 5.81 and 6.25 g/100 mL) the corresponding control group value (5.34 g/100 mL). The rabbits fed diet supplemented with *Cu-Meth* or *Cu-Gly* chelates had significantly higher ( $P<0.05$ ) albumin values (ranged between 3.45 and 3.54 g/100 mL) compared with the control value (3.15 g/100 mL). In contrast, the albumin values of rabbits fed diet supplemented with inorganic *Cu-Sul* of both studied levels showed insignificant differences ( $P>0.05$ ) compared with the control value.

Within the studied Cu sources, the total lipids values of rabbits fed different sources of Cu was significantly lower ( $P<0.05$ ), ranged between 240.8 and 266.3 mg/100 mL, than the control group value of 297.5 mg/100 mL. Likewise, the rabbits fed diet supplemented with *Cu-Meth* or *Cu-Gly* chelates had insignificant lower ( $P>0.05$ ) total

**Table 4:** Means±standard error of blood constituent of growing rabbits fed diets supplemented with different sources of copper under summer condition.

Blood constituent	Treatments							SEM	P-value
	T1	T2	T3	T4	T5	T6	T7		
RBCs ( $10^6$ /mL)	4.83 <sup>a</sup>	5.13 <sup>ab</sup>	5.20 <sup>ab</sup>	6.08 <sup>b</sup>	6.15 <sup>b</sup>	6.05 <sup>b</sup>	6.00 <sup>b</sup>	0.18	0.028
Hb (g/100 mL)	10.03	11.55	11.68	11.93	12.03	11.88	11.98	0.55	0.279
PCV (%)	33.20	35.80	37.58	41.40	41.70	40.23	40.93	3.01	0.576
WBCs ( $10^3$ /mL)	5.85	6.50	6.65	6.83	7.00	6.75	7.00	0.32	0.244
Lymphocyte (%)	35.50 <sup>a</sup>	38.75 <sup>ab</sup>	39.75 <sup>ab</sup>	42.25 <sup>bc</sup>	43.75 <sup>b</sup>	43.00 <sup>bc</sup>	45.00 <sup>c</sup>	1.36	0.002
Neutrophils (%)	58.50	54.75	52.75	50.50	49.00	53.00	52.50	2.37	0.272
Neutro/Lymph ratio	1.65 <sup>c</sup>	1.41 <sup>bc</sup>	1.33 <sup>ab</sup>	1.20 <sup>ab</sup>	1.12 <sup>a</sup>	1.23 <sup>ab</sup>	1.17 <sup>ab</sup>	0.09	0.007
T. protein (g/100 mL)	5.34 <sup>a</sup>	5.85 <sup>b</sup>	5.81 <sup>bc</sup>	6.25 <sup>d</sup>	6.20 <sup>cd</sup>	6.17 <sup>cd</sup>	6.11 <sup>bc</sup>	0.12	0.002
Albumin (g/100 mL)	3.15 <sup>a</sup>	3.19 <sup>ab</sup>	3.24 <sup>abc</sup>	3.52 <sup>c</sup>	3.54 <sup>c</sup>	3.45 <sup>bc</sup>	3.52 <sup>c</sup>	0.08	0.019
Globulin (g/100 mL)	2.19	2.66	2.57	2.73	2.66	2.72	2.59	0.11	0.091

<sup>a,b,c</sup>Different letters in the same row indicate significant differences ( $P<0.05$ ), according to Duncan test.

RBCs: red blood cells; Hb: haemoglobin; PCV: Packed cell volume; WBCs: White blood cells; T. protein: Total protein; T1: fed basal diet without any supplementation (control), T2: basal diet+copper sulphate 100 ppm (100 mg/kg), T3: basal diet+copper sulphate 200 ppm (200 mg/kg), T4: basal diet+copper methionine 100 ppm (100 mg/kg), T5: basal diet+copper methionine 200 ppm (200 mg/kg), T6: basal diet+copper glycine 100 ppm (100 mg/kg), T7: basal diet+copper glycine 200 ppm (200 mg/kg).

lipids values (ranged between 240.8 and 254.0 mg/100 mL) compared with that for both inorganic *Cu-Sul* groups (266.3 and 257.8 mg/100 mL). In regard to cholesterol trait, the values of rabbits fed different sources of Cu were significantly lower ( $P<0.05$ ), ranged between 66.50 and 81.00 mg/100 mL, than the corresponding control group value of 91.25 mg/100 mL. Similarly, the rabbits fed diet supplemented with both levels of *Cu-Meth* chelates had lower cholesterol values (70.00 and 66.50 mg/100 mL for 100 and 200 mg levels, respectively) in Table 5, with insignificant differences ( $P>0.05$ ) with the corresponding values of both level of *Cu-Gly* chelates and 200 mg level of inorganic *Cu-Sul* treatments.

Regarding HDL values, the rabbit fed different sources of Cu presented significantly higher values (ranged between 40.25 and 47.02 mg/100 mL) than the control group value (38.25 mg/100 mL). The rabbits fed diet supplemented with *Cu-Meth* or *Cu-Gly* chelates generally had higher HDL values (ranged between 42.25 and 47.02 mg/100 mL) compared with those for both *Cu-Sul* groups (40.25 and 41.75 mg/100 mL). In all studied Cu sources, the HDL value was increased slightly by increasing the level of Cu. Rabbits fed a higher level of *Cu-Meth* had, in general, the lowest total lipids and cholesterol values, and the highest HDL value among all treatments.

Supplementation with different sources of Cu had no effect on the serum AST and ALT values. Data in Table 5 showed that the rabbits fed the diet supplemented with a higher level of inorganic *Cu-Sul* had a significantly higher ( $P<0.05$ ) creatinine value (1.08 mg/100 mL) compared with the control value and other treatments.

The Ca, IP and Fe values ranged in Table 5 between 10.02 and 11.05 mg/100 mL, 4.35 and 5.96 mg/100 mL and 20.38 and 29.85 µg/mL, respectively. Data showed that the different sources of Cu had no effect on the serum Ca, IP and Fe values. For blood Cu values, the control group normally had a significantly lower ( $P<0.05$ ) Cu value (101.8 µg/100 mL) than other treatments supplemented with different sources of Cu (ranged between 118.8 and 129.5 µg/100 mL).

**Table 5:** Means±standard error of blood constituent of growing rabbits fed diets supplemented with different sources of copper under summer condition.

Blood constituent	Treatments							SEM	P-value
	T1	T2	T3	T4	T5	T6	T7		
Total lipids (mg/100 mL)	297.5 <sup>b</sup>	266.3 <sup>a</sup>	257.8 <sup>a</sup>	247.8 <sup>a</sup>	240.8 <sup>a</sup>	254.8 <sup>a</sup>	246.3 <sup>a</sup>	17.17	0.037
Cholesterol (mg/100 mL)	91.25 <sup>c</sup>	81.00 <sup>b</sup>	77.00 <sup>ab</sup>	70.00 <sup>ab</sup>	66.50 <sup>a</sup>	75.50 <sup>ab</sup>	69.25 <sup>ab</sup>	3.94	0.001
Triglycerides (mg/100 mL)	121.5	110.0	103.3	102.8	95.0	97.5	93.5	9.41	0.221
LDL (mg/100 mL)	29.50	28.75	26.75	23.50	21.50	26.50	23.75	4.06	0.074
HDL (mg/100 mL)	38.25 <sup>a</sup>	40.25 <sup>b</sup>	41.75 <sup>bc</sup>	45.75 <sup>cd</sup>	47.02 <sup>d</sup>	42.25 <sup>bc</sup>	45.50 <sup>cd</sup>	1.33	0.001
AST (U/L)	72.42	70.84	71.05	68.92	68.76	69.17	69.50	1.25	0.458
ALT (U/L)	47.55	44.50	46.00	42.35	40.90	43.70	44.75	1.50	0.173
Creatinine (mg/100 mL)	1.04 <sup>bc</sup>	1.06 <sup>cd</sup>	1.08 <sup>d</sup>	0.90 <sup>b</sup>	0.86 <sup>a</sup>	0.88 <sup>ab</sup>	0.90 <sup>b</sup>	0.05	0.022
Ca (mg/100 mL)	10.02	10.85	10.76	11.05	11.00	10.92	10.94	0.25	0.174
IP (mg/100 mL)	4.35	5.84	5.47	5.96	5.51	5.73	5.38	0.47	0.836
Cu (µg/100 mL)	101.8 <sup>a</sup>	118.8 <sup>b</sup>	121.0 <sup>bc</sup>	127.3 <sup>bc</sup>	129.5 <sup>c</sup>	126.8 <sup>bc</sup>	125.0 <sup>bc</sup>	3.30	0.001
Fe (µg/mL)	26.85	25.18	23.33	24.38	21.58	24.13	20.38	1.85	0.095
MDA (µmol/L)	126.0 <sup>b</sup>	103.5 <sup>a</sup>	102.8 <sup>a</sup>	107.5 <sup>a</sup>	101.3 <sup>a</sup>	105.8 <sup>a</sup>	102.8 <sup>a</sup>	3.88	0.049
TAC (nmol/L)	1.17 <sup>a</sup>	1.25 <sup>ab</sup>	1.31 <sup>ab</sup>	1.33 <sup>b</sup>	1.34 <sup>b</sup>	1.34 <sup>b</sup>	1.35 <sup>b</sup>	0.04	0.034
GSH-Px (mmol/L)	281.3 <sup>a</sup>	304.8 <sup>ab</sup>	310.8 <sup>b</sup>	309.0 <sup>ab</sup>	316.0 <sup>b</sup>	312.3 <sup>b</sup>	311.3 <sup>b</sup>	8.22	0.036
SOD (U/mL)	1.05 <sup>a</sup>	1.22 <sup>ab</sup>	1.20 <sup>ab</sup>	1.23 <sup>ab</sup>	1.30 <sup>b</sup>	1.35 <sup>b</sup>	1.38 <sup>b</sup>	0.06	0.030

<sup>a,b,c,d</sup>Different letters in the same row indicate significant differences ( $P<0.05$ ), according to Duncan test.

LDL: Low-density lipoprotein; HDL: High-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; Ca: Calcium; IP: Inorganic phosphorus; Cu: Copper; Fe: Iron; MDA: Malondialdehyde; TAC: Total antioxidant capacity; GSH-Px: Glutathione peroxidase; SOD: Superoxide dismutase; T1: Fed basal diet without any supplementation (control); T2: Basal diet+copper sulphate 100 ppm (100 mg/kg); T3: Basal diet+copper sulphate 200 ppm (200 mg/kg); T4: Basal diet+copper methionine 100 ppm (100 mg/kg); T5: Basal diet+copper methionine 200 ppm (200 mg/kg); T6: Basal diet+copper glycine 100 ppm (100 mg/kg); T7: Basal diet+copper glycine 200 ppm (200 mg/kg).



The different Cu sources had significantly lower ( $P<0.05$ ) MDA (ranged between 101.3 and 107.5  $\mu\text{mol/L}$ ) and in general significantly higher ( $P<0.05$ ) SOD (ranged between 1.20 and 1.38 U/mL) values compared with the corresponding control treatment value (126.0 mmol/L and 1.05 U/mL, respectively). Regarding the serum TAC indicator, each organic chelate studied had values (ranged between 1.33 and 1.35 nmol/L) that were insignificantly higher than the value of both levels of *Cu-Sul* treatments (1.25 and 1.31 nmol/L) and differed significantly from the control value (1.17 nmol/L). As for the GSH-Px indicator, the different sources studied, in general, had higher values (ranged between 304.75 and 316.00 mmol/L) compared with the corresponding control group value (281.3 mmol/L).

## DISCUSSION

The present results showed good performance (BW, DBWG, and FCR traits) of rabbits fed diets supplemented with different levels and sources of Cu under severe heat stress, and the results are consistent with the findings of Attia (2003). In general, these results were better for organic *Cu-Meth* or *Cu-Gly* chelates compared to inorganic *Cu-Sul* sources. In interpretation, Cu can play an important role in health and growth of livestock by maintaining oxidising dietary nutrients and protecting cells from free radical damage as a component of superoxide dismutase. Moreover, Cu is an essential trace element that plays a vital role in animal physiology (McDowell, 1992).

Copper is a component of several intracellular and extracellular enzymes such as cytochrome oxidase, lysyl oxidase, ceruloplasmin and superoxide dismutase (Klasing, 1998). The present results are in accordance with the findings of Aboul-Ela *et al.* (2000), who noted that organic copper has more physiological effects than inorganic copper, as it is absorbed from the gut more efficiently. Moreover, Vieira (2008) reported that trace minerals in organic form can be used better than those from inorganic sources and they can be absorbed in a consistent form by the intestinal mucous membrane through the amino acid transport system. Chelated or complex trace elements may enhance the bioavailability of minerals and also provide readily bioavailable amino acids for pigs and poultry (Miles *et al.*, 2003).

These results indicated that the studied supplemented *Cu-Meth* or *Cu-Gly* chelates or other treatment levels had a positive impact on FCR of growing rabbits under summer condition. Generally, all studied performance characteristics indicated that the copper as a trace mineral with different studied levels and sources is an encouraging element for the growth (BW and DBWG) and FCR of rabbits during summer conditions, with the advantage in favour of organic copper rather than non-organic copper.

The haematological values found in the present results across the treatments were within the normal range for rabbit's blood (Adu *et al.*, 2010). These results can be interpreted by Cronin *et al.* (2019), who indicated that it could be as a result of the subsequent production of more Cu transporting protein ceruloplasmin, which is required for normal RBC formation by allowing more Fe absorption from the small intestine and release of Fe in the tissue into the blood plasma. Ceruloplasmin had been reported to play a critical role in the haematopoietic process by facilitating the mobilisation of Fe from the reticular endothelial cells of the liver and spleen to the bone marrow cells and by catalysing the oxidation of Fe ions during the formation of ferry transferrin. Bassuny (1991) reported similar results in a rabbit experiment conducted where the values of the RBC and Hb were higher in animals fed dietary copper compared to the control.

In addition, it has been reported that red cells blood count and PCV values are influenced by stress, age, gender, season and genus in rabbits (Jenkins, 2008). Adu (2004) reported a similar result in a rabbit fed dietary Cu compared to the control. The present results match those of Chineke *et al.* (2006) who posited that a high PCV reading indicated either an increase in the number of RBCs or a reduction in circulating plasma volume. This is reflected in the good transport of oxygen and absorbed nutrients (Isaac *et al.*, 2013). Moore (2017) stated that Cu also aids in the formation of bone and RBC, and is involved in vital functions.

Increased WBCs may be a positive indicator of the activation and readiness of the body's defence and immune system, which helps increase the release of white blood cells from the bone marrow pool in the blood. These results reflect that the studied two organic Cu, enhanced the lymphocyte type synthesis of growing rabbits more than inorganic *Cu-Sul*. Also, the neutrophil/lymphocyte (N/L) ratio determines the balance between the unspecific and fast-acting defences of H and the antigen specific, slower-acting defences of L (Yousef *et al.*, 2003). Therefore, the heterophils/lymphocyte ratio is considered as a sensitive haematological indicator of stress response among groups

of chickens and as a general biomarker related to immune function in poultry. Considering the later explanations, the rabbits fed different sources of Cu in the present experiment have good health/defences, depending on their WBCs and N/L ratio values compared to the control value (Onbaşilar *et al.*, 2008).

The values of the serum protein profile of rabbits in the present study are within the range of reference values reported for rabbits in previous studies (NseAbasi *et al.*, 2014). These results indicated that studied Cu sources enhanced serum total protein values, and only the studied organic chelates enhanced albumin values. Lipids and cholesterol values of the present study are in agreement with the trend in the experiments with chickens of Skřivan *et al.* (2002), who found that lipids and cholesterol values were reduced in plasma and muscles as affected by Cu supplementation to diet. In rabbits, Adu *et al.* (2010) showed that blood serum cholesterol was not affected by dietary treatment Cu levels. In contrast to the present results, Lei *et al.* (2017) found a significant increase in the level of plasma triglycerides due to the addition of dietary Cu, but it was noted that there was a decrease in the levels of low-density lipoprotein.

The insignificant AST and ALT enzyme activities found in the present study could indicate that the studied levels and sources of Cu supplementation did not change tissue development and modifications of growing rabbit, which may be caused by high serum enzyme activity (Moniello *et al.*, 2005). Also, the normal values recorded here for AST, ALT, and ALP (which reflect insignificant differences compared with control values) were indicative of normal functioning of the livers of the experimental rabbits. These results indicated that the studied Cu sources did not affect liver functions, with an insignificant decrease in AST and ALT values for those fed *Cu-Meth* (68.76 and 40.90 U/L) and *Cu-Gly* (69.17 and 43.70 U/L) chelates compared with those of the *Cu-Sul* and control groups.

The rabbits fed diet supplemented with organic *Cu-Meth* and *Cu-Gly* chelates presented, in general, lower creatinine values, which reflect good kidney functions of organic chelates more than inorganic source. The present results agree with the previous findings of Adu *et al.* (2010), who found that the creatinine level in rabbit females was 1.20, 1.23, 1.26 and 1.25 mg/100 mL for 0, 100, 200, and 300 ppm inclusion groups, respectively. After these comparisons and in spite of the significant differences among treatments observed for creatinine values, the results of creatinine levels in the present study reflect that the rabbits under different Cu treatments have normal kidney functions.

The rabbits fed diet supplemented with organic *Cu-Meth* and *Cu-Gly* chelates had, in general, higher Cu values, which reflect higher absorption for them. The Ca, IP, Cu and Fe levels determined in this study and presented in Table 5 were within the range of corresponding reference values reported for rabbits (Silva *et al.*, 2005). The results indicated that Ca and inorganic P values increased insignificantly ( $P>0.05$ ) with the different studied sources of Cu, while the opposite trend was found with Fe values ranged between 20.38 and 25.18 ug/mL.

Oxidative stress has a pivotal role in many diseases, including those associated with animal and poultry production, reproduction and welfare (Pastorelli *et al.*, 2010). It can be said that the state of antioxidants is closely related to the health of the animal and the extent of the specific and non-specific response to the body's immune system.

Research in humans has shown that Cu-Met chelates stimulate phagocytic activity in phagocytes and increase SOD and ceruloplasmin concentrations in the organism (Das *et al.*, 2014). To reduce animal extraction of minerals which caused environmental pollution, chelates of selected minerals including Cu and amino acids are performed (Abdallah *et al.*, 2009). According to previous observations and the present results for antioxidant indicators, the experimental rabbits have better oxidative stability, compared with the control group, which may be due to dietary Cu supplementation.

High temperature is of one the most extensively studied of the various environmental conditions that can cause severe organ disorders through many mechanisms, such as the metabolic activation to highly reactive free radicals. Besides, heat stress and other forms of stress, such as hypoxia/ischaemia, oxidative stress or exposure to heavy metals, can overcome various underlying defence mechanisms such as the antioxidant defence mechanisms, intracellular glutathione concentration, superoxide dismutase (SOD) and catalase (CAT) activities, which become significantly weak and inadequate (Sangiah, 2004).

Generally, the severe heat stress conditions of this study did not affect the measured blood parameters abnormally or effectively, and this is probably because the levels used from the sources of Cu are lower (below toxic level) than those used in other research studies. Copper has even improved some blood parameters.



## CONCLUSION

Under the severe heat stress conditions of the present study, the studied Cu sources (copper sulphate, copper methionine chelate and copper- glycine chelate) had positive effects on V line rabbits, with the advantage of the organic sources over inorganic in most of the traits studied (productive and blood constituent traits). Therefore, the study could recommend the use of the studied organic Cu sources at a level between 100 and 200 mg/kg diet to improve the performance and oxidative status of rabbits without any negative impact under the severe heat stress conditions of summer in Egypt.

**Conflicts of interest:** The authors declare that they have no conflict of interest.

**Acknowledgements:** Mohamed Fesal Goodb conducted the experimental work and laboratory analysis. Farid Nassif Soliman and Osama Ahmed El-Ghalid designed the experiment and conducted the data analysis. Ahmed Mohamed Abd El-Hady preparation of the research protocol and wrote the manuscript. The authors wish to thank "nucleus breeding rabbit unit" of the Poultry Research Centre, Poultry Department, Faculty of Agriculture, Alexandria University, Egypt for helping in providing the rabbits and supervising the experiment. The authors were thankfulness acknowledge all participating research assistants and laboratory technicians for their technical and scientific researchers. This research did not receive any specific funding.

**Ethical Standards:** All animal procedures were ethically reviewed approved by Institutional Animal Care and Use Committee (IACUC) in Alexandria University, Egypt with the review report No. AU08191112349. Authors announce that the measures imposed on animals have been implemented to meet the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

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