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EFFECT OF SUPPLEMENTAL NANO *VS.* CONVENTIONAL COPPER SOURCES ON GROWTH PERFORMANCE OF NEW ZEALAND WHITE RABBITS

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ABSTRACT

The aim of this study was to compare the response to some forms of copper on growth performance, nutrient digestibility, carcass traits, some blood and histological parameters as well as economical evaluation. A total number of ninety- six NZW weaning rabbits, of 5 weeks old and weighing 634 g ±6.6 were randomly distributed into 4 similar groups (24 each). The first group (T1) was fed the control diet (5 mg Cu/kg diet), while, the other 3 groups (T2, T3 and T4) were fed the basal diet supplemented with 50mg Cu/kg diet as nano copper (Cu-nano), 200 mg Cu/kg diet as copper sulfate (Cu-SO4) or 200 mg Cu/kg diet as copper methionine (Cu-meth), respectively. The experimental period was lasted up to the 13th week of age. Results indicate that feeding rabbits a diet containing Cu-nano (50 mg Cu/kg diet) achieved the best growth performance compared to other copper forms and the control. The improvements were in terms of the highest live weight gain, least feed intake, best feed conversion ratio, performance index and reduced mortality. A corresponding significant improvements in carcass meat parts and nutrient digestibilities of dry matter %, organic matter %, crude protein % and digestible crude protein % were also recorded for the Cu-nano treatment. The digestibility of crude fiber, ether extract and nitrogen free extract as well as total digestible nutrients and digestible energy did not affected significantly by different cu-sources incorporated in dietary treatments. Sanitary status as reflected by liver and kidney histopathology revealed that the nano form is safe and reliable copper source in growing rabbit diets. Economically, the diet formulated with Cu-nano particles seemed to be performed the best one among all experimented Cu forms.

In conclusion*, supplementing NZW rabbit's diet with 50 mg Cu /kg diet as Cu-nano significantly improved growth performance, carcass traits, digestibility of crude and digestible protein, blood* *constituents and economical efficiency without any adverse effect on liver and kidneys histology.*

Key words: Nano copper, copper sulfate, copper methionine, rabbit performance.

Nowadays, there is evidence that some developing countries are beginning to utilize the rabbit as a main source of meat where, it contains highly unsaturated fatty acids (60% of the total fatty acids), rich in protein (20-21%) with high biological values amino acids, low in cholesterol and sodium, also rich in potassium and magnesium (Dalle Zotte, 2000).

Improvement in growth performance of weaning rabbits fed diets supplemented with high level of copper has been widely accepted in rabbit industry. Copper requirement is very low (3 mg/kg diet) in young rabbits according to NRC (1977), but, the literature showed that copper has a growth promoter effect at high addition rate up to 300 mg Cu/kg diet (Liang *et al.*, 1988). Different studies had been conducted to evaluate copper supplementation to rabbit diets where Onifade and Abu (1998) reported an improvement of rabbits daily gain, feed intake and feed conversion ratio when supplementing copper sulphate at level up to 300 mg Cu/kg diet. Also, Aboul_Ela *et al.* (2000) recorded an improvement in growth performance and reproductive efficiency of growing NZW rabbits by supplementation their diets with 100-300 mg Cu/kg diet as inorganic copper.

The biological availability of copper is affected by sources and animal species (Ammerman *et al.*, 1995). Many studies showed that copper bioavailability was higher when fed at organic form such as metal amino acid chelates or metal proteinates (Du *et al.*, 1996) or nano copper (Gonzales-Eguia *et al.*, 2009). Other studies reported that organic form of copper appeared to have equal bioavailability as copper sulfate (Coffey *et al.*, 1994 and Apgar *et al.*, 1995). While, Zhou *et al.* (1994) concluded that growing pigs fed diet supplemented with copper lysine grew faster than others fed dietary copper sulfate.

Copper nanoparticles (Cu-nano) have attracted more attention due to its properties that are not observed in commercial copper such as catalytic, optical, electrical, antifungal and antibacterial. The antibacterial properties of the Cu-nano have found in applications with water treatment, food storage and other different types of processing. The effects of Cu-nano have been attributed to their small particle size and high surface to volume ratio, which allows interacting closely with microbial membranes (Ramyadevi *et al.*, 2012). In this connection, Gonzales-Eguia *et al.* (2009) reported that weaning pigs fed 50 mg Nano-Cu/kg diet showed higher growth performance comparing to those fed copper sulfate supplemented diet. Also, Scott *et al.* (2016) concluded that broiler embryos' that *in-ovo* injected with Nano-Cu recorded higher metabolic rate and growth performance after hatching than others which *in-ovo* injected with copper sulfate.

Therefore, the objectives of the present study are to compare the impact of adding different forms of copper in the diet on growth performance, nutrient digestibility, carcass traits, some blood constituents', liver and kidney histology and economical efficiency of weaning rabbits.

MATERIALS AND METHODS

The experimental work was carried out at Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt.

Copper forms:

Three sources of supplemental Cu were used in the current study where: copper sulphate, $CuSO_4.5H_2O$, 98% (Cu-SO4; contains 25% Cu) working as inorganic form, copper-methionine 56.7% (Cu-meth; contains 10% Cu) as organic form and nanoparticles of copper (Cu-nano; contains 37.38% Cu) as nano form with a particle size of 70nm. Cu-nano was prepared as described by Refaie *et al.* (2015).

Experimental design, animals and diets:

Ninety-six New Zealand White rabbits (NZW) at weaning age of 5 weeks old (average weight of 634 g ±6.6), were divided equally into four experimental groups (24 rabbit in each) in 4 equal replicates. Rabbits were reared on wire battery cages and had the same managerial procedures throughout the growth period (from 5 to 13 weeks of age). Rabbits were fed *ad libitum* access to water and diets. Dietary treatments were designed to receive control diet (containing 5 mg Cu/kg diet) without copper supplementation which serves as control group (T1). While, T2, T3 and T4 fed the control diet supplemented with 50 mg Cu as Cu-nano/kg diet, 200 mg Cu as Cu-SO_4/kg diet and 200 mg Cu as Cu-meth/kg diet, respectively.

Diet was pelleted and formulated to meet the recommended nutrient requirements of rabbits according to NRC, (1977). Composition and calculated chemical analysis of the basal experimental diet are presented in Table 1. Live body weights (BW) were recorded individually at the beginning of the experiment (5 weeks of age) and every two weeks till the end of the experiment (13 weeks of age). Daily weight gains (DWG) were calculated.

Table 1: Composition and chemical analyses of the basal diet.

Ingredients	**%**	**Chemical analysis** [2]	**%**
Alfalfa hay (12%)	28.55	Crude protein	17.10
Barley	34.40	Crude fiber	12.74
Wheat bran	11.00	Crude fat	1.99
Soybean meal (44%)	17.00	Digestible energy (kcal/kg)	2500
Molasses	5.00	Calcium	1.30
Limestone	0.85	Total Phosphorus	0.80
Di-calcium phosphate	2.10	Methionine	0.63
Sodium chloride	0.40	Lysine Meth.+Cyc.	0.86 0.91
Mineral and vitamin premix[1]	0.30		
DL-Methionine	0.40		
Total	**100**		

(1) Each 3 kg contain: 6000000 IU Vit. A; 900000 IU Vit. D3; 40000 mg Vit. E; 2000 mg Vit. K3; 2000 mg Vit. B1; 4000 mg Vit. B2; 2000 mg Vit. B6; 10 mg Vit. B12; 50 mg Biotin; 10000 mg Pantothenic acid; 50000 Niacin; 3000 mg Folic acid; 250000 mg Choline; 8500 mg Mn; 50000 mg Zn; 50000 mg Fe; 200 mg I; 100 mg Se, 5000 mg Cu, and 100 mg Co.
(2) According the Egyptian Regional Center for Food and Feed (RCFF, 2001)

Feed intake (FI) was recorded, while feed conversion ratios (FCR), relative growth rate (RGR) and performance index % (PI) were calculated according to the following equations:

$$\text{Relative growth rate} = [(W2 - W1) \times 100] / [1/2\ (W2+W1)]$$

whereas: W1= Initial body weight (g), and W2 = Final body weight (g).
Performance index was calculated according to North (1981) as follow:

$$\text{Performance index (PI)} = (\text{Final live body weight (kg)} / \text{Feed conversion ratio}) \times 100.$$

Digestibility trials:

At the end of growth period at 13 weeks of age, 16 rabbits (4 per treatment) were used to determine the digestibility of dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), organic matter (OM) and nitrogen free extract (NFE) and the feeding values of dietary treatments such as total digestible nutrients (TDN), digestible crude protein (%) (DCP) and digestible energy (kcal/g) (DE) as described by Cheeke *et al.* (1982). The experimental diets and water were offered *ad-libitum*, while feces were collected daily, weighed then dried at 60 ^{o}C for 24 hrs. The diets and dried feces were analyzed according to AOAC (1990) at the Central Laboratory of Foods and Feeds, Agricultural Research Center, Ministry of Agriculture, Egypt.

Slaughter trail:

At the end of the experimental period (13 weeks of age), 4 rabbits from each treatment were chosen to evaluate carcass characteristics. Fore parts, trunk, hind parts, heart, kidneys, liver, gastrointestinal tract (GTI) and total edible parts were weighed and carcass percentage was calculated according to Cheeke (1987).

Haematology:

For each replicate, one blood sample was taken to determine packed cell volume (PCV) by spinning about 75µl of each blood sample in heparinized capillary tube in a haematocrit centrifuge for about 5 minutes and read on haematocrit reader as described by Benson *et al.* (1989), while erythrocytes (RBC) and leucocytes (WBC) counts and lymphocytes % were determined using haemocytometer as described by Lamb (1981).

The haemoglobin (Hb) concentration and the blood indexes: mean cell haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were determined using cyanethaemoglobin method and appropriate formula respectively as described by Jain (1986).

***Economical efficiency*:**

Economical evaluation (EE) was calculated according to the prices of feed ingredients, feed additives and rabbits meat prevailing, during year 2016. It was calculated as follows:

EE = Net revenue/ Total feed cost

While, Net revenue = Selling price of total weight gain – Total feed cost.

Tissues Specimens:

Tissues specimens were taken during dressing process, where the proper tissue samples from liver and kidney were obtained for histopathological examination. Tissues were immediately fixed in a 10 % formalin-saline solution. All samples were then dehydrated in ascending strengths of 70% absolute alcohol. After clearing in zylol, infiltration and embedding in paraffin wax, tissues blocks were sectioned and then stained by hematoxylin and eosin stains, according to Junqeira *et al.* (1971). The histological sections (4-5 micron) were conducted in the Faculty of Veterinary Medicine, Cairo University. All sections were examined with light microscope supplied with electrical lamp, and the magnification power was 400x.

Statistical analysis:

Data were statistically analyzed according to SAS (2001), using the following fixed model:

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

Where: Y_{ij} = The observation; µ = Overall mean; T_i = Effect of treatments (i = 1, 2, 3 and 4); e_{ij}= Random error component assumed to be normally distributed. Data presented as percentages were transformed to the corresponding arcsine values (Warren and Gregory, 2005) before being statistically analyzed. The differences among means were tested using Duncan (1955).

RESULTS AND DISCUSSION

Growth performance:

Results of growth performance and mortality rate are presented in Table (2). Rabbits fed Cu-nano supplemented diet recorded significantly the highest final body weight comparing with either control group or those fed the other forms of dietary copper. During 5-9 wks-growing period, the daily weight gain of rabbits fed dietary Cu-nano (T2) was significantly higher than that of those fed diet supplemented with Cu-So4 (T3), but insignificantly higher than of control group (T1) or diet contained the organic form of Cu (T4). Otherwise, over 9-13 wks of age, the differences among treatments respecting this item seemed to be non significant. In addition, over the whole growing period, the group of rabbits fed Cu-nano added diet had the highest value of daily gain being the differences only significant with control group but insignificant with the other tested ones (T3 and T4).

The effects of supplementing Cu-nano on growth performance are in agreement with the findings of Gonzales-Eguia *et al.* (2009) who concluded that piglets fed Cu-nano supplemented diet had the best results in daily gain compared to others fed dietary copper sulfate or control group. In a previous research, Refaie *et al.* (2015) concluded that supplementing growing rabbit diets with 50 mg Cu-nano/kg diet increased final body weight by 4.6% comparing to unsupplemented group. One reason for the increased growth rate may be due to the improvement in the digestibility of crude and digestible protein as shown in Table (3). Replacing traditional copper sources by copper nanoparticles exhibiting ultrahigh physical activity and chemical neutrality. The activity of nanoparticles results from a large surface area, exposing their atoms to direct contact with target cells (Pineda *et al.*, 2013).

Over the growing phase 5-9 wks and whole period (5-13 wks), feed intake was significantly lower with the rabbits fed Cu-nano ration rather than

Table 2. Effect of different copper forms supplementation on growth performance of NZW rabbits from 5 to 13 wks of age.

Parameters	T1 Control	Copper forms T2 Cu-nano 50 mg/kg	T3 $Cu\text{-}SO_4$ 200 mg/kg	T4 Cu-meth 200 mg/kg	SEM	Sig.
Initial body weight (g)	634	636	638	638	6.64	NS
Final body weight (g)	2045^{b}	2151^{a}	2073^{b}	2088^{b}	18.03	**
Daily weight gain (g):						
5-9 weeks	26^{ab}	28^{a}	25^{b}	26^{ab}	0.71	*
9-13 weeks	24	26	26	25	0.72	NS
5-13 weeks	25^{b}	27^{a}	26^{ab}	26^{ab}	0.32	*
Feed intake (g/ d):						
5-9 weeks	62^{a}	59^{b}	61^{a}	62^{a}	0.43	**
9-13 weeks	103^{a}	99^{b}	101^{ab}	101^{ab}	0.61	*
5-13 weeks	83^{a}	79^{b}	81^{a}	81^{a}	0.45	**
Feed conversion ratio:						
5-9 weeks	2.38^{a}	2.11^{b}	2.44^{a}	2.38^{a}	0.06	**
9-13 weeks	4.29^{a}	3.81^{b}	3.88^{b}	4.04^{ab}	0.12	*
5-13 weeks	3.32^{a}	2.93^{c}	3.11^{b}	3.12^{b}	0.04	**
Relative growth rate	105.3^{b}	108.7^{a}	105.9^{b}	106.4^{ab}	1.35	*
Performance index (%)	61.6^{c}	73.4^{a}	66.7^{b}	66.9^{b}	1.17	**
Mortality (%)	8.3	4.2	8.3	8.3	-	-

[a , b and c] Means in the same row with different superscripts are significantly different ($P<0.05$).
Cu-nano = nano copper, $Cu\text{-}SO_4$ = inorganic copper (copper sulfate), Cu-meth = organic copper (copper methionine).
** : Significant at 1% level of probability, *: Significant at 5% level of probability, NS: Non-significant.

other copper forms and control group. Whereas, during growing phase of 9-13 wks, Cu-nano group recorded insignificant lower FI compared to other tested rations (containing inorganic /organic copper forms) but had significantly lower feed intake value than that of control. It is greatly mean that despite the group of Cu-nano diet consumed lower feed, but at the meantime gained more daily gain and heaviest final body weight than the other groups and consequently this favorable effect would be also reflected on the feed conversion ratio and the economical efficiency. The present results regarding feed intake are in agreement with those obtained by Abd-El Ghany *et al.* (2016) who found that feed intake was lower with nano-copper diet than that of normal one with growing rabbits. On the other hand, Results obtained here disagreed with Polen and Sorin (2015) who reported that the daily feed intake with pigs for inorganic and organic copper compared to the control group was higher by 2.8% and 2.9%, respectively. Also, Jolliff and Mahan (2013) and Martin *et al.* (2011) did not found any effect of mineral source

(Cu-peptide vs. Cu sulphate) on feed intake in growing pigs when diets contained about 15 mg/kg of Cu.

Feed conversion ratio was improved significantly for the group fed diet supplemented with Cu-nano in comparison with control and other tested rations over 5-9 wks and 5-13 wks growing phases, while the worst values were recorded for the control group compared with the two groups fed diet supplemented with either Cu- nano or Cu-SO_4 during 9-13 wks of age. The enhancement of Cu-nano group was 11.34%, 11.19% and 11.75% compared to control group during 5-9 weeks, 9-13 weeks and 5-13 weeks, respectively. In comparison between addition of copper sulfate and copper nanoparticles to piglet diets, Gonzales-Eguia *et al.* (2009) reported an improvement in FCR by about 5.7% and 8% for the group fed dietary copper nanoparticles relative to the group fed dietary copper sulfate and control group, respectively. Also, results documented here is in accordance with those found by Polen and Sorin (2015) who concluded that feeding fattening pigs with either dietary inorganic or organic copper achieved an improvement in feed conversion ratio by 4.3% and 9.3%, respectively compared with the control group.

The highest value of relative growth rate and performance index% was recorded for Cu-nano diet while, the lowest values were recorded for groups fed either Cu-SO4 diet or control. In this respect, Coffey *et al.* (1994) evaluated the copper-lysine complex effectiveness in comparison with copper sulfate in growing pigs and found that better growth rate (11.5%, $P < 0.05$) in pigs treated with organic copper, which is an outcome of high utilization of copper from organic sources. Several researchers have attributed the improvement in Cu-nano group probably due to the antimicrobial and antibacterial properties of copper nanoparticles (Usman *et al.*, 2013), and in the meantime increasing the population of total bacterial count and lactolacillus while decreased the population of *E. coli* and *clostridium spp.* Where, the population of Lactobacillus in group of rabbits fed dietary 50 mg Cu-nano /kg were increased by 3 folds and the population of *E.coli* and *clostridium* were decreased by 63.9% and 64.1%, respectively compared to control group as reported by Refaie *et al.* (2015).

Regarding to the mortality rate, rabbits fed diet supplemented with 50 mg Cu as Cu-nano/kg diet recorded numerically the lowest value (4.2%) comparing to that of control group and the other two groups fed diets supplemented with either Cu-SO_4 or Cu-meth which recorded 8.3%. The beneficial effect of copper nanoparticles may be due to its protective effects against enteritis. Since enteritis is known to be a major problem in commercial rabbit production. These results agreed with the findings of Refaie *et al.* (2015) who reported that supplementing rabbit diet with 25 or 50 mg Cu/kg diet as Cu-nano reduced mortality rate compared to either control group or others fed 75 mg Cu/kg diet as copper nanoparticles. But disagreed with Abo El-Ezz *et al.* (1996) who observed a decrease in mortality rate % occurred in rabbits given drinking water supplemented with copper sulfate at levels 20, 60 and 180 mg/L water as compared with control. Patton *et al.* (1982) and Bassuny (1991) stated that adding copper sulfate into rabbit diets reduced mortality. On the other hand, Grobner *et al.* (1986) stated that mortality rate was not significantly differed by dietary copper.

Nutrient digestibilities and feeding values:

Results illustrated in Table (3) show that rabbits fed diet added with 50 mg Cu-nano/kg diet recorded significantly higher values of DM and CP digestibility compared to either control group or those fed CU-SO_4 diet. Moreover, nano copper group achieved significantly higher OM digestibility compared to control group. The improvement in group of Cu-nano ration was 3.7%, 2.8% and 2.8% in digestibility of DM, OM and CP, respectively in relative to control group. While, the other nutrients (CF%, EE% and NFE%) did not significantly affected by addition of any copper forms. Lastly, the group of rabbit fed organic copper diet (T4) had comparable values (insignificant differences among treatments) for all nutrient digestibilities with control (T1) and the other tested ones (T2 and T3). Results are in accordance with those obtained by Abd El-Moneim *et al.* (2013) who noticed that digestibility of CP was increased insignificantly by treatment of heat-stressed rabbits with 40 or 80 mg Cu as copper sulfate/L water. Also, Abd El-Azeem and Abd El-Reheem (2006) reported that the digestibility coefficients of DM and OM were insignificantly increased for group supplemented with copper sulphate compared to the control group, and not agreed with Attia (2003) who found that digestibility coefficients of nutrients were not significantly affected by copper sulphate supplementation. Almost, results here are in harmony with those reported by Abd-El Ghany *et al*. (2016) who compared between the addition of normal and nano copper in the diets of growing rabbits in which they were found no significant differences between them respecting nutrient digestibilities.

Concerning to DCP, group of rabbits fed diet plus nano copper achieved significantly higher value than that of control group while the others were insignificantly higher than control. Also, no significant differences were observed among all groups in TDN and DE. The results are in agreement with the findings of Attia (2003) who concluded that the feeding values as TDN and DE were not affected significantly by copper

Table 3. Effect of different copper forms supplementation on nutrient digestibilities and feeding values of diets.

Parameters	T1 Control	Copper forms			SEM	Sig.
		T2 Cu-nano 50 mg/kg	T3 Cu-SO_4 200 mg/kg	T4 Cu-meth 200 mg/kg		
Digestibility (%):						
DM	64.27^{b}	66.68^{a}	64.82^{b}	65.37^{ab}	0.383	*
OM	68.14^{b}	70.05^{a}	68.95^{ab}	69.26^{ab}	0.416	*
CP	73.32^{b}	75.38^{a}	73.99^{b}	74.45^{ab}	0.289	*
CF	24.52	25.06	24.60	24.89	0.433	NS
EE	75.10	75.47	74.70	74.68	0.336	NS
NFE	72.10	72.63	72.14	72.59	0.384	NS
Feeding values:						
TDN, %	52.17	52.85	52.29	52.61	0.250	NS
DCP,%	12.73^{b}	13.09^{a}	12.85^{b}	12.92^{ab}	0.074	*
DE (kcal/g)	2.416	2.424	2.412	2.427	0.015	NS

a, b Means in the same row with different superscripts are significantly different (P<0.05).
* : Significant at 5% level of probability, NS: Non-significant.
Cu-nano = Nano copper, Cu-SO_4 = inorganic copper (copper sulfate), Cu-meth = organic copper (copper methionine).
DM =Dry matter, OM= Organic matter, CP = Crude protein, CF = Crude fiber, EE = Ether extract, NFE = Nitrogen free extract, TDN=Total digestible nutrient %, DCP = Digestible crude protein %,(DE= Digestible energy (kcal/g)

sulphate levels. As high concentration of Cu in the diet (organic or inorganic), it could inhibit formation of proteases in gut Chen *et al.* (2002) and this may be due to strong Cu-binding sites on amino acids prevent Cu interaction with the enzymatically active sites. While, using nanoparticles of copper (50 mg/kg diet) which led to an elevated absorption rate of copper in the gastrointestinal tract (Hussain *et al.,* 2001) and also did not affect protease creation hence, improve digestible crude protein.

Slaughter traits:

Results presented in Table (4) demonstrate that hot carcass % and hind part% for dietary Cu-nano group had significantly positive rates comparing to control group or other dietary copper forms. While, groups of Cu-meth and Cu-SO_4 recorded insignificant differences between them. However, the worst value was recorded by the control group. This conclusion confirms ``the point of view in Refaie *et al.* (2015) who concluded that rabbits fed dietary 50 mg Cu-nano/kg diet recorded an improvement in carcass percentage compared to control group. In addition, Abd El-Azeem and Abd El-Reheem (2006) and Zanaty (2005) reported that

Table 4. Effect of different copper forms supplementation on carcass traits of NZW rabbits.

Parameters (%)	T1 Control	Copper forms T2 Cu-nano 50 mg/kg	T3 $Cu\text{-}SO_4$ 200mg/kg	T4 Cu-meth 200mg/kg	SEM	Sig.
Hot Carcass	49.01[c]	51.08[a]	49.69[b]	50.13[b]	0.19	**
Fore parts	12.58[b]	13.28[a]	12.80[ab]	12.91[ab]	0.15	*
Trunk	17.59[b]	18.20[a]	17.90[ab]	18.13[a]	0.13	*
Hind parts	18.84[c]	19.61[a]	18.99[bc]	19.10[b]	0.08	**
Liver	3.573	3.531	3.564	3.659	0.18	NS
Kidney	0.651	0.668	0.636	0.665	0.04	NS
Heart	0.314	0.339	0.307	0.336	0.01	NS
Total edible parts (TEP)	53.55[c]	55.62[a]	54.19[bc]	54.79[ab]	0.32	**
Abdominal fat	1.452[a]	1.092[b]	1.197[b]	1.315[ab]	0.08	*
Gastrointestinal tract (GTI)	24.19[a]	22.32[b]	23.20[ab]	22.91[b]	0.42	*

[a,b and c] Means in the same row with different superscripts are significantly different (P<0.05).
**: Significant at 1% level of probability *: Significant at 5% level of probability, NS: Non-significant
Cu-nano = Nano copper, $Cu\text{-}SO_4$ = inorganic copper (copper sulfate), Cu-meth = organic copper (copper methionine).

copper supplementation improved dressing percentage and hot carcass weight in growing rabbits. Moreover, recently Abd-El Ghany *et al.* (2016) found that similar results to those obtained in the present study, where the empty carcass weight and percentage were significant higher with nano-Cu diet than those of normal-Cu one with growing rabbits. Also, Yassein *et al.* (2011) observed that adding copper sulfate in drinking water at level of 100 mg/l, increased rabbit dressing and carcass weight. Contradicting results were obtained by Ayyat *et al.* (1995) and Bassuny (1991) who found no significant effect for copper sulfate supplementation on rabbits carcass yield.

The group of rabbits fed Cu-nano supplemented diet achieved insignificantly higher fore parts % and trunk % comparing to either dietary $Cu\text{-}SO_4$ or Cu-meth, but had a significant values comparing to control group. Our results are close to Abd El-Moneim *et al.* (2013) who concluded that carcass and its parts were significantly (P<0.01) increased with addition of 120 mg copper sulfate/l water to heat-stressed growing rabbits comparatively with un-supplemented and other levels of copper sulfate (40 or 80 mg Cu/l water). Liver%, kidney% and heart % were not significantly differing between all groups. On the other hand, total edible parts % was significantly improved in group of rabbits fed either Cu-nano or Cu-meth compared with the control group.

In the opposite trend, the group of rabbits fed either nano or inorganic copper diet recorded significantly lower percentage of abdominal fat compared with control one, but others fed organic copper diet was insignificant lower than that of control one. The reduce in abdominal fat in groups fed dietary Cu-nano, Cu-SO_4 and Cu-meth were 24.8%, 17.6% and 9.4% respectively comparing to control group. Also, according to gastrointestinal tract %, the control group recorded significantly higher value comparing to those fed Cu-nano and Cu-meth supplemented diets.

Hematological response:

Results in Table (5) show that values of blood picture including Hb, PCV, RBCs, MCV and MCHC were significantly higher ($P \leq 0.05$) in Cu-nano group than those of other copper forms or the control group. Whereas, lymphocyte % was significantly lower in Cu-nano group compared to other treatments. The levels of these components in whole blood are within the normal biological limits and the results indicated good health condition for animals from all groups.

All groups supplemented with different types of copper (Cu-nano, Cu-SO_4 or Cu-meth) recorded significantly ($P \leq 0.05$) lower white blood cells count comparing to the control group.

Table 5. Effect of different copper forms supplementation on blood hematological parameters of NZW rabbits.

Parameters	T1 Control	Copper forms			SEM	Sig.
		T2 Cu-nano 50 mg/kg	T3 Cu-SO_4 200 mg/kg	T4 Cu-meth 200 mg/kg		
Hemoglobin (g/ dl)	12.33^{b}	13.40^{a}	12.47^{b}	12.47^{b}	0.167	*
PCV (%)	34.30^{b}	37.80^{a}	34.15^{b}	33.39^{b}	0.333	**
RBCs (x10^6/ µl)	6.31^{b}	6.85^{a}	6.19^{b}	6.14^{b}	0.081	**
MCV(1) (fl)	67.05^{bc}	68.52^{a}	67.47^{b}	66.42^{c}	0.315	**
MCH(2) (pg)	23.27^{ab}	24.20^{a}	22.63^{b}	22.77^{b}	0.240	*
MCHC(3) (g/ l)	33.57^{b}	34.83^{a}	33.53^{b}	33.13^{b}	0.285	**
WBCs (x10^3/ µl)	11.73^{a}	8.58^{b}	9.87^{b}	9.80^{b}	0.459	**
Lymphocytes (%)	46.37^{a}	44.32^{b}	46.70^{a}	45.97^{a}	0.291	**

a, b and c Means in the same row with different superscripts are significantly different ($P<0.05$).

**: Significant at 1% level of probability, *: Significant at 5% level of probability, NS: Non-significant

(1) Mean corpuscular volume, (2) Mean corpuscular hemoglobin, (3) Mean corpuscular hemoglobin concentration, Cu-nano = nano copper, Cu-SO_4 = inorganic copper (copper sulfate), Cu-meth = organic copper (copper methionine).

Hemoglobin (Hb) values were increased (within a normal range) in rabbits as a result of addition of Cu-nano. The enhancement was 8.7% in

Cu-nano supplemented group comparing to control group. While, there were no significant differences in Hb values between control group and those fed diets supplemented with traditional copper sources (organic and inorganic). Our results are in line with those recorded by Mroczek-Sosnowska *et al.* (2015) who concluded that the concentration of haemoglobin was higher in chicken diet treated with Cu-NP compared with chickens diet treated with $CuSO_4$ or control group. The high level of Hb value in Cu-nano group could be due to it's unique properties that are responsible in forming major components of protein transporting O_2 into cells (hemoglobin) (Pineda *et al.,* 2013). Also, it could be speculated that because of their small size and O_2 carrying capacity, feeding Cu-nano could enhance the contribution of O_2 to cellular oxidation and improve the activities of essential enzymes (cuproenzymes such as cytochrome C oxidase) that are vital in the cellular energy generation (Pineda *et al.,* 2013). Therefore, it was hypothesised that the Cu-nano would affect the metabolic rate, moreover increasing O_2 consumption and consequently improving growth as reported previously in Table 2.

On the other hand, results here are disagree with those recorded by Angelov *et al.* (2010) who reported that the Hb levels were significantly higher ($P < 0.05$) for pigs fed diets supplemented with 20 or 40 ppm copper from Cu methionine comparing with the control group and others fed dietary copper sulfate.

Economical study:

Results presented in Table (6) show that all groups fed diets supplemented with different sources of dietary copper recorded an improvement in economical efficiency compared with control. The best value was recorded with the group fed Cu-nano supplemented diet (116.55%), followed by the group fed Cu-SO_4 (108.62%) and finally Cu-meth group (101.12%). The results are in accordance with the findings of Ayyat (1995) who concluded that addition of 100 or 200 mg copper sulfate in rabbit diets increased final, margin with 27.0 and 51.7%, respectively than the control diet. Recently, Refaie *et al.* (2015) reported that supplementing NZW rabbit diet with 50 mg Cu-nano/kg diet improved economical efficiency by 107.9%. More recently, Mohamed *et al.* (2016) and Abd-El Ghany *et al.* (2016) stated that rabbits fed nano copper diet enhanced the economical efficiency of their meat production in comparison with the others fed diet contained normal copper.

Table 6. Effect of different copper forms supplementation on economical efficiency of NZW rabbits at 13 wks of age.

Parameters	T1 Control	Copper forms		
		T2 Cu-nano 50 mg/kg	T3 $Cu\text{-}SO_4$ 200 mg/kg	T4 Cu-meth 200 mg/kg
Feed intake (kg /head)	4.648	4.424	4.536	4.536
Price /kg diet (L.E.)	3.513	3.626	3.554	3.863
Total feed cost (L.E.)	16.33	16.04	16.12	17.52
Weight gain (kg/head)	1.400	1.512	1.456	1.456
Selling price (L.E.)(1)	35.00	37.80	36.40	36.40
Net revenue (L.E.)(2)	18.67	21.76	20.28	18.88
Relative revenue (%)	100	116.55	108.62	101.12

Other conditions like management are fixed.
- Ingredients price (L.E. per ton) at 2016 were: 3700 barley; 2000 berseem hay; 3000 wheat bran; 6500 soybean meal (44%); 250 limestone; 9000 premix; 31000 methionine; 1000 di-calcium phosphate; 1000 molasses; 250 salt.
- Adding 100 L.E. /ton for pelliting.
(1) Price of kg live body weight was 25 L.E.,(2) Net revenue = Selling price – total feed cost.
Cu-nano = Nano copper, $Cu\text{-}SO_4$ = Inorganic copper (copper sulfate), Cu-meth = Organic copper (copper methionine).

Histopathological evaluation:

The results of histological investigation of the kidneys and liver sections of treated and untreated rabbits are shown in Plates 1–8. The liver of the untreated rabbits showed no visible lesion. There was normal hexagonal architecture of the hepatocytes, hepatic vein and sinusoids (Plate 1). In the 50 mg Cu-nano/kg feed for treated rabbits, there was normal granular of the hepatocytes in the liver (Plate 2) while, in the 200 mg $Cu\text{-}SO_4$/kg feed, rabbits liver showed malfunction of mononuclear cells and fiberocytes around the central vein and dilation of sinusoid, with vacuolar degeneration of hepatocytes (Plate 3). Whereas in the group treated with 200 mg Cu-meth/kg diet, liver of rabbits showed little vacuolar improvement of hepatocytes (Plate 4). The kidney sections in the untreated rabbits showed normal kidney features, having normal glomerulus, Bowman's capsule and collecting tubules (Plate 5). A cross section of kidney for rabbits treated with 50 mg Cu-nano/kg diet showing the epithelial lining of the collecting tubules (Plate 6). The trends resulting from adding Cu-nano in the present study are in agreement with study involving rats received 0.5 ml of solution containing 5, 10,100 mg/kg CuO nanoperticle (Doudi and Setorki, 2014). The kidney in the rabbits treated with 200 mg $Cu\text{-}SO_4$/kg diet showing few necrosis of the tubules and epithelial glomerulo-nephritis of the glomeruli (Plate 7). A cross section of kidney for rabbits treated with 200 mg Cu-meth/kg diet showed normal tubules and blood vessels(Plate 8).

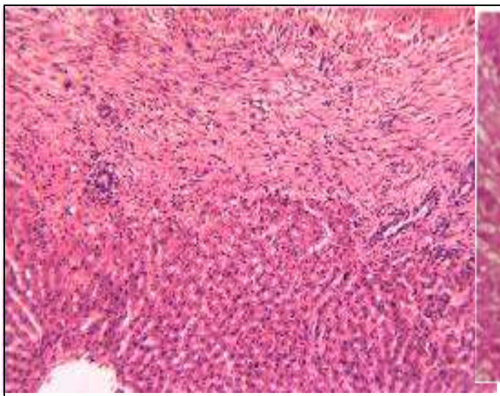

Plate 1. A cross section of liver from untreated rabbits showing normal histological structure of the hepatocytes in the hepatic cords and lobules with the central vein as well as the portal area (H.&E. X400).

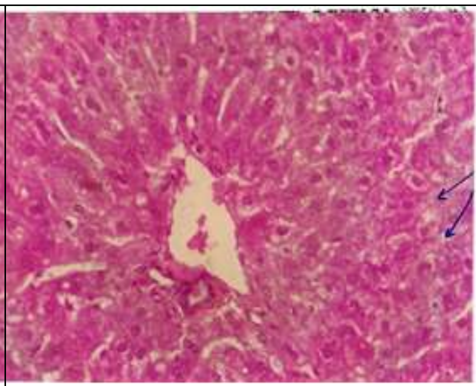

Plate 2. A cross section of liver from rabbits treated with 50 mg Cu-nano/kg diet showing normal granular of the hepatocytes (H.&E. X400).

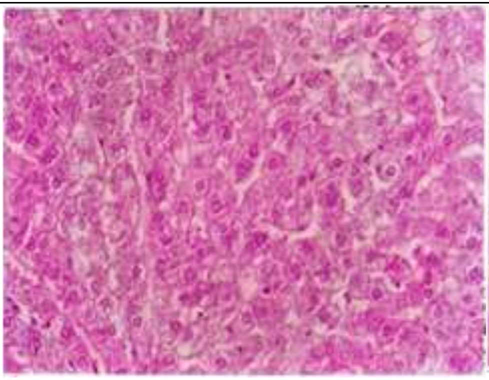

Plate 3. A cross section of liver from rabbits treated with 200 mg Cu-SO4/kg diet showing malfunction of mononuclear cells and fiberocytes around the central vein (H.&E. X400).

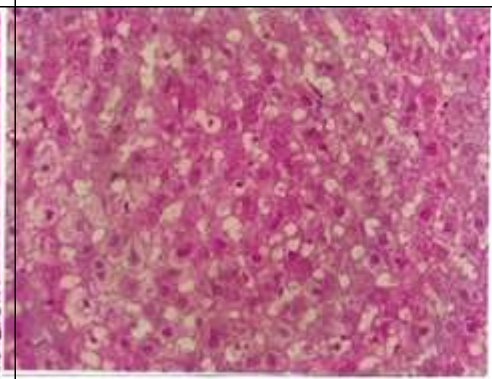

Plate 4. A cross section of liver from rabbits treated with 200 mg Cu-meth/kg diet showing vacuolar improvement of hepatocytes (H.&E. X400).

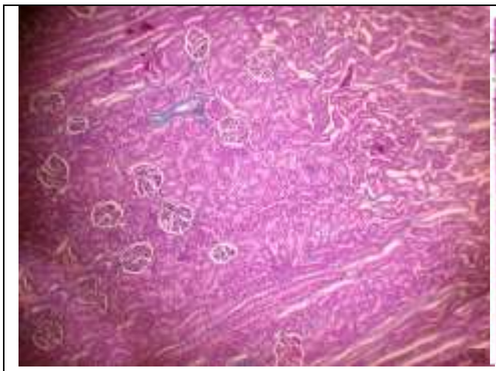	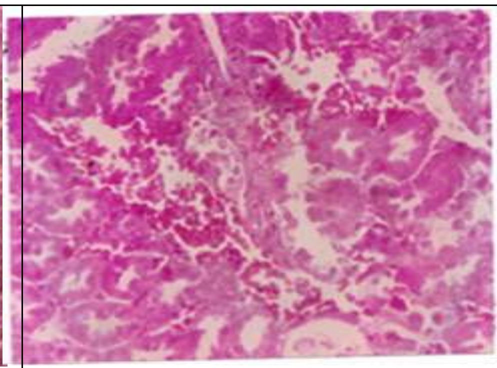
Plate 5. A cross section of kidney from untreated rabbits showing normal glomerulus, Bowman's capsule and tubules (H.&E. X400).	**Plate 6.** A cross section of kidney from rabbits treated with 50 mg Cu-nano/kg diet showing common epithelial lining tubules (H.&E. X400).
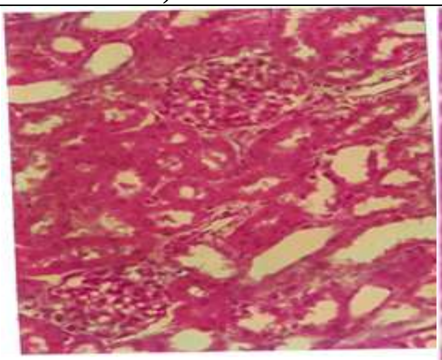	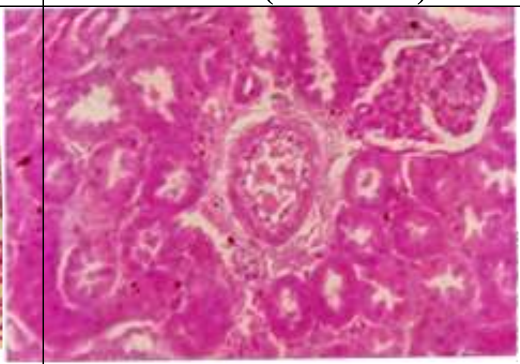
Plate 7. A cross section of kidney from rabbits treated with 200 mg Cu-SO4/kg diet showing few necrosis of the tubules, epithelial glomerulo-nephritis of the glomeruli (H.&E. X400).	**Plate 8**. A cross section of kidney from rabbits treated with 200 mg Cu-meth/kg diet showing usual tubules and blood vessels (H.&E. X400).

Conclusively, supplementing New Zealand White rabbit diets with 50 mg Cu/ kg diet as Cu-nano particles improved growth performance, enhance digestibility of nutrients, hematological response and economical efficiency without any adverse effect in histology of liver and kidney.

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تأثير اضافة النانو مقابل المصادر التقليدية للنحاس على الاداء الإنتاجي للأرانب النيوزلندي النامية

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تهدف الدراسة الحالية الى مقارنة الاستجابة لبعض المصادر المختلفة للنحاس على النمو ومعاملات الهضم وبعض مقاييس الدم والهستولوجى والتقييم الاقتصادي للأرانب النيوزلندي النامية. استخدم عدد ٩٦ أرنب نيوزلندي عمر الفطام (٥ أسابيع) تزن ٦٣٤ جم ±٦,٦ وقد وزعت عشوائيا الى ٤ مجاميع (٢٤ أرنب بكل منها). تغذت المجموعة الاولى على عليقة مقارنة بها ٥ ملجم نحاس/كجم علف بينما تغذت الثلاث مجاميع الاخرى على عليقة المقارنة مضاف اليها ٥٠ ملجم نحاس /كجم علف في صورة نانو النحاس (معاملة ٢)، ٢٠٠ ملجم نحاس/كجم علف في صورة كبريتات النحاس (معاملة ٣)، أو ٢٠٠ ملجم نحاس/كجم علف في صورة نحاس مثيونين (معاملة ٤) على التوالي. وقد استمرت الدراسة حتى ١٣ أسبوع من عمر الارانب. واوضحت النتائج ان تغذية الارانب النامية على عليقة مضاف اليها نانو النحاس (٥٠ ملجم/كجم علف) اعطت أفضل النتائج في اداء النمو مقارنة بمصدري النحاس الاخريين ومجموعة المقارنة. وقد تمثل التحسن في زيادة وزن الجسم المكتسب وانخفاض معدل الغذاء المأكول وأفضل كفاءة تحويل غذائي ودليل النمو وانخفاض اعداد النافق. بالإضافة للتحسن المعنوي في اجزاء الذبيحة ومعاملات الهضم (المادة الجافة والمادة العضوية والبروتين الخام) والبروتين الخام المهضوم سجلت للمجموعة المغذاة على نانو النحاس. بينما لم تتأثر معاملات هضم كلا من الالياف الخام ومستخلصي الاثير والكربوهيدرات الذائبة والمغذيات الكلية المهضومة والطاقة المهضومة بأي من المعاملات المختلفة.

حققت المجموعة التي تغذت على نانو النحاس أعلى عائد اقتصادي ثم تلتها المجموعتين الاخريين اللتين تغذتا على مصدري النحاس المعدني والعضوي مقارنة بمجموعة المقارنة.

اظهر الفحص الهستولوجى للكبد والكليتين أن استخدام نانو النحاس فى علائق الارانب امن على الحيوانات.

التوصية: يوصى بإضافة ٥٠ ملليجرام نحاس/كجم علف في صورة نانو النحاس لعلائق الارانب النيوزلندي والتى أدت لتحسن الاداء الإنتاجي وصفات الذبيحة ومعامل هضم البروتين وكمياء الدم والكفاءة الاقتصادية بدون التأثير على هستولوجى الكبد والكليتين.