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NANO-COPPER AS A NEW GROWTH PROMOTER IN THE DIET OF GROWING NEW ZEALAND WHITE RABBITS

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Seventy two rabbits, 35 days old and weighing 622g average body weight, were used for the present study. The rabbits were randomly assigned into 4 equal treatments (n=18). Animals in treatment 1 served as a control group and were given basal diet without supplementation, while rabbits in treatments 2, 3 and 4 were given nano-copper in the diet at levels 25, 50 and 75 mg/kg diet, respectively. The experiment lasted for 8 weeks.

***The results revealed that**, rabbits fed diets supplemented with 50 or 75 mg Cu/ kg diet as Nano-Cu recorded significantly higher ($P<0.01$) final body weight and performance index also, recorded better feed conversion ratio during all growth periods than the control group. The group of rabbits fed diet supplemented with 50 mg Nano-Cu/kg diet significantly achieved the best relative growth rate percentage. Dietary supplementation with 50 mg Nano-Cu /kg diet significantly recorded higher carcass, fore part, trunk and hind part percentages comparing with the control group, it also significantly reduced abdominal fat by 28.4%. The Nano- Cu supplementation significantly ($P<0.05$) increased activity of superoxide dismutase enzyme compared with the control group. Copper content in rabbits liver significantly increased by increasing dietary Nano-Cu.*

Dietary Nano-Cu supplementation significantly ($P<0.05$) increased the population of total bacterial count and lactolacillus counts and decreased the population of ureolytic bacteria, Escherichia coli and clostridium spp. Supplementation of Nano-Cu to rabbit diets significantly increased plasma hemoglobin, red blood cells count and lymphocytes percentage. Economical efficiency *improved for rabbits fed either 50 or 75 mg Nano-Cu supplemental diet.*

Conclusively*, Nano-Cu is considered a new substitution for high dose of inorganic copper in growing NZW rabbits feeding and can be fed up to 50 mg/kg diet without any deleterious effects on production and meat safety of rabbits.*
Keywords: Nano-copper, growth promoter, growing rabbits, productive performance.

Mortality rate in growing rabbits is about 30% throughout the year mostly occur after post-weaning during 5-10 weeks of age as reported by Nikkels *et al.* (1976). This problem may be due to non-specific enteropathy arise in weaned rabbits. Many studies Laurence *et al.* (2003) and Alves *et al.* (2004) revealed a reduction in villous height and either an increase or decrease in crypt depth. This is possible due to contributions of local inflammatory reactions to villus-crypt during the weaning (McCracken *et al.*, 1999). Also, the activity of the digestive enzymes in pancreatic tissue is low after 5 days post weaning due to interaction with other factors which may increase the risk of developing post-weaning diarrhea (Hedemann and Jensen, 2004).

Copper is an essential trace element, and plays a vital role in the physiology of animals for feotal growth and early postnatal development, bone maturation and haemoglobin formation (McDowell, 1992 and McDonald *et al.,*2002). Although copper is not essentially a constituent of haemoglobin, it is present in certain other plasma proteins such as ceruloplasmin, which is concerned with the release of iron from the cell into the plasma McDonald *et al.* (2002), and Adu and Egbunike (2010) reported that copper added at levels higher than normal requirement has a growth promoting effect because copper inhibit intestinal harmful microbs, thus it has function to stimulate growth and improve feed efficiency (Shurson *et al.*, 1987).

Dietary copper supplementation at levels of 200 to 300 mg/kg caused a growth enhancement effect, particularly in weaned rabbits (Onifade and Abu, 1998 and Aboul-Ela *et al.*, 2000). Also, Adu *et al.* (2010) found that dietary copper level up to 300 mg/kg improved feed intake, and weight gains in growing rabbits. However, (McDowell, 1992) noticed that when dietary copper level was high, its absorption low, because large amounts of copper is excreted in the feces. According to European Union Commission Health (2003) recommended the maximum concentration of copper allowed in rabbit diets has been restricted to 35 mg/kg. Copper nano particle is one of the nano metal which is recently prepared for application in various fields, so we suppose that if the copper absorption is enhanced the copper supplementation and excretion levels may be reduced. Gonzales-Eguia *et al.* (2009) concluded that the growth performance of piglets fed diets supplemented with 50 mg Nano-Cu/kg diet showed significant improvement in growth performance and copper availability. Also, Lien (2009) found that the bioavailability of copper improved in pigs fed diets supplemented with 50 mg Nano-Cu/kg diet. In broilers, Wang *et al.* (2011) showed that supplementation of Nano-Cu at level of 50 or 100 mg/kg diet improves growth performance, strengthen the immune system, enhance protein synthesis and activates the cecal microbiota.

Studies so far have indicated that the application of nano minerals in animal production and immunity is promising. However, application of nano minerals in this field is immense (Rajendran, 2013). The safety aspects of application of nano-minerals need to be addressed before being applied.

Therefore, this study aimed to evaluate the impact of dietary supplementation of Nano-Cu at different levels on productive and physiological performance of growing NZW rabbits and determine the food safety by testing copper residue on edible organ (liver) and muscles. Finally, to determine the best and beneficial level that would improve rabbit performance.

MATERIALS AND METHODS

The present study was carried out at Sakha Research Station, Animal Production Research Institute, Agriculture Research Center, Egypt.

Preparation of nano copper:

The colloid of copper nanoparticles was prepared at room temperature using $Cu(NO_3)_2$. $3H_2O$ (S.d. Fine-Chem. Ltd) as a source for Cu metal. Typically, 2.41 gm of Cu precursor were added to 100 ml solution of 75 ml deionized water and 25 ml ethylene glycol under strong magnetic stirring. The deep blue solution was subjected to additional stirring for 30 min to attain higher homogeneity. Thereafter, 300 µl of ice cold (1 M) NaBH4 (Merck) was quickly injected into the above mixture. The solution turned colorless after about 1 min, and then turned burgundy, indicating the growth of Cu nanoparticles, Zhang *et al*. (2009). The copper content of Nano- Cu was 37.38%, which was determined by flame atomic absorption spectrometry (Perkin-Elmer Analyst 100: Flame Atomic Absorption spectrometry).

Animals and experimental design:

A total number of seventy two NZW weaned rabbits at five weeks of age (average weight 622 g) were divided equally into four treatments. Each group contained 18 rabbits. The experimental period extended from 5 to 13 weeks of age. The four groups were as follows: The control group, received basal diet without nano copper supplementation, while groups 2, 3 and 4 received basal diet with 25, 50 and 75 mg nano copper/kg diet, respectively.

Diet was pelleted and formulated to meet the recommended nutrient requirements of rabbits according to NRC (1977). Composition and calculated analysis of the basal experimental diet was presented in Table 1. Feed was provided *ad libitum*. Fresh water was available from automatic drinkers with nipples for each cage. All rabbits were observed daily, kept under the same managerial, hygienic and environmental condition, and vaccinated against common diseases.

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. Feed intake (FI) was recorded, while feed conversion ratios (FCR) and, relative growth rate (RGR) was calculated according to the following equation:

Relative growth rate = [(W2 - W1) x100]/ [1/2 (W2+W1)]

Whereas: W1= The initial body weight (g), and W2 = The final body weight (g).

Performance index (PI) = (Final live body weight (kg)/ Feed conversion ratio) × 100, according to North (1981).

Carcass traits and Blood constitutes :

At the end of the experimental period, three animals randomly were taken from each group and slaughtered to study carcass characteristics. Head, heart, kidneys and liver were weighed and carcass percentage was calculated according to Cheeke (1987).

Rabbits blood samples were taken into tubes with anticoagulant (heparin) and centrifuged at 3000 rpm for 5 minutes and plasma were stored at -20^{o}C until analysis for determination of catalase enzyme, whole blood Superoxide dismutase (SOD) activities and plasma copper concentration.

Table 1: Ingredients composition (%) and calculated analysis of the basal experimental diet.

Ingredients	%	Calculated analysis**	
Yellow corn	4.10	DE, kcal/kg	2517
Soybean meal (44%)	6.97	Crude protein %	16.09
Soybean meal (48%)	3.00	Crude fat %	2.28
Wheat bran	34.25	Crude fiber %	14.15
Clover hay	36.29	Total P %	0.80
Barley grains	8.32	Lysine %	0.70
DL. Methionine	0.28	Methionine %	0.37
Molasses	4.00	Methionine +Cys.	0.60
L. lysine Hcl	0.19		
Lime stone	0.70		
Di calcium phosphate	1.20		
NaCl	0.30		
*Vit. and Min. Mixture.	0.30		
Anticoccedial	0.10		
Total	**100**		

*** Each 3 kg contain:** 6000000 IU Vit. A; 900000 IU Vit. D_3; 40000 mg Vit. E; 2000 mg Vit. K_3; 2000 mg Vit. B_1; 4000 mg Vit. B_2; 2000 mg Vit. B_6; 10 mg Vit. B_{12}; 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.

**According to NRC (1977).

All biochemical constitutes were determined using commercial kits (Biodiagnostic Company, Cairo, Egypt).

Copper analysis:

Copper analysis was based on the method of Ansari and Raissy(2011). Samples of feces were collected for 7days from three animals/ treatment, and placed in metabolic cages to analyze of total Cu concentration. Also samples for liver and muscles / each group were analyzed for total Cu concentration. The samples were prepared for Cu analysis by digestion in microwave. The determination of Cu concentration was done by atomic absorption spectrometry (Hitachi, Japan) at the Central Laboratory for Pesticides Residue and Heavy Metals in Food, Agricultural Research Center, Ministry of Agriculture, Egypt.

Cecum traits:

Samples of cecum content were taken individually from rabbits of each group and filtrated to estimate pH and cecum microflora. Total anaerobic bacteria count and *Escherichia coli* (*E. coli*) were estimated according to Collins *et al.* (1995), and lactobacilli bacteria count according to Kim and Goepfert (1971). In addition, cecum pH was measured by using pH meter in filtrate cecum content. Ammonia nitrogen concentration was determined as described by Conway (1958).

Haematology analyses:

Packed cell volume (PCV) was determined 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 differential white blood counts (neutrophils, eosinophils, basophils, lymphocytes and monocytes) were determined using haemocytometer method as described by Lamb (1981). The haemoglobin (Hb) concentration and the blood constants: 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:

Feeding economical efficiency (EE) was calculated and carried out according to the prices of feed ingredients, feed additives and rabbits meat prevailing, during year of 2014.

It was calculated as follows: EE = Net revenue/ Total feed cost

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

Statistical analysis:

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

$$Y_i=\mu + T_i + e_i$$

Where: Y_i = The observation; μ = Overall mean; T_i = Effect of treatments (i = 1, 2, 3 and 4); e_i= 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's New

Multiple Range Test (Duncan, 1955). All data are presented in least square means form.

RESULTS AND DISCCUSION

Growth performance:

Results in Table 2 show that final BW values were significantly (P<0.01) the highest with rabbits fed diets supplemented with either 50 or 75 mg Nano-Cu/kg diet compared with those fed zero or 25 mg Nano-Cu/ kg diet. The proportional increments were 4.6 and 2.9 % for the two highest levels, respectively. The same trend was noted in body weight gain either, during 5 – 9 weeks of age or during the whole growth period (5- 13 weeks of age). These results are in agreement with Wang *et al*. (2011) who showed that dietary supplementation with 100 mg/kg of copper-loaded chitosan nanoparticle (CNP-Cu) increased the average daily body gain in broiler chickens.

The increase in body weight gain may be interpreted through the unique characteristics of nanoparticles might make copper more effective in stimulating activities of some growth factors much better than the control diet or the small amount of Nano-Cu (Du, 2008).

Concerning to rabbits feed intake, it was gradually decreased by increasing Nano-Cu level in the diet during all growth periods. In this concern, Gonzales-Eguia *et al.,* (2009) found that average daily weight and feed/gain ratio were higher in piglets fed diet supplemented with 50 mg Nano-Cu/kg diet comparing with the control group and those fed diet supplemented with copper sulphate (50 mg/kg diet). also, Wang *et al.,* (2011) reported that the average daily gain of broiler chickens received 100 mg/kg of Nano-Cu diets was increased by 6.31% ($P < 0.05$) during 0 to 21d, 8.52% ($P < 0.05$) during 22 to 42 d, and 7.79% ($P < 0.05$) during 0 to 42 d, in comparison with the control group.

Feed conversion ratio was significantly improved in rabbits fed diets supplemented with 50 mg Nano-Cu/kg diet, during all growth periods while, the worst value was recorded in rabbits fed the control diet. The improvement recorded 8.6% comparing to the control group. These results are in accordance with the studies reported by Patton *et al.* (1982) and Grobner *et al.* (1986) who reported that growth performance of rabbits improved as a consequence of copper supplement in the diet or drinking water under tropical conditions, but in contrast with the results obtained by

Table 2: Effect of dietary nano copper supplementation on growth performance of growing NZW rabbits from 5 to 13 wks of age.

Parameters	Nano copper levels (mg/kg)				SEM	Sig.
	0	25	50	75		
No. of animals	18	18	18	18	-	-
Initial body weight (g)	621.50	623.50	624.00	621.80	7.72	NS
Final body weight (g)	1928.30[b]	1922.10[b]	2017.10[a]	1983.30[a]	17.91	**
Daily weight gain (g):						
5-9 weeks	23.99[b]	23.87[b]	25.95[a]	25.29[ab]	0.54	*
9-13 weeks	22.78	22.53	23.82	23.40	0.45	NS
5-13 weeks	23.38[bc]	23.20[c]	24.88[a]	24.35[ab]	0.37	**
Feed intake (g/ d):						
5-9 weeks	58.96[a]	57.63[b]	57.16[bc]	56.03[c]	0.46	**
9-13 weeks	99.04[a]	97.27[b]	96.50[bc]	95.36[c]	0.49	**
5-13 weeks	79.00[a]	77.45[b]	76.83[bc]	75.69[c]	0.56	**
Feed conversion ratio:						
5-9 weeks	2.46[a]	2.45[a]	2.21[b]	2.23[b]	0.06	**
9-13 weeks	4.38[a]	4.38[a]	4.07[b]	4.10[ab]	0.08	*
5-13 weeks	3.39[a]	3.36[a]	3.09[b]	3.12[b]	0.05	**
Relative growth rate(%)	102.50[b]	102.02[b]	105.50 [a]	104.50[ab]	1.46	*
Performance index (%)	56.90[b]	57.20[b]	65.10[a]	63.60[a]	01.03	**
Mortality (%) [(1)]	10.00	5.00	5.00	10.00	-	-

SEM = Standard error of means, Sig.= significance, ** : Significant at 0.01% level of probability,
* : Significant at 0.05% level of probabilityNS: Non-significant.
[a,b,c,] Means in the same row the different superscript are significantly different (P<0.05)., (1) Chi-square test

Adu (2004) and Adu and Egbunike (2010) who concluded that There was no significant effect of supplementing copper (P>0.05) on the feed conversion ratio of growing rabbits.

The results obtained of growth performance parameters were confirmed by the value of relative growth rate where, the group of rabbits fed diet supplemented with 50 mg Nano-Cu/kg diet was the best group. Shurson *et al.* (1990) reported that the performance enhancing effect of copper in animals may be achieved through the microbial gut flora as shown by the results of positive effect of high concentrations (283ppm) of copper. It has been reported that copper can be used as a performance enhancer in fresh water-farming because it protects fish from diseases, thereby improving the growth of fish (Berntssen *et al.,* 1999). Copper was reported to improve digestibility and utilization of nutrients in the diets of pigs and broilers which might be achieved through influencing activities of hormones such as growth and thyroid hormones as reported by Underwood and Suttle (1999).

Carcass characteristics:

The effect of different levels of supplemental Nano-Cu are shown in Table 3. Comparing with the control, carcass, fore parts, trunk and hind parts percentages in rabbits fed diet supplemented with 50mg Nano-Cu /kg diet were higher by 4.2%, 5.7%, 4% and 3.6%, respectively, while, abdominal fat was lower by 28.4%. This may be indicating that Cu is hypolipolemic and has capability of influencing lipid metabolism Idowu *et al.* (2011). In this connection Yassein *et al.* (2011) concluded that dressing, hot carcass weight, leg weight and abdominal fat percentages were significantly improved in rabbits fed diet supplemented with copper comparing to the control group. While, liver weight did not affected significantly by Cu supplementation. On the other hand, Ayyat *et al.* (1995) and Bassuny (1991) who reported that carcass traits was insignificantly affected by dietary copper levels in NZW rabbits.

Oxidative enzymes:

Results in Table 4 indicated that superoxide dismutase enzyme activity was higher in rabbits fed diets supplemented with different levels of Nano-Cu compared with control group. This result is in agreement with Lien (2009) who found that SOD activity in nanocopper supplemental pigs was significantly the highest followed by those fed inorganic copper ($CuSO_4$). McDwell (1992) reported that Cu has an important role in metalloenzyme such as superoxide dismutase (SOD) activity. Also, Gonzales-Eguia *et al.* (2009) showed that Nano-Cu supplementation significantly increased the SOD activity in the blood serum of piglets. This effect may be due to that nanoparticles with reduced size and greater surface area exhibit a high rate of absorption in the gasterointestinal tract (Hussain *et al.,* 2001). As their small size, the nanoparticles can penetrate through small capillaries and absorbed by the cells (Sahoo and Labhasetwar 2003). Plasma catalase enzyme activity and copper were not affected by Nano-Cu supplementation. On the other hand, these results disagree with these reported by Fahmy *et al.* (2009) who showed that exposed to copper nanoparticles decreased level of catalase enzyme activity.

***Copper residues in liver, muscles and feaces*:**

Effect of dietary levels of Nano-Cu on copper residues for NZW rabbits' liver, muscles and feces is shown in Table 4. Results indicated that liver and feaces copper content were significantly ($P<0.05$ and 0.01) higher

Table 3: Effect of dietary nano copper supplementation on carcass traits of growing NZW rabbits.

Parameters	Nano copper levels (mg/kg)				SEM	Sig.
	0	25	50	75		
Carcass, %	48.05^{b}	48.36^{b}	50.09^{a}	49.61^{a}	0.36	**
Fore parts ,%	11.83^{b}	11.90^{b}	12.50^{a}	12.13^{ab}	0.18	*
Trunk, %	17.62^{b}	17.68^{b}	18.32^{a}	18.23^{a}	0.12	*
Hind parts, %	18.60^{b}	18.78^{b}	19.27^{a}	19.24^{a}	0.15	*
Liver, %	3.54	03.56	4.44	4.01	0.28	NS
Kidney, %	0.58	0.52	0.55	0.52	0.04	NS
Heart, %	0.23	0.28	0.33	0.35	0.02	NS
Total edible parts ,%	52.41^{b}	52.71^{b}	55.41^{a}	54.48^{a}	0.405	**
Abdominal fat, %	1.34^{a}	1.20^{a}	0.96^{b}	1.22^{a}	0.053	**
GIT , %(1)	20.61^{a}	19.39^{b}	17.89^{c}	18.90^{bc}	0.401	**

SEM = Standard error of means, Sig.= significance a,b,c Means in the same row the different superscript are significantly different (P<0.05).** : Significant at 0.01% level of probability, *: Significant at 0.05% level of probability, NS: Non-significant (1)GIT: Gastrointestinal Tract

Table 4: Effect of dietary nano copper supplementation on oxidative enzyme activity and copper content in plasma, liver muscles and feces of growing NZW rabbits.

Parameters		Nano-copper levels (mg/kg)				SEM	Sig.
		0	25	50	75		
Superoxide dismutase (u/ml)		103.90^{b}	140.57^{ab}	195.88^{ab}	227.90^{a}	32.11	*
Catalase (mmol/ml)		20.50	28.83	28.83	34.33	4.33	NS
Copper	**Plasma (mg/dl)**	0.06	0.09	0.10	0.12	0.01	NS
	Liver(mg/kg)	1.75^{b}	1.99^{b}	2.22^{ab}	2.91^{a}	0.25	*
	Muscles (mg/kg)	0.73	0.85	0.86	0.87	0.13	NS
	Feces (mg/kg)	10.62	11.32	13.24	18.01	0.33	**

SEM = Standard error of means, Sig.= significance a,b,c Means in the same row the different superscript are significantly different (P<0.05).** : Significant at 0.01% level of probability, *: Significant at 0.05% level of probability, NS: Non-significant

in rabbits fed Nano-Cu compared to control group. A similar trend was observed by Grobner *et al.* (1986) and Adu and Egbunike (2010). The present study also showed that copper residues were greater in liver than in muscles. This result is in line with finding of Valenzuela *et al.* (2011) who showed that liver of rabbits had the highest Cu content. Nano-Cu supplementation had no significant effect on Cu concentration in plasma and muscles (Table 4). This mean that levels of Nano-Cu addition did not exceed the permissible limits. Besides that, we can recommend to get rid of liver organ from rabbits received Nano-Cu.

Microbiological assays:

Caecum content and microbial activity of growing NZW rabbits are presented in Table 5. Dietary Nano-Cu supplementation increased the population of total bacterial count and *lactolacillus* and decreased the population of *E. coli* and *clostridium spp*. in ceacum digesta, especially with 50 mg/kg of Nano-Cu. Compared with the control group, the population of *Lactobacillus* in 50 mg/kg of Nano-Cu supplemented group were increased by 3 folds and the population of *E.coli* and clostridium were decreased by 63.9% and 64.1%, respectively. Likewise, Wang *et al*. (2011) showed that dietary supplementation with Nano-Cu increased *Lactobacillus* count. It is well known that the intestinal microbiota plays a vital role in nutritional and immunological functions of the host animals (Rehman *et al.,* 2008). The lactobacillus in animal intestines is beneficial to the hosts, whereas coliforms are harmful (Santos *et al.,* 2006). This result may be due to that Nano-Cu establish a better environment for the growth of Lactobacillus (Han *et al.,* 2010). Also, Nanoparticles constitute another group of potent antimicrobials that have already been applied in medicine and pharmacology (Monteiro *et al.,* 2009). In our present microbiological assays, the effects of 50 and 75 mg/kg of Nano-Cu supplementation were similar. However, more research is needed.

Hematological response:

Results in Table 6 show that, values of blood picture including Hb, PCV, RBCs, MCH, MCHC and lymphocyte were significantly higher ($P \leq 0.05$) in Nano-Cu supplemented growing rabbits than those of the control group. However, these values are still within the normal ranges. These results disagreed with the reports of Adu (2004) and Ahmed *et al.* (1997) who reported that diets supplemented with copper had no effects on blood values when fed to rabbits. There were no significant differences ($P \leq 0.05$) observed for WBCs level among the experimental groups.

Hemoglobin values were increased (within a normal range) due to addition of Nano-Cu. This may be due to the result of subsequent production of more copper transporting protein ceruloplasmin, which is required for the normal RBC formation by allowing more iron absorption from the small intestine and release of iron from the tissue into the blood plasma as observed by Cromwell *et al.,* (1989). Ceruloplasmin had been

Table 5: Effect of dietary nano copper supplementation on caecum content and microbial activity of growing NZW rabbits.

Parameters	Nano copper levels (mg/kg)				SEM	Sig.
	0	25	50	75		
pH	6.87[a]	6.57[ab]	6.20[b]	6.17[b]	0.18	*
NH_3 (mmol/ l)	13.19[a]	10.77[b]	9.74[b]	9.68[b]	0.38	**
Total bacterial count (10^6)(1)	9.20[c]	13.13[bc]	18.87[a]	17.77[ab]	1.35	**
***Ureolytic bacteria* (10^5)(1)**	3.23[a]	2.70[a]	1.08[b]	1.13[b]	0.22	**
***Lactobacilli* (10^5)(1)**	3.27[c]	5.87[b]	9.37[a]	9.47[a]	0.35	**
***Escherichia coli* (10^4) (1)**	6.93[a]	4.36[b]	2.50[b]	2.68[b]	0.54	**
***Clostridium spp.* (1)**	4.37[a]	3.27[a]	1.57[b]	1.53[b]	0.43	**

(1) SEM = Standard error of means, Sig.= significance a,b,c Means in the same row the different superscript are significantly different (P<0.05).** : Significant at 0.01% level of probability,
(2) *: Significant at 0.05% level of probability, NS: Non-significant,(1) Germ counts expressed in CFU/g caecal digesta.

Table 6: Effect of dietary nano copper supplementation on blood hematological values of growing NZW rabbits.

Parameters	Nano copper levels (mg/kg)				MSE	Sig.
	0	25	50	75		
Hemoglobin (g/ dl)	12.13 [b]	12.4 [ab]	12.66 [ab]	13.56 [a]	0.356	*
PCV (%)	30.6 [c]	32.41 [c]	33.46 [b]	38.7 [a]	0.155	*
RBCs (10^6/ µl)	5.19 [b]	5.89 [a]	6.05 [a]	6.18 [a]	0.144	*
MCV(1) (fl)	59.75 [d]	66.83 [c]	67.1 [b]	67.76 [a]	0.078	*
MCH(2) (pg)	19.5 [d]	21.8 [c]	22.26 [b]	22.6 [a]	0.088	*
MCHC(3) (g/ l)	32.5 [c]	32.86 [b]	33 [b]	34.36 [a]	0.074	*
WBCs (10^3/ µl)	6.08	7.25	7.87	8.4	2.511	NS
Lymphocytes (%)	44.73 [d]	47.73 [c]	48.73 [b]	55.45[a]	0.302	*

SEM = Standard error of means, Sig.= significance a,b,c Means in the same row the different superscript are significantly different (P<0.05)*: Significant at 0.05% level of probability, NS: Non-significant (1) Mean corpuscular volume , (2) Mean corpuscular hemoglobin
(3) Mean corpuscular hemoglobin concentration

reported to play a critical role in the haematopoietic process, by facilitating the mobilization of iron from the reticuloendothelial cells of the liver and spleen to the bone marrow cells and by catalyzing the oxidation of ferrous iron ions during the formation of ferritransferrin (Freiden and Hsieh, 1976). Bassuny (1991) reported similar result in rabbits, where the values of the RBC and Hb were higher in animals fed dietary copper than the control. Also, Dorton *et al.* (2003) concluded that dietary supplementation with copper could stimulate body immune system.

Economical efficiency:

Effect of Nano-Cu supplementation at different levels are shown in Table 7. Rabbits fed basal diet supplemented with either 50 or 75 mg Nano-Cu/kg diet recorded high economical efficiency (107.9 and 104.5%), respectively compared with the control group. These results were in accordance with Ayyat (1995) who observed that addition of 100 or 200 mg copper in rabbit diets increased final, margin with 27.0 and 51.7%, respectively than the control diet.

Table 7: Effect of dietary nano copper supplementation on economical traits of NZW rabbits at 13 wks of age.

Parameters	Nano copper levels (mg/kg)			
	0	25	50	75
Average feed intake (kg /head)	4.42	4.34	4.30	4.24
Price /kg diet (L.E.)	2.25	2.30	2.35	2.40
Total feed cost (L.E.)	9.96	9.98	10.11	10.18
Average weight gain (kg/head)	1.31	1.30	1.39	1.36
Selling price (L.E.)(1)	35.37	35.10	37.53	36.72
Net revenue (L.E.)(2)	25.41	25.12	27.42	26.54
Relative revenue (%)	100	98.86	107.91	104.45

Other conditions like management are fixed.
- Ingredients price (L.E. per ton) at 2013 were: 2500 yellow corn; 3000 barley; 1500 berseem hay; 1800 wheat bran; 3750 soybean meal (44%); 4000 soybean meal (48%); 250 limestone; 9000 premix; 40000 methionine; 21000 lysine; 1000 di-calcium phosphate; 1000 molasses; 250 salt; 13000 choline chloride; 5000 antioxidant.
- Adding 100 L.E. /ton for pelliting.

(1) Price of kg live body weight was 27 L.E.
(2) Net revenue = Selling price – total feed cost

Conclusively, the present results indicated that the supplementation of Nano-Cu can improve growth performance, influence intestinal microbiota, and improve economical efficiency, especially with the supplementation of 50 mg/kg, and Nano-Cu could be a new substitute for high dose of inorganic copper.

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نانو النحاس كمنشط نمو جديد فى علائق الارانب النيوزلندى النامية

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استخدم فى هذه الدراسة عدد ٧٢ أرنب نيوزلندى عمر ٣٥ يوم بمتوسط وزن ٦٢٢ جرام . تم تقسيم الارانب الى أربعة مجاميع (١٨ أرنب بكل مجموعة) ، المجموعة الاولى هى مجموعة المقارنة بينما تمت اضافة نانو النحاس بالمعدلات الاتية ٢٥، ٥٠، ٧٥ ملجم/كجم علف للمجاميع الثانية والثالثة والرابعة على التوالى، وقد استمرت تجربة التغذية لمدة ٨ أسابيع.

وقد أشارت النتائج المتحصل عليها الى ان اضافة نانو النحاس بمعدل ٥٠، ٧٥ ملجم/كجم عليقة حسنت معنويا وزن الجسم والمعدل النسبى للنمو ايضا سجلت أفضل

كفاءة تحويلية للغذاء خلال مراحل النمو مقارنة بمجموعة الكنترول. حققت مجموعة الارانب المغذاة على علائق مزودة بنانو النحاس بمعدل ٥٠ ملجم/كجم عليقة أحسن نسبة لمعدل النمو النسبى.سجلت الارانب المغذاة على ٥٠ ملجم نانو النحاس/كجم عليقة أعلى نسبة لوزن الذبيحة والاجزاء الامامية والخلفية والجذع مقارنة بمجموعة الكنترول بالاضافة لان هذه المجموعة قللت من نسبة دهون البطن بمعدل ٢٨,٤% . اضافة نانو النحاس بمستوياته المختلفة حسن معنويا من نشاط انزيم superoxide dismutase والاعداد الكلية للبكتريا و بكتيريا اللاكتوباسيلس بينما قلت أعداد بكتريا ureolytic, Escherichia coli clostridium spp.

زاد تركيز النحاس فى كبد الارانب معنويا بزيادة نسبة نانو النحاس فى العليقة. حسنت اضافة نانو النحاس لعلائق الارانب معنويا نسبة الهيموجلوبين وعدد كرات الدم الحمراء ونسبة الخلايا الليمفاوية. تحسنت الكفاءة الاقتصادية لمجموعة الارانب المغذاة على علائق مزودة ب ٥٠ أو ٧٥ ملجم نانو النحاس/كجم عليقة.

التوصية: يمكن استخدام نانو النحاس بمعدل ٥٠ ملجم/كجم عليقة كبديل للجرعات العالية من المصادر الاخرى للنحاس فى علائق الارانب النيوزلندى النامية.