

## **INFLUENCE OF SOME TRACE MINERALS IN FORM OF NORMAL AND NANO PARTICLES AS FEED SUPPLEMENTATION ON GROWING RABBIT DIETS**

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### **SUMMARY**

**T**his study was conducted to evaluate effect of adding some trace minerals (copper, zinc and selenium) in Normal and Nano form in a half of amount. Seven diets adding with Control (0), 1g Normal copper Cu, 0.5g Nano-Cu, 0.8 Normal Zinc (Zn), 0.4 g Nano- Zn, 0.2 Normal selenium (Se) and 0.1mg Nano-Se /100kg of diet, respectively. Eighty four New Zealand White (NZW) growing rabbits (5 weeks old) with an average weight of 565 g were randomly assigned individually to seven treatments of twelve rabbits each. All diets were formulated to be iso-protein and iso-digestible energy, and to satisfy the nutrients requirements according to Agriculture Ministry Decree (1996) recommendation. The experimental lasted for 8 weeks. The results showed that nano form of trace minerals used have more improving in live body weight, body weight gain and feed conversion. There are no significant differences in dry matter digestibility and organic matter digestibility between groups. The digestion coefficients of different nutrients and nutritive values of diets contained trace minerals (Cu, Zn and Se) were significantly ( $P \leq 0.05$ ) better in general than control group. Groups supplemented with Nano- Cu, Nano- Ze and Nano- Se were the best one's in the digestion coefficients of different nutrients and nutritive values between all groups. Diets had significant ( $P \leq 0.05$ ) effect on carcass traits, highest cecum weight and Total edible parts were in group supplemented with Nano-Se. Also, the highest liver and heart weight were in group supplemented with Nano-Se. Data showed that rabbit fed diet supplemented with Nano-Se increased significantly ( $P < 0.05$ ) in total protein, glucose and total antioxidant capacity. Results also, showed that least cecum microbial counts, in general were noticed with Nano-Se group. Yeast counts increased when compared with the control diet except normal-Cu group. The least feed cost/ Kg body weight gain, economic efficiency and the best relative economic efficiency were for Nano-Cu supplementation, while the worst values were for control. The all diets for normal and nano trace minerals were better than the control diet. In conclusion, adding copper, zinc and selenium in normal form and nano form in even a half of amount in growing rabbit diets can be used economically without any adverse effects on growing rabbit performance with percentages used.

**Keywords:** *Nano zinc, nano copper, nano selenium, rabbits, growth performance, digestibility, carcass traits.*

### **INTRODUCTION**

Trace minerals have an important role in biological processes happened in animal body. Selenium (Se) is an essential trace mineral for animal and human. Supplementation of Se usually in livestock diet has been proved as effective element. The role of Se in production performance in animal seems to be dependent on both Se sources. Inorganic and organic forms of Se (selenate, selenide, selenium-enriched yeast, selenium-enriched algae) may be used as supplements. Selenate is the major inorganic selenocompound found in both animal and plant tissues (Guo and Wu, 1998). Se supplementation may also improve the efficiency of the antioxidant system; enhance the disease resistance and nutritional quality of the livestock product. Zinc has an important role in numerous biological processes in avian and mammalian species. For instance, zinc and copper are an essential components of many enzymes (Vallee and Auld, 1990), and they have both structural and catalytic functions in metalloenzymes (O'Dell, 1992). Using trace minerals in a nano scale (between 1 nanometer (nm) and 100 nm) maybe more active for a biological processes. The Nano-Cu used in dose 80 mg/kg in rabbit diet improved the activities of

trypsin, amylase and lipase in the small intestinal contents and maltase, sucrose and lactase of duodenum, jejunum, and ileum mucosa (Xin-Yan Han, 2012). Many researches reported that a novel elemental Se source called Nano-Se possessed a higher efficiency than selenite, selenomethionine and methylselenocysteine in upregulating selenoenzymes in mice and rats (Zhang *et al.*, 2005, 2008; Wang *et al.*, 2007), and exhibited a lower toxicity (Zhang *et al.*, 2001). The development of nanotechnology holds unique properties for this redox state Se 0, powder, because nanometer particulates exhibit novel characteristics, such as great specific surface area, high surface activity, a lot of surface active centers, high catalytic efficiency and strong adsorbing ability (Shi *et al.*, 2011). However, little was known about influence of Nano-Cu, Nano-Zn and Nano-Se on animal nutrition. Thus, the objective of this study was to evaluate the effects of Nano-Cu, Nano-Zn and Nano-Se supplemented dose half in normal form in growing rabbit performance.

## **MATERIALS AND METHODS**

This experimental study was carried out at Noharia research Station, El-Behira Government, Egypt, Ministry of Agriculture. The Laboratory work was conducted at Laboratories of By-products Research Department, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. The experiment aimed to evaluate adding copper, zinc and selenium in normal and nano form in even a half of amount in growing rabbit diets.

### ***Experimental diets***

Seven diets adding with Control (0), 1g Normal copper Cu, 0.5g Nano- Cu, 0.8 Normal Zinc (Zn), 0.4 g Nano-Zn, 0.2 Normal selenium (Se) and 0.1 mg Nano- Se/100kg diet, respectively. All diets were iso-protein and iso-digestible energy, and to satisfy the nutrient requirements according to Agriculture Ministry Decree (1996) recommendations. The experimental lasted for 8 weeks. Composition and calculated analysis of the experimental diets are presented in Table (1).

Nano trace minerals synthesized in National Research Centre, Center of Excellence for Advanced Sciences, Advanced Materials and Nanotechnology Group 12622 Dokki, Cairo, Egypt. Nano-Se was synthesized by reducing selenite in an environment containing bovine serum albumin (BSA), which is able to adhere to Se atoms and control the size of their aggregation according to Zhang *et al.* (2001). For nano copper and zinc were created by the artisans by adding copper and zinc salts and oxides together with vinegar, ochre and clay, on the surface of previously-glazed pottery. The object was then placed into a kiln and heated to about 600 °C in a reducing atmosphere. In the heat the glaze would soften, causing the copper and zinc ions to migrate into the outer layers of the glaze. There the reducing atmosphere reduced the ions back to metals, which then came together forming the nano particles according to Philip (1984).

To identify the particle size and morphology of the synthesized materials (Nano particles), transmission electron microscope (TEM) type “JEOL JEM-1230 operating at 120 kv attached to a CCD camera” was used.

### ***Animals and management***

A total number of 84 NZW weaned rabbits at 5 weeks of age about 556 g as an average body weight were assigned, individually, into 7 treatments of 12 rabbits each. Rabbits were housed in galvanized metal rabbit battery cages (60 x 50 x 40) supplied with separated feeders. Diets were offered in pellets form *ad libitum* and fresh water was available all times from automatic nipple drinkers. All animals were kept under the same managements and hygienic conditions. Both feed intake and live body weight were recorded weekly and then feed conversion ratio was calculated

### ***Digestibility trail***

At the end of the experimental period, digestibility trail was carried out using four rabbits of each treatment. Feces were collected daily, weighed and dried at 60-70 ° C for 24 hours, finely ground and stored for chemical analysis. Data of quantities and chemical analysis of feed and feces were used to calculate the nutrients digestion coefficients and the nutritive values of the dietary treatments, as described by Cheeke *et al.* (1982).

Blood samples were collected from three animals in each group, at the end of experimental period after slaughtering. Blood plasma samples were separated by centrifugation at 4000 rpm for 10 minutes,

then frozen at -20<sup>0</sup> C until analysis. Commercial kits were used to determine plasma total protein, albumin, globulin and activities of AST and ALT, glucose, urea and total antioxidant capacity (TAC).

**Table (1): Composition and calculated analysis of the experimental diets for growing rabbits.**

Ingredients	Control	Copper**		Zinc**		Selenium**	
		normal	nano	normal	nano	normal	nano
		1g/ 100kg	0.5g/ 100kg	0.8g/ 100kg	0.4g/ 100 kg	0.2 mg/ 100kg	0.1mg/ 100 kg
Clover hay (12%CP)	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Barely	29.00	29.00	29.00	29.00	29.00	29.00	29.00
Yellow corn	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soybean meal (44%CP)	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Wheat bran	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Molasses	3.00	3.00	3.00	3.00	3.00	3.00	3.00
DL-Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vit.& Min. mix.*	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Limestone	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100	100	100
<i>Calculated analysis<sup>l</sup></i>							
Crude protein %	17.02	17.02	17.02	17.02	17.02	17.02	17.02
Digestible energy (Kcal/Kg)	2500.0	2500.0	2500.0	2500.0	2500.0	2500.0	2500.0
C/P ratio	147	147	147	147	147	147	147
Ether extract %	2.72	2.72	2.72	2.72	2.72	2.72	2.72
Crude fiber %	13.25	13.25	13.25	13.25	13.25	13.25	13.25
NDF% <sup>m</sup>	37.63	37.63	37.63	37.63	37.63	37.63	37.63
ADF% <sup>n</sup>	21.52	21.52	21.52	21.52	21.52	21.52	21.52
Hemicellulose % <sup>o</sup>	16.11	16.11	16.11	16.11	16.11	16.11	16.11
Calcium %	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Total Phosphorus %	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Methionine %	0.36	0.36	0.36	0.36	0.36	0.36	0.36
TSAA	0.61	0.61	0.61	0.61	0.61	0.61	0.61
Lysine %	0.75	0.75	0.75	0.75	0.75	0.75	0.75

\* Each 1.5Kg. of Vit. mix contained: 50,000,000 IU Vit.A; 1,000,000 IU D<sub>3</sub>; 10,000 mg Vit. E; 1170 mg Vit. K<sub>3</sub>;735 mg Vit.B<sub>1</sub>; mg Vit.B<sub>2</sub>; 15000 mg Vit B<sub>6</sub>;15 mg Vit;B<sub>12</sub>; 500 mg Vit.B5 Panathonic acid; 30,000 g Nicotinic acid; 84 mg Biotin; 500 g Folic acid; 300g choline cholride. Each 1.5 Kg Min. mix contained 25 g Zn (oxid); 33.4 g Mn; 26.7 g Fe ; 2.67 g Cu; 67 mg cobalt;1mg Se and.0.334 gI.

<sup>l</sup>According to Feed Composition Tables for animal and poultry feedstuffs used in Egypt (2001) , except values of DDGS, which were determined (Table 2).

<sup>mmo</sup> Calculated according to Cheeke (1987).

<sup>m</sup>% NDF = 28.924 + 0.657 (%CF)

<sup>n</sup>% ADF = 9.432 + 0.912 (%CF)

<sup>o</sup>Hemicellulose = %NDF - % ADF

\*\*Copper, Zinc and Selenium in normal or nano form were supplemented over requirements, that the requirements were covered from vit .& min. mixture in all diets (control diet or other diets).

### Cecum activities

Samples of cecum contents from the same slaughtered rabbits under each treatment were taken and used immediately for estimation of cecum pH, cecum microflora (bacteria) Aerobic total count, Fecal coliforms, Escherichia coli count, Bacillus cereus, Enterobacter, Clostridium sp., Enterococcus, yeasts, Salmonella and Shigella. Another sample of cecum content was strained through four folds of gauze and divided into two portions. The first portion was used immediately for the estimation of ammonia nitrogen concentration. The second portion was preserved by addition of 1 ml N/10 HCL and 2 ml orthophosphoric acid to each 2 ml of cecum contents juice for determination total volatile fatty acids. The pH of the cecum contents was measured immediately by using a digital pH meter. The microbial contents

were studied in their selective media, as described by Postage (1969) for Aerobic total bacterial counts and Difco (1989) for Fecal coliforms and E.coli, while, the methods described by Baired Parker (1962) and Kim and Goepfert (1971) were used for Enterococcus and Bacillus cereus, respectively and Difco (1989) for Enterobacter and Clostridium sp.; while the method described by Lodder (1952) was used for yeasts determination. Salmonella and Shigella were enumerated according to the methods described by AOAC (1998). Technique of colony forming unit (CFU) was adopted. Incubation took place at 30 °C for 2-7 days. The ammonia nitrogen concentration was determined by applying method of Conway (1958). The total volatile fatty acids were determined by steam distillation of the distillate as mentioned by Eadie *et al.* (1967).

#### ***Economic efficiency***

The economic efficiency of the experimental diets was calculated as the ratio between income (price of weight gain) and cost of feed consumed, calculated according to the price of the Egyptian market.

#### ***Statistical analysis***

The data were analyzed using General Linear Models (GLM) procedure of SAS (2001). The statistical model was:

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

Where:  $\mu$  = overall mean of  $Y_{ij}$ ,  $T_i$  = Effect of treatment groups,  $I = (1, \dots$  and 7) and  $e_{ij}$  = Experimental error. Variables having a significant F-test were compared using Duncan's multiple rang test (Duncan, 1955).

## **RESULTS AND DISCUSSIN**

#### ***Chemical structure of nano trace elements***

Images of synthesized copper (a), zinc (b), and selenium (c) nanoparticles at two different magnifications. It is indicated that the selenium exhibits agglomerated rod-like particles with homogeneous particles having length of about 50 nm and width ranging between 8 and 26 nm. Well-dispersed copper nano particles with different morphologies i.e. rods, cuboids and cubes, are appeared (Fig. 1c). Their sizes are ranging between 25-90 nm. On the other hand, agglomerated flower shape zinc nanoparticles are detected (Fig. 1b). These agglomerates consist of several cube nanoparticles with size ranging between 6-11 nm.

#### ***Growth performance***

##### ***Live body weight and body weight gain***

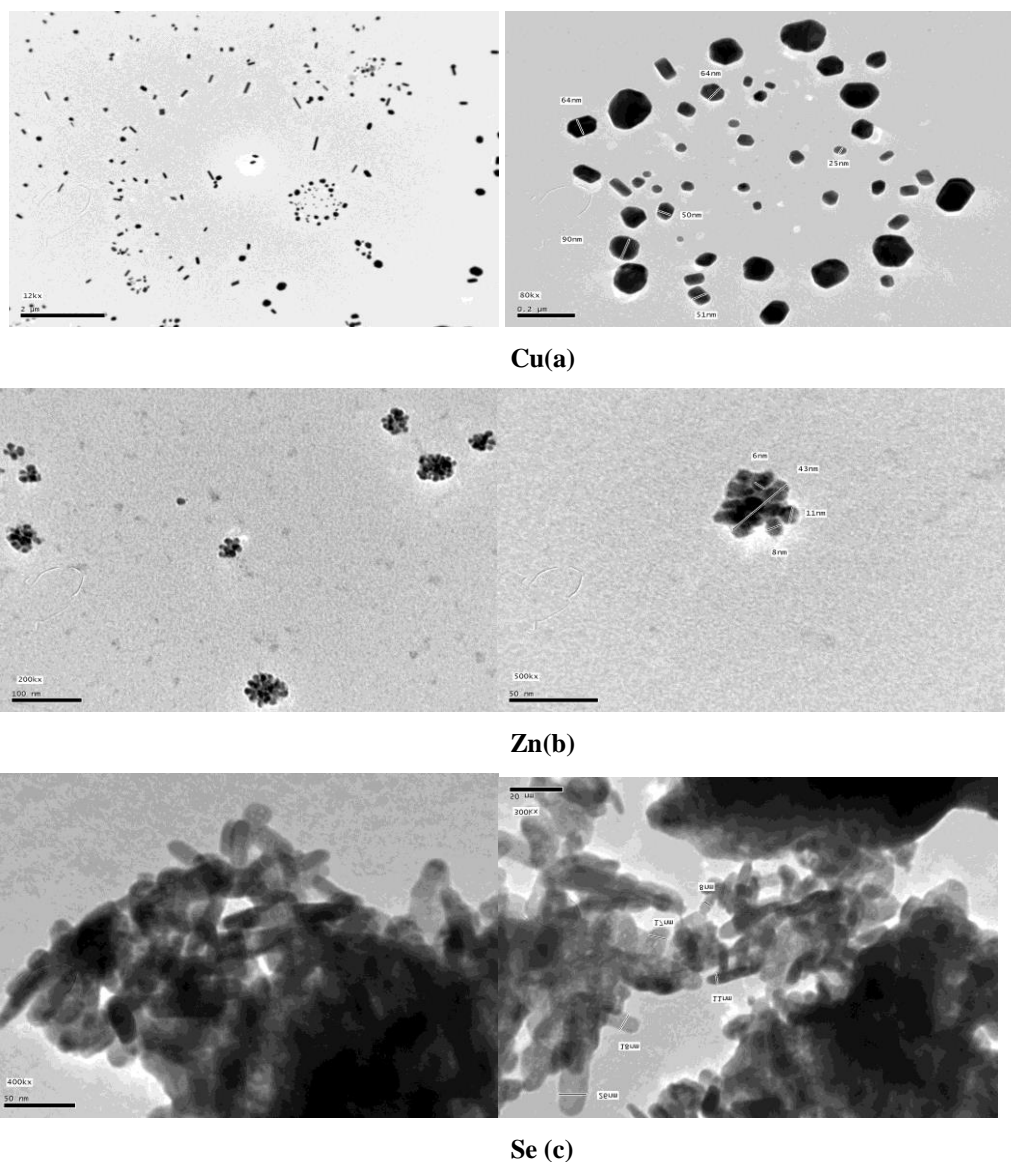
Results presented in Table (2) indicated that there is a significant ( $P \leq 0.05$ ) difference between treatments comparing with control in final live body weight and body weight gain except that there isn't a significant difference between control group and group supplemented with Normal copper in a total body weight gain. The best one's were for group supplemented with nano-Se. Also, results indicated that no significant differences between groups supplemented with Nano-Cu, Normal-Zn and Normal-Zn in alive body weight. in 9 weeks age, there no significant difference between group supplemented with Nano-Cu and group supplemented with Nano-Se in alive body weight. For weight gain 10-13 weeks and a total weight gain there a significant difference between groups supplemented with Normal Cu, Zn, Se and group supplemented Nano form of them these results agree with Okunlola *et al.* (2015).

This result indicated that Nano form of trace element has more improving in live body weight and body weight gain, that's may be related to more active for a biological processes in body, which resulting to great specific surface area, high surface activity, a lot of surface active centers, high catalytic efficiency and strong adsorbing ability of elements in a Nano form (Zhang *et al.*, 2005, 2008; Wang *et al.*, 2007).

##### ***Feed intake and feed conversion***

Also, results presented in Table (2) showed that a significant ( $P \leq 0.05$ ) difference between control group and others groups that the higher one in total feed intake and feed conversion was in the control group. There is no significant difference in total feed intake and feed conversion between groups supplemented with Normal Cu, Zn, Se Nano Cu and Zn. The best one in a total feed conversion was for group supplemented with Nano-Se.

Results indicated that nano form of trace mineral groups were more improving in feed conversion that's relating to improving in weight gain in this groups. These results were in agreement with (Hu *et al.*, 2012).



**Figure (1): Images of synthesized copper (a), zinc (b) and selenium (c).**

***Digestion coefficients of nutrients and nutritive values***

The results presented in Table (3) showed significant differences between groups in the most of the digestion coefficients of different nutrients and nutritive values. There is no significant difference in dry matter digestibility and organic matter digestibility only between groups. The digestion coefficients of different nutrients and nutritive values of diets contained trace minerals (Cu, Zn and Se) were significantly ( $P \leq 0.05$ ) better than control group. Groups supplemented with Nano- Cu, Nano- Zn and Nano- Se were the best one's in the digestion coefficients of different nutrients and nutritive values between all groups.

**Table (2): Rabbits performance values as affected by the experimental diets.**

Item	Control	Normal copper	Nano copper	Normal zinc	Nano zinc	Normal selenium	Nano selenium
<i>Live body weight (g)</i>							
Initial	559.55	569.17	565.33	567.50	562.50	563.33	568.33
Weight	±15.38	±18.98	±17.81	±16.79	±19.55	±20.28	±19.02
9 weeks	1096.25 <sup>d</sup>	1153.75 <sup>cd</sup>	1248.33 <sup>ab</sup>	1217.08 <sup>b</sup>	1229.17 <sup>b</sup>	1196.25 <sup>bcd</sup>	1305.83 <sup>a</sup>
	±26.34	±26.34	±21.81	±14.18	±24.97	±17.09	±15.40
13 weeks	1693.42 <sup>d</sup>	1761.00 <sup>c</sup>	1806.67 <sup>bc</sup>	1785.00 <sup>c</sup>	1845.00 <sup>b</sup>	1780.42 <sup>c</sup>	2.006.67 <sup>a</sup>
	±15.85	±18.12	±21.19	±23.31	±21.30	±11.94	±13.45
<i>Body weight gain (g)</i>							
5-9 weeks	536.70 <sup>e</sup>	584.58 <sup>d</sup>	683.00 <sup>b</sup>	649.58 <sup>bc</sup>	666.67 <sup>bc</sup>	632.92 <sup>c</sup>	737.50 <sup>a</sup>
	±18.47	±14.61	±21.71	±11.62	±15.19	±8.23	±9.70
10-13 weeks	597.17 <sup>bc</sup>	607.25 <sup>bc</sup>	558.34 <sup>c</sup>	567.92 <sup>bc</sup>	615.83 <sup>b</sup>	584.17 <sup>bc</sup>	700.84 <sup>a</sup>
	±17.50	±18.05	±13.97	±14.45	±10.11	±18.74	±21.51
5-13 weeks	1133.87 <sup>e</sup>	1191.83 <sup>de</sup>	1241.34 <sup>bc</sup>	1217.50 <sup>cd</sup>	1282.50 <sup>b</sup>	1217.09 <sup>cd</sup>	1438.34 <sup>a</sup>
	±7.83	±18.92	±19.44	±19.51	±18.10	±22.99	±26.02
<i>Feed intake(g)</i>							
5-9 weeks	1721.50 <sup>ab</sup>	1503.50 <sup>c</sup>	1681.58 <sup>ab</sup>	1570.25 <sup>bc</sup>	1619.00 <sup>bc</sup>	1598.33 <sup>bc</sup>	1803.92 <sup>a</sup>
	±62.10	±42.25	±62.35	±22.36	±46.55	±56.30	±47.60
10-13 weeks	2737.50 <sup>a</sup>	2195.20 <sup>bc</sup>	2093.10 <sup>c</sup>	2150.90 <sup>bc</sup>	2337.90 <sup>b</sup>	2224.80 <sup>bc</sup>	2178.50 <sup>bc</sup>
	±89.99	±103.48	±53.46	±57.31	±53.83	±91.26	±49.15
5-13 weeks	4459.00 <sup>a</sup>	3698.70 <sup>d</sup>	3374.68 <sup>bcd</sup>	3721.15 <sup>cd</sup>	3956.90 <sup>bc</sup>	3823.13 <sup>bcd</sup>	3982.42 <sup>b</sup>
	±67.34	±88.54	±62.73	±70.29	±63.61	±116.10	±67.46
<i>Feed conversion</i>							
5-9 weeks	3.21 <sup>a</sup>	2.57 <sup>b</sup>	2.46 <sup>b</sup>	2.42 <sup>b</sup>	2.43 <sup>b</sup>	2.53 <sup>b</sup>	2.45 <sup>b</sup>
	±0.08	±0.07	±0.07	±0.03	±0.03	±0.08	±0.05
10-13 weeks	4.58 <sup>a</sup>	3.61 <sup>b</sup>	3.75 <sup>b</sup>	3.79 <sup>b</sup>	3.80 <sup>b</sup>	3.81 <sup>b</sup>	3.11 <sup>c</sup>
	±0.06	±0.11	±0.08	±0.07	±0.03	±0.08	±0.04
5-13 weeks	3.93 <sup>a</sup>	3.10 <sup>b</sup>	3.04 <sup>b</sup>	3.06 <sup>b</sup>	3.09 <sup>b</sup>	3.14 <sup>b</sup>	2.77 <sup>c</sup>
	±0.06	±0.04	±0.04	±0.03	±0.01	±0.04	±0.03

a, b, c, d and e: Means in the same row with different superscripts are significantly different ( $P \leq 0.05$ ).

SE = Standard error.

**Table (3): Digestion coefficients of nutrients and nutritive values as affected by the experimental diets.**

Treatments	Digestibility (%)						Nutritive values <sup>k</sup>	
	DM	OM	CP	CF	EE	NFE	TDN	DCP
Control	64.53	59.21	71.68 <sup>c</sup>	46.57 <sup>c</sup>	77.65 <sup>b</sup>	67.61 <sup>b</sup>	52.36 <sup>b</sup>	13.56 <sup>c</sup>
	±0.96	±2.19	±0.19	±0.99	±1.29	±1.63	±1.68	±0.04
Normal Copper	64.55	64.55	76.03 <sup>b</sup>	55.11 <sup>ab</sup>	82.22 <sup>a</sup>	70.31 <sup>b</sup>	60.10 <sup>b</sup>	14.41 <sup>b</sup>
	±0.53	±0.48	±0.68	±1.48	±1.71	±1.96	±1.42	±0.13
Nano Copper	64.57	59.86	75.01 <sup>b</sup>	55.54 <sup>ab</sup>	82.81 <sup>a</sup>	68.70 <sup>b</sup>	57.38 <sup>b</sup>	14.20 <sup>b</sup>
	±0.34	±0.80	±0.89	±1.18	±0.59	±0.55	±1.13	±0.17
Normal Zinc	64.18	59.70	75.76 <sup>b</sup>	52.73 <sup>b</sup>	82.33 <sup>a</sup>	68.27 <sup>b</sup>	57.42 <sup>b</sup>	14.32 <sup>b</sup>
	±1.64	±2.37	±1.51	±2.43	±1.28	±2.76	±2.53	±0.29
Nano Zinc	64.95	60.74	75.26 <sup>b</sup>	55.30 <sup>ab</sup>	83.27 <sup>a</sup>	69.03 <sup>b</sup>	58.02 <sup>b</sup>	14.28 <sup>b</sup>
	±1.21	±1.28	±1.18	±2.49	±0.55	±0.37	±1.23	±0.23
Normal Selenium	62.44	57.78	75.22 <sup>b</sup>	50.02 <sup>bc</sup>	76.96 <sup>b</sup>	67.35 <sup>b</sup>	56.53 <sup>bc</sup>	14.20 <sup>b</sup>
	±0.19	±0.49	±0.15	±0.49	±0.67	±0.70	±0.60	±0.03
Nano Selenium	64.36	62.37	79.53 <sup>a</sup>	59.74 <sup>a</sup>	85.59 <sup>a</sup>	76.76 <sup>b</sup>	64.62 <sup>a</sup>	15.15 <sup>a</sup>
	±1.11	±4.99	±0.87	±2.22	±0.24	±0.16	±0.60	±0.16

a, b and c: Means in the same column with different superscripts are significantly different ( $P \leq 0.05$ ).

k: Calculated according to Cheeke et al. (1982).

Results indicated that nano form of trace minerals were more improving the digestion coefficients of different nutrients and nutritive values, that's may be related to more active for a biological processes and great specific surface area, high surface activity and strong adsorbing ability of elements in a nano form (Zhang *et al.*, 2005, 2008 and Wang *et al.*, 2007). That's improving soluble fiber has high water holding capacity, readily forms gel, increases luminal viscosity, and is easily degraded by micro- flora in the large bowel. On the contrarily, insoluble fiber has little water holding capacity, decreases transit time, is only partially degraded by micro- flora, and increases fecal bulk (Swanson *et al.*, 2001).

Also, results agree with Shi *et al.* (2011) who indicated that fraction of CP was quadratically ( $P < 0.01$ ) increased with increasing Nano-Se supplementation and Xin-Yan Han (2012) who reported that the Nano-Cu used in dose 80 mg/kg in rabbit diet improved the activities of trypsin, amylase and lipase in the small intestinal contents and maltase, sucrose and lactase of duodenum, jejunum, and ileum mucosa.

#### ***Carcass traits***

Effects of diets on carcass traits are shown in Table (4). Diets had significant ( $P \leq 0.05$ ) effect on carcass traits, with no clear trend. Highest cecum weight and total edible parts were in group supplemented with Nano-Se. Also, the highest liver and heart weight were in group supplemented with Nano-Se. These results were in agreement with (Hu *et al.*, 2012). There is no significant ( $P \geq 0.05$ ) difference in dressing percentage between control group and groups supplemented with normal and Nano-Zn and the lower one significantly ( $P \leq 0.05$ ) was for group supplemented with normal Cu. The highest group significantly ( $P \leq 0.05$ ) in edible parts percentage was for group supplemented with Nano-Se. These results agree with (Selim *et al.*, 2015) who reported that using Se-Yeast or Zn-Se Methionine as organic form of Se or L-Nano Se as nano form of Se at level of 0.30 ppm in broiler diets or its equivalent in drinking water are more effective to get better growth performance and quality of broiler meat.

#### ***Blood parameters***

Table (5) showed the effect of feeding rabbits on different diets on plasma constituents. There are significant effects on all parameters measured, except AST with no trend. Also, the Table showed that rabbit feeding on diet supplemented with Nano-Se increased significantly ( $P < 0.05$ ) in total protein, glucose and total antioxidant capacity. These results agree with Okunlola *et al.* (2015), who indicated that the activity of the blood enzymes measured increase with selenium supplementation whereas the cholesterol fall significantly in birds fed selenium supplemented diets. The hematological and serum characteristics were not significantly affected ( $P \geq 0.05$ ) across the treatments, except the differential count of the white blood cells.

#### ***Cecum activities***

Data in Table (6) showed the effect of using different forms of Cu, Zn and Se in rabbit diets on cecum activity pH, ammonia and TVFA's which were determined, at the end of the growing period (13 weeks of age), the results showed that had significant ( $P \leq 0.05$ ) effect on cecum pH, ammonia and TVFA's concentration. The values of pH were ranged between 6.10 - 6.23 for treatments groups vs. 6.07 for control, while ammonia concentration values ranged between 7.83 - 8.98 mg/100 ml for treatments vs. 8.99 mg/100 ml for control. Ammonia concentration decreased in rabbits fed on diets supplemented with trace elements, especially diets supplemented with Nano-Zn and Nano-Se. All rabbits fed on diets gave higher cecum pH than the control. The lowest ( $P \leq 0.05$ ) values of ammonia concentration were for Nano-Se. Results indicated that had significant effect on total volatile fatty acids (TVFA) values of cecum. However, TVFA's ranged between 9.49 -10.35 (ml eq/100ml) for treatments groups vs. 9.24 for control. The previous results may be due to treatments that yeast counts increased in diets supplemented with trace minerals when compared with the control that's agree with Lansdown (2006), that may be the reason of decreasing ammonia concentration and increased values of pH and TVFA's .

Cecum microbial counts ( $\log^{-1}$  CFU/ml) as affected by experimental diets were presented in Table (7). Results showed that lowest cecum microbial counts were noticed with Nano-Se group. Yeast counts increased when compared with the control diet, except with normal-Cu group. These results may be due to antimicrobial effect of nano form element for harmful micro-organisms Lansdown (2006).

**Table (4): Carcass traits of rabbits as affected by the experimental diets.**

Treatments	Carcass traits							
	Empty Carcass		Liver	Kidney	Heart	ceacum	Dressing	Edible giblets
	weight	%	%	%	%	weight	%	%
Control	119.33±14.53 <sup>a</sup>	68.62±1.58 <sup>a</sup>	2.80±0.14 <sup>bc</sup>	0.82±0.01 <sup>ab</sup>	0.25±0.02 <sup>c</sup>	90.67±1.76 <sup>b</sup>	68.62±1.58 <sup>a</sup>	3.87±0.17 <sup>cd</sup>
Normal Cupper	1056.67±23.33 <sup>b</sup>	59.72±1.47 <sup>c</sup>	2.92±0.07 <sup>b</sup>	0.86±0.01 <sup>a</sup>	0.29±0.002 <sup>ab</sup>	89.33±2.85 <sup>b</sup>	59.72±1.47 <sup>c</sup>	4.07±0.06 <sup>bc</sup>
Nano Cupper	1163.33±17.64 <sup>a</sup>	62.58±1.41 <sup>bc</sup>	2.58±0.09 <sup>cd</sup>	0.78±0.02 <sup>cd</sup>	0.27±0.004 <sup>bc</sup>	86.67±4.26 <sup>b</sup>	62.58±1.41 <sup>bc</sup>	3.63±0.11 <sup>de</sup>
Normal Zinc	1170.00±41.63 <sup>a</sup>	66.35±2.21 <sup>ab</sup>	2.65±0.08 <sup>bcd</sup>	0.83±0.01 <sup>ab</sup>	0.29±0.01 <sup>abc</sup>	90.10±5.08 <sup>b</sup>	66.35±2.21 <sup>ab</sup>	3.76±0.09 <sup>cde</sup>
Nano Zinc	1206.67±39.30 <sup>a</sup>	64.36±2.82 <sup>abc</sup>	2.42±0.07 <sup>d</sup>	0.79±0.002 <sup>bcd</sup>	0.26±0.002 <sup>bc</sup>	92.00±1.53 <sup>b</sup>	64.36±2.82 <sup>abc</sup>	3.47±0.07 <sup>cde</sup>
Normal Selenium	1070.00±32.15 <sup>b</sup>	58.88±0.83 <sup>c</sup>	3.21±0.01 <sup>a</sup>	0.82±0.01 <sup>bc</sup>	0.32±0.02 <sup>a</sup>	95.83±2.09 <sup>ab</sup>	58.88±0.83 <sup>c</sup>	4.35±0.03 <sup>ab</sup>
Nano Selenium	1243.33±29.06 <sup>a</sup>	61.54±1.09 <sup>bc</sup>	3.45±0.12 <sup>a</sup>	0.77±0.1 <sup>d</sup>	0.31±0.007 <sup>a</sup>	103.67±3.48 <sup>a</sup>	61.54±1.09 <sup>bc</sup>	4.53±0.13 <sup>a</sup>

*a, b, c, d and e: Means in the same column with different superscripts are significantly different (p ≤ 0.05).*

*Total edible parts wt. = Dressing wt. = Empty carcass wt. (without head) + Edible giblets wt.*

*Edible giblets wt. = Liver wt. + Kidney wt. + Heart wt.*

*Total edible parts % = Total edible parts wt. / fasted wt. \*100.*



**Table (5). Some blood plasma parameters of rabbits as affected by the experimental diets.**

Treatments	Blood plasma parameters								
	Glucose (g/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	ALT (U/I)	AST (U/I)	Urea (mg/dl)	TAC* (g/dl)
Control	55.00±2.89 <sup>b</sup>	6.03±0.23 <sup>c</sup>	3.79±0.23 <sup>c</sup>	2.24±0.42 <sup>bc</sup>	1.83±0.37 <sup>bc</sup>	24.76±3.96 <sup>cd</sup>	57.14±3.30	93.33±7.70 <sup>a</sup>	2.90±0.08 <sup>d</sup>
Normal Cupper	75.00±1.44 <sup>b</sup>	6.45±0.05 <sup>c</sup>	4.11±0.37 <sup>bc</sup>	2.35±0.34 <sup>bc</sup>	1.88±0.46 <sup>bc</sup>	45.71±1.10 <sup>a</sup>	85.71±16.50	88.56±9.28 <sup>a</sup>	3.97±0.03 <sup>a</sup>
Nano Cupper	65.00±4.33 <sup>b</sup>	7.91±0.26 <sup>ab</sup>	6.40±0.28 <sup>a</sup>	1.51±0.01 <sup>c</sup>	4.25±0.23 <sup>a</sup>	43.81±3.30 <sup>a</sup>	80.00±6.60	32.63±3.91 <sup>b</sup>	3.33±0.35 <sup>c</sup>
Normal Zinc	105.78±5.04 <sup>a</sup>	6.24±0.13 <sup>c</sup>	4.80±0.09 <sup>b</sup>	1.44±0.13 <sup>c</sup>	3.39±0.34 <sup>ab</sup>	27.94±1.68 <sup>bc</sup>	66.67±5.04	80.00±6.93 <sup>a</sup>	3.57±0.24 <sup>abc</sup>
Nano Zinc	127.17±10.74 <sup>a</sup>	6.88±0.58 <sup>bc</sup>	4.89±0.39 <sup>b</sup>	1.99±0.73 <sup>c</sup>	3.26±1.13 <sup>ab</sup>	19.05±1.10 <sup>d</sup>	51.42±3.30	42.26±12.36 <sup>b</sup>	3.36±0.12 <sup>bc</sup>
Normal Selenium	106.33±11.05 <sup>a</sup>	7.94±0.23 <sup>ab</sup>	4.48±0.09 <sup>bc</sup>	3.46±0.14 <sup>ab</sup>	1.30±0.03 <sup>c</sup>	32.77±1.39 <sup>b</sup>	68.45±9.41	29.69±1.99 <sup>b</sup>	3.91±0.04 <sup>ab</sup>
Nano Selenium	122.33±6.49 <sup>a</sup>	8.52±0.95 <sup>a</sup>	4.71±0.45 <sup>bc</sup>	3.80±0.52 <sup>a</sup>	1.26±0.07 <sup>c</sup>	25.40±1.68 <sup>cd</sup>	93.33±18.67	34.56±4.43 <sup>b</sup>	3.89±0.07 <sup>abc</sup>

*a, b, c and d: Means in the same column with different superscripts are significantly different (p ≤ 0.05).*

*\*TAC= total antioxidant capacity.*

**Economic efficiency**

Results in Table (8) showed that the least feed cost/ Kg body weight gain, economic efficiency and the best relative economic efficiency were recorded for Nano-Cu supplementation, while the worst values were for control, but the all diets for normal and nano trace elements were better than the control diet. These results were in agreement with (Hu *et al.*, 2012) who reported that using selenium in nano form was more economically than control.

Conclusively, adding copper, zinc and selenium in normal form and nano form in even a half of amount in growing rabbit diets can be used economically without any adverse effects on growing rabbit performance.

**Table (6): Cecum activity of rabbits as affected by the experimental diets.**

Treatments	Cecum activity		
	pH	Ammonia (mg/100ml)	TVFA's (ml eq/100ml)
Control	6.07 <sup>c</sup> ± 0.03	8.99 <sup>a</sup> ± 0.13	9.24 <sup>d</sup> ± 0.07
Normal -Copper	6.22 <sup>ab</sup> ± 0.01	8.58 <sup>b</sup> ± 0.06	9.49 <sup>cd</sup> ± 0.16
Nano- Copper	6.10 <sup>c</sup> ± 0.04	8.20 <sup>cd</sup> ± 0.10	9.65 <sup>bcd</sup> ± 0.08
Normal-Zinc	6.23 <sup>a</sup> ± 0.02	8.45 <sup>b</sup> ± 0.04	9.95 <sup>abc</sup> ± 0.06
Nano-Zinc	6.12 <sup>bc</sup> ± 0.04	7.83 <sup>e</sup> ± 0.03	10.35 <sup>a</sup> ± 0.23
Normal-Selenium	6.23 <sup>a</sup> ± 0.03	8.35 <sup>bc</sup> ± 0.07	10.01 <sup>ab</sup> ± 0.22
Nano-Selenium	6.15 <sup>abc</sup> ± 0.03	7.98 <sup>de</sup> ± 0.08	10.18 <sup>a</sup> ± 0.16

*a, b, c, d and e: Means on the same column with different superscripts are significantly different (P ≤ 0.05).*

± : Standard error.

**Table (7): Microbial counts (log<sup>-1</sup> CFU/ml) as affected by the experimental diets.**

Cecum microbes (CFU/ml) <sup>1</sup>	Control	Normal Copper	Nano Copper	Normal Zinc	Nano Zinc	Normal Selenium	Nano Selenium	LSD (0.05)
Aerobic total count	7.081	5.375	5.028	6.786	6.380	5.982	4.804	1.752
Fecal coliforms	6.105	3.657	3.070	4.720	4.634	4.873	4.883	1.389
E.Coli	7.298	3.657	4.565	2.363	4.820	2.546	6.435	1.433
Bacillus cereus	4.450	4.984	6.016	4.752	4.583	3.212	4.735	1.229
Enterobacter	5.696	4.183	3.293	3.028	4.930	3.235	3.720	1.344
Clostridium sp	2.314	1.993	1.637	1.940	1.851	1.734	2.064	1.746
Enterococcus	4.058	3.996	3.738	3.764	3.435	2.341	3.720	1.500
Yeasts	5.028	4.930	5.105	5.776	6.586	6.745	6.194	1.390
Salmonella & Shigella	ND	ND	ND	ND	ND	ND	ND	-

*Each value is an average of 3 observations.*

*LSD between treatments d.f (0.05).*

*ND =Not detected*

*Number of bacterial cells per gram of cecum content (log10<sup>-1</sup> CFU/ml).*

<sup>1</sup> CFU (Colony forming unite)

**Table (8): Economical efficiency of experimental diets for growing rabbits as affected by different treatments.**

Item	Weeks (5-13)						
	Control	Normal Copper	Nano Copper	Normal Zinc	Nano Zinc	Normal Selenium	Nano Selenium
Price / kg diet (pt)	265.00	265.95	266.44	265.80	266.20	265.25	265.84
Total feed intake/rabbit (gm)	4459.00	3698.70	3374.68	3721.15	3956.90	3823.13	3982.42
Total feed cost/rabbit (LE)	9.85	8.21	7.49	8.24	8.78	8.45	8.82
Total weight gain/rabbit(gm)	1133.87	1191.83	1241.34	1217.50	1282.50	1217.09	1438.34
Feed cost / kg gain	8.69	6.89	6.03	6.77	6.85	6.94	6.13
Total revenue/rabbit (LE)	19.28	20.26	21.10	20.70	21.80	20.69	24.45
Net revenue/rabbit (LE)	9.43	12.05	13.61	12.46	13.02	12.24	15.63
Economical efficiency(EE)	0.96	1.47	1.82	1.51	1.48	1.45	1.77
Relative EE%	100	153.13	189.58	157.26	154.17	151.04	184.38

*Based on prices of the Egyptian market during the experimental period (2016). Initial price of rabbit 17 LE.*

*Net revenue / rabbit (LE) = (Total revenue / rabbit (LE)) – (Total feed cost / rabbit (LE)).*

*Economical efficiency = (Net revenue/rabbit (LE)) / (Total feed cost/rabbit (LE)).*

*Feed cost / kg gain = Total feed cost/rabbit (LE) \*1000 / Total weight gain/rabbit (gm).*

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## تأثير إضافة بعض العناصر النادرة في صورة النانو والصورة العادية في علائق الأرانب النامية

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تهدف هذه الدراسة لتقييم اثر إضافة بعض العناصر النادرة في صورة النانو والصورة العادية في علائق الأرانب النامية. حيث استخدم اربعة وثمانون أرنب سلالة نيوزيلندي أبيض (عمر 5 أسابيع) بمتوسط وزن 565 جم قسمت عشوائيا إلى 7 مجموعات تجريبية بحيث احتوت كل مجموعة منها على 12 أرنب. احتوت علي عليقة ضابطة، عليقة تم إضافة 1جم من النحاس في الصورة العادية و0.5جم في صورة النانو و 0.8 جم زنك في الصورة العادية و0.4 جم في صورة النانو و 0.2 ملجم سليليوم و 0.1 و ملجم سليليوم في الصورة العادية / 100 كجم علف علي التوالي. وكانت الاحتياجات الغذائية طبقا لتوصيات القرار الوزاري لسنة 1996 وقد كانت العلائق متساوية في محتواها من الطاقة والبروتين واستمرت التجربة لمدة 8 أسابيع.

وقد أظهرت النتائج أن استخدام عناصر (النحاس والزنك والسلنيوم) في صورة النانو ادي تحسن في اوزان الحيوانات والاوزان المكتسبة ومعامل التحويل. كما أوضحت النتائج ايضا أنه لم يكن هناك اختلاف معنوي في معامل هضم المادة الجافة والمادة العضوية الا انه كان هناك اختلاف معنوي في معاملات هضم بقية العناصر الغذائية وكانت معاملات هضم المجاميع التي اضيف اليها النحاس والزنك والسلنيوم افضل معنويا من مجموعة الكنترول. وبالنسبة للمجاميع التي اضيف اليها هذه العناصر في صورة النانو كانت افضل مجموعة هي المجموعة التي اضيف اليها النانو سلنيوم في كل معاملات الهضم.

كما ادي استخدام هذه العناصر في الصورة العادية وصورة النانو الي اختلافات معنوي في خصائص الذبيحة وكان اكبر وزن للاعور و وزن الاجزاء المأكولة في المجموعة التي اضيف اليها النانو سلنيوم كما كانت ايضا الاعلي في وزن القلب و الكبد.

كما اوضحت النتائج ايضا أنه كان أقل في عدد الميكروبات الكلي في الاعور في المجموعة التي اضيف اليها النانو سلنيوم. كما زاد عدد البكتريا في جميع المجاميع بالمقارنة بمجموعة الكنترول عدا المجموعة التي اضيف اليها النحاس في الصورة العادية.

كان اقل تكلفة لكل كجم وزن مكتسب والكفاءة الاقتصادية للمجموعة التي اضيف اليها النحاس في صورة النانو وكانت المجموعة الاعلي تكلفة هي الكنترول بالمقارنة بكل المجاميع التي اضيف اليها العناصر المستخدمة سواء في صورة النانو أو الصورة العادية.

يستخلص من هذه الدراسة أنه يمكن استخدام النحاس والزنك والسلنيوم في الصورة العادية في حدود النسب المستخدمة وفي صورة النانو بنصف النسب في علائق الارانب دون حدوث تأثيرات عكسية علي النمو في الارانب وذات تأثير ايجابي من الناحية الاقتصادية.