Research Article

Reproductive and Productive Performance of APRI Primiparous Rabbit Does Influenced by Copper Sources During Pregnancy Period

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Abstract | The present study aimed particularly to study the impact of different dietary copper forms on new synthetic APRI line under Egyptian conditions. Forty primiparous APRI rabbit does aged between 5-6 months were divided into 4 equal groups; the first group was considered as control one (G1) that fed with basal diet; G2 is the second group fed basal diet supplemented with Nano-Cu particles (50mg/kg diet). The G3 and G4 are groups that supplemented with 200 mg /kg of Cu-SO4 (metal copper) and 200 mg / kg of copper-methionine (organic source), respectively. The results showed that, level of estrogen was higher ($P<0.05$) in treated groups (especially G2) than that in G1. In addition, the supplemented copper forms exhibited positive impact ($P<0.05$) on rabbit does body weight, starting from one month before insemination reaching to kits weaning age. The conception rate was higher in treated groups than that in G1 by 50%, 33% and 16.6%, in groups G2, G3 and G4 respectively. They born bunny with body weight at birth, 41.42 g, 33.63 g, 36.13 g and 45.71g in G1, G2, G3 and G4 respectively. Moreover higher ($P<0.05$) litter size at birth was recorded in G2 in comparison with control group and other treated groups. Additionally the supplemented Cu resulted in producing kits with higher ($P<0.05$) body weight (especially G2) than G1. Milk production was affected ($P<0.05$) by Cu supplementation and recorded as 150.5, 173.0, 208.0 and 212.5 g/day/doe, in G1, G2, G3 and G4, respectively at 3rd week of lactation. Milk protein, and fat percentages were also modulated ($P<0.05$) by the Cu treatment. In conclusion, supplemented rabbit does with different copper forms enhanced does and kits performance, especially the group supplemented with Nano-Cu.

Keywords | APRI Rabbit, Copper Forms, Fertility Traits, Litter Size and Weight, Milk Production

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INTRODUCTION

Rabbit farming have several advantages for small holders, which can rear small numbers in the backyard by preparing their cages from local materials and can feed them on food wastes or fresh forage, and the labor are from family itself. Therefore, in developing countries, rabbit production is an important economic project especially at small holder level (Finzi, 2000). Although of rabbit industry superiority, they are facing problems such as, heat stress and lack of knowledge about rabbit biology at level of breeders which considered the vital step in success of rabbit farming.

Rabbit's pregnancy is a critical period, it needs sufficient and balanced diets to meet their requirements for both fetus and dam (Ashour and Abdel-Rahman, 2019). The balanced nutrition is the key factor that enable rabbits to face different stressor including heat stress and unsuitable husbandry care (Abd El-Rahim, 2017).

Copper (Cu) is a micro-element, that essential for biological functions such as participating enzymes activity and gene expression regulation. In addition, Cu has an important role in developing immune system, by forming antibodies and white blood cells (Abd El-Rahim, 2017). According to McWalliams (2001), Cu dietary supplementation should not less than 5.0 ppm in growing, lactating and non-lactating does. Konrad et al. (2020) stated that, majority (about 70%) of Cu is absorbed by duodenum and the upper part of small intestine and the rest is absorbed in the stomach. Copper absorption is depending on its chemical properties soluble such as sulphate or citrate that easily absorbed, or insoluble as sulphides which hardly absorbed (Younan et al., 2020). During pregnancy, when Cu delivered to placenta, it attached to Cu-transporting P-type ATPase (ATP7A) which is responsible for delivering Cu to fetal circulation. Meanwhile, the excess of Cu is releasing to maternal circulation via transporter ATP7B (Hardman et al., 2004). Many researchers (Refai et al., 2015; Younan et al., 2020) attempted to test different forms of Cu (metal, organic and Nano- Cu) on kits growth and semen quality in rabbit bucks. They recorded enhancement in growth performance in young rabbits and semen quality in bucks. But, fewer data was available concerning the impact of Cu on reproductive and productive performance of rabbit does.

So, the present work aimed to test and compare the influence of different forms of Cu (Nano Cu particles, $CuSO_4.5H_2O$ as inorganic source and Cu methionine as organic source) on reproductive and productive efficiency of primiparous rabbit does with particular references to their progeny growth performance.

MATERIALS AND METHODS

Ethical approval

This experiment was carried out according to the guidelines of the ethical committee of Animal Production Research Institute. The experimental work was conducted during the period from September to December, 2020 in the Rabbit Research Unit, Sakha Research Station, Kafr Elsheik governorate, Egypt. This Research is belonging to Animal Production Research Institute (APRI), Agricultural Research Center (ARC).

Experimental design and animals

In this experiment, the APRI rabbit line was used which established in 2009 at Animal Production Research Institute (APRI) – Agricultural Research Center, Giza, Egypt, by crossbreeding between Red Baladi bucks with dose of V-line. A total number of 40 healthy primiparous APRI rabbit does were used (Figure- 1). They aged between 5-6 months and their initial body weight (IBW) was ranged between 2503.0 ± 50.05 to 2519 ± 43.07g. Rabbit does were divided into four equal groups, each group have 10 does and they kept continuously for four months in well ventilated indoors house under the same managerial and hygienic conditions. The first group (G1) fed with the balanced basal diet without any supplementation and served as control group. While the second group (G2) fed with same basal diets supplemented with 50 mg Nano-Cu particles /kg diet, that containing 37.38% Cu. This Nano particles sized with 60-70 nm, prepared by using source of copper metal presented by Cu $(NO_3)_2.3H_2O$ (S.D. Fine-Chem. Ltd). Copper precursor (2.41 gm) was added to 25 ml ethylene glycol and 100 solution of 75 deionized water under strong magnetic stirring. The formed solution with blue color was subjected to further stirring that lasted for 30 minutes. After that, by injecting the formed mixture with 300 µl ice cold (1M) NaBH4, the mixture became colorless then became with burgundy color, which consider as a prove of forming Nano-Cu particles according to Zhang et al. (2009).

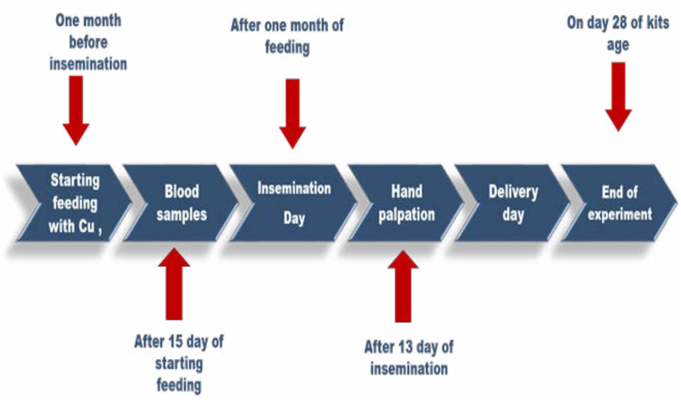

Figure 1: Experimental design for supplementation of APRI rabbit does with different copper forms.

The third group (G3) and the fourth one (G4) were supplemented with 200 mg /kg of Cu-SO4 (metal copper) and 200 mg / kg of copper-methionine (organic source), respectively. All the mentioned levels of copper sources were conducted based on Easa et al. (2018). Each group was fed (*ad libitum*) according to the recommendations of APRI and feeding ingredients are illustrated in Table-1 and had free access to water. Feeding levels with different copper sources were started before one month of APRI does insemination and lasted continuously until end of experiment (Figure 1).

Before starting feeding with different forms of Cu, IBW of each group was recorded. Then, weights were recorded continuously at, the day 15 of starting feeding with Cu, day of insemination, during pregnancy, on delivery and weaning days (28 days of kits age) as shown in Figure- 1.

Additionally, conception rate (CR), kindling rate (KR), the number of total kits (TK) and live-born kits (LBK) were calculated according to IRRG (2005) as follow:
Conception rate = (No. of pregnant doe/total No. of doe) x 100.
Kindling rate = No. of delivered does/No. of inseminated does.

Blood sampling and analyses

Before starting insemination by 15 days, 3 ml of blood samples were collected in heparinized tube and centrifuged at 3000 rpm to collect blood plasma that stored at - 20°C. The tested hormones were, progesterone (P4, expressed in ng/ml, Pl-1188), and estradiol (E2, expressed in pg/ml, pl- A21854), their levels were quantified by radio immune assay (RIA) technique. All kits were purchased from Immuno-Tech Company- France, and the analysis was done according to the outlines described by the manufactures.

Kit's performance

Both litter size (LS) and weight (LW) and bunny weight were determined from birth and at days 7, 14, 21 and 28 of kits age.

Milk yield and compositions

Average of daily milk yield per doe (DMY, g/d/doe) was estimated weekly during the lactation period (four weeks). The young was prevented from suckling for 24 hours by separation from the doe. The bunnies and does were weighed before and after suckling and the mean of increase in weight of bunnies and decrease in weight of doe were indicated as DMY. The collected samples were kept frozen until performing the chemical analysis for different milk constituents [milk protein (MP), milk fat (MF), milk lactose (ML), milk ash (MA), and milk total solids (MTS)]. Milk components were determined at the International Livestock Management Training Center at Kafr Elsheik governorates, using Milkoscan analyzer (130 B, N. Foss Electronic, Denmark).

Statistical analysis

Data were analyzed using the General Linear Model procedure of SAS Program (SAS, 2001). The used model was as follow:
$Y_{ij} = +D_i + e_{ij}$
Where Y_{ij} = the observation on ij^{th} trait, = the overall mean, D_i = the effect of diet, i^{th} (i= 1,2,3) and e_{ij} = the random error. Percentage values were transformed to arc-sin values before being statistically analyzed. Differences among treated groups were separated based on Duncan's Multiple Range (Duncan, 1955), and the level of significance was 5%. The correlation coefficient among copper forms, dam body weights, conception rate, litter size, litter weight and milk yield at birth was calculated.

RESULTS AND DISCUSSION

Progesterone and estrogen levels

After 15 days of starting feeding with different copper sources rabbit does did not significantly ($P < 0.05$) affect levels of P4 in these treated groups (Table- 2). Furthermore, treated groups recorded a slight lower level of P4 than that found in G1. Quite the opposite trend for E2 levels, which showed higher levels in treated groups than that in G1, by 37.7%, 31.9% and 23.2% for G2, G3 and G4, respectively. These elevations in E2 levels in treated groups reflect the positive impact of Cu sources (especially Nano-Cu, G2) on E2 concentration. Both P4 and E2 are reproductive hormones, they have important role in reproductive cycle in mammals. Their role is limited to maintain corpus leutum (CL). But, they are involved in maintain uterine quiescence, regulating blood flow in uterine and formation of receptors on cell surface (Botanci et al., 2012). Moreover, Han et al. (2013) stated that, P4 has a protection effect on ovary cancer, and E2 is considered as antioxidant through its ability in association with excess of free radicals (Botanci et al., 2012).

In rabbits, no literature is available that explain the relationship between Cu and both P4 and E2. However, in rats, Field et al. (1986) and Pal (2015) mentioned that, in case of Cu deficiency, E2 can elevate its level in plasma by altering hepatic subcellular Cu distribution and stimulating ceruloplasmin formation, thus increasing Cu level. So, maintaining Cu level during pregnancy is essential for dam and fetus. In an *in-vitro* study on rats, it was found that, Cu is important in early embryonic development, and in case of Cu deficiency may lead to changes in nervous system (Konrad et al., 2020).

Unfortunately, the reproductive biology of rabbits and their hormonal changes during different reproductive cycles are not received much attention and need further studies (Gonzales-Mariscal et al., 2009; Ashour and Abdel-Rahman, 2019).

Doe weights

As shown in Table-3, after feeding APRI rabbit does with copper sources by 15 days, the treated groups have higher BW than G1. The most noticeable BW increase was observed in does of G3, followed by G2 and G4. By continuing in feeding with Cu forms, the treatments caused BW elevation than that in control group (especially Nano-Cu, which significantly higher than G1). Through calculating weight gain during the period of one month of starting feeding with Cu, it was found that G2 have the highest

BW followed by G3 and G4, respectively. After that, the same trend in increasing BW has been noticed when the rabbit does get inseminated. This was confirmed through the positive correlation between averages of does BW gain from 1-30 days of starting feeding with Cu forms, which was 0.5, 0.2 and 0.4 in G2, G3 and G4, respectively.

When rabbits became pregnant, treated groups with Cu forms have been significantly higher in their BW than G1. This elevation in BW could return to many reasons; firstly, the positive impact of Cu on increasing feed intake and enhances nutrient digestibility, thus causing increase in BW. Secondly; return to presence of both fetus and fluids of pregnancy. On delivery day, both G2 and G3 were significantly ($P < 0.05$) higher than G1, but G4 was insignificantly ($P>0.05$) higher than G1. Whereas, at weaning age (28 days of their kits age), dam received Nano-Cu recorded highest BW followed by G3 and G4.

Reproductive performance of APRI does

In Table-4, the data revealed that, in G1, out of 10 does six were successfully conceived at first mating. While, in treated groups (G2, G3 and G4) number of conceived does at first mating was nine, eight and seven, respectively. Their CR was at lower level in G1, while supplemented rabbits with Cu forms showed CR elevation. As confirmed by the highest rate in G2 that surpassed G1 by 50%. The dose of G3 and G4 were the next in CR elevation, which were higher than that of G1 by 33% and 16.6%, respectively. This reflects that, CR was positively correlated with Nano-Cu (0.3), Cu SO4 (0.2) and Cu-methio (0.2). All pregnant APRI rabbits kindled after 29.30 to 30 days of gestation, with no abortion incidence. The higher borne live kits (BLK) was reported in G2 (Table 5). A single higher bunny BW at birth was noticed in G4 followed by G1, then G3 and finally G2. At 28 days of age, the bunny reached to 474.69 g for G3, 449.8 for G4 and 419.53 g for G1. But the lowest bunny BW was recorded in the group of G2. However, there was a negative correlation between bunny BW at birth and Nano-Cu which was -0.5. While, both G3 and G4 recorded positive correlation with bunny BW (0.2 and 0.5, respectively). Previously, Wilmut et al. (1990) cleared that, the higher number of born at birth, the greater total weight reduction of litter. However, the relationship between these traits is an inverse relationship, which implies a progressive reduction of individual weight of young at birth when dam born large numbers. The present data are lower than that reported by Marai et al. (2000), who found that rabbit does (NZW rabbits, during winter and summer seasons) supplemented with Cu-SO4, born bunny with 52.9 g at birth and 570 g at 28 days of age. And they attributed their results to the positive role of copper in reproduction and its ability in increasing milk yield.

Litter size and weight

Table- 5 and Table- 6 show, LS and LW as affected by different forms of Cu. Generally, the total number were higher in treated groups (G2, G3 and G4) than that in G1. Highest LS was recorded in G2 in comparison with control group and other treated groups. Additionally, the data revealed the positive correlation between LS at birth and treated groups, which was 0.3 for G2, and 0.2 for G3. Quite the opposite, G4 showed negative correlation with (-0.2) LS at birth.

But, after kindling to 28 days of age, LS dropped (litter loss) by 31.8%, 22%, 24.7% and 26.4% in groups G1, G2, G3 and G4, respectively. At weaning age, the treated groups surpassed in LS over control one by 44.9%, 38.8% and 22.4% for G2, G3 and G4, respectively. At the age of weaning, LS recorded negative correlation with Nan- Cu (-0.09), Cu-SO4 (-0.3) and Cu- Methio (-0.5). In the present study, the rates of pre-weaning loss are higher than reported by Rashwan and Marai (2000) who stated that, the percentage of litter loss is ranging between 16-20% pre-weaning. The authors mentioned that, pre-weaning loss could be related to many reasons including; diseases, crushing, cannibalism, dam abandons the litter and insufficient milk for suckling. In addition, the data are similar to those of Marai et al. (2000) who found that, LS born from dam supplemented with 50 mg/ Kg diet Cu-SO4 was 8.81, 7.26 and 6.98 at birth, 21 and 28 days of age, respectively.

Concerning LW, supplying rabbit does with different Cu forms during pregnancy period resulted in producing kits with higher body weight (especially G3) than G1. As shown in Table (6), the BW of kits in G1 increased 432 g from birth to day 21 of age. Then, a noticeable increase (1336 g) in BW was recorded during days 21 to 28 of age. Meanwhile, in treated groups, the BW increased about 644, 819 and 625 g from birth to day 21of age in G2, G3 and G4, respectively. By reaching 28 days of age BW elevated markedly by 1819.5, 2051 and 1719 g in G2, G3 and G4, respectively. These results reflect that, different Cu sources achieved progressive elevation in kits BW, which was higher, at day 28 of age than G1 by 36.5%, 55.5% and 31.3 % in G2, G3 and G4, respectively. This may attributed to the impact of Cu on increasing milk yield (will be discussed later) than G1, which reflects providing adequate of milk quantities with nutritive value for those growing kits. Additionally, Ashour et al. (2018) stated that when rabbit kits born with higher body weight, they may grow faster in comparison with the kits born with lower BW. Beyond that, the authors confirmed that, milk is the main energy source for the new borne for 18-19 days of their ages. After that, the kits starting in consuming feeds gradually to prepare them for weaning. The data are totally agree with Easa et al. (2018), who studied the impact of 50 mg/kg

Table 1: Feed ingredients and calculated chemical analysis of the reference diet.

Ingredient feed	%	Calculated chemical analysis	%
Berseem hay *(Trifolium alexandrinum)*	30.05	Crude protein	17.34
Barley	24.60	Crude fiber	12.38
Wheat bran	21.50	Ether extract	2.27
Soybean meal (44% CP)	17.50	Calcium	1.24
Molasses	3.00	Total phosphorus	0.80
Di-calcium phosphate	1.60	Lysine	0.98
Limestone	0.95	Methionine	0.46
Sodium chloride (NaCl)	0.30	Methionine + Cystine	0.76
Vitamin & mineral mixture*	0.30	Sodium	0.16
DL-Methionine	0.20	Digestible energy (Kcal/Kg diet)	2500
Total	100		

*Supplied per kilogram of diet: Vitamin A, 6000 IU; Vitamin D3, 900 IU; Vitamin E, 40 mg; Vitamin K3, 2 mg; Vitamin B1, 2 mg; Vitamin B2, 4 mg; Vitamin B6, 2 mg; Pantothenic acid, 10 mg; Vitamin B12, 0.01 mg; Niacin, 50 mg; Folic acid, 3 mg; Biotin, 0.05 mg; Choline, 250 mg; Fe, 50 mg; Mn, 85 mg; Cu, 5 mg; Co, 0.1 mg; Se, 0,1 mg; I, 0.2 mg and Zn, 50 mg.

Table 2: Effect of different copper forms supplementation on sexual hormones of APRI line rabbit does before 15 day of insemination.

Parameters	Groups			
	G1	G2	G3	G4
P4 (ng/ml)	1.53±0.09	1.16±0.17	1.20±0.17	1.30±0.21
E2 (pg/ml)	23.00^{b}±1.53	31.66 ^{a}±1.85	30.33^{a}±1.45	28.33^{a}±0.88

G1= Control group; G2= Supplemented group with 50 mg Nano-Cu/ Kg diet; G3= Supplemented group with 200 mg Cu-SO4/kg diet and G4=200 mg copper-methionine / kg diet.

Means within the same row bearing different letter superscripts (a, b) are significantly different (P<0.05).

Table 3: Effect of different copper forms supplementation on body weight of APRI line rabbit does before one month of insemination and during pregnancy period

Measurements (g)	Groups			
	G1	G2	G3	G4
Initial body weight	2519.5±43.07	2503.0±50.05	2511.5±31.90	2506.0±35.96
weight at 15 day	2844.0±39.58	2905.0±49.19	2911.0±35.57	2889.0±31.84
weight at 30 day	3095.0^{b}±36.22	3223.0^{a}±37.89	3206.5^{ab}±35.84	3190.0^{ab}±38.73
Weight gain (1-15 d)	324.50^{b}±11.60	402.00^{a}±13.64	399.50^{a} ±11.50	383.00^{a} ±10.11
Weight gain (16-30 d)	251.00^{b}±10.89	318.00^{a}±19.53	295.50^{a}±15.39	301.00^{a}±14.71
Average weight gain (1-30 d)	19.18^{b}±0.61	24.00^{a}±0.73	23.16^{a}±0.61	22.80^{a}±0.53
Weight on insemination day	3095.0^{b}±36.22	3223.0^{a}±37.89	3206.5^{ab}±35.84	3190.0^{ab}±38.73
On day 15th of pregnancy	3292.0^{b}±34.36	3499.0^{a}±47.66	3446.0^{a}±45.90	3415.5^{a}±33.50
On day 21st of pregnancy	3391.0^{c}±33.41	3674.0^{a}±48.05	3641.0^{a}±40.53	3522.5^{b}±33.33
On delivery day	3049.0^{b}±41.98	3267.0^{a}±60.90	3262.0^{a}±46.21	3150.5^{ab}±28.99
At weaning day	2826.0^{c}±51.59	3235.0^{a}±94.66	3118.00^{ab}±63.24	2974.50^{bc}±53.62

G1= Control group; G2= Supplemented group with 50 mg Nano-Cu/ Kg diet; G3= Supplemented group with 200 mg Cu-SO4/kg diet and G4=200 mg copper-methionine / kg diet.

Means within the same row bearing different letter superscripts (a, b) are significantly different (P<0.05).

Table 4: Effect of different copper forms supplementation on fertility traits of APRI line rabbit does.

Measurements	Groups			
	G1	**G2**	**G3**	**G4**
Number of inseminated does	10	10	10	10
Number of conceived does at first mating	6	9	8	7
Conception rate (%) at first mating	60±16.33	90±10.00	80±13.33	70±15.27
Gestation period (day)	30.00±0.21	29.60±0.22	29.30±0.26	29.60±0.26
Kindling rate (%)	100	100	100	100
Bunny weight at birth (g)	41.42^{ab}±1.34	33.63^{b}±0.78	36.13^{b}±2.21	45.71^{a}±4.74
Bunny weight at weaning (g)	419.53^{b}±5.20	396.8^{b}±20.21	474.69^{a} ±28.19	449.8^{ab}±8.63

G1= Control group; G2= Supplemented group with 50 mg Nano-Cu/ Kg diet; G3= Supplemented group with 200 mg Cu-SO4/ kg diet and G4=200 mg copper-methionine / kg diet.
Means within the same row bearing different letter superscripts (a, b) are significantly different (P<0.05).

Table 5: Litter size of APRI line rabbit does as affected by different copper forms supplementation

Days	Groups			
	G1	**G2**	**G3**	**G4**
At birth	6.90^{b}±0.54	9.90^{a}±0.48	8.90^{a}±0.48	7.20^{b}±0.38
7 days	6.10^{b}±0.378	9.00^{a}±0.365	8.10^{a}±0.348	6.60^{b}±0.400
14 days	5.60^{b}±0.31	8.30^{a} ±0.36	7.60^{a}±0.34	6.20^{b}±0.38
21 days	5.30^{b}±0.21	7.50^{a}±0.26	7.30^{a}±0.36	6.10^{b}±0.37
28 days	4.7±0.33^{b}	7.7±0.39^{a}	6.7±0.33^{a}	5.3±0.42^{b}

G1= Control group; G2= Supplemented group with 50 mg Nano-Cu/ Kg diet; G3= Supplemented group with 200 mg Cu-SO4/kg diet and G4=200 mg copper-methionine / kg diet.
Means within the same row bearing different letter superscripts (a, b) are significantly different (P<0.05).

Table 6: Litter weight (g) of APRI line rabbits does as affected by different copper forms supplementation.

Days	Groups			
	G1	**G2**	**G3**	**G4**
At birth	280.0±14.24	331.5±14.72	320.5±25.06	314.0±18.25
7	491.0^{b} ±28.30	703.0^{a}±35.65	747.0^{a}±34.12^{a}	561.0^{b}±33.38
14	712.00^{c}±74.12	975.50^{b}±42.31	1140.00^{a}±46.42	966.00^{b}±53.52
21	1011^{c}±36.92	1345^{b}±61.6486	1727^{a}±72.09	1417^{b}±48.645
28	2048.0^{c}±103.73	2795.0^{ab}±133.21	3191.0^{a}±156.79	2685.00^{b}±162.79

G1= Control group; G2= Supplemented group with 50 mg Nano-Cu/ Kg diet; G3= Supplemented group with 200 mg Cu-SO4/kg diet and G4=200 mg copper-methionine / kg diet.
Means within the same row bearing different letter superscripts (a, b) are significantly different (P<0.05).

Table 7: Effect of different copper forms supplementation on milk yield (g/doe/day) of APRI line rabbit does.

Lactation weeks	Groups			
	G1	**G2**	**G3**	**G4**
1^{st}	79.50^{b}±2.16	94.50^{a}±2.62	91.50^{a}±4.89	81.00^{b}±3.78
2^{nd}	120.00^{c}±2.35	132.00^{ab}±2.60	136.50^{a}±2.36	127.00^{bc}±3.59
3^{rd}	150.5^{c}±4.50	173.0^{b}±4.48	208.0^{a}±8.66	212.5^{a}±10.52
4^{th}	104.00^{b}±3.71	113.00^{a}±2.38	121.50^{a}±2.58	117.00^{a}±3.26

G1= Control group; G2= Supplemented group with 50 mg Nano-Cu/ Kg diet; G3= Supplemented group with 200 mg Cu-SO4/kg diet and G4=200 mg copper-methionine / kg diet.
Means within the same row bearing different letter superscripts (a, b) are significantly different (P<0.05)

Table 8: Effect of different copper forms supplementation on milk composition of APRI line rabbit does.

Milk components %	Groups			
	G1	G2	G3	G4
MP	13.33[b]±0.28	13.91[ab]±0.21	14.12[a]±0.10	13.83[a] ±0.19
MF	11.04[a] ±0.25	10.38[ab]±0.14	9.98[b] ±0.24	10.15[b] ±0.29
ML	3.25±0.15	3.32±0.13	3.21±0.07	3.28±0.12
MA	2.65±0.11	2.52±0.08	2.50±0.02	2.65±0.07
MTS	30.29± 0.07	30.12± 0.31	29.81 ± 0.16	29.92± 0.29

G1= Control group; G2= Supplemented group with 50 mg Nano-Cu/ Kg diet; G3= Supplemented group with 200 mg Cu-SO4/kg diet and G4=200 mg copper-methionine / kg diet.

Means within the same row bearing different letter superscripts (a, b) are significantly different (P<0.05).

MP= Milk protein, MF = Milk fat, ML = Milk lactose, MA= Milk ash, MTS = Milk total solids

diet of Nano-Cu on rabbit's growth performance. They recorded an improvement in kits BW and attributed that to the positive effect of Nano-Cu on nutrient digestibility. Abd El-Rahim (2017) cleared that, in young rabbits, most of nutrients digestion and absorption are occurred in the mucosa of small intestine that also responsible for resisting the antigenic aggression's in rabbit kits. So, increasing immunity for these cells could be achieved via available nutrients with specific additives such as Cu and this may enhance growth performance. Moreover, the data are in harmony with Espinosa and Stein (2021) who found that, supplemented pig diets with Cu can improve growth performance and increasing body weight. They attributed these findings to the ability of Cu in increasing activity of digestive enzymes in small intestine that result in improv ing growth performance. In addition, the authors cleared that, Cu has the ability to modulate microbial populations in intestine, which results in enhancing growth performance.

Milk production

As shown in Table-7, the primiparous rabbits does in all groups lactated for consecutive four weeks. Reaching their production peak at the third one of lactation. At the fourth week, milk production started to decline drastically. These data are in harmony with Cascado et al. (2006), Maertens et al. (2006) and Ashour et al. (2018). They stated that, rabbits can lactate for 4-5 weeks during the whole lactation period. The treated groups with Cu sources produced more milk than G1. During the first week of lactation, the group of Nano-Cu particles lactated more milk than control group by 18.9%. In the same context, G3 and G4 were higher in their milk production than G1 by 15.1% and 1.8%, respectively. At peak, all treated groups were significantly ($P < 0.05$) higher than G1. The higher significant ($P < 0.05$) elevation in milk yield was recorded in G4 that surpassed G1 by 41.3 % followed by G2 and G3, which overpassed G1 by 15% and 38.2%. At the end of lactation, the treated groups did not significantly ($P > 0.05$) differed between each other, but they significantly ($P < 0.05$) differed than G1. Also, the DMY declined from the third week to fourth one in all groups by 46.5g, 60 g, 87 g and 90.2 g in G1, G2, G3 and G4, respectively. Moreover, the data pointed to the positive correlation between DMY and Cu sources (0.4, 0.5 and 0.2 for G2, G3 and G4, respectively). In the next three weeks a negative correlation coefficient was recorded. For example, in the second week of lactation correlation coefficient (between DMY and Cu forms) was -0.7, -0.3 and -0.2 for G2, G3 and G4, respectively. The reduction in MY at the fourth week can be attributed to starting of mammary gland involution that resulted from declining level of reproductive hormone (prolactin) and metabolic hormones (T3, T4 and IGF-I) as mentioned by Ashour and Abdel-Rahman (2019). Besides that, Maertens et al. (2006) and Ashour et al. (2018) stated that, one of the more important factors that affecting milk yield is LS and LW (as discussed previously). In addition, when rabbit does suckling more kits (6-8 kit) they will produce more milk than those suckling 4 kits according to Mohamed (2013) and El-Deghadi (2019). Because, suckling is stimulating milk secretion through sending signals for oxytocin release (Neville et al., 2002). Previously, Kulikov et al. (1985) suggested that, when dam produce 6-8 kits they need 5.06 mg Cu/ day to enable them to produce 107 g milk/ day during 45 days of lactation period. The present data are in agreement with Marai et al. (2000) who supplemented rabbit does with copper sulphat. They found that these does produced daily more milk (170 g) than control group (101 g).

Concerning milk composition, Table- 8 generally showed that, MP was insignificantly ($P > 0.05$) higher in G2 than that in G1. But, G3 and G4 were significantly higher ($P < 0.05$) than that the control one. Meanwhile, MF was higher in G1 than that recorded in other supplemented groups with Cu. On the other hand, ML, MA and MTS were not significantly differed between all studied groups and they have almost close values of these parameters. The data are differed than that reported by Ashour et al. (2018) who found that, MF,MP, ML, MA and MTS in primiparous NZW rabbit does were; 12.2, 16.9, 2.82, 7.01 and 39, respectively. These differences may be attributed to breed differences, the NZW rabbits are exotic breed while APRI line is a new synthetic line developed by the Animal Production Research Institute (APRI).

This study assumed to be one of the fewer studies that explored the relationship between different copper sources and milk yield and its composition in rabbit because most of studies had been done on ruminants milk production. The improvement in APRI rabbit does milk production could be attributed to the positive impact of copper on feed intake, therefore providing the nutrient requirements that are important for milk production.

CONCLUSION

The results showed that, supplemented rabbit does with different copper forms, nano, sulphate and copper-methionine exhibited enhancement in rabbit does reproductive and productive performance. Nano-Cu showed superior effect on E2 level, dam body weight gain and their conception rate, litter size at birth and weaning age, and finally daily milk yield at the first week of lactation.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS CONTRIBUTION

GEY conceived the experimental design, SAB done the statistical analysis, FE assisted in the practical part, and SMA write the manuscript.

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