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EFFECT OF ZINC OXIDE LEVELS SUPPLEMENTATION ON SEMEN CHARACTERISTICS AND FERTILITY RATE OF BUCKS RABBITS UNDER SUBTROPICAL CONDITIONS

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The present study was conducted in the experimental farm of Animal Production Department, Faculty of Agriculture, South Valley University, Qena, Egypt, where there is a prevailing a subtropical climate. The experiment started in 15th of January 2012 to the end of May 2012. The work aimed to study the effect of zinc oxide (Zn O) levels supplementation on semen quality and fertility rate of buck New Zealand White (NZW) rabbits under subtropical conditions. A total number of thirty buck rabbits (16 weeks of age and average body weight 2368±14.21) were randomly divided into three equal treatments (10 bucks of each) without significant difference in average body weight between treatments. The 1st treatment (T1), rabbits saved as control (0 g Zn O supplement/ kg diet). The 2nd and 3rd treatments (T2 and T3), rabbits were fed with basal diet containing 75 and 150 ppm Zn O/ kg diet, respectively (0.096 and 0.192 g Zn O supplement/ kg diet, respectively).

***The results indicated that** all the physical semen characteristics of NZW were significantly (P<0.05) improved by Zn O supplementation of buck NZW rabbits except ejaculate volume and sperm motility. Sperm concentration, total sperm count and live spermatozoa were significantly increased (P≤0.05) in the bucks of T2 and T3 as compared to the control ones. Moreover, fertility rate and litter size at birth were higher (P<0.05) in the bucks of T2 and T3 (Zn O supplementation) as compared to the bucks in T1 (control group).*

***In conclusion**, adding 75 or 150 ppm zinc O/ kg diet improved the physical characteristics of semen quality and fertility rate of buck NZW rabbits under subtropical conditions.*

Key words: NZW rabbits, Zinc O supplementation, semen quality.

Under subtropical conditions, ambient temperature is the major determining factor controlling animal productivity. In Egypt, especially in the south Egypt (Qena) the ambient temperature begin increased from

March month to reach maximum at August month, these months are not with in the thermal neutral zone (18 - 20 ^{0}C) for male rabbits (Attia *et al*., 2011). Under such environmental conditions during these months, adversely effects on reproductive performance of male rabbits were occurring, reducing semen quality, as well as, reducing the ability of leydig and sertoli cells to respond to Luteinizing hormone (LH) and the diameter of the somniferous tubules (El-Sherbiny, 1987). In addition, exposure of buck rabbits to heat stress conditions led to negatively effects on ejaculate volume, sperm cell concentration, total sperm output, live spermatozoa and sperm abnormalities (Marai et al., 2003).

Moreover, high ambient temperature stimulates the hypothalamo–pituitary–adrenal axis activity inducing the sympathetic system functions, which increase levels of free radicals and imbalances in the antioxidant-defense system (Agarwal *et al*., 2008 and Ahmad *et al*., 2012). Accumulations of the free radicals have been associated with significant decreases in sperm motility and sperm plasma membrane integrity, and significant increases in sperm abnormality and DNA damage leading to infertility (Potts *et al*., 2000).

The role of zinc (Zn) in the animal organism began to gain special attention. This mineral is involved in over 200 proteins and enzymes which were essential for male fertility (Kumar et al., 2006).

Zn supplementation enhances physical characteristics of semen including ejaculate volume, sperm count, motility, seminal plasma antioxidants and fertility rate (Amen and Muhammad, 2016; Rahman *et al*., 2014; El-Speiy and El-Hanoun, 2013; Rafique *et al*. 2010; Ghasemi *et al*. 2009; Oliveira *et al*., 2004; Maldjian *et al*., 1998; Rode *et al*., 1995 and El-Masry *et al*., 1994) and increase the secretion of the principal sex hormone (Follicle-stimulating hormone and interstitial cell–stimulating hormone) in male rabbits. Consequently, improve reproductive among male rabbits (Ogbu and Ezeokoli, 2016). Zinc is found in high amount in male reproductive tract and semen. So, there is a strong relationship between the zinc and spermatogenesis (Chia et al. 2000). On the other hand, Zn scavenges excessive production of superoxide radical, and thus, this element has antioxidant like activities (Gavella and Lipovac 1988). Chia *et al*. (2000) suggested that Zn may bind with the free radicals in the seminal plasma, produced by abnormal spermatozoa, and thus, the concentration of this element may be decreased. Deficiency of zinc causes a lowering of testosterone level, shrinks of testicle size and produces misshapen and less healthy sperm, among other negative effects. (Hadwan *et al*. 2012 and Chia

et al. 2000). A diet deficient in Zn may cause atrophy of the primary, secondary and accessory sex glands (Martin *et al*., 1994). Most of the studies above were carried out in temperate regions and the question, which may arise the present is whether Zn supplementation can still have such effects when the climatic condition changes.

Therefore, the aim of this experiment was to determine the effects of zinc oxide supplementation upon physical semen quality characteristics and fertility rate of buck NZW rabbits under subtropical climate prevalent in the study location.

MATERIALS AND METHODS

The present study was conducted in the experimental farm of Animal Production Department, Faculty of Agriculture, South Valley University, Qena, Egypt, where there is a prevailing a subtropical climate. The experiment started in 15th of January 2012 to the end of May 2012. The work aimed to study the effect of zinc oxide supplementation on semen quality and fertility rate of buck NZW rabbits. A total number of thirty buck rabbits (16 weeks of age and average body weight 2368±14.21) were randomly divided into three equal treatments (10 bucks of each) without significant difference in average body weight between treatments. The 1st treatment (T1), rabbits saved as control (0 g Zn O supplement/ kg ration). The 2nd and 3rd treatments (T2 and T3), rabbits were fed with basal diet containing 75 and 150 ppm Zinc oxide supplement / kg diet (0.096 and 0.192 g Zn O supplement / kg diet) respectively. Chemical analyses of rations (Table 1) were done according to A.O.A.C. methods (1995). Rabbits were housed in individual cages, receiving rations and water *ad libitum*, kept under the same managerial and hygienic conditions. Lighting program consisted of a period of 14 h light and 10 h of darkness.

Indoor ambient temperature and relative humidity were recorded during experimental period by digital thermometer Figure (1 & 2). Ambient temperatures (AT, ^{0}C) ranged from 21.4 to 35.3 ^{0}C during April and May months. The mean of minimum AT was 19.9 ^{0}C, while the mean of maximum AT was 36.9 ^{0}C. Relative humidity (RH, %) ranged from 26.1 to 64.3 % with a mean daily low of 22.3 % and a mean daily high of 69.8 %. Maximum temperature humidity index was 31.5 and calculated according to Marai *et al*. (2001). The THI values were classified as absence of heat stress (<27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and

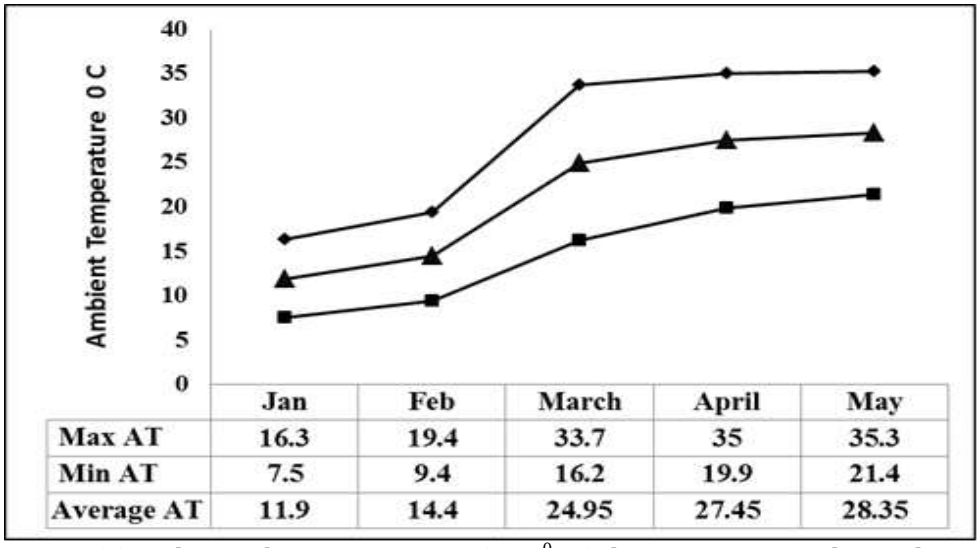
Table 1. Formulation and chemical composition of the basal diet.

Ingredients	%
Alfalfa hay	30.420
Soybean meal (44% CP)	12.500
Corn meal	22.500
Whole sunflower meal	7.000
Barley meal	14.000
Wheat bran	5.000
Beet molasses	1.200
Calcium carbonate	1.372
Calcium diphosphate	0.671
Sodium chloride	0.500
Dl-methionine	0.057
premix (Zn O-free)	1.000
Total	100.0
chemical composition of the diets	
Dry matter	89.2
Crude protein	17.3
Ether extract	5.30
Crude fibre	14.9
NFE	53.70
Ash	8.80
Digestible energy** MJ kg-1	10.90

** Estimated according to Maertens *et al.* (1984)

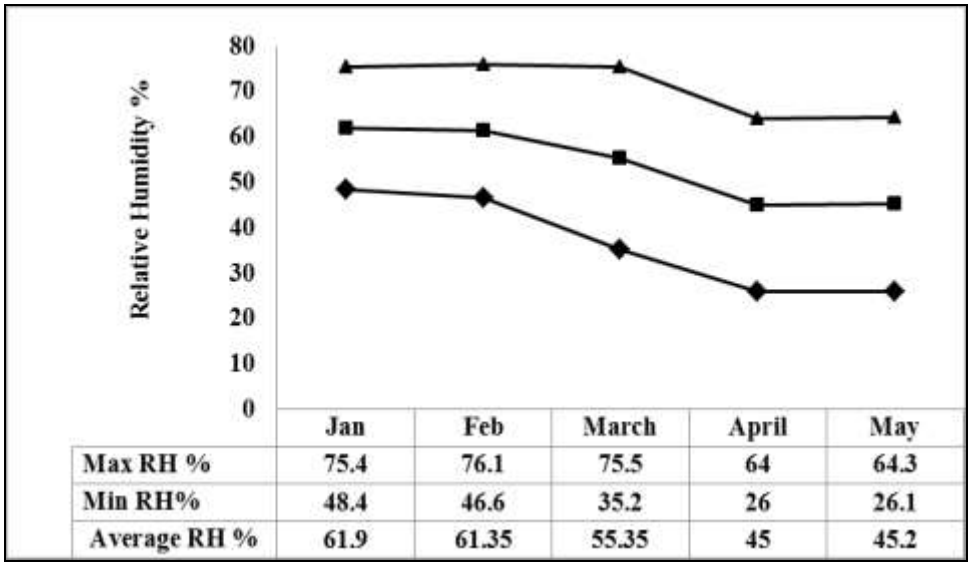
very severe heat stress (>30.0). Hence, rabbits in this study suffered from very severe heat stress during period of semen evaluation.

At 26 weeks of bucks age, started the training period with artificial vagina for 2 weeks by collected two ejaculate per buck per week. At 28 weeks of bucks age collection of semen from buck rabbits began (April - May) for semen examination. During collection period (8 weeks), two ejaculates per buck per week were collected, with an interval of 30 minutes between them. All ejaculates (average 128 samples for each treatment during semen collection period) were stored at 37 ^{0}C in a water bath until evaluation, not later than 15 minutes after collection. Ejaculate volume (ml) determined by using graduated tube. Spermatozoa concentration (number of sperms per ml) was counted using a haemocytometer according to (Smith and Mayer, 1955). For evaluation of percentage of sperm motility drop of



	Jan	Feb	March	April	May
Max AT	16.3	19.4	33.7	35	35.3
Min AT	7.5	9.4	16.2	19.9	21.4
Average AT	11.9	14.4	24.95	27.45	28.35

Figure (1). Indoor ambient temperature (AT ^{0} C) during experimental period



	Jan	Feb	March	April	May
Max RH %	75.4	76.1	75.5	64	64.3
Min RH%	48.4	46.6	35.2	26	26.1
Average RH %	61.9	61.35	55.35	45	45.2

Figure (2). Indoor relative humidity % (RH %) during experimental period

semen was examined under the low power of microscope using a hot stage at 37 ^{0}C. Progressive motility was estimated on a percentage score. Total and motile sperm output / ejaculate were calculated. Total sperm output was calculated by multiplying ejaculate volume and spermatozoa concentration Percentage of live and abnormal sperms were determined after staining with eosin and nigrosine (Blom, 1950) and then calculated as a percentage out of

randomly chosen 100 sperm counted. Percentage of motile sperm was estimated a phase-contrast microscope according to (Melrose and Laing, 1970). Total motile sperm number (TMS) was estimated by multiplying percentage of motile sperm and total sperm output. All bucks were used for natural mating (two natural mating per buck, with an interval of 30 minutes between them) of NZW does after the end of semen collection to study the effect of zinc oxide supplementation on fertility rate and litter size at birth of the different treatments. These animals represented progeny of 5 bucks and 15 does in each group. Ingredient and chemical analysis were determined according to NRC (1977).

Data were analysed according to Snedecor and Cochran (1982) by the least square analysis of variance using the General Linear Model Procedure (SAS, 2004) according to following model:

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

Where, Y_{ij}= Observations, μ = Overall mean, Tr_i = Effect of i^{th} treatment (i: 1-3), eij= Experimental error.

Duncan's New Multiple Range Test (Duncan, 1955) separated differences among treatment means.

RESULTS AND DISCUSSION

The mean values of some physical semen characteristics of NZW rabbits as affected by zinc oxide supplementation are shown in Table (2). The results indicated that all the physical semen characteristics were significantly ($P<0.05$) affected by Zn O supplementation except ejaculate volume and sperm motility. However, it is interest to note that, ejaculate volume was insignificantly increased by 10.9 and 15.5 % in the bucks fed diet supplemented with 75 (T2) and 150 ppm zinc oxide (T3), respectively, as compared to the control (T1). This result agreement with the results of Narasimhaiah *et al*. (2018), they found the semen ejaculate volumes were similar among the Zn supplemented groups and control group of bucks. In opposite , Moce *et al*. (2000) and Rahman *et al*. (2014) found that ejaculate volume was significantly higher in animals fed supplemented zinc oxide (levels from 150 to 200 ppm) as compared to non-supplemented ones, they obtained a significant difference might because of the higher level of Zn oxide supplementation.

Similar trend was observed in sperm percentage of motility which insignificantly increased by about 11% in the bucks of T3 (150 ppm Zn O) were compared with the bucks of T1 (control group). The semen of the

Table 2. Means (±SE) for some physical semen characteristics of buck NZW rabbit as affected by zinc oxide supplementation.

Traits	Treatments			
	T_1	T_2	T_3	±SE
Ejaculate volume (ml)	0.49	0.55	0.58	0.019
Sperm motility (%)	46.95	48.90	52.30	1.77
Sperm concentration (10^6/ml)	313.70 ^{c}	347.10 ^{b}	381.00^{a}	4.96
Total sperm output (10^6/ejac.)	152.63 ^{c}	190.62 ^{b}	221.61^{a}	4.85
Motile sperm /ml (×10^6)	146.69 ^{c}	169.31 ^{b}	199.37^{a}	5.10
Motile sperm /ejaculate (×10^6)	71.83 ^{c}	93.17 ^{b}	115.37^{a}	4.29
Live spermatozoa (%)	71.80 ^{b}	81.90 ^{a}	84.10^{a}	0.66
Dead spermatozoa (%)	28.20 ^{a}	18.10 ^{b}	16.90^{b}	0.66
Abnormal spermatozoa (%)	24.10 ^{a}	22.50 ^{ab}	20.78^{b}	0.64

a ,b, c Means in the same row followed by different letters are significantly different (P<0.05).

T1= Bucks fed with basal diet saved as control (0 g Zn O supplement/ kg diet), T2= Bucks fed with basal diet containing 75 ppm Zinc oxide / kg diet (0.096 g Zn O supplement/ kg diet). T3= Bucks fed with basal diet containing 150 ppm zinc oxide/ kg diet (0.192 g Zn O supplement/ kg diet)

treated bucks with zinc supplementation had significantly higher (P≤0.05) sperm concentration and total sperm count compared to the control ones. The present study demonstrated that enhancement of cellular mass volume of ejaculates in rabbits supplemented with 75 and 150 ppm zinc, leading to significant increase in spermatozoa concentration in the respective bucks. These results are in agreement with those reported in the literature by Ogbu and Ezeokoli (2016); Marai *et al*., (2003) and Moce *et al*. (2000) they reported that improve reproductive performance of buck rabbits treated with dietary Zinc recorded greater (P≤0.05) ejaculate volume, sperm motility, cell concentration and fertility rate than the control group. Also, they reported that dead and abnormal spermatozoa were decreased in treated bucks. These improvements in semen quality may returns to zinc ions are involved in processes of cell division, development and differentiation and in the control of gene expression (Danek, 2002; Viudes *et al*., 1997 and Bicudo and Paschoal, 1991).

On the other hand, fertility rate and litter size at birth were significantly increased in the rabbits of T2 (by 30.1 and 20.2 %, respectively) and T3 (by 31.8 and 21.7 %, respectively) when compared to the rabbits in T1 (Table 3). These results agreement with El-Speiy and El-

Table 3. Means (±SE) for fertility rate and litter size at birth of NZW rabbit as affected by zinc oxide supplementation

Traits	Treatment			
	T_1	T_2	T_3	±SE
Fertility rate (%)	29.20 [b]	38.00 [a]	38.50 [a]	0.64
Litter size at birth	6.90 [b]	8.30 [a]	8.40 [a]	0.84

a ,b, c Means in the same row followed by different letters are significantly different (P<0.05).
T1= Bucks fed with basal diet saved as control (0 g Zn O supplement/ kg diet), T2= Bucks fed with basal diet containing 75 ppm Zinc oxide / kg diet (0.096 g Zn O supplement/ kg diet). T3= Bucks fed with basal diet containing 150 ppm zinc oxide/ kg diet (0.192 g Zn O supplement/ kg diet)

Hanoun (2013). The reduction in fertility rate in control group is related to the low sperm count, motility and high percentage of abnormal spermatozoa level (Raji *et al*., 2003). However, Brun *et al*. (2002) found that litter size at birth was significantly influenced by concentration and number of total motile sperms. In addition, Ulkowski *et al*. (2005) confirmed that the positive effect of zinc as an enhancer of reproductive capacity of rabbit bucks could be attributed to its ability to protect mammal cells from oxidation.

In conclusion, adding 75 or 150 ppm zinc oxide / kg diet caused to improve the physical characteristics of semen quality and fertility rate of buck NZW rabbits under subtropical conditions.

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تأثير أضافة مستويات اكسيد الزنك على صفات السائل المنوى ومعدل الخصوبة فى ذكور الارانب تحت الظروف شبه الأستوائية

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أجريت هذه الدراسة بمزرعة الإنتاج الحيواني- كلية الزراعة - جامعة جنوب الوادي بقنا - مصر حيث يوجد المناخ شبة الاستوائى. بدات الدراسة فى منتصف يناير وحتى نهاية مايو ٢٠١٢. تهدف هذة الدراسة لدراسة تاثير إضافة اكسيد الزنك للعلائق على صفات السائل المنوى ومعدل الخصوبة لذكور سلالة الارانب النيوزيلندى البيضاء تحت الظروف شبة الاستوائية. استخدم في هذه الدراسة عدد ثلاثون من ذكور الأرانب (عمر ١٦ اسبوع ومتوسط وزن جسم ٢٣٦٨± ١٤.٢١ جم). وزعت الذكور عشوائيا إلى ثلاثة معاملات متساوية (عشرة ذكور في كل مجموعة) بدون وجود فروق معنوية فى متوسط وزن الجسم بين المعاملات . المعاملة الأولى غذيت الارانب فيها على العليقة الأساسية محتويه على صفر زنك اوكسيد لكل كجم عليقة واعتبرت مجموعة مقارنة ، المعاملة الثانية والثالثة غذيت الارانب على العليقة الأساسية محتويه على ٧٥ و ١٥٠ جزء في المليون من اوكسيد الزنك / كجم عليقة (٠,٠٩٦ و ٠,١٩٢جرام اكسيد الزنك / كجم عليقة) على التوالى.

اشارت النتائج الى ان كل الصفات الفيزيائية للسائل المنوى تحسنت معنوياً نتيجة امداد علائق ذكور الارانب النيوزيلندى بالزنك ما عدا حجم القذفة وحركة الحيوانات المنوية. حيث ان الذكور المعاملة زاد فيها كلا من عدد وتركيز الحيوانات المنوية فى القذفة بصورة معنوية مقارنة بالذكور الكنترول. كما ادى استخدام اكسيد الزنك فى علائق ذكور الارنب الى تحسن معدل الخصوبة وعدد الخلفة عند الميلاد لامهات الارانب النيوزيلندى التى لقحت بذكور غذيت على ٧٥ او ١٥٠ جزء فى المليون اكسيد زنك .

التوصية: من هذه النتائج يتضح أن اضافة أكسيد الزنك الى عليقة ذكور الارانب النيوزيلندى ألابيض سواء عند مستوى ٧٥ أو ١٥٠ جزء فى المليون/ كجم عليقة قد أدى الى تحسين الصفات الطبيعية للسائل المنوى ومعدل الخصوبة تحت الظروف شبه الاستوائية.