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EFFECT OF DIETARY ZINC SUPPLEMENTATION ON SEMEN CHARACTERISTICS OF RABBIT BUCKS.

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ABSTRACT

The present study was carried out at the Experimental farm of Animal Production Department, Faculty of Agriculture, South Valley University, Qena during the period from March to April 2008.Thirty matrue New Zealand White rabbit bucks aged 6 months were used, following a completely randomized design with three treatments(Zn levels). First group; fed control ration ,second group fed control ration; supplemented with 75 ppm ZnO and the third group fed control ration supplemented with 150 ppm ZnO . The rabbits were selected immediately after weaning (4 weeks of age) and used in the experiment that extended till 36 weeks of age. The treatments were respectively,0 , 75 and 150 ppm supplemental zinc per one kg ration in the diet. The animal received ZnO in their diets as soon as they were weaned. First semen collection was performed at 28 wks of age, being collected six times weekly per animal. The volume of each ejaculate was registered and an aliquot was separated for further analyses. Mean values of total ejaculate didn't differ($p>0.05$) among treatments. Animal fed 75 and 150ppm ZnO presented higher sperm cells concentration compared to the control group. In conclusion this study shows that it is possible to improve the quality of rabbit semen through dietary supplementation of zinc oxide. Key words: reproductive performance; semen, rabbit, supplementation, ZnO.

INTRODUCTION

Zinc is a micro-mineral involved in various processes of animal metabolism. Since it was originally demonstrated that zinc is necessary for healthy growth of rats (Todd *et al.*, 1934, cited by Underwood and Somers, 1977), the role of zinc in the animal organism began to gain special attention. This mineral is involved in over 200 proteins and enzymes and is essential for male fertility. Zinc is involved in the activation of key sperm enzymes, and moves into the prostate with the assistance of testosterone (Prasad, 1979). A lack of zinc causes a lowering of testosterone, shrinks testicle size and produces misshapen and less healthy sperm, among other negatives. Upon restoring a daily dose of 15 milligrams , testosterone and sperm count levels rebounded to acceptable levels within12months (Prasad, 1979).Zinc participates actively in protein synthesis and carbohydrate metabolism. The discovery that the enzyme carbonic anhydrase contains 0.33% of zinc in its molecule (Keilin and Mann, 1939, cited by Underwood and Somers, 1977) is considered the first acceptable explanation of the mechanism of action of this element. After that, many other enzymes have been identified as containing zinc: alcohol dehydrogenase, carboxipeptidase and DNA-polymerase, this latest being fundamental in cell division process. This mineral stabilizes the quaternary structure of enzymes; large quantities of zinc were found to provide stability to the structures of RNA, DNA and ribosomes (Prask and

Plocke,1971, quoted by Mc Dowell, 1992). The high concentration of zinc in organs like prostate, testicles and in the spermatozoa itself (Bertrand and Vladesco, 1921, cited by Underwood and Somers, 1977), suggests its importance in reproduction. Zinc requirement for rabbits, indicated in the literature, is 3060 mg/Kg dry matter, with suggestion of higher levels for breeders (Mateos and Blas, 1998). Zinc supplementation enhances spermatogenesis, but the mechanism is not completely known. This mineral is important for spermatogenesis, being directly involved in spermatozoa maturation and preservation of germinative epithelium (Underwood and Somers,1969). It is also essential to cellular division, synthesis and stability of DNA(Devenson *et al*.,1993) , as well as in cellular differentiation. Zinc is directly involved in anatomic development and normal function of male reproductive organs; deficiency of zinc in the diet delays testicle development, reduce testosterone production and stops spermatogenesis (Devenson *et al*.,1993).Testosterone, or male sex hormone, plays a key role in developing and maintaining masculine sexual organ, and promotes secondary sexual characteristics, including the appearance of facial hair, sexual desire, and sexual behavior(Hafez,1987) . Testosterone helps to build protein and is essential for normal sexual behavior and producing erections. It also affects bone formation, lipid metabolism, carbohydrate metabolism, and prostate gland growth. Androgens are essential for the maintenance of the cellular and integrity of the sperm duct system and may indirectly influence the viability of the spermatozoa (Hafez,1987). The increased in testosterone will stimulate glycolysis in sperm cells.The production of hormones by the pituitary gland is also affected in an animal when the diet is deficient in zinc (Hidroglou and Knipfel, 1984; Reeves and Odeel, 1988). Studies demonstrated that the use of some natural antioxidants, such as vitamins A and E, can improve semen quality, through protection of spermatozoa membrane (Rode *et al*., 1995; Maldjian *et al.*, 1998). Deficiencies of zinc in males have resulted in impaired spermatogenesis and testosterone production . Also, zinc supplementation has been showing its advantages in spermatic production (Reeves and Odeel, 1988) . The aim of this experiment was to determine the effects of dietary supplementation of zinc upon total volume and cellular mass volume of rabbit semen .

In most studies reviewed (National research council, 1980), no adverse effects occurred when dietary Zn concentration was below 600 PPM. Many factors influence Zn toxicity; dietary Pb, Cu deficiency, marginal Se intake exacerbate it, while soybean protein appears to protect against excess Zn compared with casein, perhaps due to its phytate content. Details of the toxic effects of Zn have been reviewed (National research council, 1980, 2005)

MATERIALS AND METHODS

This study was carried out at the Experimental farm of Animal Production Department, Faculty of Agriculture, South Valley University, Qena

Experimental design :-

Thirty male rabbits of New Zealand White breed were used in this investigation. After weaning (at four weeks of age), the rabbits were housed

in individual cages in the same room, receiving rations and water ad libitum with commercial rabbit pellets. Semen collection was preformed from28th week(March-April 2008)The experimental design was completely randomized . Three groups each of 10 New Zealand White male rabbits were used.

Feeding and management: -

Treatments were made up of basal diets varying in supplemental zinc contents provided as zinc oxide (ZnO) as described in table 1.

Each group was further divided and fed ad libitum as follows:

Control diet + 0 ZnO (Control group) ;

Control diet + 75ppm per one kg ration ZnO (Second group fed tested 1st ration) ;

Control diet +150 ppm per one kg ration ZnO (Third group fed tested 2nd ration) ;

Table(1).Quantity of ZnO (g/kg diet) supplied and corresponding estimated zinc supplement per treatment .

Group	ZnO supplement (g)/ kg diet	Estimated supplemental Zn (ppm)/ kg diet
Control	0.000	0
1	0.096	75
2	0.192	150

Table(2). Formulation and chemical composition of the diet (g/kg).

Ingredients	%
Alfalfa hay	3.04.2
Soybean meal (44% CP)	12.5
Corn meal	22.5
Whole sunflower meal	7.0
Barley meal	14.0
Wheat bran	5.0
Beet molasses	1.2
Calcium carbonate	1.372
Calcium diphosphate	0.671
Sodium chloride	0.5
Dl-methionine	0.057
premix (Zn-free)	1.0
Total	100.0

Calculated chemical composition of the diets .

	Percentage
Dry matter	89.2
Crude protein	17.3
Ether extract	5.3
Crude fibre	14.9
Ash	8.8
Digestible energy** MJ kg-1	10.9

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

Semen collection and analysis :-

At six months of age bucks started the training period with artificial vagina. One ejaculate was collected per male per week. At seventh month of age rabbit bucks started semen collection period. During 8 weeks, two ejaculates per male per week were collected, with an interval of 30 minutes between them. All ejaculates were stored at 37^{o}.C in a water bath until evaluation, not-later than 15 minutes after collection. Volume (ml) and pH of the ejaculate were determined by using a graduated tube and a pH-meter 507 Crison), respectively. Immediately after collection, the ejaculate volume (ml) and the spermatozoa concentration (number of sperms /ml) were recorded by using a graduated tube and haemocytometer respectively. For evaluation of percentage of sperm motility drop of semen was examined under the low power of microscope using a hot stage at 37^{o}c Progressive motility was estimated on a percentage score .Total and motile sperm out put / ejaculate were calculated . All bucks were used for natural three weeks after the end of the collection period to study the fertility rate of the different treatments .

as described in table 2. Chemical analyses of diets were done according to A.O.A.C. methods (1995) .

Statistical analyses :-

Analysis of variance was carried out using SAS (1989), Duncan Multiple range Test was used to compare the differences among means (Duncan, 1955).

RESULTS AND DISCUSSION

The mean value of some physical semen characteristics of the male rabbits of New Zealand White affected by Zinc supplementation are shown in table 3.The results indicated that most physical semen characteristics are significantly($p<0.05$)affected by supplementation of ZnO in the ration of New Zealand White (NZW) bucks which were subjected to ZnO supplementation showed improvement ($p<0.05$)in most physical semen characteristics studied.

Tables (3): Means(±SE) of some physical semen characteristics of New Zealand white rabbit bucks as affected by ZincO supplementation in the ration .

Semen characteristics	Zinc O		
	0.0 control	75 ppm	150ppm
Ejaculate volume (ml)	0.49± 0.019 a	0.55±0.031a	0.58±0.023a
Sperm motility (%)	46.95± 2.05a	48.90±1.77a	52.30±1.78a
Sperm concentration (10^6/ml)	313.70± 4.96c	347.10±8.40b	381.00±12.1a
Total sperm out put (10^6/ejac)	152.63± 4.85c	190.62±12.68b	221.61±12.5a
Motile sperm /ml ($x10^6$)	146.69±5.10c	169.31±9.11b	199.37±9.79a
Motile sperm /ejac ($x10^6$)	71.83±4.29c	93.17±7.66b	115.37±6.89a
Live spermatozoa (%)	71.80± 0.66b	81.90±0.88a	84.10±1.06a
Dead spermatozoa (%)	28.20± 0.66a	18.10± 0.88b	16.90±0.99b
Abnormal spermatozoa (%)	24.10± 1.06a	22.50±0.68ab	20.78 ±0.64b

a ,b, c means in the same row followed by different letters are significantly different ($p<0.05$) .

Results in table (3) showed that ejaculate volume was not affected significantly by zinc supplementation . However, it is of interest to note that ejaculate volume was increased insignificantly in the rabbit buck groups fed diet supplemented with zinc compared to the control group (Table 3) . The mean value of ejaculate volume ranged from 0.49 to 0.58 ml, and it is increased with zinc supplementation. In contrast, El-Masry *et al*.(1994) and Moce *et al*.(2000) found that ejaculate volume was higher in animals fed supplemental zinc (levels from35 to 100 ppm) as compared to non-supplemented ones. Increasing ZincO addition from 75 to 150 ppm per 1 kg of ration did not influence ejaculate volume of the New Zealand White(NZW) bucks. Thus, reports of other authors that ZincO is beneficial to New Zealand White(NZW) bucks ejaculate volume Moce *et al* (2000) .

The same trend was observed in sperm motility percentage which increased insignificantly by about 11% in the third group compared with the unsupplemented control group . The semen of the treatment bucks group showed significantly higher ($p \leq 0.05$) sperm concentration and total sperm count compared to the control group. Also, an increase of spermatozoa concentration in the ejaculates of animal fed the supplemental zinc was observed. The present study demonstrated enhancement of cellular mass volume of ejaculates in rabbits supplemented with 75 and 150 ppm zinc, revealing a possible increase of spermatozoa concentration in the respective animals, which is in agreement with some authors. Rabbits supplemented with 75 ppm zinc, as well as non-supplemented animals, showed low values of cellular mass volume(Table3). The same trend of positive significant effect and improvement in most semen quality due to ZincO supplementation in the level of 150ppm . This suggests that, the positive effect of zinc supplementation upon semen concentration may be valid until certain limit of concentration of the referred mineral. The present results, regarding, the effect of ZnO on reproductive performance of New Zealand White (NZW) bucks including as well as semen quality are in agreement with those reported recently in the literature by Underwood and Somers (1977) and Quarterman (1986). Moce *et al* (2000) reported that males treated with dietary Zinc showed greater ($p<0.05$) ejaculate volume, sperm motility , cell concentration and conception rate than the control group . Also , they reported that dead and abnormal spermatozoa were decreased in low Fertil bucks . They concluded that Zinc could be used successfully alone to improve reproductive performance of New Zealand White (NZW) bucks. Bicudo and Paschoal (1991) studied the effect of supplementation of ration with Zinc on semen quality of bucks , they reported that the overall mean of the ejaculate volume , live sperm percentage , sperm concentration /ml and sperm out put /ejaculate were improved , while the percentage of sperm motility and abnormalities were slightly lower in treated group than the control . Viudes *et al* (1997) found that most physical semen characteristics of bucks were improved ($p<0.05$) by the presence of Zinc on body wt. and daily gain.

Generally it was observed that both levels75 and 150 ppm ZincO subjected to ZincO supplementation exhibited higher semen quality . The present results are in harmony with those reported by Bicudo and Paschoal

(1991) , Moce *et al* (2000) , Underwood and Somers (1977) and Quarterman (1986) .

2-Effect of ZnO on some reproductive traits of New Zealand white rabbit bucks.

Table(4).Effect of ZincO on some reproductive traits of New Zealand white rabbit bucks.

Item	Zinc O		
	0.0 control	75 ppm	150ppm
Conception rate (CR)	29.20± 0.64b	38.00±1.59a	38.50±1.64a
Litter size at birth (N)	6.90± 0.48 a	8.30±0.49a	8.40±0.541a

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

Using ZincO in improving the reproductive efficiency as represented in conception rate and litter size at birth of female New Zealand white rabbits have been reported by Prasad (1979), Underwood and Somers (1977) and Mateos and Blas, (1998) and also Underwood and Somers (1969). Rashwan et al (1997) postulated that ZnicO could be used alone to improve fertility of female New Zealand white rabbits .

Conclusion

Increased addition of ZincO to balanced feed mixtures fed to New Zealand white rabbit bucks, from the currently recommended standard amounts (0.0 ppm ZincO per 1 kg) to 75 ppm ZincO per 1 kg or 150 ppm ZincO per 1 kg hast positively influences qualitative semen traits of the New Zealand white rabbit bucks.

REFERENCES

A.O.A.C ``Association of Official Agriculture Chemists``, (1995): Official Methods of Analysis. 10th Ed. Published by the A.O.A.C. , Washington , D. C., USA.

Bertrand G.; Vladesco R. C. (1921). R. Acad. Sci. 1:173-176.

Bicudo S. D.; Paschoal J. P. S. (1991).Some rabbit semen traits in spring and early summer. In: Congresso Brasileiro DE Reproduc.O Animal, 9, 1991, Belo Horizonte. Anais..., Belo Horizonte: CBRA, 2. 457-459.

Devenson D. P.; Enerik R. J. , Jost L. K. (1993). Zinc/Silicon interactions influencing sperm chromatin integrity and testicular cell development in the rat as measured by flow cytometry. J. Anim. Sci., 1, 71:955-962.

Duncan D.B.,(1955):Multiple range and multiple F-test Biometrics,11:1-42.Edinger, PH.,

EL-Masry K. A.; Nasr A. S.; Kamal T. H. (1994). Influences of season and dietary supplementation with selenium and vitamin E or zinc on some blood constituents and semen quality of New Zealand White rabbit males. World Rab. Sci., 1, 2:79-86.

Finzi, A., Moreira P., Macchioni P.(1994). Rabbit production in hot climates. In: Finzi, A. Modifications of some rabbit spermatic parameters in relationship to high ambient temperatures. Cairo:[s.n.]. 333-336.

Hafez,E.S.E.(1987)Reproduction in farm animals.5th Ed. Philadelphia: Lea & Febiger,PP.

Hambidge,K. M., and Krebs, N.F. (2001).*Annu. Rev. Nutr.* 21:429.

Hidroglou M.; Knipfel J.E. (1984). Zinc in mammalian sperm: a review. J. Dairy Sci . 1,35:1175-1185.

Keilin D.; Mann T. (1939). Nat. Mag. 144:442.

Maertens L., Moermans R., De Groote G. (1984). Prediction of apparent digestible energy content of commercial pelletted feeds for rabbits. J. Appl. Rabbit Res., 11, 60- 67.

Maldjian A.; Cerolini S.; Surai P.; Speake B. K. (1998). The effect of vitamin E, green tea extracts and catechin on the in vitro storage of turkey spermatozoa at room temperature. Poult. Avian Biol. Rev., 1, 9:143-151.

Mateos G. G.; Blas C. (1998). Minerals, vitamins and additives. In: Blas, C.;

Wiseman, J. The nutrition of the rabbit. London: Cabi Publishing, 9:145-175.

Mc Dowell L. R. (1992). Minerals in animal and human nutrition. London:

Academic,12:265-293.

Moce E., Arouca M., Lavara R., Pascual J.(2000). Effect of dietary zinc and vitamin supplementation on semen characteristics of high growth rate males during Summer

season. In: World Rabbit Congress,7,2000,Valencia. Proceedings. Valencia:[s.n.],. CD-ROM.

O'Dell, B.L.(1998) In Trace Elements in human health and Disease, Vol. I, A. S. Prasad (Ed.). Academic Press, New York.

Prasad, A. S. (1979) Zinc in Human Nutrition. CRC press, Boca Raton, FL.

Prask J. A.; Plocke D. J. (1971). Plant Phisiol, 48: 501..

Quarterman, J.(1986). In Trace Elements in human and Animal Nutrition (5th ed.), p. 281, W. Mertz (ed.). Academic Press, New York.

Reeves P.G.; Odeel B. L.(1988). Zinc deficiency in rats and angiotensin-converting

enzyme activity: comparative effects on lung and testis. J. Nutr., 1, 118:622-626.

Rode L.M.; Colter G.H.; Kastelic J. P.; Bailey D. R. C. (1995). Seminal quality and sperm production in beef bulls with chronic dietary vitamin a deficiency and subsequently re-alimentation. Theriogenology , 1, 43:1269-1277.

SAS. (1989). User's Guide. Version 6, 4. ed. Cary, NC: SAS Institute Inc., 1789.

Todd, W. R, Elvehjemc.A.; Harte.B. (1934). *J. Pysiol.* 107:146.

Underwood E. J.; Somers M. (1969). Zinc nutrition. Aust. J. Agric., 2, 20:889.

Underwood E. J.; Somers M. (1977). Zinc: trace elements in human and animal nutrition. New York: Academic . 196-242.

Viudes DE Castro M. P.; Vicente J. S. (1997). Effect of sperm count on the fertility prolificity rates of meat rabbits. Anim. Reprod. Sci., 1, 46:313-319.

تأثير أضافة الزنك للعليقة على صفات السائل المنوى فى ذكور الارانب

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أجريت هذه الدراسة بمزرعة الإنتاج الحيواني كلية الزراعة- جامعة جنوب الوادي بقنا خلال شهري مارس وأبريل ٢٠٠٨ لدراسة تأثير أكسيد الزنك على صفات السائل المنوي في ذكور الأرانب واستخدم في هذه الدراسة ثلاثون من ذكور الأرانب (النيوزيلندي) ووزعت الحيوانات عشوائيا تبعا لأوزانها وأعمارها في ثلاثة مجموعات متساوية عشرة في كل مجموعة. الأولى مقارنة حيث غذيت فيها الحيوانات على العليقة الأساسية , الثانية غذيت الحيوانات على العليقة الأساسية +٧٥جزء في المليون من عنصر الزنك فى صورة أكسيد الزنك لكل ١كجم عليقة أما الثالثة فغذيت الحيوانات على العليقة الأساسية+١٥٠جزء في المليون من أكسيد الزنك وتم جمع السائل المنوي مرة كل أسبوع طوال فترة التجربة من كل حيوان على حدة بمعدل قذفتين فى كل مرة . وأجريت الاختبارات الطبيعية على قذفات السائل المنوى لدراسة خصائصه(الحجم-الحيوية–التركيز-نسبة الشواذ-الحى والميت) . كما تم قياس وزن الحيوانات ومعدل النمو بصفة دورية طوال فترة التجربة وفى نهاية التجربة تم تلقيح عدد من الاناث لتحديد نسبة الخصوبة فى كل معاملة واتضح من الدراسة عدم وجود فروق معنوية فى معدل الوزن سواء بين مجموعتى المعاملة ومجموعة الكنترول أو بين مجموعتى المعاملة . أما بالنسبة لخصائص السائل المنوى لذكور الارانب النيوزيلندى المعاملة باكسيد الزنك ٧٥جزء فى المليون فقد أعطت حجم للقذفة(٠,٥٥سم) بينما حجم القذفة فى ذكور الارانب النيوزيلندى المعاملة باكسيد الزنك.١٥جزء فى المليون(٥٨و.سم)مقارنة بمجموعة المقارنة التى أعطت حجم للقذفة(٠,٥ سم)وكانت الفروق بين مجموعتى المعاملة ومجموعة المقارنة غير معنوية عند مستوى٥%. أما بالنسبة لتركيز الحيوانات المنوية/مل فى السائل المنوى للذكور المعاملة باكسيد الزنك ٧٥جزء فى المليون ٣٤٧,١مليون حيوان منوى/مل بينما المعاملة باكسيد الزنك .١٥جزء فى المليون٣٨١ مليون حيوان منوى/مل مقارنة بمجموعة المقارنة ٣١٣,٧مليون حيوان منوى/مل فى حين كان تركيز الحيوانت المنوية فى القذفة للذكور المعاملة باكسيد الزنك ٧٥جزء فى المليون ١٩٠٦٢مليون حيوان منوى/ القذفة بينما المعاملة باكسيد الزنك .١٥ جزء فى ٢٢١,٦١ المليون مقارنة بمجموعة المقارنة ١٥٢,٦٣ مليون حيوان منوى/ القذفة وكانت الفروق عالية المعنوية عند مستوى معنوية١% سواء كانت الفروق بين مجموعتى المعاملة ومجموعة المقارنة أو بين المعاملتين حيث أعطى المستوى ١٥٠اعلى نسبة تركيز/مل أو القذفة. وكانت النسبة المئوية للحركة الكلية للحيوانات المنوية فى مجموعة المعاملة ٧٥جزء فى المليون ٤٨,٩% بينما المعاملة باكسيد الزنك .١٥ جزء فى المليون ٥٢,٣ % مقارنة بمجموعة المقارنة ٤٦,٩٥% وكانت الفروق بين مجموعتى المعاملة ومجموعة المقارنة غير معنوية عند مستوى ٥%. أما نسبة الشواذ والميت من الحيوانت المنوية كانت أعلى فى مجموعة المقارنة وكانت الفروق بين مجموعتى المعاملة ومجموعة المقارنة معنوية عند مستوى معنوية٥% ولم يكن هنالك فروق معنوية بين استخدام مستوى٧٥ جزء فى المليون و.١٥ جزء فى المليون . كما تلاحظ أن أعلى نسبة أخصاب تم الحصول عليها من الذكور المعاملة باكسيد الزنك ١٥٠جزء فى المليون تليها للذكور المعاملة باكسيد الزنك ٧٥جزء فى المليون مقارنة بمجموعة المقارنة .من هذه النتائج يتضح أن اضافة عنصر الزنك فى صورة أكسيد الزنك الى عليقة ذكور الارانب النيوزيلندى ألابيض سواء عند مستوى٧٥ أو ١٥٠ جزء فى المليون قد أدى الى تحسين الصفات الطبيعية للسائل المنوى لذكور الارانب النيوزيلندى ألابيض التى تمت دراستها فى هذا البحث خاصة عند استخدام اكسيد الزنك بمعدل ١٥٠جزء فى المليون.