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IMPACT OF ZINC SUPPLEMENT ON SOME REPRODUCTIVE TRAITS IN EGYPTIAN BUFFALO BULLS

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SUMMARY

This work aimed at studying the effect of zinc supplementation on sexual reaction time, plasma testosterone level, scrotal circumference and semen quality of Egyptian buffalo bulls. For that, four sexually mature and clinically normal buffalo bulls were used. The bulls were almost at the same age (3.5 years) and body weight (550 kg) at the beginning of the experiment.

The data were collected throughout a one year round. This time interval comprised a pre-experimental period of three months and an experimental period of nine months. During the experimental period, each bull was orally supplemented with 0.7 g of zinc oxide (equal to 554 mg zinc) per day. The semen was collected by means of the artificial vagina at the rate of twice weekly with two successive ejaculates at each collection day.

The sexual reaction time (RT) was assessed at each semen ejaculation. In addition, the scrotal circumference (SC) and plasma testosterone levels were recorded at biweekly intervals. The results recorded during the experimental period were compared with those obtained during the pre-experimental period.

The statistical analysis showed that zinc supplementation resulted in a highly significant improvement ($P< 0.01$) in ejaculate volume, percent individual motility, percent live sperm, percent morphologically normal sperm, sperm concentration/ml, total sperm number / ejaculate, scrotal circumference and zinc concentration in blood plasma (mg %). Significant improvement ($P< 0.05$) was also recorded for reaction time, fructose concentration in whole semen (mg %), plasma testosterone level (ng/ml) and zinc concentration in whole semen (mg %).

The results of this work indicate that the current level of dietary zinc supplement (0.7 g Zn o containing 554 mg zinc /head / day) could be useful for enhancing the reproductive capacity of Egyptian buffalo bulls.

Keywords: Buffalo bulls, zinc supplement, semen quality, scrotal circumference, testosterone

INTRODUCTION

It has been well documented that zinc concentration in the mammalian semen is several folds higher than its concentration in the blood (Mann, 1964; Madding *et al.*, 1986; El- Anwar and Badr, 1996 and Khalifa, 1997). This attracted the workers' attention to investigate the possible effects of this element on male reproduction.

Zinc was reported to be essential for membrane stability and motility of bovine spermatozoa (Swarup and Sekhon, 1976). Marmar *et al.* (1975) reported that supplementary zinc sulfate may be useful in the treatment of some cases of infertility in men.

Studies on the effect of zinc supplementation on the androgenic activity and semen quality of buffalo bulls are scarce and inconclusive (Nour, 1985; Misra *et al.*, 1989; and Khalifa, 1997). Hence, the objective of this study was to investigate the influence of zinc supplementation on reaction time, scrotal circumference, plasma testosterone level and semen quality of Egyptian buffalo bulls.

Four sexually mature and clinically normal buffalo bulls were utilized throughout a one-year period. The data were collected, analyzed and the results discussed.

MATERIALS AND METHODS

This study was conducted at Mehallet Moussa Research Station, Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture. The farm is located in the North Center of Nile Delta, Kafr El- Sheikh Governorate.

Experimental Animals and Management

Four healthy and sexually mature buffalo bulls were used in this study. The average age and body weight of the bulls (at the beginning of the experiment) were 3.5 ± 0.2 years and 550 ± 32 kg, respectively.

The bulls were individually penned in 4 x 5 meters adjacent boxes with counter- slope asbestos sheds of 4 meters height. The feeding regime was similar to that regularly applied on the farm for dry and green feeding seasons (APRI, 1975). During the dry feeding season (from May 1st to November 30th, 1998) each bull received a daily ration of 5.5 kg concentrated mixture cubes, 6 kg rice straw and 2 kg berseem hay. During the green feeding season (from December 1st, 1998 to April 30th, 1999) the per head daily ration included : 15 kg berseem, 3 kg rice straw and 4 kg concentrate cubes. The concentrate cubes contained 48 % decorticated cotton-seed cake, 21.5 % wheat bran, 20 % maize, 4.5% rice polish, 3 % molasses, 2 % lime stone and 1 % sodium chloride. The bulls were allowed to drink water twice daily. In addition, they also had regular exercise and daily washing under the running water.

Experimental design

The experiment was conducted during the period extended from May 1st, 1998 to April 30th, 1999. This time interval (twelve months) comprised a pre-experimental period of three months (from May 1st to July 31st, 1998) and an experimental period of 9 months (from August 1st to April 30th, 1999). The later was subdivided into two successive intervals. The first interval was extended from August 1st to November 30th (4 months) and presented the dry feeding season. The second interval was conducted from December 1st, 1998 to April 30th, 1999 (5 months) and presented the green feeding season. Starting from August 1st, 1998 all buffalo bulls began to receive a supplementary zinc oxide at the rate of 0.7 g (equal to 554 mg Zn) / head / day. This level of zinc supplement was recommended by Mohamed *et al.* (1994) and continued until the end of the study.

A total of 784 semen ejaculates was collected by means of the artificial vagina. The semen was collected at the rate of twice / week / bull. At each collection day two successive ejaculates were obtained. The reaction time (RT) for each ejaculate was measured in seconds as the time elapsed from the first approach of the bull to the teaser animal (a yearling buffalo calf bull) until the ejaculation was completed. The scrotal circumference (SC) was measured in cm at biweekly intervals using a scrotal tape specially designed for this purpose (A. S. T, 1983).

Ejaculate volume was measured to the nearest 0.1 ml using a graduated collection tube. Percent sperm individual motility was estimated to the nearest 5% on a bright field stage microscope (at 38 °C) and a magnification of 450 X. Percent live sperm was estimated using the eosin-nigrosin staining technique (Barth and Oko, 1989). The percentage of eosinophilic (unstained) cells was calculated from a total number of 200 spermatozoa using a magnification of 650 X. Percent morphologically abnormal sperm as well as the percentages of the different types of sperm abnormalities were estimated on the same smears prepared for live/dead counts. The smears were observed on the bright field microscope using the immersion oil lens (at 1600 X magnification). The incidence of each type of sperm abnormalities as well as of the total sperm abnormalities were calculated separately (each from a total number of 100 spermatozoa). Sperm cell concentration / ml was determined according to the conventional procedure described by Sorensen (1979) using the improved type of Neubour haemocytometer. The sperm concentration / ejaculate was calculated by multiplying the ejaculate volume (ml) by sperm concentration / ml.

The initial fructose concentration (mg %) in the whole semen was determined colorimetrically according to Mann (1964) using a Spectronic 20-type spectrophotometer. Zinc concentrations (mg %) in the whole semen as well as in blood plasma were assessed according to the procedure described by Smith *et al.* (1979) using the Philips pug 100-type atomic absorption spectrophotometer. Testosterone concentration (ng / ml) in the blood plasma was determined using testosterone [I^{125}] coated tube RIA kits (Orion Diagnostic, Finland). According to the manufacturer, the cross reaction of testosterone antibody (at 40-60% binding) was 100% with testosterone. The standard curve ranged between 0-15 ng/ml. The sensitivity of the method was 0.03 ng/ml. The analysis was performed in one assay and the intra assay coefficient of variation was 7.6%.

Statistical analysis

The data were analyzed using GLM procedure/ SAS® program (1985). The actual means (± SE) as well as the ANOVA using the repeated measurements analysis were computed. The changes in the different studied traits (due to zinc treatment) were calculated in percentages.

RESULTS AND DISCUSSION

Results presented in Table 1, showed that all studied reproductive traits were improved by zinc treatment. The percentages of improvement were more pronounced for ejaculate volume (+ 30 %), total sperm number / ejaculate (+ 48.2 %) and percent morphologically abnormal sperm (- 30 %). These findings agree with the results obtained by Khalifa (1997) for buffalo bulls. However, contradictory results were early reported by Pitts *et al.* (1966) who did not find any significant improvement in the reproductive performance of zinc supplemented Holstein bulls. The variable responses to zinc supplement could be attributed to: species differences; level and form of zinc offered (Organic, non organic or ionic); dietary zinc levels (in feeds and water), interference with other metals in the intestine; type and level of feeding and the rate of its utilization by the target organs (O'Dell, 1983; El-Masry *et al.*, 1994, and Khalifa, 1997).

The sexual RT, percent sperm individual motility, percent live sperm and sperm concentration / ml were also improved by zinc treatment. This comes in complete agreement with the results reported by Johnson and Eliasson (1978) in men; El- Masry *et al.* (1994) in rabbits and Khalifa (1997) in buffalo. The former authors suggested that zinc appears to play an important role in gamete biology by protecting spermatozoa from peroxidation. They added that zinc is considered as a metabolic activator of many enzymes (e. g. acid phosphatase, carbonic anhydrase and ATPase). The significant decrease ($P< 0.05$) in RT due to zinc treatment contradicted with the results reported by Pitts *et al.* (1966) in bulls. Otherwise it agreed with the findings of Alexandrov and Zajanckovskii (1969) who demonstrated that zinc supplement improved the sexual desire of breeding bulls.

The impact of zinc treatment on the incidence of different types of sperm abnormalities is shown in Table 2. It could be seen that all types of sperm abnormalities decreased by zinc treatment. The sperm head abnormalities declined significantly ($P< 0.01$) from 2.4 % (before zinc treatment) to 0.9 % (after zinc supplementation). The percentage of decrease was 62.5 %. It is also clear that the percentages of decline in the other types of abnormalities confined to the head region (e. g., abnormal acrosomes and diadem / craters) are obvious (42.8 % and 50 %) respectively. Similar results were reported by El-Masry *et al.* (1994) in rabbits and Khalifa (1997) in buffalo. The detached heads, protoplasmic droplets, middle piece and tail abnormalities decreased by 17.3, 50, 22.6 and 25.2 %, respectively. The improvement in the picture of sperm abnormal forms could be attributed to improved spermatogenesis due to zinc treatment. Khalifa (1997) suggested that zinc might cause an activation of lysosomal enzymes which in turn reduce the incidence of protoplasmic droplets. Hence, it enhances sperm maturation, motility and fertilizing capacity. The fact that zinc is required for sperm maturation during the final stage of spermatogenesis (*i.e.,* spermiogenesis) could explain the improvement in sperm morphology due to zinc treatment (Rodriguez *et al.*, 1984).

The highly significant increase ($P<0.01$) in the SC is largely attributed to the increase in the testicular parenchyma rather than to fat deposition under the scrotal skin, known to occur in bulls above 5 years (Hahn *et al.*, 1969). The SC, testicular size, semen production, sperm motility, morphology and fertility have long been reported to be highly correlated in bulls up to 5 years of age (Hahn *et al.*, 1969; Ball *et al.*, 1983 and Larsen *et al.*, 1990). This could interpret our results as the improvement occurring in almost all studied traits coincided with the increase in SC. In the same vein, zinc deficiency was found to reduce testicular size and sexual desire in rams (Martin *et al.*, 1994).

Plasma testosterone levels as well as the fructose concentration in the whole semen increased significantly ($P< 0.05$) due to zinc treatment (Table 1). These findings agreed with the results obtained by Martin *et al.* (1994) in rams and Khalifa (1997) in buffalo. The former authors suggested that zinc-specific effect is localized within the testis and that zinc deficiency reduces the testicular capacity to produce testosterone, the critical factor for growth and function of both semineferous tubules and accessory sex organs. It was also found that the seminal vesicles of zinc-deficient male rabbits had significant low levels of fructose and zinc (El- Tohamy and Younis, 1991). It could be seen that the reproductive traits are interrelated and all of them are under the control of testosterone which in turn may be stimulated by zinc supplement.

Zinc concentration in the ejaculated semen exceeded its blood concentration by 40-80 folds (Table 1). However, the overall mean of zinc level in the blood plasma (0.08 ± 0.01 mg %) during the nine months of treatment was two times its level during the pre-treatment period (0.04 ± 0.002 mg %). This constituted 100 % increase in blood plasma zinc. The corresponding percentage of increase in the content of seminal zinc was 9.4 %. These findings are in complete agreement with the results reported by El- Anwar and Badr (1996) in goat and Khalifa (1997) in buffalo. The tremendously higher levels of zinc in the ejaculated semen as compared with its levels in the blood may suggest that zinc circulating in the blood is continually trapped by the target organs of male reproductive tract, mainly the prostate gland (Eissa *et al.*, 1992). The same authors stated that zinc content in the accessory sex glands of buffalo is under the control of testosterone.

Table 1. Impact of zinc supplementation and season of feeding on some reproductive traits of Egyptian buffalo bulls

Exp. stages	R.T. (sec.)	Ej. Vol. (ml)	Ind. Mot. (%)	Live sp. (%)	Conc./ ml (x 10^6)	Conc./ej. (x 10^6)	Total abn. (%)	Fructose (mg%)	Testost. (ng/ml)	S.C. (cm)	Blood zinc (mg%)	Semen zinc (mg%)
Pre-exp. period	**103 ± 6^{b} (208)**	**$2.7\pm.1^{B}$ (208)**	**55.2 ± 2.7^{B} (208)**	**$79.2\pm.7^{B}$ (208)**	**712 ± 53^{B} (208)**	**1959 ± 123^{B} (208)**	**19.3 ± 1^{B} (208)**	**380 ± 9^{b} (48)**	**$0.7\pm.1^{b}$ (12)**	**$33.7\pm.7^{B}$ (24)**	**$.04\pm.002^{B}$ (12)**	**$3.2\pm.1^{b}$ (12)**
Dry feeding Season	80±9 (272)	3.3±.1 (272)	65±0.6 (272)	86.9±.2 (272)	806±35 (272)	2706±215 (272)	14±.8 (272)	417±9 (64)	0.9±.1 (16)	34.7±.1 (32)	.08±.001 (16)	3.8±.4 (16)
Green feeding season	78±5 (304)	3.6±.1 (304)	67±1.1 (304)	87.8±.5 (304)	813±19 (304)	3051±129 (304)	13±.3 (304)	412±6 (80)	0.9±.1 (20)	35.0±.1 (40)	.08±.01 (20)	3.2±.3 (20)
Overall mea zinc treatment	**79 ± 3^{a} (576)**	**$3.5\pm.1^{A}$ (576)**	**$66.1\pm.2^{A}$ (576)**	**$87.3\pm.4^{A}$ (576)**	**810 ± 22^{A} (576)**	**2904 ± 109^{A} (576)**	**$13.5\pm.5^{A}$ (576)**	**415 ± 3^{a} (144)**	**$0.9\pm.07^{a}$ (36)**	**$34.9\pm.08^{A}$ (72)**	**$.08\pm.01^{A}$ (36)**	**$3.5\pm.1^{a}$ (36)**
Percent change	-23.3%	+30%	+19.7%	+10.2%	+13.8%	+48.2%	-30%	+9.2%	+28.6%	+3.6%	+100%	+9.4%

Figures in parenthesis indicate the number of observations.
Means with different capital letters within each column are statistically different at 0.01 level.
Means with different small letters within each column are statistically different at 0.05 level.
Means with blank superscripts within each column are statistically not significant.

Table 2. Impact of zinc supplementation on the incidence of the different types of sperm abnormalities

Experimental stages	Abnormal Acrosomes %	Abnormal heads %	Detached heads %	Diadem/ craters %	Protoplasmic droplets %	Middle piece %	Abnormal tails %	Total abnormality %
Pre-experimental period	**$0.7\pm.1^{b}$ (208)**	**$2.4\pm.2^{B}$ (208)**	**2.3±.1 (208)**	**$0.4\pm.3^{B}$ (208)**	**$0.6\pm.1^{B}$ (208)**	**3.1±.1 (208)**	**11.5±.01 (208)**	**$19.3\pm.1^{B}$ (208)**
Dry feeding season	0.4±.04 (272)	1.1±.2 (272)	1.9±.1 (272)	0.1±.04 (272)	0.3±.05 (272)	2.6±.08 (272)	8.2±.6 (272)	14.0±.8 (272)
Green feeding season	0.5±.07 (304)	0.8±.06 (304)	1.8±.03 (304)	0.3±.03 (304)	0.2±.04 (304)	2.2±.7 (304)	8.9±.3 (304)	13.0±.3 (304)
Overall mean zinc treatment	**$0.4\pm.04^{a}$ (576)**	**$0.9\pm.1^{A}$ (576)**	**1.9±.1 (576)**	**$0.2\pm.04^{A}$ (576)**	**$0.3\pm.03^{A}$ (576)**	**2.4±.4 (576)**	**8.6±.3 (576)**	**$13.5\pm.5^{A}$ (576)**
Percent change	-42.8%	-62.5%	-17.3%	-50%	-50%	-22.6%	-25.2%	-30%

Figures in parenthesis indicate the number of observations.
Means with different capital letters within each column are statistically different at 0.01 level.
Means with different small letters within each column are statistically different at 0.05 level.
Means with blank superscripts within each column are statistically not significant.

It should be mentioned that the differences in all studied traits between the green and dry feeding seasons lacked statistical significance. The impact of the different interactions between season of the year and zinc treatment was also lacking. However, the absence of seasonal impact on the reproductive performance of good managed buffalo bulls is not new especially in the north region of Nile Delta where the experiment was conducted (El- Fouly, 1992 and Osman, 1996). Hence, the improvement occurring in almost all traits is mainly attributed to the zinc treatment rather than to other interfering factors.

It has been well documented that the Egyptian soil is deficient in its zinc content. This constitutes a real problem resulting in a poor zinc content in most livestock rations of Egypt (Attia *et al.*, 1987). Hence, the external supplementation of zinc to the livestock of Egypt should be regarded as an urgent necessity

The findings of the present work shade light on the importance of zinc element in regulating the reproductive functions in Egyptian buffalo bulls. The use of supplementary zinc oxide at the rate of 0.7 g/bull/day could be suggested. However, experimenting the efficiency of other levels of zinc supplement in the further research might be recommended. Further studies on the combined effect of zinc with other minerals are also needed.

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