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EFFECT OF SELENIUM-VITAMIN E MIXTURE ON EGYPTIAN BUFFALO BULL SEMEN CHARACTERISTICS

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ABSTRACT

Two Experiments were conducted in Mahallet Mosa to evaluate the effect of using Selenium-vitamin E (Se-vit.E) mixture via intramuscularly injection or via the addition to the diluted semen on buffalo bull semen quality, reaction time (sec), was recorded also, semen quality criteria studied were : ejaculate volume (ml), sperm cell concentration (x 10^6), sperm output (x 10^9), appearance, pH, mass activity, initial activity, live sperms, acrosomal abnormalities and total abnormality. Results obtained indicated a significant effect for intramuscularly injection with Se-vit E mixture on each of reaction time, ejaculate volume, mass activity, initial motility and live-sperm percentage. Addition of Se-vit. E mixture to buffalo bull diluted semen do not improve initial motility, live sperm, acrosomal abnormality and total abnormality.

Under, the prevailing condition of this investigation, these findings suggest that intramuscularly injection with Se-vit. E solution exhibits a better effect on buffalo bull semen quality comparing with its addition to semen diluent.

Keywords: Buffalo-semen characters-selenium-vitamin E.

INTRODUCTION

Trace elements have a major role in livestock feeding, most of feed stuff produced in the world deficient in selenium (Hidiroglou and Jenkis, 1975). Selenium (Se) is an element necessary in optimal livestock performance. It acts senergesly with vitamin E (vit E.). Several reproductive problems have been shown to be responsive to Selenium treatment. Selenium and vitamin E difecient produced non motile sperms (McCoy and Wesing, 1969). Similarly Wu et al. (1973) stated that selenium deficient rates produced sperm with poor motility and with high proportion of broken tails. On the other hand, Alpha-tocopherol and glutathion peroxidase are believed to be the primary components of the antioxident system of the spermatozoa (Surai et al., 1998), selenium is a component of glutathion peroxidase enzyme (Rotruck et al., 1973). Vitamin E interacts with Se to prevent the oxidative breakdown of cell membrane (Putnam and Compen, 1987). Therefore, the objective of this study was to determine the effect of selenium and vitamin E mixture on Egyptian buffalo bulls semen quality.

MATERIALS AND METHODS

This study is carried out at Mahallet Mousa Experimental Farm belonging to Animal Production Research Institute. Agriculture Research Center. Ministry of Agriculture.

Two experiments involving a total of 6 mature buffalo bulls aged 36-48 months with 650 kg average body weight were used to evaluate the effect of

using vitamin E and selenium (vit E.-Se) mixture in the rate of 90:1, respectively on buffalo bull semen quality. Vit-Se (vitselen) solution was produced by ADWIA Co., Egypt. Selenium in vitselen was in form of sodium selenite (NaS_2O_3), each ml of vitselen contains 150 mg vit. E and 1.67 mg NaS_2O_3, the recommended minimum daily requirements of cow or buffalo of Se is 0.1 mg Se/kg dry mater intake (DMI) and 300 mg vit. E/kg DMI, (NRC, 1978), selenium concentration in sodium selenite is 45.6% (ACSAD, 1979). So, Se concentration in vitselen solution is 0.76 mg/ml.

The bulls were allowed into two equal groups by restricted randomization of live body weight for each experiment.

Two ejaculates per week, were obtained along the experimental period, (12 weeks post treatment period in the 1st experiment and 3 weeks in the 2nd experiment) average of each semen characteristics was recorded, reaction time (RT) as lipido test and other semen physical characters were measured according to Sorenson (1979). Bull semen was collected by mean of artificial vagina. Only RT (sec.) and semen characteristics; ejaculate volume (ml), sperm cell concentration ($x10^6$), sperm output ($x10^9$), appearance semen density using grades score from zero to 4 and semen pH were measured in the first experiment, while, mass activity using 5 grades of score (0-4 grades), initial motility (%), live sperms (%), acrosomal abnormality (Ac. Abnor. %) and total abnormality (Total abnor. %) were recorded in both of the two experiments.

All handling step of semen in the laboratory were performed under the normal hygenic standards.

Bulls in the two experiments were under similar managerial conditions. All bulls received the same ration, their requirements were calculated according to APRI, (1997), bulls were fed daily concentrate feed mixture (CFM), 4.0 kg/head, clover hay (CH) 3.0 kg/head and rice straw (RS), 5.0 kg/head, chemical analysis of feed stuff used in this study were carried out according to A. O. A. G. (1986) (Table 1) drinking water was offered to the bulls three times a day.

Table 1. Chemical composition of feedstuff.

	DM	DM basis %				
		CP	CF	EE	NFE	Ash
CFM	89.3	15.5	11.4	2.8	58.9	11.4
CH	90.2	12.9	33.7	1.1	40.1	12.2
RS	91.3	2.8	29.3	1.5	39.5	87.9

Selenium concentration (mg/kg DMI) in feedstuff used in feeding Animal Production Research Institute (APRI) livestock was given in Table 2.

Table 2. Selenium concentration in different feedstuff (Nagwa *et al.*, 2000).

Feedstuff	Selenium concentration (mg/kg DMI)
CFM	0.073
CH	0.021
RS	0.048

Table 3 indicated that actual selenium daily intake (0.537 mg/day) is about 49% of the minimum calculated recommended value (11.023 kg DMI x 0.1 mg Se/kg DMI = 1.1 mg/day).

Table 3. Daily dray matter intake (kg) and selenium intake (mg/kg DMI).

Feedstuff	DMI/day (kg)	Actual selenium intake (mg/day)
CFM	3.572	0.261
CH	2.706	0.057
RD	4.565	0.219
Total intake	11.023	0.537

To overcome this deficiency in daily actual selenium intake (0.537 mg/day) comparing with the daily minimum recommended value (1.1 g/day), two experiments were carried out:

Experiment 1:

This experiment study the effect of intramuscularly injection with vitselen on buffalo bull semen quality.

The total period of this trial was 19 weeks, it was divided into three intervals, pretreatment period (4 weeks), which bulls did not receive vitselen (control), the second period (treatment period, 3 weeks), where bulls were received 3 consecutive weekly intramuscularly injections (30 ml vitselen solution/week/head), each injection contained 22.8 mg selenium (50.0 mg $NaSe_2O_3$) and 4500 mg vitamin E. The third period (the post treatment period), extended 12 weeks after the treatment period.

Physical characteristics measured in this trial were recorded immediately in the fresh semen.

Statistical analysis in this trial was according to SPSS (1975)

Statistical model used in this trial:

$Y_{ijk} = \mu + T_i + W_j + E_{ijk}$

Where:
- Y_{ijk} an observation
- μ overall mean
- T_i treatment effect
- W_j week effect
- E_{ijk} random error

Experiment 2:

This experiment studied the effect of addition vitselen to semen diluent on bull semen quality, the whole period of this trial was 3 weeks, each fresh ejaculate obtained was splited into three equal portions, one fresh, without dilution (T1). the second portion (T2) was diluted with egg yolk citrate buffer only, (Table 4), the third portion was diluted with the same buffer supplemented with 1.0 ml vitselen solution/ml of diluent, as recommended by Rebecca *et al.* (1980), diluted rate used in this trial was 1: 10 (semen: diluent), semen characteristics were determined, immediately after

ejaculation, then it kept under the refrigeration condition (4°C) to determinate the same characteristics after 24 and 48 hours.

Table 4. Diluent composition.

Tris (hydroxy methyle amino methothan)	3.61 gm
Citric acid monohydrate	1.89 gm
Egg yolk	20.0 ml
Glycerol	5.0 ml
Penicillin G-Sodium	160,000 IU
Streptomycin sulfate	50,000 mg
Up to 100 ml distilled water	

Statistical model used in the second trial

$Y_{ijk} = \mu + T_i + B_j + TB_{ij} + E_{ijk}$

Where
- Y_{ijk} an observation
- μ overall mean
- T_i treatment effect
- B_j time effect
- TB_{ij} interaction between treatment and time
- E_{ijk} random error

RESULTS AND DISCUSSION

Experiment 1:

Effect of intramuscularly injection with vitselen on buffalo bull semen quality:

Means (X±SE) of reaction time and different physical characteristics before and after treatment (average of 12 weeks post treatment period) are shown in Table 5. It is apparent that intramuscularly injection with Se-vit. E solution have a significant ($P<0.05$) effect on the reaction time, it was decreased by 26.1 sec. of its value before treatment, also some of the studied buffalo bull semen characters were affected significantly ($P<0.05$) by treatment, since ejaculate volume increased by about 34.6%, mass activity increased by 26.9%, initial motility increased by 14.4%, live sperms increased by 8.3%. comparing to their corresponding values before treatment.

Semen characteristics which affected significantly by intramuscularly administration with Se-vit. E solution were shown in Table 6 and represented in figure 1 along 12 weeks of post treatment period. Reaction time shows its minimum value at the 5th week (33.8 sec.), comparing with its values before injection (63.5 sec.), differences were significant. The maximum ejaculate volume was observed at 6th week (6.4 ml), compared with its volume before treatment (2.6 ml), the difference was significant ($P<0.05$).

Mass activity and initial motility shows a gradual increasing in their values with advancing of post treatment period. It was observed also that the difference between pretreatment period and post treatment period starting to be significant from 6th and 4th week in mass activity and initial motility. respectively. Live sperms reached its maximum percent in 6th week (92.2%)

compared with those obtained before treatment, after which a gradual decline ensued. Meanwhile. the difference was significant (P<0.05) between pretreatment period and each of the 12th weeks of post treatment period.

Table 5. Effect of Se-vit. E intramuscularly administration on reaction time and some buffalo bull semen quality.

Traits	Before treatment	After treatment
Reaction time (sec)	63.5±7.6[a]	46.9±2.8[b]
Ejaculate volume (ml)	2.6±0.4[b]	3.5±0.5[a]
Sperm cell concentration (x10^6)	1015.0±23.0[a]	1136.0±41.9[a]
Sperm output (x10^9)	2843.0±138.0[a]	3265.0±253[a]
Appearance	2.9±0.05[a]	3.2±0.04[a]
PH	6.76±0.03[a]	6.73±0.01[a]
Mass activity	2.6±0.07[b]	3.3±0.04[a]
Initial motility (%)	66.5±1.1[b]	76.1±1.2[a]
Live sperm (%)	82.8±3.6[b]	89.7±0.4[a]
Acr. Abnor. (%)	5.1±0.5[a]	4.7±0.2[a]
Total abnor.	14.5±1.4[a]	13.8±0.5[a]

Means of the same row with different superscripts are different (P<0.05).

Table 6. Means (X±SE) of reaction time and different physical semen characters during the experiment and post-treatment period.

Weeks	Reaction time (sec.)	Ejaculate volume (ml)	Mass activity (0-4)	Initial motility (%)	Live sperm (%)
Pretreatment period *	63.5[a] ±7.6	2.6[a] ±0.4	2.6[a] ±0.1	66.5[a] ±1.1	82.8[a] ±3.6
Post treatment period					
1	79.2[a]±11.3	2.8[a]±0.5	2.8[a] ±0.1	66.7[a] ±2.1	86.0[b]±1.2
2	55.3[a]±12.3	3.2[a] ±0.3	2.8[a] ±0.2	62.5[a] ±2.8	85.7[b]±0.5
3	51.8[a]±12.9	4.0[b]±0.3	3.0[a] ±0.2	69.2[ab]±4.4	87.3[b]±1.4
4	41.3[b]±9.7	4.1[b]±0.4	3.0[a] ±0.0	70.8[b] ±2.4	88.8[b]±1.1
5	33.8[b]±11.2	4.6[c]±0.6	3.2[a] ±0.1	74.2[b] ±2.0	86.7[b]±1.4
6	40.8[b] ±5.0	6.4[c]±0.5	3.5[b] ±0.1	75.8[b] ±3.3	92.5[b]±1.1
7	51.7[b] ±5.1	4.4[b]±0.4	3.6[c]±0.1	78.3[bc]±1.7	91.2[b]±0.4
8	40.2[b] ±6.3	4.9[b]±0.7	3.5[bc]±0.1	87.5[c] ±1.1	91.5[b]±0.9
9	37.3[b] ±4.8	4.2[b]±0.5	3.6[c] ±0.1	86.7[c] ±1.7	91.3[b]±1.4
10	46.7[ab]±4.8	4.2[b]±0.6	3.6[c] ±0.1	83.8[c] ±2.1	90.7[b]±0.8
11	38.3[b] ±4.9	4.6[bc]±0.8	3.5[c] ±0.1	82.5[c] ±2.1	89.5[b]±0.8
12	39.5[b]± 4.1	4.6[b]±0.7	3.6[c]± 0.1	83.1[c]± 2.1	88.9[b]± 0.7

* Average of four weeks pre-selenium administration
a,b,c. Means in the same column with different superscript are differ (P<0.05).

The above mentioned results indicated that there is a sensible improvement in semen physical characteristics starting from six week of post treatment period. These findings are in a agreement with those of (WU et al., 1973 ; Smith *et al.*, 1978 ; Pallinui and bacci, 1979 and Udala *et al.*, 1995). They stated that treatment bulls with a mixture of Se-vit. E, improved semen quality. However, Staweta *et al.* (1988) stated that injection with Se-vit. E

mixture have a limited effect on some physical characters. The differences among authors may be due to the dose used in their experiments.

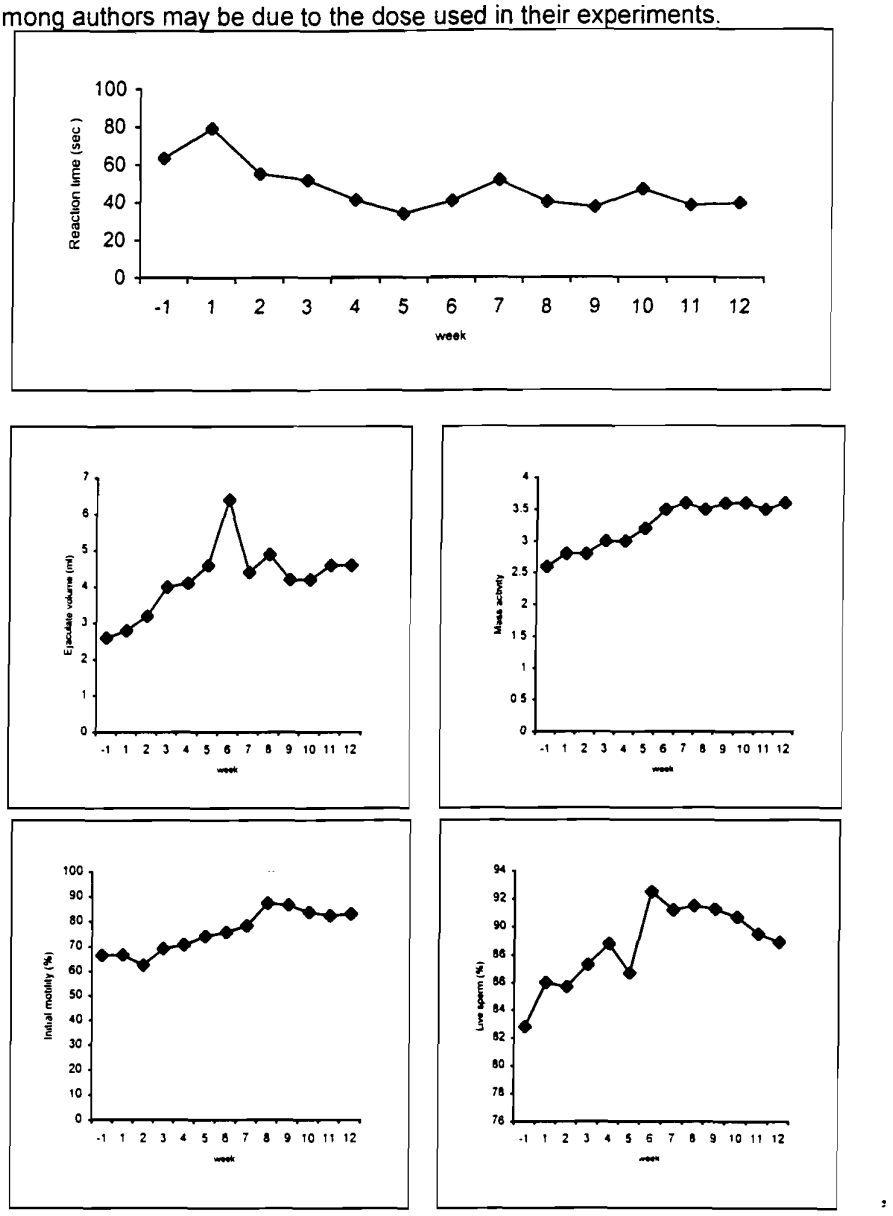

Figure 1. Effect of intramuscularly injection with Se-vit.E on reaction time and some physical bull semen characters during pretreatment period (1-) and post treatment period (1-12 weeks).

Experiment 2:

Effect of addition Se-vit. E solution to semen diluent on buffalo bull semen quality:

Means (X±SE) of buffalo bull semen characteristics in different treatment (T1, T2 and T3) during the entire experimental period (3 weeks), meanwhile the same semen characteristics at 0 time (just after ejaculation), after 24 gours and after 48 hours were presented in Table 7. It was indicated that the addition of Se-vit. E solution to semen diluent (T3) have no beneficial effect on different semen characteristics comparing to diluted semen without Se-vit. E addition (T2). Observed falling in initial motility and live sperms, and the increasing in total abnormality after dilution (T2 and T3) comparing with their values recorded as fresh (T1) may be due to the normal deterioration during dilution processes. Meanwhile, the insignificant difference between T2 and T3 may be due to that the extender may contain enough amount of selenium and vitamin E.

Referring to the interaction between treatment and the time of semen examination, it was observed a significant deterioration in initial motility, live sperms and total abnormality with advancing of examination time at different treatments. This indicates that the addition of Se-vit. E solution (at the tested dose) to bull semen have no improving effect on the normal loss in semen quality by time advancing, in this context, Rebecca et al. (1980) observed a significant reduction in semen quality when Se concentration in diluent increase than 1 mg/ml. Similarly, Marin et al. (2000) stated that sperm motility declined when Se was added to the extender, the addition of Se was demonstrated tightly adhered to the spermatozoa.

Table 7: Means (X±SE) of buffalo bull semen quality in trial 2 as affected by adding Se-vit. E solution to semen diluent.

Items	Initial motility (%)			Live sperms (5)			Acrosomal abnormality (%)			Total abnormality (%)		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Treatments	67.1^{a}±0.2	64.1^{b}±1.3	64.2^{b}±0.5	75.7^{a}±0.3	71.5^{b}±0.6	70.4^{b}±0.6	9.4±1.0	10.7±0.1	11.3±2.0	11.7^{a}±1.0	15.5^{b}±0.3	15.6b±0.3
Time (hrs)												
0	72.3^{a}±0.8	70.1^{a}±1.9	71.7^{a}±1.7	80.2^{a}±1.1	76.5^{b}±0.7	74.5^{b}±0.9	9.1±0.2	8.3±0.5	8.9±0.4	11.9^{z}±0.3	13.1^{a}±0.2	13.6a±0.9
24	65.8^{b}±0.4	62.4^{c}±0.7	63.3^{c}±1.2	75.4^{a}±.7	71.8^{b}±0.7	71.6^{b}±0.3	7.7±0.1	7.7±0.1	11.2±0.6	12.1^{a}±0.5	12.1^{a}±0.4	14.9^{ab}±0.6
48	63.2^{b}±0.6	59.9^{cd}±0.9	57.8^{cd}±0.4	71.4^{a}±0.5	66.3^{c}±0.2	63.9^{c}±1.0	10.1±0.3	10.1±0.3	13.9±0.7	11.1^{a}±0.3	11.1^{a}±0.3	18.4^{b}±0.5

a,b,c Means in the same row within each semen character with different superscript differ significantly (P<0.05).

CONCLUSION

The above resulted obtained in this study indicated the limited effect of using vetselen solution (vit. E and selenium mixture) on buffalo bull semen quality. Intramuscularly administration of vitselen improved buffalo bull semen physical characteristics while the addition of vitselen to bull semen diluent shows no beneficial effect on semen quality of buffalo bulls. Further studies are warranted concerning some general aspects, to following up the pathway of metabolism and mechanism of action of selenium intake through the different organs of Egyptian buffalo bulls.

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تأثير استخدام مخلوط السلينيوم مع فيتامين هـ على صفات السائل المنوى لطلائق الجاموس المصرى.

سامى درويش ، ابراهيم لويس ابراهيم ، ايناس رمزى الصدفى
معهد بحوث الانتاج الحيوانى - وزارة الزراعة القاهرة

تم تنفيذ تجربتين فى محطة بحوث الانتاج الحيوانى بمحلة موسى التابعة لمعهد بحوث الانتاج الحيوانى - وزارة الزراعة لتقييم تأثير استخدام مخلوط السيلينيوم مع فيتامين هـ على صفات السائل المنوى لطلائق الجاموس المصرى . وذلك عن طريق الحقن العضلى او الاضافة الى المخفف السائل المنوى وتأثير ذلك على كل من الرغبة الجنسية ، وبعض صفات السائل المنوى .

وأوضحت نتائج التجربة الاولى وجود تأثير معنوى موجب للحقن بمخلوط السلينيوم مع فيتامين هـ على الرغبة الجنسية وحجم القذفة والحركة الكلية والاولية للحيوانات المنوية وايضا تركيز الحيوانات المنوية بالقذفة .

أما نتائج التجربة الثانية فقد اظهرت انه لم يكن هناك اى تأثير لاضافة مخلوط السلينيوم مع فيتامين هـ للمخفف المستخدم على صفات السائل المنوى للطلائق الجاموسى تحت الدراسة.

من هذه الدراسة يتضح ان استخدام وسيلة الحقن العضلى لمخلوط السلينيوم مع فيتامين هـ افضل من اضافته الى مخفف السائل المنوى لطلائق الجاموس المصرى.