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Effect of dietary selenium and vitamin E supplementation on productive and reproductive performance in rams

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The studies were carried out on 32 rams of the ossimi breed (270 days of age). Sixteen rams (group 1) received a ration containing 0.2 ppm. Se , and 30 mg vit.E, the other 16 rams (group 2) received 0.5 ppm. Se, and 50 mg vit.E/kg feed mixture .The feeding test was conducted from 270 days until 360 days of age. During the experiment the rams were subjected to live evaluations, i.e. testes circumference, libido level, semen characteristics, as well as selenium concentration and glutathione peroxidase (GSH-Px) activity in blood serum and seminal plasma. Routine macroscopic and microscopic analyses of semen quality were accompanied by measurement of Se content and GSH-Px activity in blood and semen. The Se concentration in blood plasma, seminal fluid and spermatozoa was measured by fluorometric method, while the GSH-Px activity by method based on NADPH – coupled reaction .Comparing the results in animals of both groups , it was clear that the all were in good health conditions as ascertained by clinical examination. No significant variation was detected in body weight of animals of both groups. Significant elevation ($p<0.05$) in the values of semen conc., semen conc./ ejac , semen motion , sperm motility / ejac.as well as total sperms count were observed in animals of group 2 as compared to those of group 1 .

Great deal of information has recently become available for better nutrition strategies concerning feeding minerals and vitamins to livestock Selenium is an essential trace nutrient for humans and animals. It is an element fulfilling a significant function in reproduction processes, both in females and males of farm animals (Zachara *et al* .,1993a;1993b ; Wallace *et al*.,1983; Wu *et al*., 1979; Heimann *et al*.,1984; Kotowska and Kotowski,2001;Marin-Guzman *et al*, 2000a).

It was suggested that vitamins play a key role in thermoregulation of rectal and scrotal skin temperature during heat stress and maintain libido, semen quality, and fertility (El-Darawany, 1999). Selenium acts to help and prevent oxidation of the sperm cell, thus aiding in maintaining sperm cell integrity (Cohen and Takasaki,1986; Zhang *et al,*1989; Chu *et al*.,1996), Selenium represents an integral component of glutathione peroxidase (GSH-Px), an enzyme which, along with vitamin E, protects cell internal structures against free radicals and is an antioxidant for cellular membrane lipids [Ursini *et al*., 1999), Glutathione peroxidase activity has also been reported in human seminal plasma (Saaranen *et al*.,1989), and is present in the head and midpiece of sperm cells (Godeas *et al*, 1997) .Serum selenium is reported to be low in men with oligospermia ,. Selenium deficiency has been linked to reproductive problems in sheep and cattle (Combs and Combs, 1986) and leads to largely immotile and a high incidence of sperm midpiece defects. In males bred on a low selenium diet, male hypogonadism was found as well as reduced production and deteriorated semen quality (Peretz *et al.,* 1991;Kleene, 1993) and supplementation with selenium has been reported to improve reproductive performance in sheep. It was demonstrated that a diet supplemented with selenium and vitamin E improved sperm quality an effect possibly linked to the antioxidant properties of this vitamin. (Brzezinska-Slebodzinska *et al*., 1995; Marin-Guzman *et al*., 1997). The aim of the present study is to evaluate the influence of increased addition of dietary selenium and vitamin E on reproductive performance of rams, body weight as well as the general health condition of animals.

Table (1):Chemical composition and nutritive value of complete mixtures .

Item		Mixtures	
		G1	G2
Metabolizable energy	(MJ)	12.8	12.8
Dry matter	(%)	89.73	89.73
Crude protein	(%)	18.01	18.01
Ether extract	(%)	3.923	3.93
Crude fibre	(%)	9.83	9.83
N-free extractives	(%)	51.08	51.08
Ash	(%)	6.88	6.88
Vitamin E	(mg)	30.0	60.0
Selenium	(ppm)	0.20	0.50

Material and Methods

This study was carried out in the Animal Production Experimental farm Faculty of Agriculture, South Valley University:, Egypt during the period from (August till ; December 2007) . A number of the 32 rams of the Ossimi. Rams were chosen healthy and clinically free of external and internal parasites. Palpation of the external genitalia showed that they were typically normal. The rams were fed on the same diet from 230 to 270 days of age. In table (1). Chemical composition and nutritive value of complete mixtures At 270 days, Animals of the work were divided into two groups each of 16 rams. Each group of animals represents an experiment . The allocation to the groups was made with analogues method, i.e. from one litter one ram was assigned to a group. Foods were sampled weekly and dry matter intake (DM1, kg/day) was recorded daily till the termination of the experiment. Water was available all day and mineral were supplied in salt licking stones. Proximate analysis on dry matter basis (AOAC ,1990) of feed used is illustrated in Table (1).feeding test was carried out from 270 to 360 days of age. During the feeding test the rams of the group.1 were fed on standard diet, which contained 0.2 ppm. Se and 30 mg vitamin E per 1kg. While the group.2 received 0.5ppm. Se (Se yeast) and 60 mg vitamin E per 1kg of the ration. According to NRC (National Research Council, 1998),

The rams were housed in individual pens. The daily feed ration was gradually increased along with the increasing body weight. Chemical composition and nutritive value of the diets are given in table (1). The body weight of the rams, their daily gains, and feed conversion for the test period were determined at the age of 270 and 360 days. At age of 270 and 360 days the testes were measured. Upon completion of the test (from 360 days of age) on the rams were trained, and the collection of semen began, which was carried out with gloved hand technique.

Semen collection technique. The experimental rams were sexually stimulated by allowing two false mounts prior to first ejaculation. One false mount , followed by two minutes restraint, then a second false mount followed by two minutes restraint ended by the ejaculation (Almquist and Hale, 1973). When two ejaculates were collected, there was no false mount prior to second ejaculation. Semen was collected once weekly between 8.00 and 10.00 am using an artificial vagina

Semen physical characteristics. After collection, semen of each ejaculate was transferred immediately to the laboratory and was subjected to the following examinations: ejaculate volume (ml), percentage of sperm motility and sperm concentration / ml (x10 ^{9}). Ejaculate volume was measured directly in milliliters to the nearest 0.1 ml using a transparent graduated glass tube. For evaluation of percentage of motility a drop of semen was examined under the low power of a microscope using a hot stage at 37^{0}c. Progressive motility was estimated on a percentage score .Number of spermatozoa / ml of semen was determined using a heamocytometer . Total and motile sperm out put / ejaculate were calculated. Routine macroscopic and microscopic analyses of semen.

Adopted methods. The seminal fluids as well as the blood plasma samples were separated and stored at – 20 C till analysis. The activity of GSH – PX in both blood plasma and seminal fluids were determined according to Pagila and Valentine, (1967), while selenium concentrations were estimated with the fluorometric method of Watkinson, (1966). The basic nutrient concentrations in the feeds were determined with standard methods, and amino acids were measured using an automatic analyzer (Beckman Instruments Inc.).

Tables (2): Body weight and testes circumference of control and treated rams.

Traits	Groups	
	Group 1	Group 2
Body weight (kg) :		
Initial Body weight(kg)	38.63±2.34 [a]	38.41±2.12 [a]
Final Body weight(kg)	49.23±1.09 [a]	49.53±1.03 [a]
Daily gain to 380 day of life (g)	106.0 ±33.97[a]	111.2 ±36.0 [a]
Testes circumference (cm):		
At 270days of age	23.29 ±0.22 [a]	23.12 ±0.14 [a]
At 360 days of age	25.54±0.25 [a]	26.83 ±0.31 [a]

A, b means in the same row followed by the same letter are not significantly different (p>0.05).

Table(3): Semen traits, selenium(Se)content and activity of glutathione peroxidase in the semen plasma and blood plasma of Rams as affected by Selenium and vitamin E supplementation in the ration.

Semen characteristics	Selenium and vitamin E level		
	Group 1 0.2ppm Se+30mgE	Group 2 0.5ppmSe+60mgE	
Ejaculate volume (ml)	0.81±0.02	0.89±0.04	Ns
Concentration /ml ($x10^9$)	2.79±0.18	3.02±0.53	*
Concentration /ejac ($x10^9$)	2.26±0.39	2.69± 0.35	*
Semen motility (%)	82.50±0.01	86.67±2.50	*
Motile sperm /ml ($x10^9$)	2.30. ± 2.95	2.61. ± 3.11	*
Motile sperm /ejac ($x10^9$)	1.86 ± 1.21	2.33 ± 1.63	*
Total sperm count (n $x10^9$)	20.2 ± 8.0	26.2±9.3	*
Se in semen plasma ($\mu g \cdot ml^{-1}$)	0.042 ± 0.017	0.050± 0.00	*
GSH-Px in semen plasma ($U \cdot ml^{-1}$)	0.310 ±0.146	0.233±0.085	*
Se in blood plasma ($\mu g \cdot ml^{-1}$)	0.267 ± 0.049	0.280 ± 0.045	Ns
GSH-Px in blood plasma ($U \cdot ml^{-1}$)	4.00± 0.014	4.28 ±0.012	Ns

Ns= non significant * =Significantly different (p< 0.05).

Statistical analysis. Data were statistically analyzed according to the General Linear Model (G L M) and the differences between means were detected by Duncan's Multiple Range Test (Duncan, 1995), S A S (1992).

Results and Discussion

Table 2, showed that Body weight gains and feed efficiency between animals of group 1 and 2 correspond to the results of Mahan and Parrett, 1996, who did not find any influence that adding Se to rations for rams but in contrast with the findings of Gabbedy 1971., Wilkins *et al.,* 1982, who found an increase in body weight of rams supplemented with selenium

Rams of group 2 exhibited by 26.83±0.31 cm larger testes circumference compared with those of the group 1, 25.54± 0.25cm. The differences found between the groups were, however, statistically non-significant that agrees with. Marin-Guzman *et al.,* 2000a, but , Wallace *et al*., 1983, observed underdevelopment of testes and reduced production of spermatozoa in mice receiving selenium-deficient diets. On the other hand , Zachara *et al.,*1993a,1993b reported that selenium has a good effect on the sexual activity in rams .

As in table (3). The semen of rams in group 2 showed significantly higher ($p \leq 0.05$) sperm concentration and total sperm count compared to animals of group 1 ,Morphological evaluation of spermatozoa in the ejaculates of the rams demonstrated that an addition of 0.5 ppm Se + 60 mg vitamin E to 1 kg of feed reduced considerably the percentage of morphologically changed spermatozoa.

A positive effect of selenium on sperm concentration and morphology has been previously confirmed by Heimann *et al*., 1984; Behne, 1996; Marin-Guzman *et al*., 2000b, Buchanan-Smith *et al.(* 1969) mentioned the lack of univocal dependencies between the concentration of the studied microelements on

the reproductive organs , blood , semen and morphological changes in bull sperms .

It was demonstrated in studies on rams Marin-Guzman *et al* 1997, that adding vitamin E alone in an amount of 220 IU/kg of ration was of little effect in relation to morphological changes in spermatozoa or other traits. This implies that antioxidants, Se + vitamin E, which act synergistically, should be administered jointly (Buchanan-Smith *et al*., 1969; and Mahan and Parrett., 1996). The elevation in the values of blood plasma selenium and GSH-PX in rams of group 1 , table 3 agree with the previous reported findings of Marin-Guzman *et al*, (1997), in boars , and Aberden, *et al*., (1980) in bulls .

On the other hand, GSH-Px activity was by 20% lower ($p \leq 0.05$) in seminal plasma of group 2 rams, despite the fact that the latter exhibited much better semen quality than the rams of the group 1. The obtained results confirm the suggestion that most selenium in an organism occurs in proteins other than, as it was once assumed, in GSH-Px (Norton and McCarthy., 1986). Higher selenium concentration in seminal plasma of the animal of group 2 enables a conclusion that the element is a component of other selenoproteins that positively influence the rams' reproductive performance. New selenoproteins have been identified over the recent years, e.g. selenoprotein-P, which is beneficial to animal reproduction (Arthur , 1994). Vitamin E is a potent antioxidant that works primarily to stabilize dangerous free radicals that are formed in fatty tissues and membranes. However, Se is a component of the enzyme GSH-PX that protects cellular membranes and lipid containing organelles from peroxidative damage. Thus the synergistic action of these two elements seems to be very essential and important for the well production and reproduction efficiencies in rams. Increased addition of selenium and vitamin E to balanced feed mixtures fed to rams, from the currently recommended standard amounts 0.2 ppm Se + 30 mg vitamin E per 1 kg to 0.5 ppm Se + 60 mg vitamin E per 1 kg has a minor effect on testes size and libido traits of the ram, positively influences both quantitative and qualitative semen traits, and increase body weight.

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تأثير إضافة السيلينيوم و فيتامين هـ إلى علائق الخراف على الصفات الإنتاجية و التناسلية لذكور الأغنام الاوسيمي

أجريت هذه الدراسة بمزرعة الإنتاج الحيواني بكلية الزراعة جامعة جنوب الوادي بقنا في الفترة من شهر أغسطس حتى شهر ديسمبر 2007 و استخدم في هذه الدراسة عدد اثنان و ثلاثون ذكر من أغنام الاوسيمي من سن 9 تسعة أشهر حتى سن 12 اثني عشر شهرا , و كان الهدف دراسة تأثير إضافة عنصر السيلينيوم بنسبة 0.5 جزء من المليون و فيتامين هـ 60 مللي جرام لكل جرام , وذلك من وزن العليقة على الصفات الإنتاجية و التناسلية لذكور الأغنام الاوسيمي , وقسمت الحيوانات إلى مجموعتين المجموعة الأولى و التي غذيت الحيوانات بها على العليقة الأساسية مضافا إليها عنصر السيلينيوم بنسبة 0.2 جزء في المليون و فيتامين هـ 30 مللي جرام و المجموعة الثانية و غذيت الحيوانات بها على العليقة الأساسية مضافا إليها عنصر السيلينيوم بنسبة 5 جزء من المليون و 60 مللي جرام من فيتامين هـ و تم اخذ القياسات الخاصة بوزن الجسم و معدل الزيادة اليومي و حجم الخصية و تم جمع السائل المنوي بواقع قذفه كل أسبوع بواسطة المهبل الصناعي وتم قياس خصائص السائل المنوي و تركيز عنصر السيلينيوم في بلازما الدم و السائل المنوي .

وقد أوضحت النتائج وجود فروق معنوية بين المجموعتين في خصائص السائل المنوي و عدم وجود فروق معنوية في وزن الجسم ومعدل الزيادة اليومي.