

Effect of Adding Selenium-enriched Dried Algae to Ration on Productive and Reproductive Performance of Male Barki Sheep

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ABSTRACT: This study was set for more than one year to evaluate the effects of dietary addition of selenium-enriched dried algae on productive and reproductive performances of Barki sheep. Twenty eight ram-lambs (aged 4-5 months) were randomly divided into four equal groups (n=7). Basal concentrate mixture was prepared and supplemented with or without Se-algae (selenium enriched micro-algae *Spirulina*, *Atrhrospira platensis*). Four experimental concentrate mixtures were prepared, the control (basal concentrate mixture without any supplementation) (I) and the other three experimental concentrate mixtures (II, III and IV) were supplemented by Se- algae at levels of 0.2; 0.4 and 0.6 mg/kg DM of the diet, respectively. Animals of the four groups were randomly assigned to feed on one of these four concentrate mixtures. In addition of concentration mixture animals of all groups were fed *ad libitum* on berseem hay. Feed supplementation with Se-algae significantly increased animal's final live body weights and weight gains compared with control group. Significant increases in white blood cells counts were observed in animals fed diets supplemented with Se-algae when compared with the control group. Animals receiving concentrate mixture supplemented with Se-algae at 0.4 mg recorded the highest ($P<0.05$) value of packed cell volume when compared with the other two treatment groups. Dietary inclusion of different levels of Se-algae resulted in significant increases in blood total protein and albumin relative to the control group. The lowest value ($p<0.04$) of plasma globulin was observed in group fed diet containing Se-algae at 0.6 mg. Plasma urea nitrogen was significantly increased as a result of supplementing diets with 0.4 mg of Se-algae. However, Se-algae supplementation reduced plasma cholesterol ($p<0.046$) and total lipid ($p<0.0001$) contents as compared with the control group. The plasma aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels of ram-lambs were significantly affected by adding Se-algae to their diets. Significant increases in T-AOC, GSH-Px and SOD as Se-algae level increased. Conversely, plasma malondialdehyde (MDA) was significantly decreased in a dose-dependent manner. Sperm ejaculate volume, progressive motility, normal sperm, live sperm, total sperm output, total motile sperm and total functional sperm fraction (TFSF) were increased ($P\leq 0.05$) in groups supplemented with Se-algae comparing with the control group. As opposed to the previous effect, sperm concentration, abnormal sperm and dead sperm significantly ($P\leq 0.05$) decreased comparing with the control group. Seminal plasma total protein increased ($P<0.05$) in the groups given 0.4 and 0.6 mg Se-algae. Seminal plasma albumin increased ($P<0.05$) in the group that received 0.4 mg Se-algae diet as compared to the control group, while seminal plasma globulin was decreased ($P<0.05$). Seminal plasma total lipids decreased ($P\leq 0.02$). Seminal plasma cholesterol and triglycerides were not significantly affected. Seminal plasma ALT and ALP significantly ($P\leq 0.01$) decreased due to including Se-algae in ram-lambs diet as compared to the control group. In conclusion, addition of Se-algae to the diet of ram-lambs improved their growth performance, semen quality, antioxidant status and blood constituents during rearing periods.

Keywords: Sheep, Hematology, Serum constituents, Semen characteristics, Oxidative status

INTRODUCTION

Sheep production, as most agricultural enterprises, is affected by economic forces as well as environmental factors (Marai, 1987). Exposure to these factors causes a series of strong changes in the sheep biological

functions that include depression in feed intake, efficiency and utilization, disturbances in metabolism of water, protein, energy, and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites. Such changes end with low live body weight and impaired reproduction, i.e. depression in age at puberty, reproductive activity and fertility (Habeeb *et al.*, 1992). In sheep, sexual behavior and semen quality are the main factors that limit male reproduction efficiency along the year (Aller *et al.*, 2012). The efficiency of sperm production, libido and quality of sperm tend to remain uniform throughout the reproductive life of an animal but may be significantly altered by age, nutrition, environment, health status, drugs, and chemicals (Togun and Egbunike, 2006). Reproductive functions are highly demanding in both nutrients quality and quantity; in this way, nutritional status is a very important modulator of reproduction in sheep and goats (Blache *et al.*, 2008).

Several studies have demonstrated interaction between nutrition and reproduction in sheep and goats. For example, flushing or minerals have been shown to improve production and reproduction parameters (Madibela *et al.*, 2002; Griffiths *et al.*, 2007). Selenium (Se) occurs in all cells and body tissues; its content in an organism varies according to the amount of the element in the feed ration (Kim and Mahan, 2001). The role of selenium as an antioxidant indicated importance for maintaining or improving semen quality in animals (Irvine, 1996). The increase in the formation of reaction oxygen species (ROS) was noted to decrease fertility, as the ROS will attack the membranes of the spermatozoa and decrease their viability (Irvine, 1996). Selenium increases the antioxidant glutathione peroxidase (GSH-Px) activity, which decreases the ROS and preserves semen quality and, subsequently, fertility in rams (Kendall *et al.*, 2000). Moreover, organic Se is a highly available form of Se for livestock and provides antioxidant protection at a level greater than inorganic Se (Mahan, 1999; Mahmoud and Edens, 2003; Al-Waeli *et al.*, 2013).

It is necessary to mention that *Spirulina (Atrhrospira platensis)* is a rich source of phycocyanin, as antioxidant biliprotein pigment and carotenoids (Cheong *et al.*, 2010). However, scientific information available on performance and antioxidative status in Barki ram-lambs fed combinations of algae and Se are still limited.

In view of these facts, the present research was conducted on weaned ram-lambs to study the effect of dietary selenium-enriched micro-algae *Spirulina (Atrhrospira platensis)* supplementation on growth performance, blood biochemical constituents, semen characteristics and anti-oxidative status, under summer environmental condition of Egypt.

MATERIALS AND METHODS

Animals and housing

This trial was carried out at the Experimental Station of Animal Production, Borg-El Arab, Alexandria Governorate, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. Twenty eight Barki ram-lambs 4-5 months of age and weighing 26.83 – 27.67 kg were divided according to age and weight into four

similar groups of six animals each and were housed in separate stalls. The experimental animals were kept under the same managerial and hygienic conditions. Before beginning the experiment animals were treated against internal and external parasites and intero-toximia.

Source of selenium-enriched dried algae

The selected cyanobacterium *Spirulina platensis* (*Arthrospira platensis*) were obtained from Agric. Microbiology Dept. National Research Centre (NRC), Giza, Egypt.

Experimental diets

Basal concentrate mixture consists of 37% crushed corn, 30% crushed barley, 20% wheat bran, 10% soybean meal, 2% lime stone and 1% salt. Basal concentrate mixture was supplemented with or without Se-algae (selenium enriched micro-algae *Spirulina*, *Arthrospira platensis*). Four experimental concentrate mixtures were prepared, the control (basal concentrate mixture without any supplementation, I, and the other three experimental concentrate mixtures (II, III and IV) were supplemented by Se- algae at levels of 0.2, 0.4 and 0.6 mg/kg DM of the diet, respectively. Animals of the four groups were randomly assigned to feed on one of these four concentrate mixtures. In addition to these concentrate mixtures animals of all groups were fed *ad libitum* on berseem hay.

Algae contain 1 mg Se/g algae; the control diet, thus contains only the endogenous Se contained in the ingredients of the diet. Control was the basal diet containing 0.08mg Se/kg diet. The four groups of lambs were randomly assigned to fed *ad libitum* on the four experimental diets in feeding experiment that lasted until sexual maturity. Animals were kept for two weeks prior to the start of the experiment for adaptation.

Growth performance and body weight gain

Lambs were individually weighed monthly in the morning before access to feed and water. Individual live weights of every treatment group were totaled and the average monthly weight gain was calculated by subtracting the average initial body weight of a certain period from the final average body weight of the same period.

Blood collection and analyses

Blood samples were collected monthly from the jugular vein of each lamb in the morning before access to feed and water. A part of blood samples were immediately withheld into heparinized tubes to analyze red blood cell (RBCs) counts and white blood cell (WBCs) counts according to Provan *et al.* (2004). Hemoglobin (Hb) and packed cell volume (PCV) were determined according to conventional methods (Hepler, 1966). Semen are obtained from coagulated blood samples centrifugation at 3000 rpm for 20 min, and serum were harvested and stored at -20°C for later analyses. Serum total protein, albumin, glucose, urea-N, creatinine, total cholesterol, total lipids, triglycerides, ALT, AST, ALP, MDA, T-AOC, SOD and GSH-Px enzymes activities, were

assayed using chemical commercial kits of Diamond Diagnostics, Egypt. Globulin was estimated by subtraction of albumin from total protein.

Sampling and semen traits

Semen samples were collected weekly over 8 weeks using an artificial vagina and the samples of each week were subjected to chemical analysis. Semen collection and handling were carried out and evaluated according to the international guidelines (IRRG, 2005). Ejaculated volume was measured to the nearest 0.01 ml immediately after collection, semen was maintained at 35 °C in a water bath for evaluation. Fresh semen samples were used for measure went of sperm concentration by using the improved Neubauer hemocytometer slide method as described by Smith and Mayer (1955). Total sperm output (TSO) was calculated by multiplying semen ejaculate volume by semen concentration. Individual sperm motility was estimated at 400× magnification (Kamar, 1960). Assessment of live and abnormal spermatozoa was performed using an eosin–nigrosin blue-staining mixture (Blom, 1950). The percentages of live, dead and abnormal spermatozoa were determined using stains that penetrated cells with damaged membranes. Normal live sperm exclude the eosin stain and appear white in color, whereas dead sperm take up eosin and appear pinkish in color because of loss of membrane integrity. Normal sperm have an oval head with a long tail. Abnormal sperm have head, midpiece or tail defects, such as a large or a misshapen head or a crooked or a double tail. The total number of motile sperm was calculated by multiplying the percentage of motile sperm by the total sperm outputs. The total functional sperm fraction (TFSF) was also calculated by multiplying the total sperm output and sperm motility and normal-morphology sperm (Correa and Zavos, 1996). Evaluation of seminal initial fructose was carried out immediately after collection according to Mann (1948).

Seminal plasma was separated by centrifugation at 3000 rpm for 20 minutes and stored at -20 ° C in Eppendorf tubes for further analysis of total protein and albumin. Globulin values were obtained by subtracting albumin values from corresponding values of total protein. Total lipids, triglycerides, cholesterol alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lipid peroxidation (Malondialdehyde) (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were determined in seminal plasma calorimetrically using commercial kits obtained from (Bio-Diagnostics, Egypt) according to the procedure outlined by the manufacturer.

Statistical analysis

Data of each growing and slaughter experiments were analyzed using general linear model (GLM) procedure (SAS, 2002). Duncan's Multiple Range Test (1955) was used to detect any variations between means.

RESULTS AND DISCUSSION

Body weight and weight gain

Data on the effect of dietary Se-algae supplementation on growth performance of Barki ram-lambs are presented in Table (1). The data indicated

that Se-algae significantly increased their final body weight ($P<0.05$) and total body weight gain at the end of the feeding experiment ($P<0.002$) as compared to the control group. Dietary supplementation of Se-algae at 0.2, 0.4 and 0.6 mg Se-algae/kg had positive effects on growth performance. These improvement agree with previous studies on the effects of selenium on the digestibility of nutrients. Naziroglu *et al.* (1997) reported that combined supplementation of Se and vitamin E increased acetic, propionic, butyric and total volatile fatty acids concentration, and the total counts of protozoa of the ruminal fluid of lambs *in vivo*. Our results suggest that the good effectiveness of Se from Se-enriched alga can be explained by the high bioavailability of selenomethionine. Although inorganic selenite can be utilized for selenoprotein biosynthesis, only selenomethionine can be incorporated nonspecifically into body proteins in place of methionine (McConnell and Hoffman 1972), because tRNA Met does not discriminate between Met and Se-Met (Schrauzer, 2000). Mahan and Parrett (1996) reported that selenium retention in the organism is higher when Se-enriched yeast rather than sodium selenite is used as a dietary Se source. About 64% of consumed Se is excreted in case of sodium selenite while only 47% of Se is excreted when Se-enriched yeast is fed. Also, Se from Se-enriched yeast does not have higher absorption from the gastrointestinal tract compared to sodium selenite. Therefore the high bioavailability of Se from Se-enriched yeast is not caused by higher absorption.

Table (1). The effect of different levels of dietary Se-algae supplementation on the body weight and weight gain of Barki ram-lambs (n=7)

Parameters	Se-algae supplementation (mg/kg DM)				P- value
	Control	0.2	0.4	0.6	
Initial body weight (kg)	24.98±1.65	24.62±0.90	23.65±1.26	23.00±0.73	NS
Final body weight (kg)	40.31 ^b ±1.50	46.58 ^a ±6.16	43.68 ^{ab} ±0.81	42.42 ^{ab} ±1.25	0.05
Body weight gain (kg)	15.33 ^b ±0.36	21.70 ^a ±1.28	20.03 ^a ±1.08	19.42 ^a ±1.20	0.002

^{a,b}Means within rows with different superscript letters differ significantly ($P<0.05$).

NS=not significant.

Blood hematology

The effects of dietary Se-algae supplementation levels on blood hematological characteristics of ram-lambs are presented in Table (2). Jones and Allison (2007) reported that hematological values of sheep were $9-15 \times 10^6$ cells/ μ l for RBC's, 27-45% for PCV and 9-15g/dl for Hb. Hemoglobin concentrations and red blood cell counts obtained from this study were between (11.45 and 11.62 g/dl) and (10.94 and 11.68×10^6 mm³), respectively. The hemoglobin and red blood cells level of the control rams were similar to those of rams supplemented with Se-algae. However, dietary Se-algae supplementation caused a significant increase in white blood cell counts when compared with the control group. Rame-lambs supplemented with Se-algae at levels 0.4 and 0.6 mg recorded the highest ($P<0.0001$) values of white blood cell counts (10.64 and $10.47 \times 10^3/\text{mm}^3$ respectively). These results agree with those reported by Ali *et al.* (2009) who found that after 3 months of treated Awassi rams with 175 mg/ram vitamin E and 70 mg/ram vitamin E plus 2800 mg selenium, the

percentage of lymphocytes was increased compared with the untreated group. Leucocytes play an important role in non-specific or innate immunity and their count can be considered as an indicator of relatively lower disease susceptibility (Matanović *et al.*, 2007).

Table (2). The effect of different levels of dietary Se-algae supplementation on blood hematologic parameters of the experimental ram-lambs (n=7)

Parameters	Se-algae supplementation (mg/kg DM)				P- value
	Control	0.2	0.4	0.6	
Hb (g/dl)	11.62±0.11	11.59±0.25	11.58±0.15	11.45±0.48	NS
RBC _s (x10 ⁶ /mm ³)	11.46±0.17	10.94±0.36	11.68±0.27	11.41±0.22	NS
WBCs (x10 ³ /mm ³)	9.75 ^c ±0.06	10.14 ^b ±0.09	10.64 ^a ±0.05	10.47 ^a ±0.08	0.0001
PCV (%)	29.63 ^{ab} ±0.62	28.73 ^b ±0.16	30.53 ^a ±0.23	29.06 ^b ±0.60	0.05

^{a-c}Means within rows with different superscript letters differ significantly (P < 0.05).

NS = not significant.

In addition, supplementation of Se-algae at 0.4 mg was the highest (P<0.05) group in packed cell volume when compared with control and the other treatment groups. The increase in the PCV might be due to the boosting effects of moderate levels of selenium on the immune system which enhance the process of the production of red blood cells in the body while decline in the PCV with increased selenium could probably be due to inhibitory constituents of the fed which was not determined in this case.

Blood constituents

The effects of dietary Se-algae supplementation levels on blood constituents of Barki ram-lambs are presented in Table (3). Results of the present study demonstrated that dietary Se-algae supplementation caused significant increases in plasma total protein and albumin when compared with the control group. Plasma albumin of the ram-lambs on 0.6 mg Se-algae supplementation was significantly (p<0.0001) higher than those of lambs on 0.2 Se-algae diets. On the other hand, animals fed on diet supplemented with Se-algae at 0.6 mg recoded the lowest (P<0.05) value of plasma globulin (3.02 g/dl). McConnell and Hoffman (1972) attributed these increases in total protein and albumin to the fact that seleno-methionine was incorporated into liver polypeptides via the methionine pathway. Selenium is present in two biologically active forms, Se containing enzymes and Se-containing proteins in animals (Zhang *et al.*, 2011). The control group (without supplementation of Se-algae) showed the lowest total protein and albumin, this reduction might be due to oxidative damage in proteins ranges from specific amino acid modifications and peptide breakage to loss of enzyme activity (Stadtman and Levine, 2003). Little information on the selenium form in Se-enriched Spirulina is available for the present time. Selenium is assumed to be built into the protein structure similar to that into Se-enriched yeast (Machat *et al.*, 2005), so Se-enriched spirulina may be used as a potential form of organic selenium supplemented to sows diets (Kotrbaček *et al.*, 2004).

The results of Table (3) revealed that plasma concentrations of glucose, creatinine, triglycerides and enzyme alanine aminotransferase (ALT) were not affected significantly by dietary supplementation with different levels of Se algae. Data in Table (3) indicated that dietary Se-algae supplementation significantly reduced plasma cholesterol and total lipid contents compared with the control group. Ram-lambs fed diets supplemented with 0.2 and 0.4 mg Se-algae had the lowest values of cholesterol content (78.37 and 77.72 mg/dl, respectively). This may be attributed to the anabolic role of Se on fat deposition. The effects of this supplementation were to modulate fatty acid composition in the whole body. These alterations could be also due to the *Spirulina* components such as polyunsaturated fatty acids, phycocyanin which are thought to be compounds with antioxidant abilities according to Nagaoka *et al.* (2005). Similarly, Ebied *et al.* (2012) noted that rabbits fed diets supplemented with 0.15 or 0.3 ppm organic Se significantly reduced total lipid compared with the control groups.

Ram-lambs on 0.4 mg Se-algae diet had plasma ALT level less than that of the groups on 0.2 mg and 0.6 mg Se-algae or control group, with insignificant differences ($p > 0.05$) between them. However, plasma AST level of the ram-lambs fed diet containing 0.4 mg Se-algae was significantly ($p < 0.05$) higher when compared to those of other groups. In addition, plasma ALP of the control group was significantly ($p < 0.0001$) higher as compared to those of groups fed diets supplemented with different levels of Se-algae. Abood *et al.* (2012) reported that Awassi ewes and their newborn lambs suffering from deficiency of vitamin E and selenium in their diet, AST levels reaching (143.71 ± 4.28 U/L) and (145.40 ± 7.94 U/L) in deficient groups of ewes and lambs, respectively compared with non-deficiency groups in which levels reached (69.14 ± 2.78 U/L) and (72.85 ± 2.33 U/L) respectively.

Table (3). The effect of different levels of dietary Se-algae supplementation on blood constituents of the experimental ram-lambs (n=7). (Mean \pm SE)

Parameters	Se-algae supplementation (mg/kg DM)				P-value
	Control	0.2	0.4	0.6	
Total protein (g/dl)	6.43 ^b \pm 0.03	6.61 ^a \pm 0.05	6.71 ^a \pm 0.05	6.65 ^a \pm 0.05	0.001
Albumin (g/dl)	3.23 ^c \pm 0.06	3.46 ^b \pm 0.03	3.55 ^{ab} \pm 0.02	3.63 ^a \pm 0.03	0.0001
Globulin (g/dl)	3.20 ^a \pm 0.07	3.15 ^a \pm 0.02	3.16 ^a \pm 0.02	3.02 ^b \pm 0.02	0.04
Glucose (mg /dl)	59.22 \pm 1.23	57.91 \pm 1.19	61.02 \pm 1.00	59.14 \pm 1.32	NS
Urea -N (mg/dl)	57.80 ^b \pm 1.13	60.57 ^{ab} \pm 0.63	63.16 ^a \pm 1.20	60.13 ^b \pm 0.80	0.0085
Creatinine (mg/dl)	1.05 \pm 0.01	1.02 \pm 0.02	1.02 \pm 0.02	1.01 \pm 0.02	NS
Total Lipids (mg/dl)	990 ^a \pm 0.008	904 ^b \pm 0.005	900 ^b \pm 0.004	909 ^b \pm 0.009	0.0001
Cholesterol (mg/dl)	82.45 ^a \pm 1.54	78.37 ^b \pm 0.66	77.72 ^b \pm 1.74	81.01 ^{ab} \pm 0.62	0.046
Triglycerides (mg/dl)	42.70 \pm 0.27	39.26 \pm 0.24	41.91 \pm 2.23	39.60 \pm 0.45	NS
AST (u/l)	55.13 ^b \pm 1.17	57.74 ^b \pm 1.30	61.69 ^a \pm 0.94	55.20 ^b \pm 1.69	0.03
ALT (u/l)	47.55 \pm 2.15	47.87 \pm 1.36	43.86 \pm 1.54	46.86 \pm 2.55	NS
ALP (u/l)	571.23 ^a \pm 10.03	452.25 ^b \pm 10.48	391.85 ^c \pm 9.24	359.14 ^d \pm 3.41	0.0001

^{a-d} Means within rows with different superscript letters differ significantly ($P < 0.05$).

NS=not significant.

AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase

Animal's antioxidative status

Data on the effect of dietary inclusion of Se-algae at different levels on antioxidative status of Barki ram-lambs are presented in Table (4). Dietary Se-algae supplementation resulted in significant effects on plasma blood antioxidative properties as measured by T-AOC and MDA as an index of oxidation and antioxidant enzymes such as GSH-Px and SOD. There were gradual and significant increases in T-AOC, GSH-Px and SOD as Se-algae level increased. Ram-lambs fed diet supplemented with 0.6 mg Se-algae recorded the highest levels of T-AOC., GSH-Px and SOD as compared with other levels of Se-algae supplementation. These results are in agreement with those of Cao *et al.* (2014) who found that the T-AOC of serum from pig fed on 0.3 mg/kg DL-Se Met supplemented diet was significantly higher compared with the low-Se and 0.3 mg/kg sodium selenite groups ($P < 0.05$).

Yue *et al.* (2009) found that serum SOD activity was higher ($p < 0.05$) in goats supplemented with both 0.30 and 0.50 mg Se/kg DM than in control goats and goats supplemented with high level of selenium 1.00 mg Se/kg DM. In accordance with our results, Faixova *et al.* (2007) found that lambs fed diet supplemented with 0.3 mg·kg⁻¹ DM in the form of Se-enriched yeast and giving a total daily intake 278 µg of Se per animal recorded the highest GSH-Px (830.85 ± 69.23 U/g Hb) when compared with the control group (that received a basic diet providing a daily intake 50.6 µg of Se only). A high and linear parallel between the Se concentration and GPx activity of blood has been reported by several authors (Pavlata *et al.* 2001; Rock *et al.*, 2001). Our results prove the positive correlation between blood Se content and the activity of this selenoenzyme. Opposite results were reported by Svoboda *et al.* (2009) who found that GSH-Px activities of the whole blood in both sows and piglets did not differ between groups fed on diet supplemented with inorganic Se (sodium selenite, 0.3 mg/kg) and those fed on diet supplemented with organic Se from Se-enriched alga (0.3 mg/kg). Also, Mahan and Peters (2004) and Yoon and McMillan (2006) found that both Se from Se-enriched yeast and inorganic form are adequate for the synthesis of GSH-Px.

Results shown in Table (4) also indicated that dietary Se-algae supplementation significantly ($P < 0.0001$) reduced MDA compared with the control, and this reduction was dose-dependent reaching 31.1 % as compared with the control group at 0.6 mg Se-algae. MDA is one of the final products of polyunsaturated fatty acid peroxidation in cells and is considered as a marker of oxidative stress (Gawel *et al.*, 2004). Zhan *et al.* (2007) reported that supplementing 0.3 mg/kg of Se to the diet of finishing pig significantly decreased the content of MDA both in liver and muscle. However, the content of tissue MDA in selenomethionine-treated group was slightly lower than that in sodium selenite group, especially in muscle (Wang *et al.*, 2011). Increased GSH-Px and decreased MDA are good indicators of improved oxidative defense of animal tissues (Celli, 2010).

Table (4). The effect of different levels of dietary Se-algae supplementation on antioxidative status of the experimental ram-lambs (n=7). (Mean±SE)

Parameters	Se-algae supplementation (mg/kg DM)				P- value
	Control	0.2	0.4	0.6	
MDA (mmol/l)	14.65 ^a ±0.56	13.40 ^b ±0.28	12.26 ^c ±0.30	10.10 ^d ±0.13	0.0001
T-AOC (mmol/l)	133.92 ^d ±1.59	144.85 ^c ±1.23	150.21 ^b ±1.55	159.64 ^a ±0.34	0.0001
SOD (u/l)	24.83 ^b ±0.6	30.22 ^a ±0.30	32.91 ^a ±0.80	35.71 ^a ±0.50	0.0001
GSH-Px (u/l)	456.04 ^d ±7.24	530.42 ^c ±6.37	563.02 ^b ±11.01	650.52 ^a ±14.30	0.0001

^{a-d} Means within rows with different superscript letters differ significantly (P < 0.01).

NS = not significant.

MDA=Malondialdehyde, T-AOC=Total antioxidant capacity, SOD=Superoxide dismutase and GSH-Px = Glutathione peroxidase.

Semen characteristics

The influence of addition different levels of Se-algae to the diet of lambs on their semen traits are presented in Table (5). Results showed that ejaculate volume of Barki rams increased as a result of supplementing their diets with se-algae. This increase ($p < 0.01$) reached 7.45% for animals receiving diet containing 0.6 Se-algae compared to the control group, while the increases were insignificant and amounted 3.33 and 4.40% for animals fed diets supplemented with 0.2 and 0.4mg Se-algae, respectively. Semen volume is an important factor in semen evaluation and reproductive performance in males (Ax *et al.*, 2000). Hashem (2014) showed that the average volume of semen was 1.03±0.001 ml in Siwa Barki sheep during a 12-month period (four seasons).

Hydrogen ion concentration (pH) was not affected significantly by adding different levels of Se-algae to the diet. However, semen hydrogen ion concentration showed numerical decrease due to inclusion of Se-algae in the diets as compared to the control group. This decrease in pH of the selenium supplemented group may be due to the decrease in percentage of dead sperm.

The highest values of Sperm progressive motility were recorded in the group receiving 0.6 mg Se-algae containing diet. Selenium supplementation improved ($p < 0.0001$) motility of ram semen. Sperm motility is a fairly reliable indication of the viability of fresh semen (Grahman *et al.*, 1980). The motile spermatozoa provide strong evidence for sperm maturation. Ali *et al.* (2009) found that treating Awassi rams for 90 days twice weekly with 175 mg/ram vitamin E and 70 mg/ram vitamin E plus 2800 mg selenium caused individual motility to increase compared with untreated group.

Sperm concentration in semen of Barki ram-lambs showed significant ($P \leq 0.05$) decrease in treated groups comparing with the control group. The lowest values of sperm concentration were recorded in the group receiving 0.4% Se-algae containing diet. In this respect,

selenium supplementation was reported to significantly decrease the sperm concentration in semen of boars supplemented by 0.3 and 0.6 mg Se/kg of feed mixture in inorganic form (Hork 2012). Hashem (2014) showed that average sperm concentration was 347×10^9 ml in Siwa Barki sheep. Hafez and Hafez (2000) reported that the sperm concentration of an ejaculate ranges from 3.5 to 6.0×10^9 ml in ram. Opposite to aim results was conducted by Ali *et al.* (2009) reported that after 3 months of treating Awassi rams with 175 mg/ram vitamin E and 70 mg/ram vitamin E plus 2800 mg selenium, the Sperm concentration was increased compared with untreated group.

Normal sperm and live sperm showed significant ($P \leq 0.05$) increases in treated groups comparing with the control group. The highest values of normal sperm was recorded in the group receiving 0.6 mg % Se-algae containing diet, but the highest values of live sperm was recorded in the group receiving 0.4 and 0.6 mg Se-algae containing diet. Beneficial effects of supplementary trace minerals (Zn, Co and Se) (Kendall *et al.*, 2000) and selenium (Anderson *et al.*, 1996) on percentages of live sperm in ram lambs have been reported. Abnormal sperm and dead sperm showed opposite trends. The best values of abnormal sperm and dead sperm was recorded in the group receiving 0.6 mg% Se-algae containing diet. These results agree with these of Ali *et al.* (2009). The present data showed that inclusion of Se-algae in the ram-lambs diets significantly ($P \leq 0.05$) increased total sperm output and total motile sperm. Results also showed that TFSF was significantly ($P \leq 0.0002$) increased by different levels of Se-algae. The best values for total sperm output, total motile sperm and TFSF were recorded in the groups of ram-lambs fed diet containing 0.6 mg Se-algae as compared to the control or the other experimental groups.

Table (5). Means \pm SE of semen traits of mature Barki rams fed diets supplemented with different levels of Se-algae (n=7)

Parameters	Se-algae supplementation (mg/kg DM)				P-value
	Control	0.2	0.4	0.6	
Ejaculate volume(ml)	0.87b \pm 0.02	0.90ab \pm 0.02	0.91ab \pm 0.02	0.94a \pm 0.02	0.01
Semen pH	6.69 \pm 0.02	6.51 \pm 0.03	6.51 \pm 0.02	6.50 \pm 0.02	NS
Progressive motility (%)	67.90b \pm 0.84	77.90a \pm 1.42	82.80a \pm 1.78	83.10a \pm 2.65	0.0001
Sperm concentration(x107/ml)	312.70a \pm 8.02	285.30b \pm 3.84	282.40b \pm 2.87	293.30b \pm 4.54	0.05
Normal sperm (%)	77.06b \pm 0.66	78.83b \pm 0.74	79.13b \pm 0.37	81.31a \pm 0.57	0.003
Abnormal sperm (%)	22.94a \pm 0.66	21.17b \pm 0.74	20.88b \pm 0.37	18.69c \pm 0.57	0.0005
Live sperm (%)	79.66c \pm 0.74	83.00b \pm 2.44	85.33a \pm 3.40	86.50a \pm 0.76	0.0001
Dead sperm (%)	20.83a \pm 2.76	17.00b \pm 2.47	14.83c \pm 3.33	13.83c \pm 2.78	0.0001
Sperm output(x107/ ejaculate)	288.09ab \pm 6.79	271.80b \pm 7.03	270.96b \pm 7.99	294.36a \pm 7.48	0.05
Motile sperm(x107/ ejaculate)	195.01c \pm 4.32	213.07bc \pm 3.77	229.94ab \pm 10.37	250.45a \pm 13.07	0.0018
TFSF (x107/ejaculate)	151c \pm 3.24	170bc \pm 8.64	185ab \pm 12.05	206a \pm 13.61	0.0002

^{a-c} Means within rows with different superscript letters differ significantly ($P < 0.05$).
NS = not significant. TFSF = Total functional sperm fraction.

Effect of dietary addition different levels of Se-algae on seminal plasma constituents

Data on the influence of feeding Barki ram-lambs on diets supplemented with different levels of Se- algae on seminal plasma constituents are presented in Table (6). Results showed that seminal plasma total protein, albumin and globulin are significantly ($P \leq 0.05$) affected by dietary addition of different levels

of Se-algae as compared to the control group. Seminal plasma total protein showed significant increase in the groups given 0.4 and 0.6 mg Se-algae diets as compared to the control group. Seminal plasma albumin showed insignificant increase in the groups receiving 0.4 and 0.6 mg Se-algae diet as compared to the control group. On the other hand, seminal plasma globulin showed significant decrease in the group receiving 0.4 mg Se-algae diet as compared to the control group.

Concentration of total lipids in semen plasma significantly decreased with inclusion of Se-algae in the diets and these decreases reached 7.15, 6.25 and 9.69% for diets containing Se-algae at levels of 0.2, 0.4 and 0.6 respectively, as compare to control group. However, seminal plasma cholesterol and triglycerides were not significantly affected by the inclusion of Se-algae in the diets.

Seminal plasma analysis presented in Table (6) showed significant ($P \leq 0.01$) decreases in concentrations of ALP and ALT as a result of dietary inclusion of Se-algae when compared with the control group. The decline in transaminase enzyme in supplemented groups may be due to the presence of Se ions (Underwood et al., 1977) which may had a protective action to reduce the damage of cell membrane of sperms and sperm dead count which leads to a decline in the release of transaminase enzyme in seminal plasma.

Transaminase enzyme levels of whole semen showed significantly higher activity during stress. These enzymes may rise as a result of the destruction of spermatozoa due to stress factors and the high levels of transaminase enzymes are used as indicator of the degree of membrane damage of spermatozoa (Corteel, 1980). Moreover, Se-algae treatments resulted in a decrease in seminal plasma alkaline phosphates which may play a role in enhancing semen properties as high seminal alkaline phosphates activity in the buffalo was associated with lower sperm numbers, decreased motility and percent of live cells, depressed dehydrogenase activity and a slight and non-significant decrease in fructolytic rate (Abdou *et al.*, 1978). The improvement in biochemical constituent's concentration of seminal plasma of ram-lambs on Se-algae supplementation found herein generally are in agreement with those reported by Kamel (2012) who found that administration of selenium and folic acid or their combination increased ($P < 0.05$) seminal plasma total proteins, globulins, alkaline phosphatase, acid phosphatase and lactate dehydrogenase.

Conversely, seminal plasma aspartate aminotransferase, alanine aminotransferase were significantly decreased compared to the control group. Although, initial fructose was non-significantly affected by different treatments, the results showed numerical increase in initial fructose in the groups fed all levels of Se-algae diets. The numerical increases of initial fructose were 2.1, 3.8 and 8.9 % for 0.2, 0.4 and 0.6 mg Se-algae fed groups, respectively.

Table (6). Means \pm SE of semen constituents of Barki mature rams fed diets supplemented with different levels of Se-Algae (n=7)

Parameters	Se-algae supplementation (mg/kg DM)				P-value
	Control	0.2	0.4	0.6	
Total protein (g/dl)	6.10 ^b \pm 0.09	6.22 ^{ab} \pm 0.12	6.38 ^a \pm 0.13	6.33 ^a \pm 0.11	0.0192
Albumin (g/dl)	3.09 ^c \pm 0.04	3.16 ^c \pm 0.06	3.57 ^a \pm 0.07	3.37 ^b \pm 0.06	0.0001
Globulin (g/dl)	3.01 ^a \pm 0.12	3.04 ^a \pm 0.06	2.82 ^b \pm 0.06	2.96 ^{ab} \pm 0.05	0.05
Total lipids (mg/dl)	908 ^a \pm 0.005	843 ^b \pm 0.011	851 ^b \pm 0.011	820 ^b \pm 0.024	0.0288
Triglycerides (mg/dl)	238.10 \pm 4.50	225.70 \pm 9.19	221.60 \pm 11.02	220.00 \pm 17.05	NS
Cholesterol (mg/dl)	213.60 \pm 3.49	204.80 \pm 10.67	201.70 \pm 11.71	200.60 \pm 9.35	NS
ALT (u/l)	65.09 ^a \pm 1.39	60.74 ^b \pm 3.83	60.15 ^b \pm 4.55	61.55 ^b \pm 3.14	0.05
AST (u/l)	84.62 \pm 0.61	86.75 \pm 2.03	86.86 \pm 2.06	87.25 \pm 1.63	NS
ALP (u/l)	17.48 ^a \pm 0.08	15.80 ^b \pm 0.43	13.63 ^c \pm 0.25	12.70 ^d \pm 0.37	0.0001
Initial fructose (mg/dl)	269.40 \pm 0.21	275.16 \pm 0.24	280.01 \pm 0.23	295.60 \pm 0.26	NS

^{a-d}Means within rows with different superscript letters differ significantly (P < 0.05).

NS = not significant.

AST=aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phosphatase

In conclusion, results of the present study demonstrated that supplemented diets of Barki ram-lambs with different levels of Se-algae increased final body weight and weight gain also; it improved their anti-oxidative status and semen quality.

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الملخص العربي

تأثير إضافة الطحالب المجففة الغنية بالسيلينيوم الى العلائق على الأداء الإنتاجي والتناسلي لذكور أغنام البرقي

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يهدف البحث لدراسة تأثير إضافة طحالب *Spirulina platensis* المجففة الغنية بالسيلينيوم الى علائق ذكور أغنام البرقي وذلك على النمو وبعض مكونات الدم وصفات السائل المنوي وايضا بعض إنزيمات الكبد المضادة للاكسدة . وقد أستمرت هذه الدراسة لمدة أكثر من عام وتم إجرائها بمحطة بحوث الإنتاج الحيواني ببرج العرب بالإسكندرية. استخدم في هذه التجربة ثمانية وعشرون من الحملان البرقي متوسط أعمارها (٤ - ٥ شهور) تم تقسيمها عشوائيا إلى أربع مجاميع متساوية كل مجموعة تضم ٧ حيوانات تم تحضير مخلوط علف مركز أساسي يتكون من ٣٧% ذرة مجروشة ، ٣٠% شعير مجروش ، ٢٠% ردة ، ١٠% كسب فول صويا ، ٢% حجر جيرى ، ١% ملح طعام. وقد تم تحضير أربع علائق تجريبية العليقة الاولى (مجموعة المقارنة) وهى عبارة عن مخلوط العلف المركز الاساسى بدون أى إضافات مع دريس البرسيم وكانت العلائق الثلاثة الأخرى (٢، ٣، ٤) تتكون من العليقة الأولى مع إضافة الطحالب المجففة الغنية بالسيلينيوم الى مخلوط العلف المركز بمستويات ٠.٢ ، ٠.٤ و ٠.٦ ملجم / كجم علف مركز ، على التوالي. وقد تم تغذية مجاميع الحيوانات الأربعة عشوائيا على العلائق التجريبية الأربعة .

وكانت أهم النتائج المتحصل عليها كالتالى:-

١- أظهرت النتائج أن إضافة الطحالب الغنية بالسيلينيوم أدت الى زيادة معنوية فى وزن الجسم ومعدل النمو مقارنة بالمجموعة المقارنة. كما لوحظ ارتفاع معنوى فى عدد كرات الدم البيضاء ومستوى البروتين الكلى وانخفاض مستوى الكوليسترول والدهون الكلية مقارنة بالمجموعة المقارنة. لوحظ أيضا ارتفاع قيمة الهيماتوكريت فى الحملان المغذاة على العليقة المحتوية على ٠.٤ ملجم سيلينيوم / كجم عليقة مقارنة بالمجاميع الاخرى بينما ارتفع البيومين الدم وانخفض الجلوبيولين عند مستوى ٠.٦ ملجم / كجم عليقة.

٢- أظهرت نتائج تحليل الدم أن هناك تأثيراً ملحوظاً لإضافة الطحالب الى العليقة على مستوى إنزيمات الكبد (AST) وايضا أنزيم ALP ولو أن هذا التأثير كان فى الحدود الطبيعية المتعارف عليها كما لوحظ حدوث ارتفاع معنوي فى مستوى انزيمات الكبد المضادة للأكسدة (T-AOC,GSH-Px,SOD) بينما إنخفض مستوى المركب MDA معنوياً وذلك عند المقارنة بالمجموعة المقارنة.

٣- أوضحت نتائج تحليل السائل المنوى حدوث زيادة معنوية فى حجم القذفة والعدد الكلى للإسبرمات فى القذفة الواحدة وكذلك معدل الحركة التقدمية للحيوانات المنوية بالنسبة للحيوانات المغذاة على العلائق المضاف لها السيلينيوم مقارنة بالمجموعة المقارنة. أيضا لوحظ ارتفاع معنوى فى مستوى البروتين الكلى فى بلازما السائل المنوى فى الحيوانات التى على علائق محتوية على مستوى ٠.٤ و ٠.٦ ملجم سيلينيوم مقارنة بباقي المعاملات الاخرى.بينما لوحظ انخفاض معنوى فى مستوى الالبيومين والدهون الكلية وكذلك مستوى ALT, ALP فى الحيوانات المعاملة مقارنة بالمجموعة المقارنة.

من النتائج السابقة نستنتج أنه يمكن استخدام الطحالب المجففة الغنية بالسيلينيوم فى علائق ذكور الاغنام النامية حيث تؤدي الى زيادة معنوية فى معدلات النمو وتحسين جودة السائل المنوى ومضادات الاكسدة ومكونات الدم الهامة والتي تنعكس بالأيجاب على الحالة الصحية والانتاجية للحيوان بصفة عامة.

