THE EFFECT OF SOME ANTIOXIDANT ON THE PHYSICAL AN BIOCHEMICAL CHARACTERISTIC OF SEMEN IN AWASSI RAMS

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Abstract
The current study was designed to evaluate the effect of some antioxidant substances on the macroscopical, microscopical and biochemical properties of semen and the concentration of some antioxidant enzymes in the seminal plasma of the Awassi rams Twenty-four Awassi rams were used in this study. The animals were divided into four groups (6 rams/group); the first group was the control group. Group II the rams were given the Curcuma 3 g/kg feed, group III were administered vitamin E Supplemented orally (Capsule) at a dose of 400 IU / animal, three doses per week, group IV were given the Coenzyme Q10 Supplemented orally (Capsule) at a dose of 400 mg / animal, three doses per week.. Semen samples were collected using an electroejaculaor once every 15 days along the duration of the experiment. Semen samples were evaluated to determine the volume, viscosity, pH, mas and individual motility of sperm, percentage of live, dead and abnormal sperms, seminal plasma content of total protein, albumin, globulin, cholesterol, triglyceride and the level of MDA, AST, ALT, SOD and CAT.
The results showed a significant effect ($P<0.05$) of the treatments on some semen parameters; these includes; the volume of the ejaculates were increased in the group of Curcuma and Coenzyme Q10. The viscosity was significantly affected by Curcuma treatment, while the individual motility and the percentage of live sperm were increased significantly in the three treatments groups. Vitamin E and Coenzyme Q10 were decrease the percentage of sperm abnormalities in comparison with control group. There was a limited effect of treatments on the seminal plasma components; the level of albumin and cholesterol were decreased significantly in the group of Coenzyme Q10, while the level of the globulin was increased in the three treatment groups than in control group. There has also a significant decrease in the level of cholesterol in the Curcuma group and a significant increase in the level of triglycerides in the vitamin E treatment compared to the control group. There was a limited effect of the treatments on the enzyme activity; The MDA was **Part of ph.D Thesis submitted by the first author**

increased in the three treatment groups, increased GSH in and vitamin E groups, and increased SOD in the Curcuma and Coenzyme Q10 groups in comparison to the control group. It be concluded that the addition of Curcuma to feed has an improved effect of semen properties; and increased the concentration of fructose in seminal plasma. Oral administration of vitamin E was increased the level of GSH and the globulin, also administration of Coenzyme Q10 was increased the level of SOD in the seminal plasma.
Key word : Awassi Rams, Semen Characters,Antioxidant.

INTRODUCTION

Sheep are important source of milk, meat and wool (1). It is one of the most bred animals and the most tolerant of the dry climate in Iraq (2). The economic characterization of sheep is necessary for livestock development and breeding programs (3).

. Among these problems, the land has witnessed in the recent period of major changes in environmental conditions, the most important of which is global warming. (4) added that Environmental stress has an effect on the chemical and biological properties of blood. During the past twenty years, there became a general idea of the Reactive Oxygen Specious (ROS) classes formed and their negative role in cellular processes (5). In addition, heat stress works to cause male infertility

(6). Vitamins are important elements for the performance and development of physiological functions in the body (7). Animal feeds need food additives such as vitamins, especially animals that live in harsh climatic conditions (8). Vitamin E is one of the main antioxidants (9).

indicated that vitamin E plays a major role in reproduction (10). Medicinal plants have also become of great importance through their use in the treatment and prevention of diseases, including the turmeric plant, which has increased in use in farm animal diets. It works to scavenge free radicals through its antioxidants such as Phenolic, B-Diketone, and Methoxy (11). Coenzyme Q10 increases the thickness of the germ cells of the seminiferous tubule

(12). It can be manufactured inside the body in small quantities and it has an important role in the cell (13). The current study was designed to To know the effect of antioxidants on reproductive efficiency, semen characteristics of rams and the level of antioxidants in semen plasma of Awassi ram .

MATERIALS AND METHODS

The study was conducted in the fields of the Department of Animal Production/College of Agriculture and Forestry/ University of Mosul, and for the period from 10/12/2019 to 10/12/2020, 24 male Awassi Sheep, with an average weight of 46,415 ± 1.54, were used on four treatments in a homogeneous manner, and one treatment contained 6 males. Each group was placed in a room with a dimension of 5 x 4 (m^2) containing feeders and water for sheep. The experiment's treatments included the first group representing a group of control animals, the second group: animals that were given turmeric in an amount of 3 g/kg feed during the duration of the experiment, and the third group: group III were administered vitamin E Supplemented orally (Capsule) at a dose of 400 IU / animal, three doses per week produced by the Canadian company ADRIEN GAGNON for the pharmaceutical industry, and the group IV were given the Coenzyme Q10 Supplemented orally (Capsule) at a dose of 400 mg / animal, three doses per week. produced by AMS company The American Pharmaceutical Industry used concentrated diets of 2.5% of the weight of the live animal, in addition to daily grazing (1-2) hours/day. Table 1 shows the proportions and components of the diet.

Table 1 : Ingredient and chemical composition of basal diet .

Ingredient *	Percentage
Barley grain	53
Wheat bran	32
Soya bean meal	5
Urea	0.25
Limestone	1.0
Salt	1.0
Sodium bicarbonate	0.25
Wheat straw	7.5
Chemical composition**	%
Crude protein	15.2
Dry mater	92.16
Organic mater	90.40
Ether Extract.	2.52
Crud fibers	7.86
Met. Energy (K.cal./Kg)	2721.5

***Ingredient of diet according to (14).**

**** Chemical composition according to (15)**

Met. energy is calculated according (16).

Animal weights were taken using a sheep scale once a month.

Veterinary care was provided according to the field program, and semen was collected once every 15 days during the experiment period using an electrical stimulation device (17) by inserting the electrode of the device into the ram's rectum and electrically stimulating it every 5-10 seconds to obtain an ejaculate, which They were collected in a graduated tube. After the process, all samples were placed in a water bath at a temperature of 37°C. Semen tests were performed on ejaculate volume (ml) and it was estimated using the graded (10) ml glass tube used to collect semen. The texture through the collection tube directly, which ranged between three degrees, which are watery (0-1), milky (1-2), and creamy (2-3) according to (18). The pH was also done using a digital pH Meter of Italian-made Hanna Instroments and the mass motility was measured as indicated by (19) by taking a drop of semen by a special plastic pipette for each sample and placing it on a glass slide fixed on a hot plate at a temperature (37°C) was examined under a microscope under magnification (×100). The individual motility of the sperm was measured as indicated by (20) by placing a drop of semen on a glass slide fixed on a warm surface 37 ˚C and adding a drop of Sodium citrate 2.9% to dilute the drop of semen and cover it with a thin slide and examine it under the microscope with a magnification force (×400), as the sperm concentration was calculated as indicated by (20) using a hemocytometer. The sperms are counted using the equation mentioned by (21) and the live, dead and distorted sperms were counted according to (22) and a slide was made for counting live, dead and distorted sperms by placing a drop of semen on a clean, heated slide and adding to it a drop of eosin dye and two drops of necrocin dye, and the mixture was mixed quietly and left for one minute and then I prepared a smear of the mixture on top of another slide and left to dry. It was examined with the oil lens ×100, and 200 sperms were counted from each sample to calculate the percentage of live, dead and primary deformed sperm. Some components of semenal plasma, such as protein, were measured using the Biuret method to estimate total protein and according to the instructions of the French company Biolabo (23) The albumin concentration was measured using the Bromocresol green method and according to the instructions of the French company Biolabo (24) and the concentration of Globulin according to the equation indicated by (25).

Globulin concentration = total protein concentration – albumin concentration

The level of cholesterol and triglycerides was estimated by the enzymatic method (26) using a ready-made kit (kit) manufactured by the French company Biolabo, and the concentration of the enzyme (ALT) in semenal plasma was measured according to (27) by following the work steps that It was referred to by the Biolabo company that produced the analysis kit (Kit) and the activity of the enzyme (AST) was measured according to (27) by following the work steps indicated by the British company (RANDOX) that produced the analysis kit (kit) and the concentration of reduced glutathione in the semenal plasma was measured using the method The Ellman reagent (28) used the modified thiobarbituric acid (TBA) reaction method. By researchers (29) to measure the level of lipid peroxidation (MDA) in semenal plasma. The enzymatic activity of catalase enzyme was estimated as indicated by (30) and the enzymatic activity of superoxide dismutase (SOD) in semenal plasma was estimated according by (31). Statistical analysis was carried out using a one-way complete random design (CRD) according to the following mathematical model $Yij=\mu+ti+eij$ (32) and the differences between the means were tested using Duncan's polynomial method (33) and using the statistical program (34).

RESULTS AND DISCUSSION

Table No. 2 that there is a significant effect ($A \leq 0.05$) of the treatments on semen volume,

as it increased significantly in the turmeric treatment (2.059 ml) compared with control group
(1.691 ml) while it reached 1.928 and 1.83 in the vitamin E and coenzyme Q10 treatments respectively, which did not differ significantly from the turmeric and control treatments, and this result was in agreement with the results of (35) who did not find a significant effect on semen volume when adding vitamin E to the Feed of Awassi rams and it differed with the result of (36) who They noticed a decrease in the semen volume of dogs when they were given Coenzyme Q10. Perhaps the increase in semen volume in the treatment of turmeric is due to its positive effect on the Sertoli cells that support and support the formation and nutrition of sperm. It was also observed that there was a significant effect ($A \leq 0.05$) of the treatments on the consistency of the semen, which increased significantly in the turmeric treatment and amounted to 2.231 in comparison with the consistency in the control (2.015), while the strength in the treatments of vitamin E and coenzyme Q10 was 2.202 and 2.197, respectively, which was no Significantly differs with turmeric and control treatments, and we notice the positive effect of turmeric in raising the value of the semen texture to the improvement of semen characteristics in this treatment compared to other treatments, especially the control treatment, which gave the lowest values (Table 2). And there was no significant effect of the treatments on mass movement, which amounted to 3.549, 3.976, 3.933 and 4.108 in the control treatments, turmeric, vitamin E and coenzyme Q10, respectively, and this result was different from the results of (35), (37). Who found a significant increase in the mass movement of the sperm of Awassi rams and roosters when they were given vitamin E and coenzyme Q10, respectively (Table 2). The results showed a highly significant effect ($a \leq 0.01$) of the treatments on the individual motility of the sperm, as it increased significantly in the treatments of turmeric, vitamin E and coenzyme Q10 (82.736, 81.794 and 4.108%) respectively compared to the moral decline in the control group (70.977%). The result is agreement with the results of (38) who observed high individual motility of semen of Awassi rams and African stunted goats when given vitamin E and consistent with the result of (39) when adding Coenzyme Q10 to horse ration. (40) indicated that curcumin It works to protect the sperm and raise the efficiency of the mitochondria, thus raising the efficiency of the sperm. Vitamin E also promotes sperm motility and Plasma membrane function and prevention of lipid peroxidation (41). Vitamin E prevents external oxidation of the sperm, which leads to the perpetuation of the movement of the sperm for a longer period (42). The results showed that there was no significant effect of the treatments on the sperm concentration/ml (2.557, 2.791, 2.371 and 2.263 x 10^9/ml) in the control treatments, turmeric, vitamin E and coenzyme Q10 respectively, and this result was in agreement with the results of (38) who They did not find a significant effect on the sperm concentration when giving vitamin E to male African goats and it did not agree with the results of (37) and (36) who noticed an increase in the sperm concentration of roosters and dogs when they were given Coenzyme Q10, respectively (Table 2).

Table 2 : Effect of antioxidants treatments on some semen quality in Awassi rams (Mean ± SE).

Treatment	Ejaculation Volume(ml)	Consistency of semen	Mass motility	individual motility%	Sperm c concentrat (m l /10^9 x)
Cont. gr.	1.691 b ± 0.082	2.015 b ±0.058	3.549 a ± 0.124	70.97 b ± 2.489	2.557 a ± 0.21
Turmeric gr.	2.059 a ± 0.117	2.231 a ±0.059	3.976 a ± 0.094	82.736 a ± 1.949	2.791 a ± 0.23
Vit.E gr.	1.928 ab ± 0.093	2.202 ab ±0.052	3.933 a ± 0.090	81.794 a ± 1.720	2.371 a ± 0.174
CoQ10 gr.	1.881 ab ± 0.106	2.197 ab ± 0.059	4.108 a ± 0.093	83.119 a ± 1.454	2.263 a ± 0.172

Means within different letters within grouping differ significantly (p<0.05).

There was no significant effect of the treatments on the pH semen)7.078, 6.841, 6.932 and 6.796) in the control, turmeric, vitamin E and coenzyme Q10 treatments, respectively (Table 3). It was also observed that there was a significant effect (A ≤ 0.05) for the treatments In the percentage of live sperm, which amounted 83.551% in the control group, it was significantly low compared to the increase in the treatments of turmeric, vitamin E and coenzyme Q10 (90,195, 87.696, and 89.356%) respectively, and this result was in agreement with the results of (39) who found a high In the proportion of live sperms of horses when giving Coenzyme Q10, it also indicated that the positive effect of the enzyme continues even after the dose is interrupted. The effect of antioxidants on raising the percentage of live sperms and its positive effect on improving semen characteristics compared with the control group (Table 3). It was also noted that there was a significant effect (a ≤ 0.01) of the treatments on the percentage of dead sperms, where the percentage of sperms decreased significantly. The mortality rates in the treatments of turmeric, vitamin E and coenzyme Q10 (9.745, 12,300 and 10.743%) respectively compared to increase in the control group (17.530%). Results showed a significant affect (P≤ 0.05) In abnormal sperm which increase in the control group (8.242%) compared to the two treatments of vitamin E and coenzyme Q10 (7.94 and 6.302%) respectively, while the percentage of distorted sperms in the turmeric treatment was 6.733%, which did not differ significantly from the rest of the experimental treatments. Deformed sperms when rams and roosters were given vitamin E and coenzyme Q10, respectively, and differed from the results of 43)), who did not

ind a significant effect on the proportion of distorted sperms for people when they were given coenzyme Q10.The decrease in the percentage of distorted sperms in the two treatments of vitamin E and coenzyme Q10 may be attributed to the protective role of them as antioxidants in improving the function of the epididymis tissue, the maturation of the sperm and the removal of the protoplasmic droplet, leading to raising Vitality of the sperm and the decrease in the percentage of distorted sperms. (44)indicated that vitamin E works to reduce the percentage of distorted sperms (Table 3).

Table 3 : : Effect of antioxidants treatments on some semen quality in Awassi rams (Mean ± SE).

Treatment	pH	live sperm (%)	dead sperm (%)	Abnormal (%) Sperr
Cont. gr.	7.078 a ± 0.061	83.551 b ± 1.408	17.530 a ± 1.496	8.242 a ± 0.988
Turmeric gr.	6.841 a ± 0.071	90.195 a ± 0.773	9.745 b ± 0.759	6.733 ab ± 0.761
Vit.E gr.	6.836 a ± 0.078	87.696 a ± 0.646	12.300 b ± 0.704	5.794 b ± 0.631
CoQ10 gr.	6.796 a ± 0.972	89.356 a ± 1.60	10.743 b ± 1.543	6.302 b ± 0.552

Means within different letters within grouping differ significantly (p<0.05**).**

Table No. 4 indicates a highly significant effect (A ≤ 0.01) of the treatments on the concentration of fructose in the semenal plasma, as it increased significantly in the turmeric and coenzyme Q10)9.073 and 8.325 mg/100ml(respectively, compared to the vitamin E treatment (6.408 mg/100ml) While the concentration of fructose in the control was 8.257 mg/100 ml, which did not differ significantly from the rest of the experimental treatments and this result was different with the result of (45), which found a significant increase in the fructose concentration in the semen

plasma of rams when they were given vitamin E. Several studies have indicated a link between sperm motility and mitochondrial activity in the middle of the sperm, due to its high energy requirement.

(46) that the seminal vesicles secrete mainly fructose, and no significant effect of the treatments on semenal plasma protein concentration was recorded, (3.305, 3.466, 3.360 and 3. .397 g/100 ml) in the control treatments, turmeric, vitamin E and coenzyme Q10, respectively (Table 4). While there was a significant effect (a ≤ 0.01) of the treatments on semenal plasma albumin concentration, which increased significantly in the control and turmeric treatments, (0.825 and 0.815 g/100 ml) respectively, compared with Coenzyme Q10 treatment (0.672 g/100 ml). Whereas in the vitamin E treatment (0.776 g/100 ml) and it did not differ significantly with the experimental treatments. A highly significant effect (a ≤ 0.01) of the treatments on the semenal plasma globulin concentration was recorded. It significantly increased in the treatments of turmeric, vitamin E and coenzyme Q10, (3.016, 2.488 and 2.664 g/100 ml) respectively, compared decrease with the control group (2.081 g/100 ml), and the treatment of coenzyme Q10 did not differ significantly from the treatments of turmeric and vitamin E (Table 4) The increase in semenal plasma globulin concentration in turmeric and vitamin E treatments may be due to the role of turmeric in enhancing immunity by raising IgG, IgM and IgA (47). Vitamin E enhances the functioning of the animal's immune system (48). (49) indicated that the increase in semenal plasma globulins have positive effects in raising and improving male fertility. Through the results of the study, it was noted that there was a significant effect (a ≤ 0.01) of the treatments on the concentration of cholesterol in semenal plasma, which increased significantly in the control group (54,645 mg/100 ml) compared decrease with the treatments of turmeric and coenzyme Q10 (46.364 and 37.465 mg/100 ml) respectively, while it was 48.738 mg/100ml in the vitamin E treatment, which did not differ significantly from the control and turmeric treatments and was significantly higher compared to the coenzyme Q10 treatment. The decrease in the

semenal plasma cholesterol concentration in the turmeric and coenzyme Q10 treatment may be due to their role as antioxidants in lowering the cholesterol concentration. And prevent high cholesterol and its negative significantly in the vitamin E treatment (384.717 mg/100 ml) compared with the control treatments, turmeric and coenzyme Q10 (330,514, 319.588 and 276.95 mg/100 ml) Respectively, the increase in triglycerides in the plasma of semen may be due to the effect of vitamin E on the activity of the sex glands and the secretion of fats necessary for impact on sperm. (Table 4) A highly significant effect (a ≤ 0.01) of the treatments on the concentration of triglycerides in the semenal plasma was observed, as it increased

the activity of sperm and its relationship with the increase in the percentage of single motility and live sperm. The sem en of bulls increases the percentage of live sperms (Table 4). of roosters treated with Coenzyme Q10 and possibly Its increase in coenzyme Q10 treatment is due to the increase in the vital activities of the testicles (50).

Table 4 : Effect of antioxidants treatments on some Biochemical Charecyerstics in Semenal plasma. .(Means ± St. Error

Treatment	Fructose (mg/100ml)	Total protein (g/100ml)	Albumin (g /100 ml)	Globulin (g/ 100ml)	Cholesterol (mg/100 ml)	Triglyceride (mg/ 100 ml)
Cont. gr.	8.257 ab ± 0.678	3.305 a ± 0.135	0.825 a ± 0.058	2.081 c ± 0.144	54.645 a ± 2.804	330.514 b 16.367
Turmeric gr.	9.073 ab ± 0.620	3.466 a ± 0.213	0.815 a ± 0.064	3.016 a ± 0.224	46.364 b ± 1.99	319.58 b ± 20.62
Vit.E gr.	6.408 b ± 0.419	3.360 a ± 0.156	0.776 b ± 0.030	2.488 b ± 0.147	48.738 ab ± 2.114	384.717 a ± 24.93
CoQ10 gr.	8.325 a ± 0.592	3.397 a ± 0.157	0.672 b ± 0.032	2.664 ab ± 0.158	37.465 c ± 2.096	276.9 b \± 16.609

Means within different letters within grouping differ significantly ($p<0.05$).

Where the individual movement increased in the treatment of enzyme facilities Q10 was followed by the
treatments of turmeric and vitamin E, and the individual movement was the least in control, as well as the increase in sperm production, which is directly proportional to the occurrence of stress and the production of free radicals, which can cause an increase and lipid peroxidation as a final product (Table 5).

The results of this study in Table No. 5 showed that there was a significant effect (A ≤ 0.01) of the treatments on the concentration of the level of Malondialehyde (MDA) in the semenal plasma, where the highest concentration of Coenzyme Q10 was 1.745 nmol/mol compared with decrease in turmeric and vitamin E treatments (1.300 and 1.455 nmol/mol) respectively, compared with the controle group (1.010 nmol/mol) , this result was different from the results of (37) who found a significant decrease in the concentration of (MDA) in the semenal plasma It was also observed in this study that there was a highly significant effect (A ≤ 0.01) of the treatments on the concentration of reduced glutathione (GSH) in the semenal plasma, which increased significantly (15.410 and 15,951 mmol/L) in control and coenzyme Q10 respectively compared to its concentration in Treatments of turmeric and vitamin E (13.967 and 14.985 mmol/L) respectively on The decrease in the concentration of reduced glutathione (GSH) in

semenal plasma in the turmeric and vitamin E treatments may be due to the high consumption of it durin metabolic reactions. Table 5 did not record a significant effect of the treatments on the concentration of enzyme AST in the semenal plasma, which increased 66,442, 57.995 and 65 −06 and 62,792 IU/L in the control treatments, turmeric, vitamin E and coenzyme Q10, respectively (Table 5). It is clear from the data in Table No. 5 that there was no significant effect of the treatments on the concentration of ALT enzyme in the semenal plasma (264.67, 253.657, 261.259 and 262.137 IU/liter) in the control treatments, turmeric, vitamin E and coenzyme Q10, respectively, and this result did not agree with the results of (37) who found a decrease in the concentration of ALT enzyme in the plasma of roosters when they were given Coenzyme Q10, and there was no significant effect of the treatments. On the concentration of catalase enzyme catalase (CAT) in semenal plasma (12.582, 11.832, 12.028 and 11.474 units/mg protein) in the control treatments, turmeric, vitamin E and coenzyme Q10, respectively, and this result was different from the result of (43), which indicated a high concentration of CAT when subjects were given Coenzyme Q10 (Table 5). It activates the conversion of ADP and inorganic phosphate to ATP (44).

Table 5 : Effect of antioxidants treatments on some Biochemical charecyerstics in semenal plasma. .(Means ± St. Error)

Treatment	MDA mol) /(nmol	GSH L) /(mmol	AST L) /(UI	ALT L) /(UI	CAT)Unit/mg protein)	SOD)Unit/ml)
Cont. gr.	1.010 c ± 0.084	أ 15.410 ± 5.011	a 66.442 ± 5.45	264.67 a ± 15.731	12.582 a ± 0.769	127.68 b ± 4.784
Turmeric gr.	1.300 b ± 0.10	ب 13.967 ± 0.5514	57.995 a ± 5.655	253.6 a ± 13.634	11.832 a ± 0.541	152.94 a ± 3.095
Vit.E gr.	1.455 b ± 0.255	ب 14.985 ± 0.484	65.906 a ± 5.703	261.25 a ± 15.095	12.028 a ± 0.4866	133.542 b ± 4.418
CoQ10 gr.	1.745 a ± 0.127	15.951 a ± 0.453	62.792 a ± 4.658	262.13 a ± 15.420	11.474 a ± 0.375	112.05 c ± 4.460

Means within different letters within grouping differ significantly ($p<0.05$).

Table No. 5 there is a highly significant effect ($A \leq 0.01$) of the treatments on the concentration of the enzyme Superoxidedismutase(SOD) in the semenal plasma, as it increased in the turmeric treatment (152.9 units/ml) compared to a decrease concentration in the control and vitamin E groups (127,689 and 133,542 Unit / ml) respectively, followed by the treatment of Coenzyme Q10 (112.05 units/ml) and this result came to clarify the role of turmeric as an antioxidant in raising the concentration of SOD enzyme in the plasma of rams semen in the treatment of turmeric, as it stimulates the effectiveness of antioxidant enzymes such as SOD (51). There is a significant negative correlation between antioxidants in seminal plasma and the percentage of dead sperm (52). Thus, reducing the consumption of the enzyme and raising its level in the seminalplasma. It be concluded that the addition of Curcuma to feed has an improved effect of semen properties; and increased the concentration of fructose in seminal plasma. Oral administration of vitamin E was increased the level of GSH and the globulin, also administration of Coenzyme Q10 was increased the level of SOD in the seminal plasma.

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