



**POTENTIAL IMPACT OF SPIRULINA ALGA AS AN
ANTIOXIDANT ON IMPROVING SEMEN PRODUCTION AND
OXIDATIVE STRESS IN BLOOD AND SEMINAL PLASMA OF
RABBIT BUCKS**

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ABSTRACT: This study aimed to evaluate the effect of *Spirulina platensis* on sexual desire, semen quality, and biochemical and antioxidant parameters in blood and seminal plasma of APRI rabbit bucks. Thirty bucks were divided into three groups (10 in each). Bucks in the 1st, 2nd and 3rd groups daily received drinking water supplemented with 0, 150, 300 mg spirulina/l for one month (treatment period), respectively. About one month of treatment, semen was collected and evaluated twice/week for ten weeks (20 ejaculates/group). At the end of the experiment, biochemical constituents, enzymatic activity and antioxidants status in blood and seminal plasma, as well as blood plasma testosterone were analyzed. Also, does (n=20/group) were naturally mated with five bucks from each group. Net semen volume, pH value, individual motility, livability, membrane integrity and concentration of spermatozoa, total sperm output per ejaculate and semen initial fructose increased ($P<0.05$), while reaction time, abnormality and acrosomal damage decreased ($P<0.05$) by both spirulina levels compared with control group. In blood and seminal plasma, total proteins, globulin, glutathione, glutathione peroxidase, glutathione S-transferase and superoxide dismutase increased ($P<0.05$), while aspartate aminotransferase and thiobarbituric acid-reactive substances ($P<0.05$) decreased by both spirulina levels. Testosterone and albumin in blood plasma increased ($P<0.05$) by both spirulina levels. Pregnancy rate and litter size improved ($P<0.05$) in does mated by bucks treated with both spirulina levels. *Spirulina platensis* administration at a level of 150 mg/l in drinking water for one month, could be effectively used for improving semen quality, oxidative status and the fertility of rabbit bucks.

Keywords: Spirulina, rabbit bucks, semen quality, antioxidants, fertility

INTRODUCTION

Buck reproductive efficiency is of economic importance, and using semen with high characteristics helps to avoid the valuable genotypes losses (Vizzarri *et al.*, 2019). Under oxidative stress, reactive oxygen species (ROS) generation rise the normal physiological process in animal tissue and organs including the testes. Rabbit spermatozoa display high metabolic activity and rich in polyunsaturated fatty acids (Castellini *et al.*, 2006), leading to increasing lipid peroxidation (Attia *et al.*, 2017) and thus they are sensitive to ROS attacks. Increasing lipid peroxidation results in reducing motility (Opuwari and Henkel, 2016), fragmentation of DNA and reducing sperm fertilizing ability (Attia *et al.*, 2019). Also, an excessive ROS production exceed the antioxidant capacity of the seminal plasma, leading to damaged mitochondria and membranes (acrosomal and plasma) of spermatozoa (Mizeraa *et al.*, 2019).

New alternative approaches to improve buck fertility kept under unfavorable reproductive seasons are demanded (Okab *et al.*, 2013). Practically, safe and economical application of several natural antioxidants resources could be useful to reduce negative impacts of oxidative stress on semen quality, thus improving reproductive efficiency of rabbit bucks (El-Desoky *et al.*, 2017).

An interest in spirulina alga as one of microalgae focused mainly on its rich contents of vitality important compounds, such as protein with all essential amino acids (60-70% by dry weight), vitamins (B₁₂ and β -carotene) and provitamin A (α -carotenes), polyunsaturated fatty acids, minerals and

phytopigments (Farag *et al.*, 2016). Spirulina alga posses impact as antioxidant, anti-inflammatory, antiviral, immune-modulatory, antitumor and probiotics properties (Farag *et al.*, 2016). In comparing with other synthetic products, animal reproduction was improved by spirulina treatment with low cost and good health status (Shanmugapriya *et al.*, 2015).

In rabbits, impact of spirulina on reproductive efficiency of does was reported by (El-Ratel, 2017). Recently, spirulina oral administration, at a level of 750 mg/buck for 5 weeks as a treatment period pre-semen collection, has importance as a treatment strategy for improving the buck reproductive performance (Fouda and Ismail, 2017). It is well known that the positive effects of spirulina are in dose depended manner and differ with treatment method, dietary, drinking water or oral administration (Bashandy *et al.*, 2016; El-Ratel, 2017). Therefore, the present study was designed to evaluate the antioxidant action of *Spirulina platensis* (SP) at different levels (0, 150 and 300 mg/l) in drinking water for one month pre-semen collection on reproductive performance, and biochemical properties and oxidative stress in blood and seminal plasma of rabbit bucks.

MATERIALS AND METHODS

This study was conducted at a private commercial rabbit farm, Mansoura City, Dakahlia Governorate, Egypt. The laboratorial work was carried out at Physiology and Biotechnology Laboratory, Animal Production Department, Faculty of Agriculture, Mansoura University, Egypt.

Spirulina platensis samples

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The SP in powder form was obtained from Alga Biotechnology Unit, National Research Center, Dokki, Egypt. Nutritional analysis and active composition of SP (amino acid and fatty acid profiles, and mineral and pigments composition) is summarized in Table 1. All analyses were performed at the Regional Centre for Food and Feed (RCFF), belonging to Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. All analyses were expressed on the basis of 100 g edible portion of SP. The nutritional analysis of SP samples was determined according to AOAC (2006), while active composition was determined according to AOAC (2000). Amino acid profile, vitamins and pigments were accomplished by using high performance liquid chromatography (HPLC), while fatty acid profile was analyzed using Gas-Chromatographic model GC-17A.

Animals and experimental design

Thirty healthy and adult APRI rabbit bucks with an average live body weight (LBW) of 3.05 ± 0.22 kg were housed individually in stainless steel cages batteries (40 × 50 × 35 cm) accommodated with feeders for pelleted rations and automatic fresh-water drinkers in a naturally ventilated and lighted rabbitry. The experimental bucks were fed *ad libitum* on a commercial pellet diet (17% crude protein, 14% crude fiber and 2850 Kcal digestible energy/kg), covering their daily nutritional requirements according to NRC (1977).

Rabbit bucks were divided into 3 experimental groups (10 bucks in each) according to LBW. Bucks in the 1st group received drinking water without any

supplementation (control group, G1), while those in the 2nd (G2) and 3rd (G3) groups were daily received drinking water supplemented with 150 or 300 mg SP/l, respectively, for 31 days (1 October to 31 October 2018) as a treatment period.

Live body weight and water consumption

Daily water consumption (ml/buck/day) and LBW (g/buck) were recorded as initial and final weight during the treatment period (1 October to 31 October 2018).

Semen collection and evaluation

Total of 10 bucks in each group were used as semen donors and semen was collected twice/week for 10 weeks (1 November 2018 to 8 January 2019) as a collection period of semen (200 ejaculates) in the morning by using an artificial vagina and a teaser doe. The reaction time as the time elapsed from introducing buck to teaser up to complete ejaculation was recorded. It was measured in seconds using a stopwatch.

All collected ejaculates were individually transferred after collection in graduated test tubes in a water bath (37°C) to the Laboratory. Ejaculate semen volume without gel and pH values (pen pH meter) was determined, semen characteristics were evaluated thereafter. Percentage of individual motility was determined in five microscopic fields for each semen sample using the aid of a microscope (Nikon E 200, China) at magnification of ×40 and assessed from 0 to 100%. Aliquots of raw semen (5 µl) were fixed using a vital eosin-nigrosin stain to allow later measurements of semen quality traits by examining 200 spermatozoa under microscope (Avishkar AVI-504 Advance

Research Binocular Microscope, Avishkar International, India), then live sperm (unstained sperm cells) and morphological abnormality (head, mid piece or tail defects) percentages were calculated. Sperm cell concentration ($\times 10^6/\text{ml}$) was determined after semen dilution (1: 99) by using the improved Neubauer hemocytometer slide (GmbH +Co., Brandstwierte 4, 2000 Hamburg 11, Germany).

Semen sample (10 μl) was gently mixed with 2 ml sucrose solution (osmolarity of 50 mOsm/kg) in a water bath (37 °C) for 30 minutes to assess hypo-osmotic swelling test (HOS-t). Spermatozoa with curled tails (membrane integrity) were considered responded to HOS-t. After incubation, 20 μl of the mixture was placed on a microscope slide and covered with a glass cover. In five sperm fields (100 sperm cells in each), number of spermatozoa with curled tails was counted at 1000 \times magnification, then membrane integrity was expressed as percentage of spermatozoa with curled tails (El-Seadawy *et al.*, 2017).

Acrosome integrity was determined by Giemsa stain according to Watson (1975). In briefly, fresh semen was extended (1:5) with warm normal saline, then smeared on glass slides, air-dried, fixed (10% neutral formal saline for 15 min), washed (running water for 20 min), stained with Giemsa solution overnight. The stained smear was rinsed in two changes of distilled water and air-dried. For acrosome integrity percentage, 100-sperm cells/sample were examined at 1000 \times in each field. Total sperm output (TSO) was calculated (El-Ratel, 2017b) as the following:

TSO = Ejaculate volume (ml) without gel \times sperm cell concentration/ml.

Testicular and epididymal characteristics

At the end of semen collection period, five bucks from each group were weighed and slaughtered, then testes and epididymis were immediately removed, trimmed of adhering connective tissue and fats. The separated testes and epididymis were dried and weighed to determine their relative weights. Also, testicular measurements (length, width and thickness) were recorded.

Semen and blood plasma constituents

In the last week of semen collection, blood samples were collected from ear vein of five bucks in each group into clean test tubes containing EDTA. Blood plasma as well as seminal plasma were separated by centrifugation at 3000 rpm for 20 min and stored at -20 °C, pending analysis. In blood and seminal plasma, total proteins (TP, g/dl) and albumin (Alb, g/dl) was determined using commercial kits purchased from Egyptian company for biotechnology (Obour City industrial area, Cairo, Egypt). Globulin (Glb, g/dl) concentration was calculated by subtracting Alb from TP. Activity of Aspartate (AST) and alanine (ALT) aminotransferase was determined using commercial kits (Diamond Diagnostics, Egypt) and spectrophotometer. In addition, total antioxidant capacity, glutathione content, glutathione peroxidase, glutathione S-transferase, superoxide dismutase and thiobarbituric acid-reactive substances were assayed using commercially available kits (Bio-diagnostic Co., Recycling Crusher-SBM®) and spectrophotometer. After collection, concentration of initial semen

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fructose was assayed immediately according to Mann (1948).

Blood testosterone

Testosterone concentration in blood plasma was determined by immunoassay commercial kits (Biosource-Europe S.A. 8, rue de L'Industrie.B-1400 Nivelles, Belgium).

Fertility trail

Total of 60 APRI nulliparous doe rabbits were naturally mated by randomly five treated bucks in each group. On day 10-12 post-mating, pregnancy diagnosis was performed abdominally to calculate pregnancy rate (Number of pregnant does/number of mated does x 100). Two days pre-expected date of parturition, doe cages were provided with nest boxes. After parturition, kindling rate (KR) was calculated as the following: $KR = (\text{Number of kindled does}/\text{number of pregnant does}) \times 100$. Total litter size at birth and live litter size after 12 h of kindling as well as litter size at weaning were recorded, and then viability rate of bunnies at birth and weaning was calculated. Bunnies were left with their dams during the suckling period and weaned on day 28 of age. Average bunny and litter weights at birth and weaning were recorded.

Statistical analysis

Data were analyzed as a randomized design using the General Linear Model procedure of SAS (2000), according to the following statistical model (one way analysis):

$Y_{ij} = \mu + A_i + e_{ij}$. Where: Y_{ij} = observed values, μ = general mean, A_i = effect of treatment (0, 150 and 300 mg/l water) and e_{ij} = random error.

Before data analysis, all sperm characteristics percentages were

subjected to logarithmic transformation ($\log_{10} x+1$) to normalize data distribution. Chi-Square test was used for analyzing the rates of pregnancy, kindling and viability. The group differences were set at $P < 0.05$ using Multiple Range Test (Duncan, 1955).

RESULTS

Body weight and water consumption

During treatment period, final and change in body weight of bucks increased ($P < 0.05$) only in G3, while these results showed that there was non-significant difference between G1 and G2. While, SP administration in the treated groups (G2 and G3) did not affect significantly the WDC values compared to (G_1) group (Table 2).

Testicular and epididymal characteristics, and sexual desire

Data in Table 3 indicated that there was non-significant difference between experimental groups (G2 and G3) and the control group (G_1) in testicular characteristics. Rabbits received SP levels caused to increase significant in epididymal weight compared to control group. These increases were pronounced with the high levels of SP (G3) being, 31%. Reaction time decreased ($P < 0.05$), while plasma testosterone increased ($P < 0.05$) in G2 and G3.

Semen quality

Net semen volume, pH value, percentages of individual motility, livability and membrane integrity of spermatozoa, sperm cell concentration, total sperm output and initial fructose concentration in raw semen increased ($P < 0.05$), while percentage of abnormality and acrosomal damage of spermatozoa decreased ($P < 0.05$) in G2 and G3 (Table 4).

Blood and seminal plasma constitutes

In blood plasma, total proteins increased ($P < 0.05$) as a result of increasing ($P < 0.05$) Alb and Glb in G2 and G3, reflecting similar Alb/Glb ratio in all groups. Activity of AST ($P < 0.05$) and ALT ($P \geq 0.05$) decreased in G2 and G3, respectively. In seminal plasma, TP increased ($P < 0.05$) in G2 and G3, as a result of increasing Glb ($P < 0.05$) and Alb ($P \geq 0.05$), reflecting marked reduction ($P < 0.05$) of Alb/Glb ratio in G3 only. Activity of AST and ALT reduced ($P < 0.05$) in G2 and G3 (Table 5).

The effect of SP treatment on TAC was not significant. However, GSH, GPx, SOD and GST levels increased ($P < 0.05$), and TBARS decreased ($P < 0.05$) in blood and seminal plasma of bucks in G2 and G3.

Fertility trail

Pregnancy rate, litter size at birth (total and live) and at weaning, viability rate at birth, bunny and litter weights at birth and at weaning of does mated with bucks were improved ($P < 0.05$) in G2 and G3 as compared to G1. However, kindling rate and viability rate at weaning were not affected ($P \geq 0.05$) by SP treatment (Table 7).

DISCUSSION

To achieve the aim of this study, the obtained results indicated positive effects of SP administration on the growth of bucks only at the highest level. This may be due to high poly-nutrients value and active composition of SP, in term of high contents from crude protein (57.40%), vitamins, minerals, essential amino acids, carotenoids, chlorophyll, and essential polyunsaturated fatty acids (Table 1). The present contents in SP were supported by Nedeva *et al.* (2014) and Farag *et al.*

(2016). Additionally, SP had antioxidant properties in similar pattern with the positive effect of dietary antioxidants, such as selenium, folic acid and their combinations on body weight of rabbit bucks (Kamel, 2012). Also, impact of dietary addition of SP was reported on doe rabbits (El-Ratel, 2017a). Regarding the effect of SP in the present study, both levels of SP indicated marked improvement in sexual desire parameters of bucks. Similar results were reported on bucks orally treated with SP (750 mg/buck/d) for five weeks pre-semen collection (Fouda and Ismail, 2017) or with red algae (Ali and Mervat, 2013). As expected, potential SP effects on semen characteristic, in terms of increasing net semen volume, and motility, viability, membrane integrity, concentration and total output/ejaculate of spermatozoa, initial fructose level in semen; decreasing sperm abnormality and acrosomal damage. These findings were supported in rabbit (Fouda and Ismail, 2017) and in rats (Bashandy *et al.*, 2016).

Enhancement in semen quality of bucks in treated groups in the present study explained to be due to antioxidant components (Table 1), which have the ability to prevent the cell damage through increasing of the enzymes of the sperm antioxidant defense system as mentioned by El-Tohamy *et al.* (2012) of SP. It is worthy noting that improving semen quality parameters in the present study were paralleled with increasing ($P < 0.05$) the sexual desire, reflecting positive effects on production of buck semen. It is well known that, testosterone is required for spermatogenesis and maturation of sperm cells (Walker, 2009), resulting in marked reduction in abnormality and

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acrosomal damage. Increased pH value in semen of treated groups was associated with increasing sperm cell concentration and semen volume. However, increasing semen volume may be attributed to increasing testosterone of bucks in treatment groups, which increase accessory sex glands activity. Concentration of semen initial fructose was found to have a positive relationship with most sperm characteristics (Ayoub *et al.*, 2000). This was proved in the present study when semen fructose concentration increased in treatment groups (Table 4). In addition, for measuring sperm membrane stability, activity of AST and ALT in the seminal plasma is good indicator of semen quality (Dogan *et al.*, 2009). The observed reduction in AST and ALT activities in the seminal plasma was in strong relationship with improving semen quality. Moreover, the decreased AST and ALT activities was in relation with decreasing sperm damage and increasing sperm livability and sperm cell concentration (Al-Daraji *et al.*, 2010). Similarly, Fouda and Ismail (2017) found increased TP concentration and decreased ($P<0.05$) AST and ALT activity in seminal plasma of rabbits treated with SP. Also, rabbit treated with red algae significantly reduced activity of AST and ALT in semen (Ali and Mervat, 2013). Improving sexual desire and consequently semen quality was in association with increasing ($P<0.05$) TP and their fraction as well as decreasing AST activity in blood plasma in G2 and G3, in term of increased protein metabolism and improvement of liver function. In this context, Bhattacharyya and Mehta (2012) mentioned that SP may have protective

property against dysfunction of the liver. Also, pronounced impact of SP as a dietary supplementation was shown on protein metabolism and liver function of doe rabbits (El-Ratel, 2017a) and pigs (Nedeva *et al.*, 2014). This improvement in protein metabolism was attributed to containing SP high crude protein, essential amino acids, vitamins, minerals, phospholipids and antioxidants (Farag *et al.*, 2016). It is of interest to note marked relationship between level of TP, Alb and Glb in blood and seminal plasma. Both treatments increased level of TP and their fraction and decreased AST activity in blood and seminal plasma. Results of antioxidant status showed similar trend of change in blood and seminal plasma of each group, but their levels were almost higher in blood plasma than in seminal plasma. These results indicated marked increase in the antioxidant enzyme activity and reducing lipid peroxidation for bucks as affected by SP. In accordance with the present results, SP treatment had improved oxidative capacity in blood of doe rabbits (El-Ratel, 2017a) and fattening lambs (El-Sabagh *et al.*, 2014) as compared to the control. Also, SP administration significantly improved antioxidant status and decreased lipid peroxidation in seminal plasma of rabbit bucks (Fouda and Ismail, 2017) and mice (Hwang *et al.*, 2011). The observed reduction in lipid peroxidation of bucks in G2 and G3 is parallel with improving in their semen quality. Reducing ROS generation from lipid peroxidation improves motility, integrity of plasma membrane and function of mitochondria in sperm cells (Attia *et al.*, 2019). Animal spermatozoa have two systems of defense against the

ROS generation: 1) SOD for catalyzing the O₂ anion remove, and 2) GP_X required for intracellular reduced GSH (Storey, 2008). Seminal plasma contains SOD, catalase and some reductases, as endogenous antioxidants, for inactivating generation of ROS, leading to protecting the spermatozoa (Faustini *et al.*, 2004). Semen quality of bucks is the main factor for determining the reproductive efficiency of rabbit does (Attia *et al.*, 2017). The obtained results indicated higher fertilizing ability of spermatozoa of bucks treated with SP as antioxidants. This improvement was associated with pronounced reduction in the oxidative stress for decreasing injury sperm damage, resulting in an enhancement in male fertility (Attia *et al.*, 2015; Calogero *et al.*, 2017). Similar results were reported by Fouda and Ismail (2017) on semen of bucks treated with SP.

CONCLUSION

Based on the foregoing results, spirulina treatment improved sexual desire, semen production, and antioxidant capacity of APRI rabbit bucks. This study recommended the importance of supplying drinking water of breeding rabbit bucks with *Spirulina platensis* (150 mg/l) for one month at least before mating of semen collection of artificial insemination.

Spirulina, rabbit bucks, semen quality, antioxidants, fertility

Table (1): Nutritional analysis and active composition of *Spirulina platensis* powder

Item	g/100 g	Item	g/100 g
Nutritional analysis:			
Crud protein	57.40	Carbohydrate	23.52
Crud fat	1.50	Fiber	21.85
Amino acid profile:			
Aspartic acid	5.65	Tyrosine	3.62
Threonine	1.90	Phenylalanine	2.45
Serine	2.62	Histidine	0.98
Glycine	3.10	Lysine	2.78
Glutamic acid	7.34	Arginine	3.09
Alanine	2.40	Proline	2.35
Valine	4.25	Cystine	0.57
Isoleucine	3.30	Methionine	1.20
Leucine	4.79		
Vitamins composition:			
Vitamin (A)	98.24 µg	Vitamin (B1)	3.74
Vitamin (B2)	5	Vitamin (B3)	11.20
Vitamin E	6.12	Phanthothenic acid	0.3
Fatty acid profile:			
Caprylic acid	1.52	Heptadecenoic acid ω-9	0.26
Capric acid	30.00	Hexa decatrienoice acid	0.35
Myristic acid	0.37	Vaccinic acid	4.20
Tetradecenoic acid	0.60	Linoleic acid	15.20
Pentadecenoic acid	0.30	Gamma-Linolenic acid	10.21
Palmitic acid	25.19	Eicosaenoic acid	0.27
Palmitoleic acid	0.82	C18:2 ω5	1.90
Stearic acid	1.82	Hexadecenoic ω7	2.63
Oleic acid ω-9	3.25	Non identified fatty acid	1.11
Mineral composition:			
Calcium	600	Phosphorus	700
Copper	1.1	Potassium	1200
Iron	100	Sodium	900
Manganese	6.00	Zinc	3.00
Pigment composition:			
Carotenoids	400	C-Phycocyanin	13,000
Chlorophyll	950		

Table (2): Average body weight, and consumption from drinking water and spirulina of rabbit bucks as affected by *Spirulina platensis* administration during the treatment period

Item	<i>Spirulina platensis</i> level			±SEM	P-Value
	G1 (Control)	G2 (150 mg/l)	G3 (300 mg/l)		
Initial buck body weight (kg)	3.060	3.063	3.040	0.222	0.8465
Final buck body weight (kg)	3.313 ^b	3.379 ^{ab}	3.460 ^a	0.760	0.0516
Change in body weight (kg)	0.253 ^b	0.316 ^{ab}	0.424 ^a	0.047	0.0510
Daily water consumption (ml/buck/day)	192.20	195.41	196.15	5.651	0.4213
Daily spirulina intake (mg/buck/day)	-	29.31	58.85	-	-

Different lower case letters indicate significant differences (P<0.05).

Table (3): Testicular and epididymal characteristics, libido and blood plasma testosterone concentration of rabbit bucks as affected by *Spirulina platensis* administration

Item	<i>Spirulina platensis</i> level			±SEM	P-Value
	G1 (Control)	G2 (150 mg/l)	G3 (300 mg/l)		
Pre-slaughtered weight of bucks (g)	3300.67	3360.60	3406.67	-	-
Relative testicular weight (%)	0.198	0.208	0.209	0.013	0.0824
Average testicular length (cm)	3.15	3.16	3.17	0.050	0.9858
Average testicular width (cm)	1.14	1.16	1.17	0.041	0.8915
Average testicular thickness (cm)	0.91	0.91	0.92	0.016	0.9549
Sexual desire					
Reaction time (s)	22.20 ^a	15.30 ^b	12.10 ^c	0.828	0.001
Plasma testosterone (ng/ml)	1.75 ^b	2.64 ^a	2.66 ^a	0.113	0.001

Different lower case letters indicate significant differences (P<0.05).

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Table (4): Semen quality parameters of rabbit bucks as affected by *Spirulina Platensis* administration

Item	<i>Spirulina platensis</i> level			±SEM	P-Value
	G1 (Control)	G2 (150 mg/l)	G3 (300 mg/l)		
Net semen volume (ml)	0.645 ^b	0.730 ^a	0.736 ^a	0.008	0.001
Semen pH value	7.24 ^b	7.37 ^a	7.43 ^a	0.028	0.001
Progressive sperm motility (%)	71.50 ^b	82.00 ^a	82.50 ^a	1.244	0.001
Live sperm (%)	73.40 ^b	80.60 ^a	81.90 ^a	0.913	0.001
Abnormal sperm (%)	21.70 ^a	12.30 ^c	13.70 ^b	0.809	0.001
Sperm cell concentration (×10 ⁶ /ml)	428.00 ^c	591.40 ^a	577.90 ^b	13.788	0.001
Acrosomal damage (%)	14.80 ^a	11.50 ^b	11.80 ^b	0.375	0.001
Sperm membrane integrity (%)	24.60 ^c	36.20 ^a	32.50 ^b	0.971	0.001
Total sperm output/ejaculate (×10 ⁶)	275.95 ^b	431.72 ^a	425.33 ^a	13.486	0.001
Initial semen fructose (mg/dl)	160.60 ^b	242.80 ^a	236.20 ^a	10.071	0.001

Different lower case letters indicate significant differences (P<0.05).

Table (5): Biochemical constituents and enzymatic activity in blood and seminal plasma of rabbit bucks as affected by *Spirulina platensis* administration

Item	<i>Spirulina platensis</i> level			±SEM	P-Value
	G1 (Control)	G2 (150 mg/l)	G3 (300 mg/l)		
Blood plasma					
Total proteins (g/dl)	5.82 ^b	6.37 ^a	6.30 ^a	0.048	0.0004
Albumin (Alb, g/dl)	3.05 ^b	3.25 ^a	3.23 ^a	0.032	0.008
Globulin (Glb, g/dl)	2.77 ^b	3.12 ^a	3.07 ^a	0.065	0.0141
Alb/Glb ratio	1.10	1.04	1.05	0.032	0.413
AST (IU/l)	20.22 ^a	12.83 ^b	12.75 ^b	1.386	0.014
ALT(IU/l)	16.02	11.09	11.13	1.992	0.213
Seminal plasma					
Total proteins (g/dl)	3.69 ^b	4.23 ^a	4.28 ^a	0.096	0.009
Albumin (Alb, g/dl)	2.09	2.25	2.20	0.051	0.162
Globulin (Glb, g/dl)	1.60 ^b	1.98 ^a	2.08 ^a	0.08	0.015
Alb/Glb ratio	1.31 ^a	1.15 ^{ab}	1.06 ^b	0.056	0.050
AST (IU/l)	27.400 ^a	18.800 ^b	16.400 ^b	1.400	0.001
ALT(IU/l)	19.600 ^a	14.180 ^b	14.156 ^b	0.801	0.001

Different lower case letters indicate significant differences (P<0.05). AST: aspartate aminotransferase and ALT: alanine aminotransferase.

Table (6): Oxidative capacity in blood and seminal plasma of rabbit bucks as affected by *Spirulina Platensis* administration

Item	<i>Spirulina platensis</i> level			±SEM	P-Value
	G1 (Control)	G2 (150 mg/l)	G3 (300 mg/l)		
Blood plasma					
TAC (mmol/l)	1.01	1.18	1.19	0.051	0.0800
GSH (mg/dl)	16.45 ^b	21.78 ^a	21.80 ^a	1.371	0.0517
GPx (mg/dl)	6.93 ^b	7.85 ^a	7.87 ^a	0.135	0.0041
GST (IU)	1.20 ^b	1.33 ^a	1.34 ^a	0.025	0.0119
SOD (IU)	6.45 ^b	7.16 ^a	7.14 ^a	0.039	0.0001
TBARS (nmol/ml)	1.52 ^a	1.08 ^b	1.11 ^b	0.043	0.0006
Seminal plasma					
TAC (mmol/l)	0.92	1.10	1.12	0.063	0.1231
GSH (mg/dl)	12.99 ^b	17.52 ^a	18.12 ^a	0.701	0.001
GPx (mg/dl)	6.142 ^b	7.572 ^a	7.664 ^a	0.188	0.001
GST (IU)	1.09 ^b	1.32 ^a	1.27 ^a	0.029	0.001
SOD (IU)	6.68 ^b	8.71 ^a	8.83 ^a	0.271	0.001
TBARS(nmol/ml)	1.44 ^a	1.07 ^b	1.05 ^b	0.050	0.001

Different lower case letters indicate significant differences (P<0.05). TAC: Total antioxidant capacity, GSH: glutathione content, GPx: glutathione peroxidase, GST: glutathione S-transferase, SOD: superoxide dismutase and TBARS: thiobarbituric acid-reactive substances.

Table (7): Reproductive performance of rabbit does naturally mated by bucks in the experimental groups

Reproductive parameter	<i>Spirulina Platensis</i> level			±SEM	P-Value
	G1 (Control)	G2 (150 mg/l)	G3 (300 mg/l)		
Pregnancy rate	14/20 (70) ^b	17/20 (85) ^a	16/20 (80) ^{ab}	-	-
Kindling rate	11/14(78.6)	14/17(82.4)	12/16(75.0)	-	-
Total litter size at birth (n)	6.73 ^b	8.17 ^a	8.36 ^a	0.219	0.01
Live litter size at birth (n)	5.18 ^b	7.57 ^a	7.08 ^a	0.229	0.001
Viability rate at birth	77.89 ^b	90.68 ^a	87.54 ^a	-	-
Litter size at weaning (n)	4.91 ^b	7.29 ^a	6.75 ^a	0.224	0.001
Viability rate at weaning	94.85	96.41	95.49	-	-
Average bunny weight at birth (g)	44.09 ^b	52.50 ^a	50.92 ^a	0.685	0.001
Average bunny weight at weaning (g)	420.82 ^c	555.43 ^a	547.58 ^b	10.024	0.001
Litter weight at birth (g)	228.37 ^b	397.43 ^a	360.51 ^a	14.394	0.001
Litter weight at weaning(g)	2066.22 ^b	4049.08 ^a	3696.17 ^a	162.479	0.001

Different lower case letters indicate significant differences (P<0.05).

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Spirulina, rabbit bucks, semen quality, antioxidants, fertility

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الملخص العربي

التأثير المحتمل لطحلب الاسبيرولينا كمضاد للأكسده على تحسين انتاج السائل المنوى والاجهاد التأكسدى فى الدم والبلازما المنوية لذكور الارانب

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هدفت هذه الدراسة الى تقييم تأثير طحلب الاسبيرولينا على الرغبه الجنسيه، جودة السائل المنوى وخصائص الدم البيوكيميائية وحالة مضادات الأكسده فى الدم والبلازما المنوية لذكور الارانب. استخدم فى هذه الدراسة عدد 30 من ذكور الأرانب (ابري) الناضجه جنسيا، تم تقسيمها الى 3 مجموعات (10 ذكور فى كل مجموعة). كانت الذكور فى المجموعة الاولى (كنترول)، الثانية و الثالثة تشرب ماء مضاف اليه صفر، 150 و 300 ملجرام طحلب الاسبيرولينا/ لتر، يوميا ولمدة شهر (مدة المعاملة)، على التوالى. بعد المعاملة مباشرة، تم جمع السائل المنوى وتقييمه مرتين اسبوعيا لمدة 10 اسابيع (200 قذفه/مجموعه). فى نهاية التجربة تم تقدير مكونات الدم البيوكيميائية، نشاط الانزيمات وحالة مضادات الاكسده فى الدم والبلازما المنوية وكذلك تقدير تركيز هرمون التستوستيرون فى بلازما الدم. تم تلقيح 20 أم ابرى فى كل مجموعة طبيعيا بعدد خمس ذكور من كل مجموعة. ادت المعاملة بطحلب الاسبيرولينا (150 و 300 ملجم/لتر) الى:-

- 1- زيادة معنوية ($P<0.05$) فى حجم القذفة المنوية ، قيمة الاس الهيدروجينى والنسبة المئوية للحركة التقدمية، الحيوية، سلامة الغشاء وتركيز الحيوانات المنوية، التركيز الكلى للحيوانات المنوية فى القذفة وتركيز الفركتوز الاولى فى السائل المنوى.
 - 2- انخفاض معنوى ($P<0.05$) فى وقت رد الفعل والنسبة المئوية للحيوانات المنوية الشاذة وشذوذ الاكروسوم.
 - 3- زيادة معنوية ($P<0.05$) فى تركيز البروتين الكلى، الجلوبيولين، ونشاط الجلوتاثيون، الجلوتاثيون بيروكسيداز، الجلوتاثيون اس- ترانسفيراز و السوبر اوكسيد ديسميوتاز.
 - 4- انخفاض معنوى ($P<0.05$) فى نشاط الأنزيمات الناقله لمجموعة الأمين ومواد حمض الثيوباربيتوريك التفاعلية.
 - 5- زيادة معنوية ($P<0.05$) فى تركيز هرمون التستوستيرون والاليومين فى بلازما الدم.
 - 6- تحسن معنوي ($P<0.05$) فى معدل الحمل وحجم البطن فى الامهات الملقحة طبيعيا.
- نستخلص من هذه الدراسة: معاملة ذكور الارانب بـ 150 ملجرام طحلب الاسبيرولينا / لتر ماء شرب يوميا ولمدة 30 يوم على الأقل يمكن استخدامها بشكل فعال لتحسين جودة السائل المنوي، حالة الاجهاد التأكسدى والخصوبة لذكور الارانب .