# EFFECT OF *SPIRULINA PLATENSIS* ON REPRODUCTIVE PERFORMANCE OF RABBIT BUCKS

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## SUMMARY

This study was designed to investigate reproductive performance of adult New Zealand White (NZW) rabbit bucks as affected by oral administration of *Spirulina platensis* (*S. platensis*). Bucks were randomly distributed into 2 homogeneous groups (10 in each). The first group were orally received 3 ml distilled water (control), while those in the second group were orally treated with 3 ml distilled water containing *S. platensis* at a level of 700 mg/buck as daily oral for 5 weeks before semen collection period. Semen was collected twice weekly for 10 successive weeks by artificial vagina. On day of semen collection, reaction time (RT) and physical semen characteristics were determined. During the last week of collection, concentration of testosterone in blood serum (STC), and some biochemicals and antioxidant activity in seminal plasma were estimated. Results showed that the *S. platensis* orally administration bucks improved ($P<0.01$) LBW, testicular ($P<0.001$) and epididymal ($P<0.01$) weights, testicular length ($P<0.01$) and STC ($P<0.001$), and decreased RT ($P<0.01$) of bucks as compared to controls. Ejaculate volume and percentages of progressive sperm motility, livability, normality, and acrosomal damage as well as sperm cell concentration and total sperm output (motile, motile normal, motile live and total functional) significantly improved by *S. platensis* compared with controls. Also, semen pH value decreased ($P<0.05$) by *S. platensis* treatment. Concentrations of total fructose and total proteins were significantly higher in seminal plasma as affected by *S. platensis* treatment, while, concentrations of albumin (AL), globulin (GL) and AL: GL ratio were not affected by *S. platensis* treatment. Activity of total antioxidant capacity, glutathione S-transferase, superoxide dismutase, and glutathione peroxidase significantly increased, while TBARS concentration significantly decreased in seminal plasma of bucks treated with *S. platensis*, however, glutathione concentration was not affected. Conception rate and kindling rate, litter size and average bunny weight at birth improved in doe rabbits mated with bucks treated with *S. platensis*. In conclusion, *S. platensis* at a level of 700 mg/buck as oral administration for 5 weeks period to be used in natural mating or artificial insemination is recommended to be serving as a supportive treatment in the nutritional management to improve semen production of rabbit bucks, and fertility of doe rabbits mated with these bucks.

***Keywords:*** *Rabbit bucks, spirulina platensis, semen quality, fertility.*

## INTRODUCTION

Reproduction of rabbits plays a vital role in profitability of rabbits breeding (Ghazal *et al.*, 2016). Artificial insemination (AI) is widely used on commercial scale in rabbit production and semen quality is the guarantee of successful AI in breeding rabbits (Castellini, 2008). Several endogenous and exogenous factors were reported to affect semen quality of rabbit (Sheteifa and Morsy, 2014). Appropriate protocols are needed to improve different characteristics of rabbit spermatozoa (Brun *et al.*, 2002).

For improving the reproductive performances of animals, there was a development in biological farm system producers to use natural active compounds such as microalgae (Kistanova *et al.*, 2009). Recently, microalga production such as blue-green alga (*Spirulina*), which is considered a promising microorganism, has a great attention to be a good alternative dietary source of proteins (Alvarenga *et al.*, 2011). These microorganisms are classified into *Spirulina platensis (S. platensis), Spirulina fusiformis* and *Spirulina maxima* (Karkos *et al.*, 2011).

*Spirulina* alga (SA) is a primitive planktonic photosynthetic filamentous cyanobacterium that has a simple structure but a complex composition. It has high feeding value with wide range of medicinal applications (Abu-Elala *et al.*, 2016). The SA contains important compounds, in term of high protein

content (60-70% on dry matter basis) with all essential amino acids (Farag *et al.,* 2016), vitamins ($B_{12}$ and β-carotene), poly-unsaturated fatty acids (γ-linolenic acid), and minerals (Ca, Cr, K, Mg, Cu, Fe, Na, P, Mn, Zn and Se (Hoseini *et al.,* 2013). It also contains many photosynthetic pigments (phycocyanobilin chlorophyll and xanthophyll phytopigments) as reported by Gong *et al.* (2005); Bermejo *et al.* (2008), that makes SA a promising new dietary resource supporting the future production requirements of animals. The SA has various biological activities, impact effects as antioxidant (Kurd and Samavati, 2015), anti-inflammatory (Vide *et al.,* 2015), antiviral, immune-modulatory (Sahan *et al.,* 2015), antitumor (Konickova *et al.,* 2014) and probiotics (Shanmugapriya *et al.,* 2015) properties. In addition, *SA* is believed to reduce toxicity, increase palatability and digestibility, and protected many organs against many drugs and toxic chemicals (Abdel-Daim *et al.,* 2013). *Spirulina* treatment was reported to improve productivity and reproduction of animals and poultry, without side effects and more costs as compared to other synthetic products (El-Sabagh *et al.,* 2014; Shanmugapriya *et al.,* 2015).

The reproductive efficiency of rabbits depends on semen quality of bucks, environmental factors and the physiological status of the does (Theau-Clement and Roustan, 1992). The exposure of males to cold or heat stress, wind, ventilation, moisture, light and solar radiation can affect negatively on fertility by inducing the oxidative stress (Alejandro *et al.,* 2014). This stressor leads to increasing free radicals accumulation, which causes damage of cell membrane DNA fragmentation of spermatogenic cells (El-Desoky *et al.,* 2013). Normal sperm function needs a low level of reactive oxygen species (ROS) (El-Tohamy and El-Nattat, 2010). However, ROS levels more than the total antioxidant capacity caused oxidative stress in the semen occurs, which clearly impair fertility (El-Tohamy and El-Nattat, 2010). Antioxidants protect cellular components from the damaging by cellular free radicals and ROS. When antioxidants are absent, at suboptimal level or not available at the precise place within the cell, where free radicals are formed, damage can occur (El-Tohamy *et al*., 2012).

Therefore, the specific aim of the present study was to investigate the efficacy of oral *S. platensis* administration on reproductive performance of New Zealand White (NZW) rabbit bucks, in terms of physical semen characteristics, and biochemical properties, antioxidant activity in seminal plasma.

## MATERIALS AND METHODS

### *Animals:*

Twenty NZW rabbit bucks at 6-7 months old and of 2750-2850 g live body weight (LBW) were used in this study. Bucks were randomly distributed into 2 homogeneous groups (10 in each) and allowed to acclimatize for one week before the beginning of experiment. Bucks were individually housed in galvanized wire mesh cages provided with feeders and automatic stainless steel nipple drinkers. All bucks were fed *ad libitum* on a commercial complete pelleted diet throughout the experimental period.

Bucks in the first group were orally received 3 ml distilled water (control), while those in the second group were orally treated with 3 ml distilled water containing *S. platensis* at a level of 700 mg/buck daily for 5 weeks as a treatment period.

### *Collection and evaluation of semen:*

Semen was collected twice weekly for 10 weeks as a collection period from all bucks using an artificial vagina maintained at 42-45°C and a teaser doe. On day of semen collection, reaction time (RT) was estimated as the time elapsed from introducing buck up to complete ejaculation. Immediately after semen collection, ejaculates were kept at 37 °C in water bath and transferred to the laboratory.

Semen was evaluated for ejaculate volume (EV) without gel mass and for pH value determination using a pH paper (Spezial-Indikatorpapier pH 5.5-9.0, MACHEREY-NAGEL. Germany). Also, percentages of progressive motility (PSM), livability (LS), and abnormality of spermatozoa (AS) were determined in each ejaculate at 37°C. Sperm cell concentration (SCC) was estimated using Neubauer heamocytometer slide. Percentage of sperm with acrosomal damage (ASD) was determined by Giemsa stain procedure (Watson, 1975). In each ejaculate, total sperm output (TSO) was calculated by multiplying semen EV (ml) by SCC/ml; motile sperm output (MSO) was calculated by multiplying PSM (%) by TSO; motile normal sperm output (MNSO) was calculated by multiplying normal sperm (%) by TSO; motile live sperm output (MLSO) was calculated by multiplying LS by TSO, while total functional sperm fraction (TFSF) as the product of TSO by multiplying percentage of PSM x normality x livability (Correa and Zavos, 1994**).**

***Serum testosterone concentration:***

Blood samples were collected from ear vein of 3 bucks in each group. Serum was obtained by blood centrifugation at 3000 rpm for 15 minutes and carefully decanted into tubes and stored in a deep freezer at -20 $^{o}$C until analysis. Serum testosterone concentration was analyzed by immunoassay (Biosource-Europe S.A. 8, rue de L'Lndustrie.B-1400 Nivelles. Belgium).

***Biochemical analysis of seminal plasma:***

Seminal plasma samples from all bucks were taken at the end of collection period by centrifugation of fresh semen for 15 minutes at 3000 rpm, then seminal plasma was separated and stored in deep freezer at -20$^{o}$C until analyses of concentration of total protein, albumin, globulin and total fructose as well as enzyme activity of aspartate (AST) and alanine (ALT) aminotransferases, acid (ACP) and alkaline (ALP) phosphatases using commercial kits (Bio-diagnostic Co., Recycling Crusher-SBM®).

***Oxidative capacity in seminal plasma:***

Lipid peroxidation biomarkers, including total antioxidant capacity, TAC (Erel, 2004), glutathione content, GSH (Beutler *et al.,* 1963), glutathione peroxidase, GPx (Chiu *et al.*,1976), glutathione S-transferase, GST (Habig *et al.*,1974), superoxide dismutase, SOD (Misra and Fridovich, 1972) and thiobarbituric acid-reactive substances, TBARS (Tappel and Zalkin, 1959) were assayed in seminal plasma using commercially available kits (Bio Diagnostic Research).

***Testicular measurements:***

At the end of semen collection period, three 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 and width) were recorded.

***Fertility trails:***

For fertility evaluation, five bucks from each group were used for natural mating of 20 NZW nulliparous doe rabbits. Palpation of mated doe rabbits was carried out 10-12 days post-mating to determine conception rate (%). Also, kindling rate, total number of borns (live and dead/doe) and viability rate at birth, and bunny and litter weights were recorded at birth.

***Statistical Analysis:***

The data obtained were analyzed using independent T- test using SAS (2002) software for control and treated groups.

## RESULTS AND DISCUSSION

***Effect of S. platensis administration on:***

***Live body weight and reproductive organ characteristics:***

Rabbit bucks orally administrated with *S. platensis* at a level of 700 mg/buck daily were significantly ($P<0.01$) heavier than those in control group (Table 1). Impact of *S. platensis* dietary addition on growth performance of rabbits was indicated also in broiler chicks (Shanmugapriya *et al.,* 2015; Pandav and Puranik, 2015), fattening lambs (El-Sabagh *et al.,* 2014) and Nile tilapia (Abu-Elala *et al.,* 2016). The observed positive effect of *S. platensis* as an antioxidant on growth performance of rabbits in the present study is in agreement with the effect of different dietary additives such as selenium, folic acid and their combinations on increasing LBW of rabbit bucks (Kamel, 2012). Such effect of *S. platensis* on animal growth may be due to high contents of digestible protein, vitamin $B_{12}$, amino acids and minerals (Farag *et al.,* 2016). Also, bioactive constituents of *S. platensis* such as β-carotene, phycocyanin, γ-linolenic acid and phenolic compounds give this type of macrophytes its powerful antioxidant, antimicrobial, immunostimulant, and resistance against diseases (Lin *et al* ., 2010).

Regarding the testicular and epididymal characteristics, absolute and relative weights of testes and epididymis of rabbit bucks increased significantly due to oral administration of *S. platensis c*ompared with control group ($P<0.001$ and $P<0.01$, respectively). Only, testicular length of treated bucks was significantly ($P<0.01$) longer than those of control bucks, while testicular width was not affected significantly by *S. platensis* treatment (Table 1).

**Table (1): Effect of *S. platensis* treatment on live body weight and reproductive organ characteristics of NZW bucks.**

| Item | Control group | Treatment group | T-Value | P-Value |
|---|---|---|---|---|
| Initial live body weight[1] | 2835±26.93 | 2839±26.04 | 0.12 | 0.90 $^{NS}$ |
| Final live body weight[1] | 3067±20.63 | 3175.0±34.36 | 0.12 | 0.01** |
| Testicular weight (g) | 6.13±0.08 | 6.92±0.06 | 7.34 | 0.001*** |
| Relative testicular weight (%) | 0.200±0.00 | 0.213±0.01 | 4.00 | 0.01** |
| Epididymal weight (g) | 2.22±0.02 | 2.82±0.04 | 12.73 | 0.001*** |
| Relative epididymal weight (%) | 0.070±0.00 | 0.083±0.01 | 4.00 | 0.01** |
| Testicular measurements (cm): | | | | |
| Length | 2.85±0.03 | 3.08±0.04 | 4.43 | 0.01** |
| Width | 1.15±0.03 | 1.22±0.02 | 2.00 | 0.10 $^{NS}$ |

*$^{NS}$: Not significant. ** Significant at P<0.01. *** Significant at P<0.001. [1]Initial and final weights of treatment period.*

The noted increase in LBW and testicular weight (absolute and relative) is in association with increasing their length not in width. In this respect, testicular weight is largely dependent on the mass of the differentiated spermatogenic cells and reduction in the testicular weight indicates germ cell loss (Sarkar *et al.,* 2003). In rabbit bucks, Ghazal *et at.* (2016) also showed that supplementing doum (*Hyphaene thebaica*) to diets caused significant improvement of testicular and epididymal weights, sexual accessory glands and pituitary gland. In male rats, Sudha *et al.* (2010) found that methanolic extract of Moringa leaves enhanced seminiferous tubules, testis and epididymal weight. These findings may indicate that administration of *S. platensis* improved LBW, testicular characteristics and epididymal weight, which may reflect an impact on semen production of rabbit bucks.

***Sexual desire:***

Results regarding the sexual desire of rabbit bucks indicated significant reduction in reaction time (RT, P<0.001) and marked increase in serum testosterone concentration (STC, P<0.01) in treated than in control bucks. This means that *S. platensis* treatment improved libido of rabbit bucks (Fig. 1).

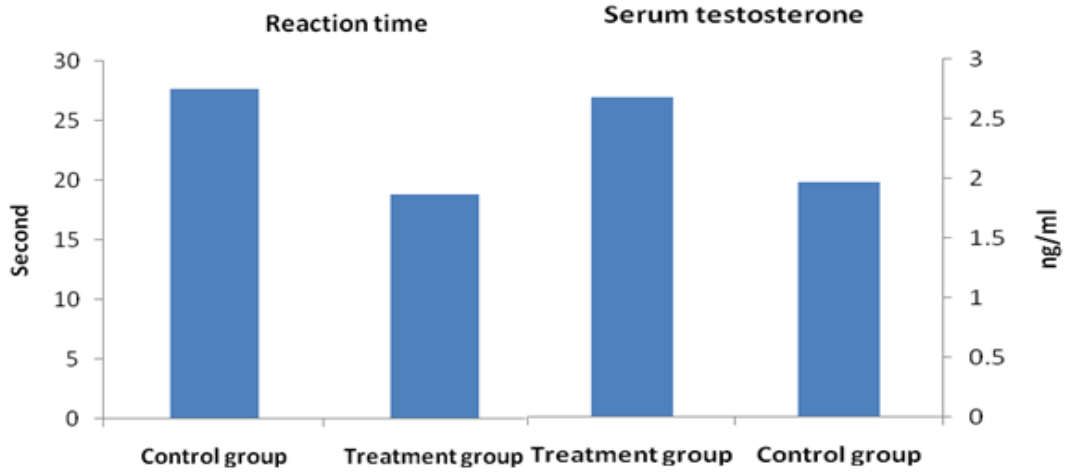


**Fig. (1): Effect of *S. platensis* treatment on sexual desire NZW bucks.**

In this respect, an improvement in total sexual libido (RT and STC) was reported in rabbit bucks treated with selenium plus vitamin E combination (Yassen *et al.*,2016), royal jelly as natural active compounds (El-Hanoun *et al.,* 2014), *red algae* (Ali and Mervat, 2013) or lycopene addition as natural antioxidants (Mangiagalli *et al.,* 2012). The improvement in libido of treated bucks may be due to antioxidant components of *S. platensis* (Rezvanfar *et al.,* 2008). Testosterone is main male sex hormone, which plays a crucial role in the suitable development of reproductive organs and in the maintenance of male sexual characteristics.

***Physical semen characteristics:***

Oral administration of *S. platensis* significantly improved all physical semen characteristics, including ejaculate volume (EV), progressive sperm motility (PSM), live sperm (LS), abnormal sperm (AS), acrosomal damage (ASD) and sperm cell concentration (SCC), and sperm output, involving total, motile, motile normal, motile live and total functional spermatozoa compared with untreated group. On the other hand, semen pH value significantly (P<0.05) decreased in treated than in control buck semen (Table2). The positive influence of *S. platensis* on semen volume and sperm quality was established in boars (Kistanova *et al.,* 2009), and on sperm motility and semen concentration in rats (El-Desoky *et al.,* 2013; Bashandy *et al.,* 2016).

**Table (2): Effect of *S. platensis* treatment on physical semen characteristics and sperm output of NZW bucks.**

| Item | Control group | Treated group | T-Value | P-Value |
|---|---|---|---|---|
| Physical semen characteristics: | | | | |
| Ejaculate volume (ml) | 0.52±0.08 | 0.71±0.02 | 2.30 | 0.04* |
| Progressive sperm motility (%) | 67.14±2.64 | 79.29±1.30 | 4.12 | 0.001*** |
| Live sperm (%) | 73.57±1.43 | 84.14 ±0.70 | 6.64 | 0.001*** |
| Live : dead sperm ratio | 2.86±0.23 | 5.37±0.24 | 7.65 | 0.001*** |
| Abnormal sperm (%) | 19.14±0.51 | 13.57±0.65 | 6.75 | 0.001*** |
| Normal: abnormal sperm ratio | 4.22±0.51 | 6.37±0.65 | 6.75 | 0.001*** |
| Acrosomal damage (%) | 18.14±0.34 | 13.43±0.57 | 7.09 | 0.001*** |
| Sperm cell concentration ($x10^6$/ml) | 484.3±6.49 | 577.1±14.9 | 5.71 | 0.001*** |
| Semen pH value | 7.41±0.05 | 7.14±0.05 | 3.70 | 0.03* |
| Sperm output ($x10^6$/ejaculate): | | | | |
| Total spermatozoa | 255.2±39.39 | 406.5±9.04 | 3.74 | 0.002** |
| Motile spermatozoa | 170.4±27.09 | 322.3±9.07 | 5.32 | 0.001*** |
| Motile normal spermatozoa | 205.7±31.81 | 351.1±6.82 | 4.47 | 0.001*** |
| Motile live spermatozoa | 186.7±28.37 | 341.9±7.39 | 5.29 | 0.001*** |
| Total functional spermatozoa[1] | 100.3±15.58 | 234.2±6.02 | 8.02 | 0.001*** |

** Significant at P<0.05. ** Significant at P<0.01. *** Significant at P<0.001. [1]: Motility x normality x livability*

In addition, treatment of rabbit bucks by royal jelly as natural active compounds in water (El-Hanoun *et al.,* 2014), red algae (Ali and Mervat, 2013), lycopene addition as natural antioxidants to drinking water (Mangiagalli *et al*., 2012), dietary addition of enzymes (Gado *et al.,* 2015) or organic selenium and folic acid (Kamel, 2012) had a positive effect on EV, semen quality and sperm output as compared to control bucks.

The improvement in semen characteristic of treated bucks may be due to antioxidant components of *S. platensis* (Rezvanfar *et al.,* 2008), which has the capability to prevent cell damage through containing enzymatic and non-enzymatic antioxidant defense system that counteract the effects of ROS and protect cellular components from the damaging under normal or stress conditions (El-Tohamy *et al.,* 2012).

It is of interest to note that the observed reduction in semen pH value in treated group may be attributed to higher SCC and PSM in semen of treated than in control bucks. It is well known that increasing sperm concentration with high viability was almost associated with remarkable increase in metabolic lactic acid as a result of sperm activity (Ayoub *et al.,* 2000; Abdel-Khalek *et al.,* 2001). Generally, improving all physical semen characteristics of treated bucks was in relation with increasing the level of STC as affected by S. *platensis.* Based on the foregoing results of sexual desire and semen characteristics in our study, rabbit bucks treated with *S. platensis* has impact on reproductive performance of rabbit bucks used for natural mating or those used for semen collection of AI.

***Biochemical analysis of seminal plasma:***

Chemical analysis of the seminal plasma revealed that concentrations of total fructose (TF) and total proteins (TP) were significantly higher in seminal plasma of treated than control bucks. While, albumin

(AL), globulin (GL) concentrations and AL: GL ratio was not affected significantly by *S. platensis* treatment (Table 3). These results may indicate that *S. platensis* had positive effect on some biochemical contents in seminal plasma of NZW bucks. A similar trend was obtained by Nedeva *et al.* (2014), who found that the *S. platensis* addition in diets of pigs significantly increased the levels of TP and GL. The same effect was also obtained by El-Harairy *et al.* (2016), who showed that *Moringa oleifera* extract treatment significantly increased TP, AL and TF concentrations in seminal plasma of NWZ rabbits. Moreover, Kamel (2012) showed that feeding rabbit bucks on organic selenium and folic acid significantly increased TP, GL and TF in seminal plasma compared with those fed control diet. El-Masry *et al.* (1994) observed that TP and GL concentrations in rabbit seminal plasma significantly increased in response to selenium plus vitamin E supplements.

**Table (3): Effect of *S. platensis* on concentration of some biochemicals and enzymatic activity in seminal plasma of NZW rabbit bucks.**

| Item | Control group | Treated group | T-Value | P-Value |
|---|---|---|---|---|
| Biochemicals: | | | | |
| Total fructose (mg/dl) | 176.7±2.40 | 241.3±4.09 | 13.62 | 0.002*** |
| Total protein (g/dl) | 3.77±0.15 | 4.57±0.12 | 4.24 | 0.01** |
| Albumin (g/dl) | 2.33±0.20 | 2.87±0.09 | 2.41 | 0.07 $^{NS}$ |
| Globulin (g/dl) | 1.44±0.19 | 1.70±0.05 | 1.37 | 0.24 $^{NS}$ |
| Albumin/globulin ratio | 1.62±0.30 | 1.69±0.06 | 0.04 | 0.96 $^{NS}$ |
| Enzyme activity (IU/l): | | | | |
| Alanine aminotransferase | 37.67±1.45 | 25.67±1.20 | 6.36 | 0.003** |
| Aspartate aminotransferase | 51.33±1.86 | 40.67±1.20 | 4.82 | 0.01** |
| Acid phosphatase | 31.67±0.88 | 23.66±0.88 | 6.41 | 0.003** |
| Alkaline phosphatase | 41.67±0.88 | 35.67 ±1.20 | 4.02 | 0.01** |

*$^{NS}$: Not significant. ** Significant at P<0.01. *** Significant at P<0.001*

In general, biochemical components in seminal plasma play a pivotal role in providing substrate energy forming essential link in the energy generating cycles in sperm metabolism in the process of fertilization and in the maintenance of constant osmotic pressure during semen preservation (Dhami and Kodagali, 1987). In this respect, fructose concentration in the semen has a positive relationship with the EV, SCC/ml, and percentages of PSM, LS, AS and acrosome status of spermatozoa (Ayoub *et al.*, 2000).

On the other hand, enzymatic activity of AST, ALT, ACP and ALP was significantly (P≤0.01) lower in seminal plasma of treated than control group (Table 3). Similar effect was reported on activity of AST, ALT, ACP and ALP in semen of NZW bucks fed diet contained red algae (Ali and Mervat, 2013). Also, El-Masry *et al.* (1994) found that AST and ALT activities in whole rabbit semen significantly decreased as affected by selenium and vitamin E supplement.

The transaminase activities in semen are good indicators of semen quality because they measure sperm membrane stability (Dogan *et al.*, 2009). Increasing the abnormal spermatozoa percentage in ejaculate may reflect high activity of transaminase enzymes into the extra-cellular fluid due to sperm membrane damage and ease of leakage of enzymes from spermatozoa (Seleem and Rowida, 2005; Zeidan *et al.*, 2008). Therefore the present results indicated that *S. platensis* treatment may reduce the leakage of these enzymes and may emphasize that *S. platensis* plays an important role in sperm membrane integrity in semen of treated rabbit bucks.

***Oxidative capacity in seminal plasma:***

Activity of total antioxidant capacity (TAC), glutathione S-transferase (GST), superoxide dismutase (SOD), and glutathione peroxidase (GPx) significantly increased, while TBARS concentration significantly (P<0.001) decreased in seminal plasma of treated than in that of control group. However, *S. platensis* treatment did not influence glutathione (GSH) in seminal plasma of rabbit bucks (Table 4).

In accordance with the present results, Hwang *et al.* (2011) found that mice administrated with *S. platensis* significantly increased activity of GPx as compared to control. Also, the organic selenium, folic acid and their combination supplementation in rabbit feeds improved oxidative capacity in seminal plasma (Kamel, 2012). Treatment of bucks with royal jelly significantly increased TAC, GST, GPx, GSH and SOD, and significantly decreased TBARS in seminal plasma compared with control group (El-Hanoun *et al.*, 2014).

**Table (4): Effect of *S. platensis* treatment on oxidative capacity in seminal plasma of NZW bucks.**

| Item | Control group | Treated group | T-Value | P-Value |
|---|---|---|---|---|
| TAC (mmol/L) | 1.03±0.04 | 1.17±0.03 | 2.82 | 0.04* |
| GST (IU) | 1.17±0.09 | 1.77±0.09 | 4.81 | 0.01** |
| SOD (IU) | 7.90±0.21 | 8.79±0.09 | 3.89 | 0.01** |
| GSH (mg/dl) | 13.67±1.45 | 17.33±1.45 | 1.78 | 0.1NS |
| GPx (mg/L) | 4.70±0.36 | 6.30±0.15 | 4.09 | 0.01** |
| TBARS (nmol/ml) | 1.33±0.03 | 0.96±0.04 | 7.71 | 0.001*** |

*NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01. *** Significant at P<0.001*

Mammalian sperm included rabbit display high rates of metabolic activity and are rich in poly-unsaturated fatty acids rendering them particularly susceptible to ROS-induced oxidation (Castellini *et al.,* 2006). Lipid peroxidation is one of the major reactions leading to phospholipid loss, membrane damage and the loss of motility in mammalian spermatozoa (Mann and Lutwak-Mann, 1981). Based on these finding, the obtained results of *S. platensis* treatment on semen quality of rabbit bucks can be used for production of high non-enzymatic antioxidant contents (carotenoids, tocopherols, ascorbic acid, and chlorophyll derivatives) and enzymatic antioxidant (SOD, catalase and GPX) according to (Abd El-Baky, 2003; Abd El-Baky *et al.,* 2007). Carotenoids and tocopherols in *S. platensis* can repair the oxidizing radical directly by its free radical scavenging activity through which they could inhibit the chain propagation steps during lipid peroxidation (Karpinski *et al.,* 1999). The antioxidant activity of *S. platensis* could be also attributed to the presence of two main phycobiliproteins ingredients (phycocyanin and allophycocyanin) that are acting mainly against superoxide radicals (Chaiklahan *et al.,* 2010). The polysaccharides from *S. platensis* had strong scavenging activities on hydroxyl radicals (Kurd and Samavati, 2015).

***Fertility trails:***

Fertility rate of doe rabbits naturally mated by bucks from treated and control groups, in terms of rates of conception and kindling, litter size and average bunny weight at birth improved by oral administration of *S. platensis*. The differences were significant only in total and live litter size as well as litter weight at birth (Table 5).

In agreement with the present results, fertility rate of multiparous doe rabbits did not significantly affected by mating with semen of bucks treated with lycopene addition (Mangiagalli *et al.,* 2012). Also, hatchability, fertility and embryo mortality of female breeder turkeys were not affected after AI by semen enriched in vitamin E (Zaniboni *et al.,* 2006). Although, Brun *et al.* (2002) reported that the mass motility significantly influenced the kindling rate, semen volume, percentage of motile sperm and concentration did not influence the kindling rate, but the number of motile sperm per ejaculate did.

**Table (5): Reproductive performance of NZW doe rabbits naturally mated by bucks treated with *S. platensis*.**

| Item | Control group | Treated group | T-Value | P-Value |
|---|---|---|---|---|
| Conception rate | 00±42.16 | 100±0.00 | 1.50 | 0.15 NS |
| Kindling rate | 00±48.30 | 80±42.16 | 0.49 | 0.62 NS |
| Total litter size/doe at birth | 43±0.37 | 7.00±0.46 | 2.60 | 0.01** |
| Live litter size/doe at birth | 36±0.26 | 6.75±0.45 | 3.48 | 0.004** |
| Bunny weight at birth (g) | .86±1.87 | 52.50±1.04 | 1.76 | 0.10NS |
| Litter weight at birth (g) | '.0±15.42 | 354.6±25.49 | 3.95 | 0.001*** |

*NS: Not significant. ** Significant at P<0.01. *** Significant at P<0.001*

In addition, Kamel (2012) found improved fertility of rabbit bucks supplemented with natural active and antioxidants components such as organic selenium, folic acid and their combination as indicated by increasing litter size (total and alive) at birth for doe rabbits mated by these bucks as compared to control does. Similarly, NZW rabbit bucks fed diets supplemented with *red alega* recorded significant improvement in litter size and weight at birth compared with control group (Ali and Mervat, 2013). Improving litter size (total born) was significantly may be associated with increasing sperm cell

concentration and all variables depending on it (sperm ouputs), particularly the number of total and motile sperm (Brun *et al.,* 2002). Also, it was reported that reproductive efficiency of rabbits depends on semen quality of bucks, environmental factors and the physiological status of the does (Theau-Clement and Roustan, 1992).

## CONCLUSION

The current study may suggest that *Spirulina platensis* at a level of 700 mg/buck daily as oral administration for 30 days period to be used in natural mating or artificial insemination is recommended to be serve as a supportive treatment in the nutritional management to improve semen production of rabbit bucks, and fertility of doe rabbits mated with these bucks.

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# تأثير فطر الاسبيرولينا على الأداء التناسلى لذكور الارانب

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يهدف هذا البحث الى دراسة تأثير التجريع بفطر الاسبيرولينا عند مستوى 700 ملليجرام/ذك يوميا على الاداء التناسلى لذكور الارانب النيوزيلاندى البيضاء البالغة (6-7 شهور). قسمت الذكورعشوائيا الى مجموعتين متجانستين (10ذكور فى كل مجموعة). المجموعة الاولى جرعت يوميا بـ 3 مللى ماء مقطر(كنترول) بينما جرعت المجموعة الثانية يوميا بـ3مللى ماء مقطر يحتوى على 700 ملليجرام/ذكر من فطر الاسبيرولينا لمدة 5 اسابيع قبل جمع السائل المنوى (المعاملة). تم جمع السائل المنوى مرتين اسبوعيا باستخدام المهبل الصناعى لمدة 10 اسابيع متتالية .تم حساب الرغبة الجنسية وتركيز هرمون التستيسترون فى سيرم الدم وتقييم خصائص السائل المنوى الفيزيائية والخصائص البيوكيمائية ونشاط مضادات الاكسدة فى بلازما السائل المنوى. وقد اظهرت النتائج ان تجريع فطر الاسبيرولينا ادى الى تحسن معنوى فى وزن الجسم الحى ($P<0.01$) والوزن المطلق والنسبى للخصية والبربخ ($P<0.001$ و $P<0.01$على التوالى) . وقد لوحظ زيادة معنوية ($P<0.01$) فى طول الخصية فقط ولم يكن هناك تأثيرمعنوى لعرض الخصية فى الذكورالمعاملة مقارنة بالكنترول. وقد تحسنت الرغبة الجنسية معنويا ($P<0.001$) وزيادة تركيز هرمون التستسترون فى سيرم الدم معنويا ($P<0.01$) وكذلك ظهر تحسن معنوى فى خصائص السائل المنوى الفيزيائية ( حجم القذفة – النسبة المئوية للحركة التقدمية والحى والشواذ وتركيز الحيوانات المنوية وشواذ الاكروسوم و التركيز الكلى لكل قذفة) مع وجود انخفاض معنوي فى قيمة درجة الحموضة ($P<0.05$) للذكور المعاملة مقارنة بالكنترول. وقد لوحظ ارتفاع معنوى فى تركيز الفركتوزالكلى والبروتين الكلى بينما لم تكن هناك فروق معنوية فى تركيز الالبيومين و الجلوبيولين ونسبة الالبيومين : الجلوبيولين فى بلازما السائل المنوى للذكور المعاملة عن مجموعة الكنترول. اظهرت الدراسة ايضا زيادة معنوية فى انزيمات مضادات الاكسدة مع انخفاض معنوى فى تركيز الاصول الحرة ($P<0.001$) فى بلازما السائل المنوى للذكور المعاملة مقارنة بالكنترول. اما بالنسبة لأمهات الأرانب التى تم تلقيحها بواسطة الذكور المعاملة بفطر الاسبيرولينا فقد حدث لها تحسن معنوى فى أدائها التناسلى حيث زاد معدل الحمل والولادة فيها ولكن بصورة غير معنوية بينما زاد معنويا العدد الكلى للخلفات وعدد الخلفات الحية ووزنها عند الولادة لكل ام. نستخلص من هذه الدراسة ان تجريع ذكورالارانب النيوزيلاندى البيضاء بمستوى 700ملليجرام/ ذكر يوميا لمدة 5 اسابيع له دور فعال فى تحسن الصفات البيوكيمائية والحيوية ومستوى انزيمات مضادات الاكسدة وبالتالى تحسن انتاج صفات السائل المنوى مصاحبا بتحسن القدرة الاخصابية للذكور.