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Semen Characteristics and Blood Metabolites of Hi-Plus Buck Rabbits Fed on Microalgae *Nannochloropsis oculata* Meal during the Summer Season

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ABSTRACT

Feeding tiny amounts of micro-algae meal to animals enhances animal physiology by improving immune response, disease resistance, and gut function, as well as enhancing anti-inflammatory and antibacterial protection, reproductive performance, feed conversion ratio, and weight gain. The purpose of this study was to identify the impact of dietary microalgae meal (*Nannochloropsis oculata*) on physical semen quality, serum biochemical parameters, and oxidative status of Hi-Plus buck rabbits for 12 weeks during the summer. A total of 45, Hi-Plus buck rabbits aged 20-24 weeks were divided into three equally comparable experimental groups. Bucks in the first, second, and third groups were daily supplemented in their diets with 0% (control), 0.50% (T1), and 1.0% (T2) microalgae meal, respectively. Semen and blood samples were collected to evaluate semen quality traits and some serum biochemical constituents, and oxidative status, as well as serum triiodothyronine (T_3) and testosterone (Ts) hormones concentrations. The obtained data revealed that dietary supplementation of *Nannochloropsis oculata* meal significantly improved most physical semen characteristics, including ejaculate volume, progressive sperm motility, semen pH value, sperm cell concentration, total sperm output, live sperm, and semen quality factor. Blood serum glucose, total proteins, and their fractions increased significantly in T1 and T2, compared with the control group, while total serum cholesterol and hepatic enzymes concentrations recorded a significant decrease in bucks supplemented with T1 and T2, compared with the control group. The total antioxidant capacity of serum significantly increased in both two levels of microalgae, compared with the control group. Serum T_3 concentration significantly increased in both levels of dietary microalgae compared with the control group. In conclusion, dietary supplementation with *Nannochloropsis oculata* meal (1.0%) was advised to improve semen quality, serum constituents, and antioxidative status without any adverse effects on the liver and kidney functions of rabbits.

Keywords: Bucks rabbits, Microalgae, Semen quality, Serum metabolite

INTRODUCTION

Microalgae is a superfood with various impacts on growth, antioxidant systems, health, and livability (Nasirian et al., 2017), rendering it important for cell regeneration and growth. *Spirulina* algae, commonly referred to as blue-green algae, is a highly nutritious feed source for various essential animal species (Holman and Malau-Aduli, 2013). The microalgae have significant substances, such as a high protein content (60-70% dry matter) and amino acids (Jung et al., 2019), 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). Microalgae contain numerous substances has biological activities and serve as antioxidant factors (Kurd and Samavati, 2015), anti-inflammatory (Vide et al., 2015), antiviral, and immune-modulatory (Sahan et al., 2015). Microalgae improve animal welfare, health, and physiological responses, which potentially enhances the reproductive performance and fertility of farm animals, including rabbits (Abd El-Hamid et al., 2022). As a result, the positive effects of various amounts of microalgae supplement on productive performance, physiological responses, and health status of various farm animals have previously been reported (Bonos et al., 2016; Mirzaie et al., 2018).

Buck's reproductive efficiency is important in the rabbit economy, and using semen with high traits avoid the loss of valuable genotypes (Vizzarri et al., 2019). Under oxidative stress, reactive oxygen species (ROS) generation enhances the normal physiological process in animal tissue and organs, including the testes. Rabbit's spermatozoa have high metabolic activity and are abundant in poly-unsaturated fatty acids, which increases lipid peroxidation (Attia et al., 2017) and makes them vulnerable to ROS attacks (Castellini et al., 2006). In roosters, increased lipid peroxidation reduces motility, fragments DNA, and reduces sperm fertilization capacity (Opuwari and Henkel, 2016; Attia et al., 2019; Okab et

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al., 2013). Practically, the safe and cost-effective administration of numerous natural antioxidant resources could help to diminish the detrimental effects of oxidative stress on rabbit buck reproductive efficiency by reducing the negative effects of oxidative stress on sperm parameters (El-Desoky et al., 2017).

Rabbit's reproductive success is affected by the semen quality of buck's rabbit, the climate changes, and the physiological state of the does (Ahmed et al., 2006 and Elnagar, 2010). Summer temperatures in Egypt can exceed more than 40°C. In addition, relative humidity (RH) and metabolic heat are also causatives of heat stress. Cold or heat stress, wind, ventilation, moisture, light, and solar radiation can deleteriously impact male fertility by causing oxidative stress (Córdova-Izquierdo et al., 2014). This stressor induces an increase in free radical accumulation, which damages spermatogenic cells (El-Desoky et al., 2013). A low amount of -ROS is required for normal sperm activity (El-Tohamy and El-Nattat, 2010). However, oxidative stress in the sperm occurs when ROS- levels exceed the total antioxidant capacity, which reduces fertility (El-Tohamy and El-Nattat, 2010). Antioxidants protect cellular components from damage caused by cellular free radicals and ROS. Damage can occur when antioxidants are absent, at a sub-optimal amount, or not accessible at the precise location within the cell where free radicals develop (El-Tohamy et al., 2012). Natural active ingredients such as microalgae might enhance animal reproductive performance (Kistanova et al., 2009). It is well known that the positive effects of microalgae, such as *Spirulina* in buck's rabbit, depend on treatment methods, pelleted diet, drinking water, and oral administration (Bashandy et al., 2016).

Abd El-Hamid et al. (2022) found that under heat stress conditions, supplementation of marine microalgae *Nannochloropsis oculata* at a level of 0.5 or 1 % to the doe rabbit's diets might improve serum progesterone and triiodothyronine profiles, some blood metabolites, oxidative status, and reproductive and productive performances. Therefore, the present study was designed to examine the effect of microalgae *Nannochloropsis oculata* on semen quality, some blood serum constituents, and total antioxidant capacity of Hi-Plus buck rabbits during the summer.

MATERIALS AND METHODS

Source of animals

The field portion of this study was conducted in a privet rabbits farm (Latitude 31° 29′ N; Longitude 32° 34′ E), North Sinai governorate, Egypt, during the summer season (from June to August 2020). Laboratory analyses were carried out at the Animal and Poultry Physiology Laboratory, Animal and Poultry Production Division, Desert Research Center, Ministry of Agriculture and Reclamation, Cairo, Egypt.

Ethical approval

This experiment was performed according to all ethics and animal rights (Desert Research Center). This work considered all rules and regulations in conformity with the European Union directive for the protection of experimental animals (2010/63/EU).

Experimental design and management

Experimental animals

Forty-five of Hi-Plus buck's rabbits at 5 months of age with an average initial live body weight (LBW) of 2686.0 ± 37.09 g were used in this study. Bucks were randomly distributed into three homogeneous groups (15 in each) based on the similarity of their LBW. 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 (3 months).

Diet and experimental design

The basal diet contained 24.60% Barley grain, 31.00% alfalfa hay, 13.25% soybean meal, 28.00% wheat brain, 1.60% dicalcium phosphate, 0.95% limestone, 0.30% sodium chloride, and 0.30% minerals-vitamins premix. The nutrient composition of the basal diet (% on dry matter basis) included 17.08% crude protein, 2.20% ether extract, 12.55% crude fiber, and 2416 digestible energy (DE, kcal/kg diet), and it was manually offered twice daily. The calculated analysis of the basal diet was done according to the feed composition tables for rabbits' feedstuffs used by De Blas and Wiseman (2010) and Villamide et al. (2010). The requirements of DE (kcal/kg diet) and crude protein (CP) were provided according to FEDIAF (2013). In this study, two levels of microalgae meal produced by the National Research Center, Dokki, Cairo, Egypt, were used. Microalgae were prudently added to the experimental basal diets while mixing the diet ingredients.

Bucks were fed on the experimental basal diet without supplementation in the first group and served as a control group. However, in the second and third groups, bucks were supplemented with a basal diet containing 0.50% (5g/kg diet) and 1.0 % (10g/ kg diet) of microalgae meal, respectively.

All rabbits were kept under the same experimental conditions. The composition of *Nannochloropsis oculata* as a fraction of dry weight (DW) biomass is presented in Table 1.

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Table 1. The composition of *Nanochloropsis oculata* constituents by Gass Chromatography mass

The composition (g/100g) of microalgae (*Nannochloropsis oculata*)	
Moisture	7.15
Crude protein	55.78
Fat	6.61
Ash	12.29
Total carbohydrates	18.17
Quantitative constituents of minerals profile (mg/100g) in microalgae (*Nannochloropsis oculate*)	
Fe	29.35
Zn	1.02
Sodium	1862.70
Calcium	229.00
Potassium	798.00
Magnesium	173.00
Quantitative constituents of amino acids profile (mg/g) in microalgae (*Nannochloropsis oculate*)	
Methionine	69.52
Cystine	17.30
Phenylanlanine	16.24
Lysine	15.20
Isoleucine	55.95
Leucine	65.11
Aspartic acid	30.16
Glutamic acid	15.07
Histidine	13.22
Tyrosine	87.69
Threonine	39.21
Valine	50.36
Serine	11.64
Glycine	9.98
Proline	31.52
Alanine	20.24
Arginine	8.56

Source: Abd El-Hamid et al. (2022)

Table 2. Overall means of indoor ambient temperature, relative humidity, and temperature humidity index throughout the experimental period, North Sinai, Egypt (according to Abd El-Hamid et al., 2022).

Month	**AT (^{o}C)**		**RH (%)**		**THI**	
	Minimum	**Maximum**	**Minimum**	**Maximum**	**Minimum**	**Maximum**
June	28.7	32.8	42.2	54.0	26.7	29.5
July	28.2	33.6	43.0	63.8	26.7	30.2
August	28.9	34.1	42.2	58.6	27.0	30.6
Overall	28.6	33.5	42.4	58.8	26.7	30.1

AT: Ambient temperature RH: Relative humidity, THI: Temperature humidity index

Climatic conditions

Ambient temperature (°C) and RH, were measured in percentage three days/week between 12 pm to 2 pm using automatic thermos-hygrometer (HANNA Instrument, Italy). Temperature Humidity Index (THI) was calculated using the following equation:

THI= db°C – [(0.31 - 0.31 × RH) × (db°C - 14.4)] according to Marai et al. (2001).

Where, db°C is dry bulb temperature in centigrade. The THI values were classified as the absence of heat stress (< 27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9), and very severe heat stress (> 30.0). The overall means of monthly climatic conditions are found in Table 2.

Growth indices

Bucks were individually weighed to the nearest ±1.0 g by a digital weighing scale at the beginning of June and at the end of August 2020 as initial and final weights, respectively. The total weight gain per animal in grams was calculated individually by subtracting the final from the initial weights using the following equation:

Total weight gain (g/buck) = (LBW_F–LBW_I)

While the relative growth rate (GR, %) was calculated using the following equations:

Growth rate (GR, %) = LBW_F – LBW_I / LBW_I × 100

Where, LBW_F is inal buck weight (g) and LBW_I denotes initial buck weight (g)

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Blood collection and serum biochemical parameters

At the end of the experimental period, individual de-coagulated blood samples (approximately 5 ml) were collected from the lateral ear vein. Blood samples were centrifuged at 4000 rpm for 20 minutes to separate the clear serum in Eppendorf tubes, and then stored in a deep freezer at -20℃ till biochemical analyses. The determined biochemical parameters of serum samples included total protein (TP), albumin (ALB), total cholesterol (CHO), glucose (GLU), liver enzymes activity (ALT and AST), calcium (CA), and phosphorous (P) which were calorimetrically analyzed using commercial kits (produced by Bio-diagnostic, Egypt), according to the procedure outlined by the manufacturers. Serum globulin (GLO) was calculated by subtracting the values of ALB concentration from the corresponding values of TP, and then the albumin/globulin (A/G) ratio was calculated.

Serum oxidative capacity

The total antioxidant capacity (TAC, µmol/mL) as lipid peroxidation biomarker was assayed in serum samples using commercially available kits (Bio Diagnostic Research, Erel, 2004).

Serum hormones

Serum testosterone (Ts) concentration was determined by immunoassay (Biosource-Europe S.A. 8, rue de L'Lndustrie. B-1400 Nivelles. Belgium). Moreover, serum triiodothyronine (T_3) concentration was determined with enzyme immunoassay using commercial kits obtained from immunotech crop, Boston, MA 02134**.**

Semen collection and evaluation of its physical characteristics

An individual semen sample was collected (Three times during the experiment) using an artificial vagina maintained at 42-45°C and a teaser doe. The reaction time (RT, sec.) was estimated as the time elapsed from introducing a teaser doe to the buck till to complete ejaculation of the artificial vagina. Immediately after semen collection, ejaculates were kept at 37 °C in the water bath and transferred to the laboratory. The ejaculated semen sample from each rabbit buck was evaluated for ejaculate volume (EV) without gel mass and for pH value using a pH paper (Spezial-Indikatorpapier pH 5.5-9.0, MACHEREY-NAGEL. Germany). In addition, the percentages of progressive sperm motility (PSM), live sperm (LS), and abnormal sperm (AS) were determined. The sperm cell concentration (SCC) was estimated using Neubauer hemocytometer slide. The 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, and sperm quality function (SQF) was calculated by multiplying SCC by EV and by LS/100. The percentages of LS, dead sperm (DS), and (AS) were determined using stains that penetrated cells with damaged membranes. Normal LS excluded the eosin stain and appeared pinkish in color because of loss of membrane and integrity. Normal sperm showed an oval head with a long tail, while abnormal sperm showed head, mid-piece, or tail defects, such as a large or misshapen head or a crooked or double tail (Correa and Zavos, 1994).

Statistical analysis

All numerical data were statistically analyzed using General Linear Model's procedure of the SAS (2009) program. A one-way ANOVA design was used to investigate the effect of different levels of dietary SA on the tested parameters by using the following model:

$Y_{ij} = \mu + T_i + e_{ij}$

Where, Y_{ij} is an observation, µ denotes the overall mean, T_i signifies the effect of treatment (i: control, 0.5%, and 1.0% microalga, respectively), and e_{ij} refers to random error. Differences between means among all treatments were subjected to Duncan´s Multiple Range-test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth indices

Table 3 shows the positive effects of microalgae administration on the LBW, total gain, and growth rate of bucks supplemented with 0.50% or 1.0% microalgae meal, compared to the control group. Numerically, the final body weight, total gain, and growth rate were of the highest values for bucks fed 1.0% microalgae (2964 g, 276 g, and 10.27%), followed by bucks fed 0.5% microalgae (2950 g, 270 g, and 10.07%). However, the lowest values of LBW and total gain were recorded in the control group bucks (2944 g, 263 g, and 9.80%). As seen in Table 3, results showed that the total gain and growth rate values did not differ fundamentally between 0.50% and 1.0% microalgae groups after 12 weeks of treatment. In accordance, El-Ratel (2017) found that the final body weight of doe rabbits was higher ($p < 0.05$) in the group that received oral 300 mg of *Spirulina platensis*/doe in drinking water than both of the doe rabbits that received oral 600 mg of *Spirulina platensis*/doe in drinking water and control does for 4 weeks period. Similarly, it was found that the dietary addition of *Spirulina platensis* increased the growth performance and feed intake of growing rabbits

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(Gerencser et al., 2012; El-Desoky et al., 2013). The positive effect *of Spirulina platensis* on growth indices may reflect the nutritive value of algae, which contains essential amino and fatty acids, photosynthetic pigments, vitamins, minerals, carotenoids, chlorophyll, pigments, and essential poly-unsaturated fatty acids in amounts ranging from 50 to 70% (on DM basis, Hoseini et al., 2013; Jung et al., 2019).

Table 3. Effect of dietary microalgae meal (*Nannochloropsis oculata)* on growth indices of Hi-Plus buck rabbits during the summer season, North Sinai, Egypt

Experimental groups / Growth indices	Control group	Level of dietary microalgae meal		± SE
		0.5 %	1.0 %	
Initial body weight (g)	2681	2680	2688	66.57
Final body weight (g)	2944	2950	2964	82.87
Total gain (g)	263	270	276	45.60
Growth rate %	9.80	10.07	10.27	2.32

SE: Standard error

Plasma biochemical parameters

Plasma proteins responses

As can be seen in Table 4, bucks fed 0.5% and 1.0% microalgae meal in their pelleted diets for 12 weeks period significantly showed an increase in serum TP, ALB, GLO, and GLU concentrations, compared with bucks in the control group ($p < 0.05$). However, there was a significant decrease in total CHO concentration as compared to bucks in the control group ($p < 0.05$). Similar results were reported regarding the concentrations of serum TP and ALB in rabbits and fed diet containing microalgae (Abd El-Hamid et al. (2022). The recorded increase in serum TP, ALB, and GLU concentrations may be related to high contents of protein, essential amino acids, vitamins, minerals, phospholipids, and antioxidants in microalgae meal (Jung et al., 2019). Similarly, El-Ratel and Gabr (2020) found a significant increase in plasm TP as a result of the rise in A/G concentrations for buck rabbits treated with 150 or 300 mg *Spirulina platensis*/liter drinking water, respectively. This result reflects a similar A/G ratio in treated groups compared with the control group. According to Moor et al. (2017), these results indicated that algal extracts could activate the enzyme lecithin cholesterol acyltransferase, which inhibits cholesterol biosynthesis and may play a role in the transverse cholesterol pathway when cells are unable to metabolize cholesterol. Previous studies performed by Hamed et al. (2015) revealed that marine *Spirulina* spp. acted as biological material by the dietary treatment for decreasing blood lipid concentrations.

Table 4. Effect of dietary microalgae meal (*Nannochloropsis oculata)* on blood serum metabolites of Hi-Plus buck rabbits during the summer season, North Sinai, Egypt

Experimental groups / Blood Serum constituents	Control group	Level of dietary microalgae meal		± SE
		0.5 %	1.0 %	
Total proteins (g/dl)	6.38^{b}	7.22^{a}	7.10^{a}	0.119
Albumin (g/dl)	4.39^{a}	4.19^{b}	4.15^{b}	0.075
Globulin (g/dl)	1.99^{b}	3.03^{a}	2.95^{a}	0.166
A/G ratio	2.32^{a}	1.50^{b}	1.50^{b}	0.164
Glucose (mg/dl)	70.82^{b}	88.34^{a}	85.10^{a}	2.59
Total cholesterol (mg/dl)	137.94^{a}	114.23^{b}	114.56^{b}	2.22
ALT (IU/L)	27.37^{a}	23.59^{b}	23.38^{b}	0.460
AST (IU/L)	91.11^{a}	88.73^{b}	89.03^{b}	0.568
T_3 (ng/ml)	0.678^{b}	0.744^{a}	0.722^{a}	0.01
Ts (ng/ml)	7.69^{b}	9.88^{a}	9.55^{a}	0.27
Calcium (mg/dl)	9.89^{b}	11.68^{a}	11.00^{a}	0.197
Phosphor (mg/dl)	4.63^{b}	5.56^{a}	6.44^{a}	0.204
TAC (µmol/L)	0.697^{b}	0.866^{a}	0.860^{a}	0.017

ALT: Alanine amino transaminase, AST: Aspartic amino transaminase, T_3: Triiodothyronine, TAC: Total antioxidant capacity, A//G: albumin/globulin, Ts: Total testosterone, SE: Standard error, ^{a,b}: Different superscript letters on the same row indicates significant differences ($p < 0.05$).

Regarding the effect of *Nannochloropsis oculata* meal on blood serum GLU concentration, the results could be found in Table 4. The results indicated that dietary supplementation with *Nannochloropsis oculata* significantly increased the GLU concentrations to 70.82, 88.34, and 85.10 mg/dl for control and 0.50, and 1.0 % microalgae-treated groups, respectively. This result may be due to the cell wall of *Nannochloropsis* being rich in several polysaccharides, and can interfere with the solubilization and digestion of the cell compounds. However, the cell wall polysaccharides of

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Nannochloropsis oculata contained almost 68% glucose along with about 4-8% rhamnose, mannose, ribose, xylose, fructose, and galactose (Brown, 1991).

Liver and kidney functions

Alanine amino transaminase and aspartate amino transferase enzymes

Regarding the effect of microalgae on ALT and AST, the results presented in Table 4 indicated that bucks fed 0.50% or 1.0% microalgae in their pelleted diets recorded a significant decrease ($p < 0.05$) in ALT (23.59 and 23.38IU/L) and AST (88.73 and 89.03IU/L) enzymes activities, compared with a control group (27.37 and 91.11 IU/L). These results may indicate that microalgae had a positive effect on protein metabolism, lipid profile, and liver functions of treated buck rabbits, and consequently, better health status compared with the control group. In accordance, Abd El-Hamid et al. (2022) demonstrated a significant increase in serum AST and ALT concentrations of doe rabbits supplemented with 0.5 or 1.0% microalga meal in their diets during the summer season. Bhattacharyya and Mehta (2012) mentioned that microalga might play a protective role against liver dysfunctions. Thus, our results suggest that dietary microalgae to 1.0% did not trigger liver impairment but had a protective effect on the biological functions of liver cells.

Thyroid hormone (tri-iodothyronine) response

The means of blood circulating concentrations of T_3 and total Ts in buck rabbits supplemented with two levels of microalgae meal for three months during the summer season are seen in Table 4. The obtained results revealed that serum T_3 significantly increased ($p < 0.05$) in both treatment levels (0.774 and 0.772 ng/ml), compared to the control group (0.678 ng/ml). The recorded rates were 9.73 and 6.45% for 0.50 and 1.0% of microalgae meal, respectively as compared with the control group. In accordance, Abd El-Hamid et al. (2022) reported that blood (T_3) and thyroxine (T_4) hormones increased significantly as the increased dietary supplementation ratio of sea woods (*Sargassum* meal) of Leghorn layers or in doe rabbits supplemented with 0.5 or 1.0% *microalga* meal in their diets during the summer season. It is well known that thyroid hormones affect spermatogenesis (Zarifkar et al., 2007). Moreover, the thyroid hormone receptor expresses in the germ cells from spermatogonia to primary spermatocytes (Buzzard et al., 2000).

Blood mineral absorption

The means of serum C and P concentrations are listed in Table 4. The obtained results indicated that dietary microalgae significantly increased serum concentrations of C and P values ($p < 0.05$). The recorded values for C were 9.89, 11.68, and 11.0 mg/dl for the control, 0.5, and 1.0% groups, respectively, while the corresponding values for P were 4.65, 5.56, and 6.44 mg/dl, respectively. Similarly, Recently, Abd El-Hamid et al. (2022) reported a significant increase in serum C and P concentrations in doe rabbits supplemented with 0.5 or 1.0% of microalgae meal in their diets during the summer season.

Antioxidant capacity status

According to Table 4, dietary microalgae significantly increased the total antioxidant capacity in both treatment levels, compared to the control group ($p < 0.05$). The results of TAC showed that bucks supplemented with microalgae showed a significant increase in TAC with a similar value (0.82 µmol/l) for both two levels compared with non-treated bucks (0.69 µmol/l). This result is in accordance with previous results indicating that the increase of serum TAC values in bucks treated microalgae meal may be due to their richness in natural biological substances, which may contribute to mitigating oxidative stress via enhancing enzymes and non-enzymes antioxidants (Abdelnour et al., 2020a; Abdelnour et al., 2020b; Abd El-Hamid et al., 2022).

Typically, the body's metabolism generates oxygen free radicals in a dynamic balance controlled by the antioxidant system. However, this balance can be disrupted by a rise in oxygen free radicals or deterioration of the antioxidant mechanism, resulting in oxidative damage to cells and lipid peroxidation (Xu and Pan, 2013). Thus, the antioxidant enzymes revealed the condition of the body's antioxidant mechanism, which reflects the body's capacity to metabolize oxygen free radicals and protect animal tissues from oxidative stress. Some biological functions are related to sugar complexes, such as glucose, a variety of mannose, galactose, rhamnose, N-acetylglucosamine, N-acetylgalactosamine, and arabinose residues, which are described by immune activity (1, 3-glucan) in all microalga species. The polysaccharides from *Spirulina platensis* had strong scavenging activities on hydroxyl radicals (Kurd and Samavati, 2015), in addition; they reduce blood lipid levels, such as triglycerides and cholesterol (Hamed et al., 2015).

Physical semen characteristics

Regarding physical semen trait responses, Figure 1 reveals that dietary supplementation of microalgae meal significantly improved most physical semen characteristics, including EV, PSM, semen pH value, SCC, TSO, LS, and SQF ($p < 0.05$). These results indicated a linear relationship between the level of microalgae meal and these traits. The best significant improvement of these traits was recorded for buck rabbits on 1.0% of microalgae meal supplementation.

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In contrast, each of the DS and AS percentages decreased significantly on both 0.5 and 1.0% levels, compared to the control group ($p < 0.05$), as this decrease was classified as a complementary part of the improvement of semen quality traits. The semen quality of bucks is the main factor in determining the reproductive efficiency of rabbit does (Attia et al., 2017). The obtained results indicated a higher fertilizing ability of spermatozoa of bucks treated with *Nannochloropsis oculata* as an antioxidant. This improvement was associated with a pronounced elevation in the ejaculate volume and sperm concentration and a reduction in DS and AS percentages, which resulted in enhancement in physical semen traits. Similar results were reported by Calogero et al. (2017), Fouda and Ismail (2017), and El-Ratel and Gabr (2020). Increased pH value in the semen of treated groups was associated with elevated levels of sperm cell concentration and semen volume. The increase in semen volume may be attributed to an increase in testosterone in treatment groups. The antioxidant components of *Spirulina platensis* may be responsible for the improved semen characteristics of treated bucks (Rezvanfar et al., 2008). *Spirulina platensis* can prevent cell damage through antioxidative defense systems that counteract the effects of ROS and protect cellular functions from damage under stress conditions (El-Tohamy et al., 2012).

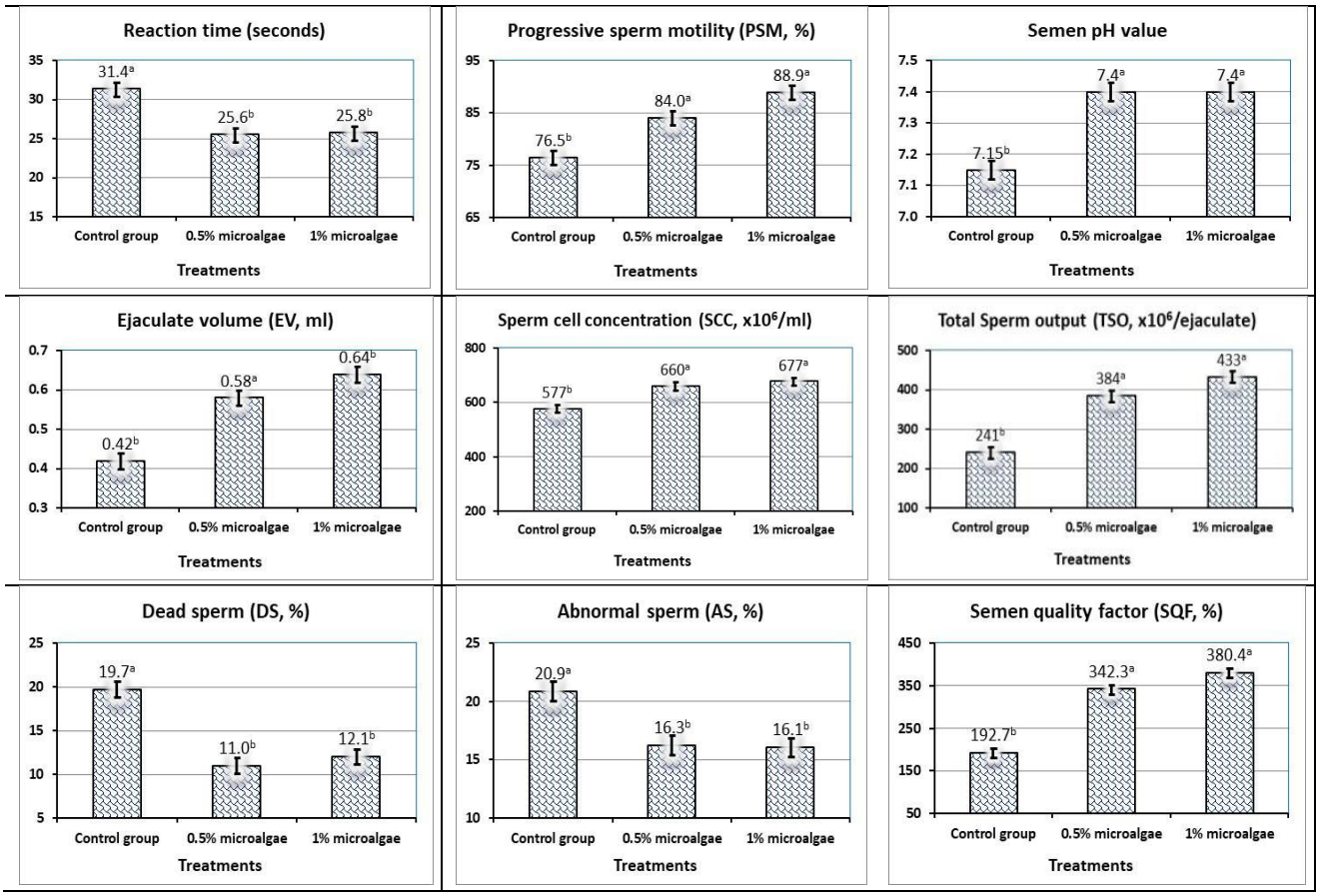

Figure 1. Effects of dietary microalgae meal (*Nannochloropsis oculata*) on physical semen characteristics and sperm output of Hi-Plus buck rabbits during the summer season, North Sinai, Egypt. [a,b]: Different superscript letters on the same row indicates significant differences ($p < 0.05$).

Sexual desire response

Regarding the effect of dietary *Nannochloropsis oculata* on sexual desire response, the obtained results in Table 4 and Figure 1 indicated that bucks fed a diet supplemented with microalgae showed a significant decrease ($p < 0.05$) in their RT and serum (Ts) concentration as indicators of sexual desire in bucks under both two levels of microalgae meal supplementation. With respect to RT, the results indicated lower values in both treated groups (25.6 and 25.8 sec) compared with the control group (31.4 sec). In contrast, the serum T_3 concentration indicated a significant ($p < 0.05$) increase in both treated groups (9.88 and 9.55 ng/ml), compared to the control group (7.69 ng/ml). Testosterone is the main male sex hormone, which plays a crucial role in the suitable development of reproductive organs and the maintenance of male sexual characteristics. These obtained results indicated that microalgae meal treatment for 12 weeks during summer months improved the libido of buck rabbits and subsequently markedly enhanced the sexual desire response of treated bucks compared with control ones. Similar results were reported in bucks treated orally with *Spirulina platensis* (750 mg/buck/day) for five weeks pre-semen collection (Fouda and Ismail, 2017) or with (200 and 400 grams of red algae per ton diet for 3 months (Ali and Mervat, 2013). Recently, El-Ratel and Gabr (2020) reported

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that increasing semen's pH value was associated with increased sperm cell concentration and ejaculate volume of buck rabbits fed microalgae (*Spirulina)*. However, increasing ejaculate volume may be attributed to an increase in the testosterone hormone of bucks fed *Spirulina*, which increases accessory sex glands activity.

CONCLUSION

It could be concluded that the supplementation of marine microalgae *Nannochloropsis oculata* at a level of 1.0 % to the buck rabbit's diets improves semen quality and blood serum constituents during the summer season. Future research on the effect of microalgae on the reproductive organs (morphology and histology) is needed.

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Competing interests

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article. The authors declare that they have no competing interests.

Authors' contribution

All the authors were collaborated in work planning, experimental design, measurement of parameters, and writing of the manuscript. Dr. Ahmed Sobhy El-Hawy designed the experiment, article writing, and revision. Dr. Moharram Fouad El-Bassiony designed the experiment, statistical analysis, tabulation of experimental data, manuscript writing, commenting, and approval. Dr. Ibrahim Samir Abd El-Hamid designed the experiments, measured the parameters, statistically analyzed data, wrote and revised the manuscript. Dr. Hesham Attia Shedeed designed the experiments, measured the parameters, wrote and revised the manuscript. Dr. Wafaa Adel Fouda designed the experiments, measured the parameters, statistically analyzed data, wrote, and revised the manuscript. Dr. Safaa Ali Mostafa designed the experiments, measured the parameters, wrote, and revised the manuscript. Dr. Reda Abd-Ellattif Abd-Elazem designed the experiments, measured the parameters, wrote, and revised the manuscript. Dr. Ali Saber Morsy designed the experiments, collected the samples, performed the experiments, and wrote and revised the manuscript. Dr. Khamis Refaay Said Emam helped in the field study, data collection, tabulation of experimental data, and article writing and revision. All the authors read and approved the final manuscript.

Data availability

All the data generated or analyzed during this study are included in this published article.

Ethical considerations

All authors admitted that they followed ethical issues concerning plagiarism, approval to publish, errors in fabrication, double publication, and submission.

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