

Research Article



Influence of Microalgae *Nannochloropsis oculata* on Blood Constituents, Reproductive Performance and Productivity in Hi-Plus Doe Rabbits Under North Sinai Conditions in Egypt

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Abstract | The goal of this study is to determine the effect of supplementation microalgae *Nannochloropsis oculata* on blood constituents, total antioxidant capacity, reproductive performance and productivity of Hi-Plus doe rabbits under North Sinai conditions. Forty-five Hi-Plus doe rabbits aged 5 months and body weight with an average of 3060.2±21.0 g were used from June to September 2020. Does were randomly divided into three equal groups, n= 15 for each group. The 1st treatment (Tr1), served as control. The 2nd treatment (Tr2), received 5 g of microalgae *N. oculata* / kg basal diet. The 3rd treatment (Tr3), received 10 g of microalgae *N. oculata* /kg basal diet during experimental period. Blood samples were monthly collected from all animals to determine some blood biochemical constituents, blood minerals and hormonal profiles. Reproductive and productive performance were evaluated. The results showed that total protein (TP) and globulin (GLO) concentrations increased (P<0.05) in treatment groups compared with control. Levels of albumin (ALB) and aspartate aminotransferase (AST) decreased (P<0.05) in Tr2 compared with Tr3 or Tr1. While alanine aminotransferases (ALT) and creatinine (CRA) concentrations decreased in treated groups compared with control. Value of total antioxidant capacity (TAC) increased (P<0.05) in treated groups compared with control. Serum phosphorus (P) increased (P<0.05) in treated groups compared with control, while calcium (Ca) level increased slightly in treated groups. Progesterone (P₄) hormone increased (P<0.05) in treated groups compared with control, while triiodothyronine (T₃) increased (P<0.05) in Tr2 compared with other groups. Reproductive performance disparity improved significantly in doe rabbits treated by microalgae *N. oculata* including conception, mortality rates, gestation length and litter size. Productive efficiency index and feed conversion of does significantly improved in treated rabbits as compared to control group. Litter weight at birth and weaning were higher (P<0.05) in rabbits treated with microalgae *N. oculata* compared to control. In conclusion, supplementation of microalgae *N. oculata* to rabbit diets has a positive effect on blood constituents and enhance reproductive, productive performance and oxidative status of Hi- Plus doe rabbits.

Keywords | Rabbits, microalgae *Nannochloropsis oculata* sp., Reproductive performance, Productivity, Blood constituents.

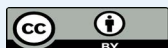
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In Egypt, rabbit's production is considered one of the most important projects that achieve a great economic return and contribute to a considerable percentage in bridging the food gap. Therefore, there is an expansion in this industry, especially in the tropical and subtropical regions. However, there are some obstacles facing the increase of production in these regions, the most important challenge is heat stress (Sakr et al., 2019) which stimulates a sequence of drastic changes lead to an increase of reactive oxygen species ROS which lead to deterioration of reproductive and production performance (Ganaie et al., 2013; Habeeb et al., 2018). Several studies discussed the beneficial application of microalgae as natural feed additives for animals because of their content of natural antioxidant compounds such as ascorbic acid, α -tocopherol, biotin, folic acid, and pantothenic acid (Durmaz et al., 2007), wide range of essential amino acids, fatty acids, digestible and non-digestible polysaccharides, glycerol, immune stimulates, essential vitamins and minerals (Zn, Fe, Ca, Se, Mg) (Borowitzka, 1997; Martins et al., 2013) which play an important role in reproduction (Abd El-Hamid et al., 2019). Thus, it may contribute in the improving of the reproductive and productive performance. *Nannochloropsis* is a unicellular microalga species with a polysaccharide cell wall structure that contains one chloroplast, this genus contains 6 different species namely as *N. gaditana*, *N. salina*, *N. limnetica*, *N. granulata*, *N. oceanica*, and *N. oculata* (Hibberd, 2008). Numerous studies (*in-vitro* and *in-vivo*) have verified the positive role of *N. oculata* on palatability, lack of toxicity (Kafaie et al., 2012), easy digestion (Kholif et al., 2020), antioxidant actions (Elsheikh et al., 2018), immunity (Derner et al., 2006; Colla et al., 2007), Anti-inflammatory and anti-cancer (Sanjeeva et al., 2016) on several animal, additionally to possibility to use as a substitute source of the conventional protein on animals diet, they also constitute a good alternative source of Eicosapentaenoic acid (EPA, C 20:5 n3) (Lacaz-Ruiz, 2003; Becker, 2007). This study aims to study the efficacy of microalga *N. oculata* as natural antioxidants on the attenuation of oxidative stress and enhances reproductive and productive performance as well as its blood constituent's effects in Hi-Plus doe rabbits under North Sinai conditions.

MATERIALS AND METHODS

ETHICAL APPROVAL

Experiments were carried out in accordance with the guidelines laid down by the Institute of Animal Ethics Committee for the use of animals (2010/63/EU of the European Parliament and of the Council of September 22, 2010).

STUDY AREA

This investigation was implemented at private rabbit's farm (Latitude 31° 29' N; Longitude 32° 34' E), North Sinai Governorate, in cooperation with department of Animal and Poultry Physiology, Desert Research Center, Ministry of Agriculture, Cairo, Egypt, Systel Telecom Company and Egyptian Center of Excellence for Bio-Saline Agriculture.

CULTURE AND PREPARATION OF *NANNOCHLOROPSIS OCVLATA*

The microalga *Nannochloropsis oculata* (*N. oculata*) used in the current study was prepared and kindly provided by the Biotechnology Microalgae Culture Unit, National Research Center (NRC), Giza, Egypt. Microalgae were maintained in standard F/2 Guillard's media (Guillard and Ryther, 1962). The collected microalgae were stored in the refrigerator at 4 °C until the culture period was finished and then harvested by centrifugation.

ANIMALS, MANAGEMENT AND EXPERIMENTAL DESIGN

A total number of 45 version Hi-Plus doe rabbits, 5 months old with an average body weight of 3060.2±21.0 g (mean ± standard error) were used. The study was conducted from June to September, 2020. The rabbits were housed in standard dimensions (50×60×40 cm) wired metallic cages attached with nest box (40×30×27 cm) for kindling and nursing. All doe rabbits were fed on a commercial concentrate pelleted diet containing 18.0% crude protein, 16.0% crude fiber, 2.5% fat, 0.6% minerals mixture and 2600 kcal/kg digestible energy according to NRC (1994) and provided drinking water *ad libitum*.

Rabbits were divided into three equal treatments: The 1st treatment served as control (Tr1, n=15) and received the basal diet. The 2nd treatment (Tr2, n=15) does received the basal diet plus 0.5% (5 g/kg diet) of marine microalgae *N. oculata* powder (Algal Biotechnology Unit, National Research Centre, Dokki, Giza, Egypt). The 3rd treatment (Tr3, n=15) does received the basal diet plus 1% (10 g/kg diet) of marine microalgae *N. oculata*. powder.

MICROALGAE *NANNOCHLOROPSIS OCVLATA* EXTRACTION, PURIFICATION AND CHEMICAL COMPOSITION DETERMINATION

The technique for microalgae *N. oculata* extraction was used as described by Hassan et al. (2015). The chemical composition of microalgae *N. oculata* extract was determined by gas chromatography-mass at complex laboratories of National Research Centre, Dokki, Giza, Egypt. The identification and quantitative measurements of microalgae *N. oculata* extract constituents are presented in Table 1.

AMBIENT TEMPERATURE AND RELATIVE HUMIDITY

Meteorological data including ambient temperature (°C),

Table 1: The quantitative measurements of *Nannochloropsis oculata* constituents by GC mass.

Chemical composition (g/100g) of microalgae <i>Nannochloropsis oculata</i>	
Moisture	7.15
Crude protein	55.78
Fat	6.61
Ash	12.29
T. carbohydrates	18.17
Quantitative constituents of minerals profile (mg/100g) in microalgae <i>Nannochloropsis oculata</i>	
Fe	29.35
Zn	1.02
Sodium	1862.70
Calcium	229
Potassium	798
Magnesium	173
Quantitative constituents of Amino acids profile (mg/g) in microalgae <i>Nannochloropsis oculata</i>	
Methionine	69.52
Cystine	17.30
Phenylalanine	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

relative humidity (RH, %) were recorded using hygro-thermometer. The temperature-humidity index (THI) was calculated according to the equation: $THI = (0.8 \times AT^{\circ}C) + [(RH/100) \times (AT^{\circ}C - 14.4)] + 46.4$ according to [Marai et al. \(2002\)](#) and shown in table 2. Mean values of environmental conditions temperature and temperature humidity index were recorded (32.9 °C and 29.6, respectively).

BLOOD SAMPLING

At the end of experiment, blood samples were collected in serum vacutainer tubes from all doe rabbits. Serum was then harvested after centrifugation at 5000 g for 10 min and then stored at -20°C for later analysis.

BLOOD BIOCHEMICAL CONSTITUENT'S ANALYSIS

Serum total proteins (TP), Albumin (ALB), Glucose

(GLC), Cholesterol (CHOL), Creatinine (CRA) were analyze using commercial kits (Spectrum company, Egypt) according to ([Weichslbaum 1964](#); [Scheletter and Nussel 1975](#); [Trinder 1969](#); [Finley et al., 1978](#); [Doolan et al., 1962](#)), respectively. Alanine aminotransferases (ALT) and aspartate aminotransferase (AST) were determined according to ([Henry, 1964](#)). Calcium, (Ca) and phosphorus (P) were determined using colorimetric kits (Biodiagnostic Research, Egypt), according to ([El-Merzabani et al., 1977](#)). Globulin (GLO) concentration was calculated according to the formula: Globulin = (Total Protein - Albumin) reported by [Howe, \(1921\)](#). Total antioxidant capacity (TAC) was calorimetrically assayed using commercial kits (Biodiagnostic Research, Egypt) according to ([Koracevic et al., 2001](#)).

Table 2: Indoor ambient temperature (AT), relative humidity (RH) and temperature-humidity index (THI) during experimental period.

Month	Ambient temperature (AT, °C)		Relative humidity (RH, %)		Temperature-Humidity Index (THI, %)	
	Min	Max	Min	Max	Min	Max
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
Aug.	28.9	34.1	42.2	58.6	27.0	30.6
Sep.	27.3	31.3	39.8	47.7	25.2	28.1
Overall	28.2	32.9	41.8	56.0	26.3	29.6

Table 3: Changes in some blood metabolites in Hi-Plus rabbit does fed a basic diet or a diet supplemented with algae *Nannochloropsis oculata*.

Items	Groups			SEM	P value
	Tr1	Tr2	Tr3		
Total protein (TP, g/dl)	5.9 ^b	6.5 ^a	6.8 ^a	0.16	0.05
Albumin (ALB, g/dl)	4.9 ^a	4.5 ^b	4.7 ^a	0.65	0.05
Globulin (GLO, g/dl)	1.0 ^b	2.0 ^a	2.1 ^a	0.15	0.05
Glucose (GLU, mg/dl)	86.5	97.6	102.3	5.34	0.11
Cholesterol (CHO, mg/dl)	147.1	141.7	139.3	3.75	0.32
Alanine aminotransferase (ALT, IU/L)	24.5 ^a	22.2 ^b	22.4 ^b	0.41	0.05
Aspartate aminotransferase (AST, IU/L)	98.1 ^a	92.7 ^b	95.5 ^{ab}	1.35	0.05
Creatinine (CRA, mg/dl)	1.6 ^a	0.80 ^b	0.67 ^b	0.10	0.05
Calcium (Ca, mg/dl)	11.2	12.1	11.7	0.31	0.14
Phosphorus (P, mg/dl)	4.7 ^b	5.3 ^{ab}	5.6 ^a	0.23	0.05
Total antioxidant capacity (TAC, mM/L)	0.58 ^b	0.76 ^a	0.78 ^a	0.02	0.05

Tr1=control, Tr2=rabbit fed 5 g microalgae *Nannochloropsis oculata* /kg diet) and Tr3= rabbits fed 10 g microalgae *Nannochloropsis oculata* /kg diet.

^{a, b} Means bearing different superscripts in the same row are significantly different (P < 0.05)

HORMONAL ASSAY

Progesterone (P₄) and Triiodothyronine (T₃) hormones were analyzed using ELISA kits (Monobind, USA) according to (Abraham 1974; Wheeler et al., 1994), respectively. The intra -and inter-assay CV's are 9.3 and 8.83, respectively.

REPRODUCTIVE PERFORMANCE AND PRODUCTIVE TRAITS

Reproductive performance including conception rate (CR, %), number of services per conception value (NSC) and gestation period (GL, day) were recorded. Productive traits including litter size at birth (LSB), litter size at weaning (LSW), litter weight at birth (LWB, gm), litter weight at weaning (LWW, gm), mortality rate from birth to weaning (MRBW%), Stillbirth (%) and overall of mortality rate (Overall MRBW%) were recorded.

ECONOMIC INDICATORS

Economic indicators were recorded as follow: Total feed intake (TFI, Kg) = daily feed intake×120 days. Productive efficiency index (PEI, kg live weight) = litter size at wean-

ing× number of parities × total weaning weight (kg). Also feed conversion (FC) was recorded.

ECONOMIC EFFICIENCY

The data was calculated based on the Egyptian market prices of diets at (2021) as follows:

Net revenue = Total feed cost - Price of total weight gain.

Economic efficiency = Net revenue / Total feed cost.

Cost of feed / 1 Kg live weight = feed conversion × price of one kg feed

Feed conversion = TFI (kg) / PEI (kg, live weight).

All costs were calculated have turned into international price (USD).

STATISTICAL ANALYSIS

Data was analyzed by the least square analysis of variance using the General Linear Model Procedure (SAS, 2004).

The model was as follows: $Y_{ij} = \mu + T_i + e_{ij}$

Y_{ij} = Any observations of ith rabbit within jth treatment

μ = Overall mean

T_i = Effect of ith treatment, (i: 1-3)

e_{ij} = Standard error

All statements of significance are based a probability of less than 0.05. Significant differences among means were tested using Duncan multiple range test (Duncan, 1955). Mortality rate of does was analyzed by Chi square analysis.

RESULTS AND DISCUSSION

CHANGES IN BLOOD BIOCHEMICAL CONSTITUENTS

The results displayed that TP and GLO concentrations increased ($P < 0.05$) in the doe rabbits of Tr3 and Tr2 ($6.8, 6.5 \pm 0.16$ g/dL and $2.1, 2.0 \pm 0.15$ g/dL), respectively compared with does of Tr1 (5.9 ± 0.16 and 1.0 ± 0.15 respectively), while mean value of ALB concentration decreased ($P < 0.05$) in the rabbits of Tr2 (4.5 ± 0.65 g/dL) compared to rabbits in Tr1 and Tr3, respectively. The higher values of serum TP and GLO concentrations might be due to the good quality and quantity of amino acids and protein in microalgae *N. oculata* (Table 3). In agreement with our results, several studies used the different types of microalga to feed different species animals, such as rabbits (Salim et al., 2019) and Holstein calves fed on microalgae *Spirulina platensis* (*S. platensis*), serum TP, GLO and ALB concentrations increased significantly (Heidarpour et al., 2011). Similar results were found in sheep (Bezerra et al., 2009), while in laying hens had a significant increase in plasma TP, ALB and GLO when fed on microalgae *S. platensis* (Mariey et al., 2012).

No significant differences were found in serum GLU and CHO concentrations among groups (Tr1, Tr2 and Tr3). Similar results showed that serum GLU concentration was not affected by diet supplemented with *N. oculata* in rabbits (Barbara Howe, 2012). Serum CHO was not change in rabbits drank water supplemented with 0.5ml microalgae *Amphora coffeaeformis* extract (Salim et al., 2019). Also, supplementation different levels (5 or 10 g/h /d) of microalgae *N. oculata* in goats' diet had no effect on lipids metabolites (Kholif et al., 2020).

Serum P concentration increased ($P < 0.05$) in treated does compared with control does. While Ca concentration was numerical increased in treated groups. The increasing of serum P and Ca concentrations in rabbits might be attributed to that microalgae *N. oculata* is rich in phosphorous and calcium, this led to good transfer of trace elements to the tissues, including blood (Spears, 1996; Olson et al., 1999; Huert et al., 2002).

Mean values of ALT, AST and CRA concentrations increased ($P < 0.05$) in Tr1 (24.5 ± 0.41 , 98.1 ± 1.35 IU/L and 1.6 mg/dL, respectively) compared with both treated groups. These results are in agreement with (Hassanein et al., 2014), they concluded that using both microalgae of *Chlorella vulgaris* and *Spirulina platensis* supplement into

the diet of rabbits led to increase of serum creatinine and AST, ALT enzymes activities. Salim et al. (2019) showed that supplementation of microalgae *Amphora coffeaeformis* extract in drinking water of rabbit led to numerical increment in levels of the ALT, AST and CRA. Additionally, Seyidoğlu and Galip, (2014); Khanna et al. (2016) and El-Ratel (2017) indicated that a significant change in serum concentration activities of AST and ALT in the rabbit that fed on *Spirulina platensis* microalgae.

The activities of AST and ALT work as an indicators of hepatotoxicity condition (Azab et al., 2013). The reduction occurred in serum ALT and AST activities in our results indicating that microalgae *N. oculata* supplements may play a protective role for liver. Higher serum creatinine in animals was previously observed in the arid regions because of heat stress in control doe rabbits (Sakr et al., 2019).

While TAC value increased ($P < 0.05$) in the all treated doe rabbits compared with control does (Table 3). It is well known that mitochondria are complex organelles capable of generating intracellular reactive oxygen species (Vakifahmetoglu-Norberg et al., 2017). When mitochondrial ROS production exceeds the cellular antioxidant capacity, the increase in ROS levels can lead to oxidative stress (Liemburg-Apers et al., 2015). Our results were in agreement with (Nacer et al., 2020), they reported that microalgae *Nannochloropsis* can help to alleviated oxidative stress by reducing of oxidant markers, such as MDA and carbonyl proteins (Bendimerad, 2018). It also has the ability to retrieve free radicals and inhibit lipid peroxidation to contain its antioxidant components such as carotenoids, fucoxanthin, astaxanthin and vitamins (Turrens, 2003).

CHANGES IN HORMONAL PROFILES

Mean value of P_4 hormone increased ($P < 0.05$) in the doe rabbits of Tr2 (8.6 ± 1.53 ng/mL) compared to doe rabbits in Tr1 or Tr3.

A few researches focused on studying the impact of microalgae on hormonal profiles, whatever many studies were reported that microalgae contain on abundant quantities different forms of long and short chain unsaturated fatty acids (Kholif et al., 2020; Salim et al., 2019; Susana et al., 2018 and Yu et al., 2019) especially linoleic acid (C18:2) and arachidonic acid (C20:4) which are precursors of P_4 hormone synthesis (Abayasekara and Wathes, 1999), this explain the observed increase in serum P_4 concentration in treated rabbits' does (Figure 1).

Similar trend was found in T_3 hormonal value (Figure 1). Triiodothyronine hormone plays an important role in regulating metabolism (Tao et al., 2006). There is a negative relationship between concentration of T_3 hormone and

feed intake, heat stress conditions, hence, exposure of rabbits to heat stress conditions lead to decrease level of T_3 (Uni et al., 2001; Attia et al., 2016). Microalgae contains active biological compounds such as carotenoids, fucoxanthin, and astaxanthin which have an important role in improving oxidative stress by inhibition of lipid peroxidation (Turrens, 2003) and release of HSP70 which might play a role for reducing the harmful effects of stress (Sakr et al., 2019). This leads to increase incorporation of iodine into thyroglobulin for the production of T_4 and T_3 , and promote the level of secretion.

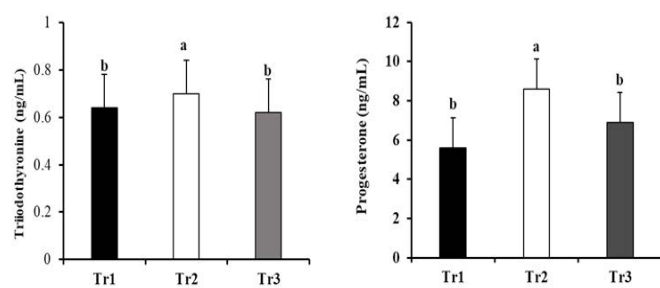


Figure 1: Changes of triiodothyronine (T_3) and progesterone (P_4) profile hormones in Hi-Plus rabbit does fed a basic diet or a diet supplemented with algae *Nannochloropsis oculata*

Tr1, control, Tr2, rabbit does feed (5 g/kg diet microalgae *Nannochloropsis oculata*) and Tr3 feed (10 g/kg diet microalgae *Nannochloropsis oculata*).

^{a, b} Means bearing different superscripts on the bars are significantly different ($P < 0.05$).

REPRODUCTIVE PERFORMANCE AND PRODUCTIVE TRAITS

The obtained results revealed that no significant effects in number of parities, CR (%) and NSC value among three groups (Tr1, Tr2 and Tr3) were noticed. While, value of GL (d) increased ($P < 0.05$) in control doe rabbit's (31.4 ± 0.20 d) as compared with all treated females (Table 4). These results might attribute to that, rabbits exposed to heat stress conditions led to increase of ROS which led to increase of gestation period (Sakr et al., 2019).

Rabbits does of Tr3 had highest ($P < 0.05$) values of LSB and LSW (8.0 and 7.1 %, respectively) followed by Tr2 (7.4 and 6.2%, respectively) than does of Tr1. Mean value of LWB increased ($P < 0.05$) in Tr3 (405.4 ± 23.87 gm) followed by Tr2 (338.5 ± 23.87 gm) compared with control does (266.2 ± 23.87 gm). On the other hand, values of LWW increased ($P < 0.05$) in all does treated with microalgae *N. oculata* compared to control does. while, mean values of MRBW and stillbirth percentage were not changed, the overall MRBW percentage was the highest ($P < 0.05$) in Tr2 ($19.8 \pm 7.44\%$) followed by Tr3, and then Tr1. (Table 4). In the present study, supplementation of microalgae *N. oculata* to the rabbits' diet improved reproductive performance

and productivity, included gestation length GL, total litter size at birth LSB, litter size at weaning LSW, litter weight at birth LWB, litter weight at weaning LWW and mortality rate from birth to weaning MRBW of the rabbits. This may be due to many positive factors, such as content of microalgae on bioactive compounds including S-nucleotide adenosyl peptide complex, polysaccharides and phenolic compounds. The ability to increase concentration of probiotics in the intestinal tracts (Janczyk et al., 2006; Mahmoud et al., 2017). Additionally, the capacity of microalgae on suppression the pathogenic bacteria in animals (Rania and Hala, 2008). Enhance of IGF-1 and T_3 secretion, this has an important role in protection and development of mammalian embryos exposed to heat stress (Jousan and Hansern, 2004; Abd-El Kafy, 2006). These factors lead to be responsible for these gains of improving immune functions, growth promotion and fertility (Maertens et al., 2006; Kunnath et al., 2018).

ECONOMIC INDICATORS

Value of TFI was not affected by different treatments (Table 5). Agree with these results, Raach-Moujahed et al. (2011) mentioned that total intake was not affected by dietary *Spirulina* supplementations. However, Hassanein et al. (2014) concluded that feed intake was significantly affected by the addition of high level (1.5g/Kg diets) of *Spirulina platensis* to NZW rabbits. Ragab et al. (2019) found similar results in doe Red Baladi rabbits fed a *Spirulina platensis* (0.6 g SP / kg diet).

Productive efficiency index was higher ($P < 0.05$) in all treated rabbit's does compared with control ones. This improvement in productive traits might be attributed to the ability of algae to create balance between generation reactive nitrogen species and elimination reactive oxygen species (ROS) of doe rabbits exposed to heat stress in arid regions (Abdullah, 2015; Morsy, 2013), and hence it reflected on improvement of metabolic function and productive performance (Emam, 2013).

In opposite trend, FC value decreased in treated groups Tr2 and Tr3 (2.42 and 1.92, respectively) compared with Tr1 group (Table 5). The possible reason for this important might be due to the efficiency of good and abundant content of microalgae protein *N. oculata* (Lum et al., 2013; Cavonius, 2016) supplemented to diets.

ECONOMIC EVALUATION

The data on economic efficiency is presented in Table 6. It shows that rabbits fed diets supplemented with both levels (5 or 10 g/kg diet) of microalgae *N. oculata* had lower economic performance than the control rabbits. However, this cost is not much in order to obtain meat productivity of high quality in content for examples they constitute a

Table 4: Reproductive performance and productive traits in Hi-Plus rabbit does fed a basic diet or a diet supplemented with algae *Nannochloropsis oculata*.

Items	Groups			SEM	P value
	Tr1	Tr2	Tr3		
Number of parity	1.88	2.11	1.94	0.15	0.55
CR (%)	62.2	72.5	68.6	5.55	0.45
NSC	1.73	1.6	1.6	0.15	0.85
GL (day)	31.4 ^a	31.1 ^{ab}	30.94 ^b	0.20	0.05
LSB	6.0 ^b	7.4 ^{ab}	8.0 ^a	0.58	0.05
LSW	4.9 ^b	6.2 ^a	7.1 ^a	0.48	0.05
LWB (g)	266.2 ^c	338.5 ^b	405.4 ^a	23.87	0.05
LWW (g)	3168.9 ^b	4178.1 ^a	4932.9 ^a	324.71	0.05
MRBW (%)	13.8	15.2	10.8	3.19	0.61
Stillbirth (%)	16.5	4.6	0.65	6.92	0.24
Overall MRBW (%)	6.0 ^b	19.8 ^{ab}	11.4 ^b	7.44	0.05

Tr1=control, Tr2=rabbit fed 5 g microalgae *Nannochloropsis oculata* /kg diet) and Tr3= rabbits fed 10 g microalgae *Nannochloropsis oculata* /kg diet.

CR, conception rate, number of services per conception, NSC, gestation length GL, litter size at birth LSB, litter size at weaning LSW, litter weight at birth LWB, litter weight at weaning, LWW, mortality rate from birth to weaning MRBW.

^{a, b, c} Means bearing different superscripts in the same row are significantly different (P< 0.05)

Table 5: Economic indicators of Hi-Plus rabbit does fed a basic diet or a diet supplemented with microalgae *Nannochloropsis oculata*.

Items	Groups			SEM	P value
	Tr1	Tr2	Tr3		
TFI (kg)	16.04	15.26	15.89	0.37	0.30
PEI (kg, live weight)	5.86 ^b	8.77 ^a	9.20 ^a	0.87	0.05
FC	16.04 ^a	2.42 ^b	1.92 ^b	0.34	0.05

Tr1=control, Tr2=rabbit fed 5 g microalgae *Nannochloropsis oculata* /kg diet) and Tr3= rabbits fed 10 g microalgae *Nannochloropsis oculata* /kg diet.

^{a, b} Means bearing different superscripts in the same row are significantly different (P< 0.05)

Table 6: Economic efficiency in Hi-Plus rabbit does fed a basic diet or a diet supplemented with algae *Nannochloropsis oculata*.

Traits	Groups		
	Tr1	Tr2	Tr3
FC	3.37	2.42	1.92
Cost of Kg feed (USD)	0.38	0.38	0.38
Cost of kg algae (USD)	0	0.095	0.19
Feed cost /1 kg live weight (USD)	1.29	1.16	1.10
Selling price of one kg live weight (USD)	2.35	2.35	2.35
Net revenue (USD)	1.07	1.20	1.26
Economic efficiency	82.98	103.85	114.12
Relative economic efficiency (%)	100	125.15	137.52

The price of diets was based on the price of ingredients in the Egyptian market during (2021).

Tr1, control, Tr2, rabbit does feed (5 g/kg diet microalgae *Nannochloropsis oculata*) and Tr3 feed (10 g/kg diet microalgae *Nannochloropsis oculata*).

good alternative source of Eicosapentaenoic acid (EPA, C 20:5 n3), which is a valuable polyunsaturated fatty acid for

prevention of several human diseases such as blood pressure (Chini Zittelli et al., 1999) also it has been shown the

effects on skin-tightening (Spolaore et al., 2006), antioxidant and anticancer activities (Mekdade et al., 2016; Nacer et al., 2019) and anti-inflammatory (Nacer et al., 2020).

Nannochloropsis oculata has been used in human food and diet products. For instance, it has been incorporated in noodles to improve their nutritional profile (Schwartz et al., 1991). Generally, the extensive in the culture of microalgae will lead to reduce the cost of production (Rocha et al., 2003).

CONCLUSION

It could be concluded 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 and oxidative status. Also, reproductive and productive performance were enhanced.

CONFLICT OF INTEREST

None of the authors has any conflict of interest to declare.

NOVELTY STATEMENT

The current study demonstrated that the microalgae *Nannochloropsis oculata* can be used as a non-traditional resource to feed animals in a safe and economic relatively, with no adverse effects on blood constituents or total antioxidant capacity, as well as improved reproductive and productive performance in Hi-Plus doe rabbits, which encourages the intensification of production in Egypt's arid conditions.

AUTHORS' CONTRIBUTIONS

Abd El-Hamid IS carried out serum biochemical analysis in DRC Complex Laboratories and wrote this article. Shedeed HA helped in blood serum biochemical analysis. Morsy AS and Amal HM performed the statistical analysis of the results and contributed in drafting the manuscript and revision. Emam KRS, Mousa BH, Yasein SA, Elbaz AM, Wafaa AA and Safa AM have carried out field execution to all experiment stages and collect blood samples and field data. All authors planned the research and approved the final manuscript.

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