Using Marine Algae (*Nannochloropsis oculata*) as Natural Feed Additives to Improve Reproductive Performance in Rabbits

El-Sayed M.; F. El-Azzazi; H. Khalil and M. El-Gayar
Department of Animal Production, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt

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Abstract: The present study was carried out to investigate the effect of using marine algae (*Nannochloropsis oculata)* instead of equine chorionic gonadotrophin (eCG) on improving productivity and reproductivity of rabbits. Thirty secondiparous Hy-plus female rabbits, aged from 6 –7 months and weighed 3.3 kg in average were used. Animals were divided randomly into three equal groups (10 does each). The 1st group was served as a control group. The 2nd, hormonal group, was intramuscularly injected with 25 IU per doe of eCG 48 hours before natural mating. The 3rd one, algae group, was supplemented with *Nannochloropsis oculata* powder (2Kg/100Kg diet). *In vitro* evaluation of early embryonic measurements was conducted on 15 matured rabbit does. Results clearly showed that eCG and algal groups achieved significant (P≤0.05) variances on most of the studied traits compared to control animals. Results showed that conception rates were higher in animals treated with eCG hormone and algae than control (100, 80.33 and 70%, respectively). Overall mean of milk yield during the lactating period decreased by 2.5% in hormonal group, however increased by 39% in algal group, compared to control group. Average total litter weight at weaning had increased by 12.4 and 42.5% in animals treated with eCG hormone and algae, respectively, compared to control. Also, litter size of does at weaning was significantly (P≤0.05) improved in eCG and algae groups by 11.3 and 24.3 %, respectively, compared with control group. On the other hand, the mucin layer was thicker in embryos of eCG hormone and marine algae groups by 13.3 and 39.9 %, respectively, than control. Number of collected embryos had increased in algae group compared with hormonal and control groups (9, 5.5 and 1.4, respectively). Results clearly showed that supplementation of *Nannochloropsis oculata* at a concentration of 2 kg / 100 kg diet had significantly improved some productive and reproductive performance traits of Hy-plus rabbits. In order to serve as a useful technique, the reproductive performance of using marine algae in feeding of other farm animals need to be addressed.

Keywords: Rabbits, marine algae, eCG hormone, multiple ovulations, embryos, productive and reproductive performance

INTRODUCTION

Recently, scientists have sought unconventional ways to upgrade the performance of rabbits. Among these substitutes, natural feed additives were used than other hormones, antibiotics, probiotics, and prebiotics to get better weight gain, improve feed efficiency, superior physiological functions of the animal body and boost the immune responses. Marine microalgae are used as a dietary supplement in animal feeding and these additives improves productivity, reproductivity, prolificacy and raise the animal's immune responses (Hemaiswarya *et al.,* 2011; Altomonte *et al.,* 2018; Ljubic *et al.,* 2020). Furthermore, microalgae have the potential to produce important energy compounds from their efficiency in harnessing solar energy, surpassing even higher organisms such as plants (Chew *et al.,* 2017). These characteristics led to the development of constant research on the use of this biomass for food and feed and biofuels. Several species of microalgae have high nutritional value and at the same time, are rich in important bioproducts and source of minerals such as copper, iodine, iron, potassium, and zinc, as well as vitamins (Christaki *et al.,* 2011). Also, microalgae have been used as a sustainable resource for domestic livestock, poultry, and aquaculture production due to their diverse nutritional profiles, carbohydrates, essential fatty acids, amino acids, carotenoids and vitamins (Saadaoui *et al.,* 2021).

Nannochloropsis oculata, a marine-water single-celled alga, is one of six species of algae found in the genus Nannochloropsis and was originally isolated of the coast of Scotland (Ribeiro *et al.,* 2020). It contains many important and essential nutrients for farm animal feeding (Spolaore et al., 2006 and Archibeque et al., 2009). And it could improve animal's conception rates and number of births (Ghazal *et al.,* 2013**;** Kovač *et al.,* 2013; Abu Hafsa *et al.,* 2021).

In compliance with the EU prohibition of using substances having a hormonal action for growth promotion in farm animals, council directive 81/602/EEC of 31 July 1981 concerning the prohibition of certain substances having a hormonal action and of any substances having a thermostatic action. In addition to the challenges facing the use of hormones for managing reproduction/fertility in farm animals, animal health and welfare and environmental related issues are also raising challenges. For example, a shortage of eCG availability is expected due to aspects related to animal welfare, as this hormone is obtained by bleeding pregnant mares. The ongoing societal pressure against companies manufacturing the hormone may prevent further hormone production in the near future (Hashem and Gonzalez, 2021). Therefore, the present study was conducted to investigate the using a natural feed additive "marine alga- *Nannochloropsis oculata* "instead of traditional reproductive hormone "eCG" on productivity and in vitro embryonic quality in Hy-plus rabbit breed.

MATERIALS AND METHODS

The present study was carried out at a private rabbitry Farm, Sahl El-Tina, North Sinai, Egypt. It was executed from October 2020 to December 2021. The farm was in northwestern part of Sinai Peninsula, between longitudes 30° 51' 13" east and 32° 27' 31" north.

Animals and management

In vivo experiment was executed from October 2020 to March 2021. Thirty secondiparous female rabbits from the Hy-plus breed were used in this experiment. The age of animals was ranged from 6-7 months with an average body weight 3.3 kg. Animals were healthy and free of any internal or external parasites. Animals were individually housed in galvanized wired cages (50 x 50 x 40 cm), where feed and water were provided *ad libitum*. Animals were fed on basal pellet ration contained yellow corn, soybean meal, corn gluten, minerals and vitamins premix, and molasses. The calculated chemical components of the diet were 17% crude protein, 2.8% fat, 10**%** crude fiber, and 2600 kcal digestible energy/kg diet according to NRC (1977). A lighting system was 14 hours light/10 hours dark in the rabbitry during the experimental period. Ten healthy Hy-plus bucks aged from 9-12 months were used for natural breeding. Does were transferred to the rabbit bucks' cages and kept under behavioral examination until natural mating was completed.

All animals were kept continuously under the same managerial and environmental conditions during the experimental period. Ambient temperature and relative humidity inside rabbitry were recorded daily during the experimental period by using a thermometer and hygrometer.

Experimental design

Animals were divided randomly into three equal experimental groups (10 does each). The 1st group was served as a control group, while the 2nd was the hormonal group, and the 3rd group was marine algae (*Nannochloropsis oculata*) group. In the hormonal group, 25 IU of equine chorionic gonadotrophic (eCG) hormone (INTERVIT, GERMANY) was injected intramuscularly 48 hours before natural mating per doe. The powder of *nannochloropsis* added with the concentration of 2 kg/100 Kg of diet. All groups were reared and fed basal and treated diets two weeks as a preliminary period before starting the main experimental period. Three consecutive parities were obtained and means of all traits were estimated from overall means of the three parities.

Productive and reproductive parameters

Live body weight of does. Live body weight was recorded at mating and parturition for each parity.

Vulva coloration. Vulva coloration and vaginal status were visually examined before the mating process for each doe. According to visually examination, the does were classified into four categories as pale color (score=1), red pale color (score = 2), rose (score = 3), and dark red (score = 4), as reported by Kishk *et al.* (2006)**.**

Gestation period and milk yield. The gestation period (day) was recorded for each doe from the date of successfully mating to parturition for each parity. Milk yield was recorded weekly for each doe at 8:00 am as the difference in the weight of kids before and after suckling. The kids were separated once a week 12 hours before suckling as described by Lukefahr *et al.* (1983)**.**

Sexual receptivity and conception rates. Receptivity was determined using a timer on the day of natural breeding. It was recorded in terms of receptive time in seconds, and it was estimated as the time between introducing the doe to the buck's cage up to until natural mating was completed (Ola and Olatunbosun, 2013). Pregnancy was diagnosed after 12 days of successfully mating using the traditional abdominal palpation method. Conception rate was calculated according to the following equation:

$$Conception\ rate\ (\%) = [\frac{\text{Number of pregnant does}}{\text{Number of mated does}}] \times 100$$

Litter size and weight. The litter size and total litter weight were recorded at birth for each doe. The average kid weight was recorded at weaning (30 days of age) for each doe.

Mortality rate of kids. The mortality rate of does was recorded during the experimental period. Also, the mortality rate of kids was calculated at birth and weaning by the following equations:

$$Mortality\ rate\ at\ weaning\ (\%) = \left[\frac{(\textbf{Total litter size at birth} - \textbf{Alive kids number at weaning})}{\textbf{Total litter size at birth}}\right] \times 100$$

$$Mortality\ rate\ at\ birth\ (\%) = \left[\frac{\textbf{Dead kids at birth}}{\textbf{Total litters at birth}}\right] \times 100$$

Mortality rate at weaning = [(total litter size at birth – alive kids number at weaning) total litter size at birth] × 100

Physiological measurements of pregnant does

Hematological parameters. Blood samples were obtained from each doe at the time of natural mating from the ear vein in heparinized tubes to estimate hematological parameters. Blood samples were collected in the morning between 8:00-10:00 am to avoid diurnal variation. Red blood cells (RBCs), white blood cells (WBCs) and platelets were counted, hemoglobin concentration were counted in samples using HMX (Coulter HMX Analyzer Operator's Guide. 21 January 1992).

Behavioral activity traits. Sexual and basic behavioral activity traits of males and females for each treatment were recorded using a video camera for half an hour during the natural mating process. Each doe in each treatment was transferred into a buck's cage. From the videotapes, at 2.5 min intervals (time sampling), the basic behavioral activities (percentage of bucks and does standing, sitting, or walking) were recorded and scored according to Khalil *et al.* (2014). In addition, sexual behavioral patterns of both sexes such as frequency of male circling around female, female circling around male, mounting male-female, mounting female-male, and actual mating were recorded and evaluated according to Khalil *et al.* (2021).

Ovarian examination and embryos collection of treated does

In vitro experiment was carried out at the same place and managerial conditions and was executed from November to December 2021. Fifteen healthy multiparous does and five bucks from the same breed were used. The age of animals was ranged from 12-15 months with an average body weight 3.3 kg. After 86 hours from natural mating all treated and control females were slaughtered, and genital organs were immediately removed. Morphology of each ovary was visually examination and number of corpora lutea (CL) was counted (Figure 1).

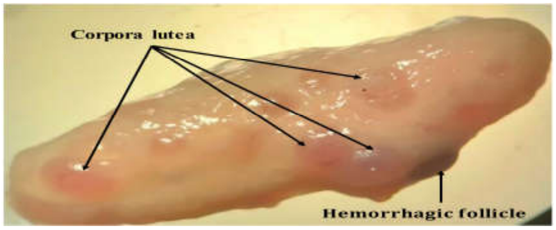

Figure (1): Morphology of the ovary indicating the corpora lutea and hemorrhagic follicles

Embryos were collected from uterus in glass petri dishes by retrograde flushing with 10 ml of phosphate-buffered saline (PBS) containing 1 ml of bovine serum albumin (BSA) according to Gutting *et al.* (2002).

Thickness of mucin and zona pellucida layers and diameter of embryos. Each embryo was immediately examined under the stereoscope. Also, the thickness of the mucin layer (μm) and zona pellucida layer (μm) were measured and the diameter of the embryos without mucin and with mucin (μm) were measured by stereoscope

Evaluation and recovery rate of embryos. The embryos were classified into three morphological grades according to Kondo *et al.* (1996): grade 1 (transferable embryos), blastomere uniform in size and shape and little or no fragmentation; grade 2, fragmentation of 10–30% of the embryonic surface; and grade 3, fragments >30% of the embryonic surface. Recovery of embryos (%) and transferable embryos (%) were calculated by the following equations:

$$Recovery\ of\ embryos\ (\%) = \left[\frac{\text{Number of total embryos}}{\text{Number of corpora lutea}}\right] \times 100$$

$$Transferable\ embryos\ (\%) = \left[\frac{\text{Number of grade1} - \text{embryos}}{\text{Numbers of total embryos}}\right] \times 100$$

Statistical analyses

Data were analyzed using One-Way ANOVA procedure of SPSS, V.25 (IBM Corp, 2017). Differences among means were tested according to Duncan's multiple range test (Duncan, 1955). Conception rate and behavioral measurements data were analyzed by the chi-square test. Pearson correlation coefficients among some traits were estimated.

RESULTS

Body weight and reproductive traits of does

Results in Table (1) show that there were significant differences (p≤0.05), due to the treatments in all studied traits except in vulva coloration. At mating and parturition, live body weights of algal female group were significantly heavier than of females in control and hormonal groups. Conception rate had significantly (p≤0.05) increased in females treated with eCG hormone compared with algae and control groups. However, the lowest values were recorded in both females treated with eCG hormone and algae. The highest kindling interval was recorded in females treated with algae (p≤0.05). However, the lowest value was obtained in females treated with eCG hormone. The females treated with eCG hormone showed increase in vulva coloration compared with control and algae groups.

Table (1): Body weight and reproductive traits of does as affected by treatments (Mean ± SE)

Parameters	Treatments		
	Control	Hormone*	Algae**
Doe body weight (g)			
at mating	3220.7^{b}±23.5	3231.5^{b}±30.1	3318.8^{a}±23.9
at parturition	3225.8^{b}±25.8	3232.9^{b}±29.7	3398.3^{a}±24.4
Body weight changes (%)	5.06^{b}±10.1 (0.15^{b})	1.43^{b}±8.2 (0.06^{b})	79.50^{a}±4.8 (2.39^{a})
Reproductive traits			
Conception rate (%)	70^{b}±15.2	100^{a}	80.33^{b}±13.3
Vulva coloration (score)	3.23±0.1	3.80±0.1	3.53±0.1
Kindling interval (day)	35.30^{ab}±0.1	35.25^{b}±0.2	35.85^{a}±0.2

[a, b] Means within the same row with different superscripts are significantly different *(P≤0.05)*

*Hormone; equine chorionic gonadotrophin (eCG) i.m. injected with 25 IU/doe. **Algae; *nannochloropsis oculate*

Productive performance

Average total litter size at birth per doe had significantly (p≤0.05) increased in females treated with eCG hormone compared with control and algae groups (Table 2). Average live litter size at birth per doe had significantly decreased (p≤0.05) in control group compared with females treated with eCG hormone and algae groups. Females injected by eCG hormone had significantly lower (p≤0.05) average kid weight/doe at birth compared with control and algae groups. On the other hand, the algae females were significantly higher (p≤0.05) in average litter size at weaning, average kid weight at weaning and total litter weight at weaning than those observed in other treatment groups. Mortality rate had opposite trends. Results revealed that milk yield had significantly (P≤0.05) increased throughout the lactating period in females fed algae compared with females treated with eCG and control groups. On the other hand, the results revealed that there were highly significant (p≤0.01) positive correlations between average milk yield and both total litter size (r = 0.562**) and total litter weight at weaning (r = 0.808**).

Table (2): Some productive performance of does as affected by treatments (Mean ± SE)

Parameters	Treatments		
	Control	Hormone	Algae
Average total litter size/doe (No)			
at birth	9.03^{c}±0.2	12.16^{a}±0.3	10.10^{b}±0.1
live at birth	8.63^{b}±0.2	10.56^{a}±0.4	9.86^{a}±0.1
at weaning (changes %)	7.93^{c} ± 0.2 (100)	8.83^{b} ± 0.3 (114.67)	9.86^{a} ± 0.1 (128.22)
Average kid weight/doe (g)			
at birth	52.70^{a}±1.1	47.16^{b}±1.8	54.65^{a}±0.9
at weaning	249.80^{b}±6.7	241.92^{b}±9.8	282.75^{a}±4.5
Litter weight at weaning/doe (g)			
Average	1946.33^{c}±39.2	2187.70^{b}±79.9	2774.06^{a}±39.4
Total	5839^{c}±104.9	6563.30^{b}±210.1	8322.20^{a}±105.1
Mortality rate of kids			
at birth (%)	0.40^{b}±0.1	1.60^{a}±0.4	0.23^{b}±0.1
At weaning (%)	0.70^{b}±0.1	1.73^{a}±0.2	0.00^{b}±0.1
Total (%)	1.10^{b}±0.2	3.33^{a}±0.4	0.23^{b}±0.1
Milk yield (g/day/doe)	129.58^{b}±1.1	126.38^{b}±2.7	180.16^{a}±1.6

a, b,c Means within the same row with different superscripts are significantly different *(P≤0.05)*
* Hormone; equine chorionic gonadotrophin (eCG) i.m. injected with 25 IU/doe. ** Algae; *nannochloropsis oculate*

Sexual and basic behavioral activity traits

No statistically significant differences among treatments in all sexual and basic behavioral traits except in time of female receptivity and frequency of actual mating (Table 3). The earliest time of female receptivity was recorded in females treated with algae (p≤0.05). Moreover, the highest frequency of actual mating was recorded in does treated with eCG hormone (p≤0.05). However, the lowest value was recorded in females of control group. On the other hand, frequency of female circling around male and male mounting female had increased insignificantly in animals treated with algae compared with control and eCG hormonal group. Frequency of males circling around females and females mounting males had the same trends. Moreover, males had spent more time walking in algae group than those recorded in both other groups. Females injected with eCG hormone had spent more time standing than those recorded in both other groups.

Table (3): Sexual and basic behavioral activities of Hy-plus rabbits as affected by treatments (Mean ± SE)

Parameters	Treatments		
	Control	Hormone	Algae
Sexual behavior activities			
Sexual Libido (Sc)	7.4±2.2	3.4±0.8	6.2±2.6
Female receptivity (Sc)	51.3^{a}±12.2	49.1^{ab}±8	25.4^{b}±4.4
Male circling around female (No)	13.3±1.5	13.9±1.7	10.6±1.8
Female circling around male (No)	1.7±0.2	2.1±0.3	2.2±0.6
Male mounting female (No)	3.0±0.6	3.2±0.8	4.9±0.8
Female mounting male (No)	1.1±0.2	1.4±0.3	0.9±0.2
Actual mating (No)	2.0^{b}±0.2	2.8^{a}±0.2	2.3^{ab}±0.2
Basic behavioral activities			
Males			
Walking (%)	52.7±6.6	54.5±6.4	59.9±4.6
Standing (%)	30.9±4.6	27.2±7.6	21.8±4.6
Sitting (%)	16.3±3.4	18.1±4.9	18.1±2.8
Females			
Walking (%)	36.3±5.8	38.1±4.4	38.0±3.4
Standing (%)	17.2±2.8	25.2±3.9	20.6±3.2
Sitting (%)	44.5±5.3	38.3±4.9	41.3±1.4

a, b Means within the same row with different superscripts are significantly different *(P≤0.05).*
* Hormone; equine chorionic gonadotrophin (eCG) i.m. injected with 25 IU/doe. ** Algae; *nannochloropsis oculata*

Embryo parameters and measurements of treated does

Females treated with 2% algae had significantly (P≤0.05) higher number of corpora lutea, embryo yield and transferable embryos than those obtained in control and hormonal groups. Also, recovery rate of embryos had decreased significantly in control group compared with those recorded in algae and hormonal groups (Table 4). Analysis of variance showed significant differences (P≤0.05) among treatments in all studied traits. Embryos collected from algae group had significantly thicker mucin layer thickness and zona pellucida than those recorded in control and hormonal groups. Also, the highest embryo diameter without mucin and total diameter of embryo were recorded in algae group (P≤0.05). However, the lowest values were recorded in control (Table 5 and Figure 2).

Table (4): Embryo parameters of Hy-plus rabbits as affected by treatments (Mean ± SE)

Parameters	Treatments			Overall
	Control	Hormone	Algae	
Corpus luteum (No)	$2.60^{c}±0.7$	$7.40^{b}±1.2$	$11.20^{a}±1.2$	7
Embryos (No)	$1.40^{c}±0.4$	$5.50^{b}±0.9$	$9.00^{a}±0.5$	5.3
Recovery rate (%)	$53.84^{b}±11.2$	$74.32^{a}±10.3$	$80.35^{a}±9.1$	69.5
Transferable embryos (%)	$48.77^{c}±13$	$68.33^{b}±10.6$	$80.00^{a}±8.2$	65.7

$^{a, b, c}$ Means within the same row with different superscripts are significantly different *(P≤0.05)*.
* Hormone; equine chorionic gonadotrophin (eCG) i.m. injected with 25 IU/doe. ** Algae; *nannochloropsis oculata*

Table (5): Embryos measurements (μm) of Hy-plus rabbit does as affected by treatments (Mean ± SE)

Parameters (μm)	Treatments		
	Control	Hormone	Algae
Mucin layer thickness	$39.48^{b}±3.1$	$44.74^{b}±1.5$	$55.23^{a}±1.7$
Zona pellucida layer	$7.04^{c}±0.9$	$8.68^{b}±0.3$	$10.03^{a}±0.3$
Embryo diameter without mucin	$103.23^{b}±13.8$	$116.17^{ab}±3.7$	$127.53^{a}±3.3$
Total diameter of embryo	$145.37^{b}±18.9$	$166.84^{ab}±4.6$	$191.08^{a}±4.9$

$^{a, b}$ Means within the same row with different superscripts are significantly different *(P≤0.05)*
* Hormone; equine chorionic gonadotrophin (eCG) i.m. injected with 25 IU/doe. ** Algae; *nannochloropsis oculate*

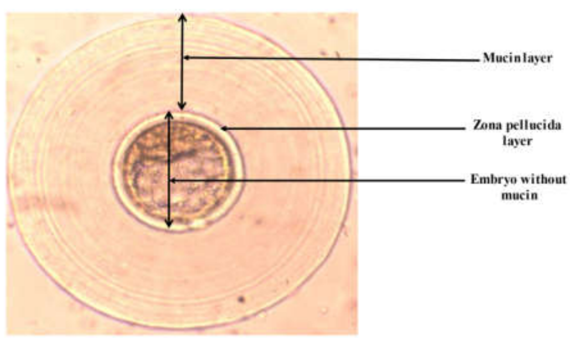

Figure (2): The components of the embryo and the thickness of different layers

Control **Hormone** **Algae**

Figure (3): The effect of treatments on embryos development at 86 hr after mating

DISCUSSION

Results of this study showed clearly that using microalgae as natural feed additives improved significantly (P≤0.05) most of the studied traits. Body weight of does at mating and parturition, and litter size at birth and weaning had significantly (P≤0.05) increased in the microalgae group compared with hormonal and control groups. These results are in agreement with the previous findings by Abu Hafsa *et al.* (2021). Earlier reports were recorded that microalgae were a good source of protein and amino acids (Archibeque *et al.,* 2009), which are responsible for the significant improvement in growth performance observed in rabbits. Furthermore, microalgae were a good source of iodine, which is an essential element for thyroid functions (Spolaore *et al.,* 2006). Also, Ali *et al.* (2021) found that supplementation of microalgae in the Boer goat's diet improved thyroid gland functions, which are responsible for the metabolism process and health of animals. In this respect, microalgae bioactive components could function as alternatives to antibiotics in feed because of their health and growth-promoting properties. Ali *et al.* (2021) found that nutrient digestibility had significantly higher in treated animals with microalgae than in the control ones. This improvement of internal environment of animals may explain the role of microalgae to increase body weight at mating and at parturition in treated females compared with hormonal and control groups.

Reproductive traits like conception rate and vulva coloration were significantly (P≤0.05) improved, however, pregnancy period had significantly (P≤0.05) decreased in females treated with microalgae compared with control group. These results agreed with that recorded by Ghazal *et al.* (2013), who reported that conception rate had significantly improved by using sea algae. Furthermore, sexual and basic behavioral activity traits were improved in females feed microalgae compared with control group. Time of female receptivity had significantly (P≤0.05) decreased, however, frequency of female circling around male, male mounting female and actual mating had insignificant increased compared with control group. Also, time of both walking and standing behavioral activity were insignificant higher in treated females than control group. This improvement in reproductive traits and sexual behavioral activity might be attributed to increase of gonadotropin and sexual hormones in treated females. These results were confirmed with Khalil *et al*. (2021), who recorded that highly significant positive correlations were found between estrogen levels and sexual behavioral activity such as frequency of male circling around female, male mounting female and actual mating in NZW rabbit breed. Also, Senosy *et al.* (2017) found that Boer goat fed diet supplemented with microalgae had higher concentration of estrogen during estrous cycle compared with control group. This improvement of reproductive traits and both basic and sexual behavioral activity traits may explain the role of microalgae to increase the production of litter size in treated females compared with control animals.

Microalgae treatment had significantly (P≤0.05) increased litter size, viability of kids and milk yield compared to hormonal and control groups. These results are in agreements with Ghazal *et al.* (2013), Kovač *et al.* (2013) and Abu Hafsa *et al.* (2021). This improvement might be attributed with the role of microalgae in improvement of nutrient digestibility and blood metabolites (Ali *et al.,* 2021). Microalgae contain several essential nutrients, which could contribute to the observed increase in milk yield (Burtin, 2003; Spolaore *et al.,* 2006). Furthermore, microalgae in rabbit diets improve gut function (Camacho et al., 2019), enrich the colonization of probiotics (Camacho *et al*., 2019; Verschuere *et al*., 2000) and intestinal contents of lactic acid-producing bacterial populations (Zheng *et al.,* 2012), increase the varieties of microflora in the intestinal tract (Hafeez *et al.,* 2016) and increase nutrient digestibility (Abu Hafsa *et al.,* 2021). Also, polysaccharides in microalgae can act as prebiotics, promoting growth and improve productivity in animals through beneficial effects in digestive tracts (Spolaore *et al.,* 2006; Archibeque *et al.,* 2009). Ali *et al.* (2021) found that immunoglobulin fractions like immunoglobulin A and immunoglobulin G were significantly higher in treated goat with microalgae compared with control group. The higher milk yield of treated females reflects good critical role of microalgae in milk production. This increment of milk yield and immunoglobulin fractions may be important factors to reduce the mortality rate of kids from birth to weaning and increase litter size and litter weight at weaning.

Number of both corpora lutea and embryos and quality of embryos were significantly (P≤0.05) improved in the microalgal group compared with hormonal and control groups. These results are in agreements with the previous findings by (Sales *et al.,* 2008; Mohammed, 2017; Senosy *et al.,* 2017). Microalgae are rich in antioxidant, minerals and vitamins, particularly selenium and vitamin E. Selenium and vitamin E act as an antioxidant element and help in detoxification and immunity, leading to increase reproductive function (Archibeque *et al.,* 2009; Surai and Fisinin, 2015). Moreover, microalgae contain high concentrations of essential fatty acids including Omega 3 (Kagan and Matulka, 2015). These lipids support normal reproductive function and absorption of carotenoids, vitamin E and other fat-soluble nutrients. Nutritional supply may be a valuable substitute to hormonal treatments to enhance reproductive performance. Also, microalgae are rich in carotenoids especially β-carotene (Spolaore *et al.,* 2006). Some reports concluded that short term supplementation of β-carotene increase the number of total follicles and corpora lutea in cyclic goat and cows (Sales *et al.,* 2008; Arenas-Herrera *et al.,* 2013). The beneficial effect of β-carotene on embryo quality, may be explained by the: (1) increase in progesterone synthesis through the stimulatory role of vitamin A on the cholesterol side chain cleavage enzyme (Weng *et al.,* 2000), (2) increase in the intra-uterine secretion of important factors linked to embryonic initial development (Harney *et al.,* 1993), (3) gap junction formation (Zheng *et al.,* 2012) that may be important to coordinate luteal cell function (Bilska *et al*., 1996). Also, β-carotene in microalgae is strongly associated with high steroidogenic activity in luteal and follicular ovarian structures (Haliloglu *et al.,* 2002). Senosy *et al.*

(2017) recorded that feed supplementation with microalgae improved blood plasma metabolites especially glucose that may act directly at the ovarian tissue (Viñoles *et al.,* 2005), consequently increase ovarian follicle numbers and size in addition to size of corpora lutea. Higher protein content in microalgae has positive effect on ovarian performance (Herrera *et al.,* 2008). Ali *et al.* (2021) found that Boer goat fed diet supplemented with microalgae had a significantly higher concentration of T_3 and T_4. The overall effects of thyroid hormones are to increase the basal metabolic rate, to make more glucose availability, to stimulate protein synthesis and increase lipid metabolism (Todini *et al*., 2007). Mucin layer thickness had significantly ($P \leq 0.05$) improved in the microalgae group compared with hormonal and control groups. Mucin layer consists mainly mucopolysaccharides and some proteins (Denker, 2000). The thickness of the mucin coat is essential for rabbit embryos to develop at term because it physically prevents the embryos from direct exposure to a deleterious uterine environment and allows them to expand until the appropriate time for implantation (Murakami and Imai, 1996). Microalgae contain high percentage of polysaccharides and proteins (Spolaore *et al.,* 2006). This may lead to an increase in growth of mucin layer thickness.

CONCLUSION

Results of this study clearly showed that *Nannochloropsis oculata* at a concentration of 2 kg / 100 kg diet could be safely used as feed additives to improve some productive and reproductive performance traits of Hy-plus rabbits. In order to serve as a useful technique, the reproductive performance of using marine algae in feeding of other farm animals need to be addressed.

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استخدام الطحالب البحرية (*Nannochloropsis oculata*) كأحد الإضافات الغذائية الطبيعية لتحسين الأداء التناسلي في الأرانب

محمود رشدي السيد، فخري الهادي حسن العزازي، حسن عبدالغفار خليل، معتز أحمد عبده الجيار

قسم الإنتاج الحيواني والثروة السمكية، كلية الزراعة، جامعة قناة السويس، الإسماعيلية ٤١٥٢٢، جمهورية مصر العربية

أجريت هذه الدراسة بهدف تقييم استبدال الطحالب البحرية (*Nannochloropsis oculata*) بهرمونات التناسل التقليدية مثل هرمون eCG، وذلك في إحداث التبويض المتعدد في الأرانب. تم استخدام خمسة وأربعون أنثى أرنب من سلالة هاى بلاس متعددة الولادات، تتراوح أعمارهم بين ٦-١٥ شهرًا ووزنها في المتوسط ٣.٣ كجم. تم تقسيم الحيوانات بشكل عشوائي إلى ثلاث مجاميع تجريبية متساوية (١٥ أنثى لكل مجموعة). استخدمت المجموعة الأولى كمجموعة ضابطة (كنترول). والمجموعة الثانية تم حقنها بهرمون eCG في العضل قبل ٤٨ ساعة من التلقيح الطبيعي بتركيز ٢٥ وحدة دولية / أرنب. أما المجموعة الثالثة تم إضافة مسحوق الطحالب بتركيز ٢ كجم/١٠٠ كجم من العلف. أظهرت نتائج هذه الدراسة بوضوح أن كل من الحقن بهرمون eCG والتغذية على الطحالب كان لهما تأثيرات معنوية (P≤0.05) على معظم الصفات المدروسة مقارنة بحيوانات مجموعة الكنترول. زادت معدلات الحمل بمعدل ١١.١و١٧.٦٪ في مجموعتي الهرمونات والطحالب، على الترتيب، مقارنة بمجموعة الكنترول. وانخفض المتوسط العام لمحصول اللبن بمعدل ٢.٥٪ في مجموعة الهرمونات بينما زاد بمعدل ٣٩٪ في مجموعة الطحالب مقارنة بمجموعة الكنترول. زاد متوسط الوزن الكلى للخلفات عند الفطام بمعدل ١٢.٤ و ٤٢.٥٪ في مجموعتي الهرمونات والطحالب على الترتيب مقارنة بمجموعة الكنترول. كما تحسن عدد الخلفات للأمهات عند الفطام معنويا بمعدل ١١.٣ و٢٤.٣٪ في مجموعتي الهرمونات والطحالب علي الترتيب مقارنة بمجموعة الكنترول. زاد سمك طبقة الميوسين في أجنة مجموعتى الهرمون والطحالب بنسبة ١٣.٣ و ٣٩.٩٪، على الترتيب، مقارنة بمجموعة الكنترول. زاد عدد الأجنة المتحصل عليها في مجموعة الطحالب مقارنة بمجموعة الهرمون والكنترول (٩، ٥.٥، ١.٤ على الترتيب). أظهرت نتائج هذه الدراسة بوضوح أن التغذية علي الطحالب البحرية من جنس (*Nannochloropsis oculata*) بتركيز ٢ كجم/١٠٠ كجم علف حسنت معنويا من بعض مقاييس الأداء الإنتاجي والتناسلي لأرانب الهاي بلاس، وبالتالي فقد يوصي باستخدامها لتحسين التبويض المتعدد في الأرانب. ولكننا نحتاج لدراسات متعددة حول استخدام الطحالب البحرية في علائق حيوانات مزرعية أخري، حتى ندعم استخدام هذه التقنية في رفع الكفاءة التناسلية.

الكلمات المفتاحية: الأرانب، الطحالب البحرية، هرمون eCG، التبويض المتعدد، الأجنة، الأداء الإنتاجي والتناسلي