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Effect of *Spirulina platensis* Supplementation to Rabbits' Does Diets on Reproductive and Economical Performance

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ABSTRACT

Spirulina platensis (SP) is the powder of microscopic green algae produced and consumed for times all over the world, besides in Africa also for its nutritional and pharmacological properties. Thus, the present study was conducted to evaluate the effects of dietary SP on the reproductive and economic efficiency parameters of adult Red Balady rabbits for 65 days. The results revealed that 0.6 g SP / kg diet led to insignificant increased of the productive performance of adult and kids' rabbits, serum biochemical and economic efficiency parameters. However, this level of SP significantly improved all tested semen quality parameters and serum lipid profiles of adult rabbits compared to those fed the low tested level of SP (0.3g / kg diet) or SP-untreated group. Based in these findings, 0.6g SP / kg diet is more useful level to potentially improve the productive and reproductive performance parameters, which sequentially led to increase the economic efficiency parameters of breeding the adult Red Balady rabbits.

Keywords: Rabbits, Spirulina, Feed additives, Production, Growth

INTRODUCTION

Microalgal species are of great value because of their high bioactive materials content, including polyunsaturated fatty acids, β-carotene and other pigments (antioxidants) (Bhat and Madyastha, 2000; Reddy *et al*., 2000), sulfated polysaccharides (anti-virals) and sterols (antimicrobials) (Ötleş and Pire, 2001). *Spirulina platensis* (SP) is a commercially powder of multicellular and filamentous blue green microalga, produced and consumed as human food and animal feed for its nutritional and pharmacological properties all over the world, including Africa countries (Kambou *et al*., 2015), as well as in Asia as a healthy food for human (FAO, 2008). SP not only primarily used as a human food supplement due to its potential antiallergenic, antiviral, antioxidant, hepato protective and immunomodulator properties (Khan *et al*., 2005), zeaxanthin and myxoxanthophyll has been reported to have pharmaceutical potential (Morist *et al*., 2001; Li *et al*., 2003), but also for animal feed, due to its highly source of complete proteins (60–70% w/w), polyunsaturated fatty acids, group B, and C vitamins, polyphenols, phycocyanin and various minerals (Khan *et al*., 2005; Seyidoglu *et al*., 2017a).

SP is gaining acceptance as a promising functional feed additive (Kapoor and Mehta, 1993). SP is called as a super food which has several effects on growth, antioxidant mechanism, health and life quality (Park *et al*., 2008; Nasirian *et al*., 2017), thus it's also imperative for growth and cell regeneration. SP is an edible microalga and it is a highly nutritious feed resource for many important animal species (Holman and Malau-Aduli, 2013). Where, SP is leading to improve animal welfare, health, and physiological responses, which it potentially enhancing the reproductive performance and fertility of farm animals, including rabbits. Hence, the beneficial impact of different levels of SP supplementation on productive performance, physiological responses and health status of different farm animals were previously reported as in broiler chicken (Bonos *et al*., 2016; Mirzaie *et al*., 2018), growing pigs (Simkus *et al*., 2008; Nedeva *et al*., 2014), African catfish (Promya and Chitmanat, 2011), fattening lambs (El-Sabagh *et al*., 2014; Malau-Aduli and Holman, 2015), and Holstein calves (Heidarpour *et al*., 2011), as well as in different strains of adult rabbits (Colla *et al*., 2008; Seyidoglu and Galip, 2014; Kambou *et al*., 2015; El-Ratel, 2017). Consequently, the present study aimed to investigate the effects of dietary supplementation of low levels of blue–green algae, *S. platensis* (SP) on the reproductive and economic performance of adult Red Balady rabbits for 65 days.

MATERIALS AND METHODS

This study was conducted at El-Serw Poultry Research Station, Animal and Poultry Research Institute (APRI), Agriculture Research Center (ARC), Egypt. Adult Red Balady rabbits were obtained from APRI, ARC, Ministry of Agriculture, Giza, Egypt. Thirty-six rabbits 6 months of age were haphazardly allocated into three experimental groups (4 does + 2 males of each) based on similarity of their live body weight (BW), with an average initial BW 736.4g / rabbit. Each treatment had two replicates. The rabbits in each replicate were kept in grower cages and fed the experimental diet *ad-libitum* for 65 days.

The experimental diet:

The experimental basal diet (BD) was obtained from APRI, ARC, Ministry of Agriculture, Giza, Egypt. The BD is contained 24.60% Barley grain, 31.00% alfalfa hay, 13.25% soy bean meal, 28.00% wheat brain, 1.60% di-calcium phosphate, 0.95% limestone, 0.30% sodium chloride, and 0.30% minerals-vitamins premix. Where, the nutrients composition of BD (% on dry matter basis) are contained 17.08% crude protein, 2.20% ether extract, 12.55% crude fiber and 2416 digestible energy (DE, kcal / kg diet) and was manually offered twice daily. The calculated analysis of BD was done according to the feed composition tables for rabbits' feedstuffs used by De Blas and Wiseman (2010) and Villamide *et al.* (2010). While, the requirements of DE (kcal / kg diet) and crude protein (CP%) were done according to FEDNA (2013). The price of one kg (Egyptian pound / kg) for different ingredients of BD according to the Egyptian local market at 2018 is; Barley grain, 4.6.; Alfalfa hay, 2.8.; Soy bean meal, 8.0.; Wheat bran, 2.1.; Di-calcium, 10.8; limestone, 0.20; Premix, 60.0; Sodium chloride, 0.50 and kg of SP powder 300 (LE). Thus, the total price of one kg of the experimental BD is 4.68 LE.

The different levels SP powder were used in the present study, where SP algae was produced by National Research Center (NRC), Dokki, Cairo, Egypt. It was prudently added to the experimental BD during the mixed

of its ingredients as follow; T_1: Rabbits fed BD *ad-libitum* without supplemented SP, T_2: Rabbits fed BD *ad-libitum* and supplemented with 0.3g SP/ kg diet, T_3: Rabbits fed BD *ad-libitum* and supplemented with 0.6g SP/ kg diet. All rabbits were kept under the same experimental conditions.

Does' reproductive traits:

During gestation and suckling periods:

The change in live BW (as BW gain) was calculated by the difference between the live BW at the beginning and at the end of the experiment. The amount of feed consumed was calculated. Litter size, and litter weight were recorded at 21 and 35 days of age. Mean bunny weight was measured at birth, 21days and 35 days of age and thereafter daily weight gain calculated for the periods from birth till 21 days, from 21 till 35 days of age and for the whole period from birth till weaning (at 35 days of age). Viability (%) as one of the sensitive parameters related to the economical return of production was also recorded in the present investigation at birth, 21 days (from birth up to 21 days of age) and 35 days (from birth up to 35 days of age). In addition, semen quality parameters were also estimated.

Serum biochemical parameters:

At the end of the experiment (65 days), three rabbits (n = 3) fasted for 12 hrs. were randomly taken from each treatment to obtain the blood samples. Blood samples were collected from ear vein without anticoagulant and kept at room temperature, then the tubes were centrifuged at 4000 rpm for 20 minutes to separate the clear serum, which stored in a deep freezer at –20^{o} C until analysis. Serum samples were used to determine the different biochemical parameters as total protein, albumin, globulin, total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), aspartate transaminase (AST) and alanine aminotransferase (ALT), which were calorimetrically analyzed using commercial kits (produced by Bio-diagnostic, Egypt), according to the procedure outlined by the manufacturers.

Economic efficiency:

To evaluate the economic efficiency of the experimental feed additive in rabbits' diets, total feed consumption / dam and feed consumption for does with their litter were recorded. Total weight rabbits / dam, average of parity / dam, average of feed consumed (kg / dam / parity) and average weight rabbits / dam / parity used to calculate the economic efficiency and relative economic efficiency dependent on the market prices for both costs and return, during the experimental period. Economic efficiency (EE) was calculated as followings:

Total feed cost / dam (LE) = Total feed intake (kg) × price / kg feed (LE).

Total return / dam (LE) = Total weight rabbits / dam (kg) × price / kg live body weight (LE).

Net return / dam (LE) = Total return / dam (LE) - Total feed cost / dam (LE)

Economic Efficiency (EE) = Net return/dam (LE) / Total feed cost/dam (LE)

Statistical analysis:

All numerical data were statistically analyzed using General Linear Models procedure of the SPSS (2008) program. A one-way analysis was used to investigate the effect of different levels of dietary SP on the tested parameters by using the following model: $\mathbf{Y_{ij} = \mu + T_i + e_{ij}}$

where: Y_{ij} = an observation, μ = overall mean, T_i = effect of treatment (i=1, 2, and 3), and e_{ij} = Random error.

Differences between means among all treatments were subjected to Duncan´s Multiple Range-test (Duncan, 1955).

RESULTS AND DISCUSSION

Feed additives improved the digestive system of animals and enable them to capture their genetic potential in growth performance. In recent years SP has a considerable place among these natural additives (Seyidoglu *et al*., 2019). Rabbits fed free SP diet had significantly ($P \leq 0.05$) increased of weight gain, total and daily feed intake compared to those fed diet supplemented with SP. However, no significant ($P \geq 0.05$) differences in other productive performance traits between the different experimental groups were detected (Table 1). Similarly, with the obtained findings herein Peiretti and Meineri (2008) observed no significant differences in growth performance of rabbits or broiler chickens (Bonos *et al*., 2016) fed SP.

Inversely, addition of SP significantly augmented growth performance of growing rabbit (Gerencser *et al*., 2012; El-Desoky *et al*,. 2013); APRI doe rabbits (El-Ratel, 2017); broiler chicks (Kharde *et al*., 2012; Zeweil *et al.*, 2016). Additionally, SP had valuable effects on final BW, and FCR of fattening lambs as compared to the control group (El-Sabagh *et al.*, 2014). In this respect, Fouda and Ismail (2017) also reported that adult New Zealand White (NZW) rabbit bucks fed 700 mg SP / diet were significantly ($P < 0.01$) increased the growth performance parameters than those in the control group. Where, SP can be digested simply due to its non cellulose structure on its cell wall, and thus it enhances of animal growth performance (Moreira *et al*., 2011; Seyidoglu and Galip, 2014; Seyidoglu *et al*., 2017b). Contradictory results are possibly due to the different tested levels of SP and quality in the present study. In addition, other secondary reasons, such as feed composition, housing conditions and production systems. As in the present findings, Nedeva *et al*. (2014) also reported that feed intake of pigs fed SP addition was higher with 6.98% and with 7.56% in comparison with those in the control group. Where, SP is supposed to improve palatability and digestibility, as well as protected farm animals against different toxic agents (Abdel-Daim *et al*., 2013).

Litter survival and litter size are imperative indicators for reproductive performance (Rothschild, 1996). In the present study, there was a positive but insignificant effect of the dietary SP on the productive performance parameters of rabbits' kids (Table 2). In agreement with the obtained results herein, Fouda and Ismail (2017) also reported that there were insignificant differences of the productive performance parameters of rabbits' kids except in total and live litter size, as well as litter weight at birth of NZW rabbit bucks treated with SP. A similar study carried out by Mangiagalli *et al.* (2012) who stated that fertility rate of doe rabbits did not significantly affected by mating with semen of bucks treated with lycopene addition. Contrariwise, Odeyinka *et*

al. (2008) showed that rabbits receiving 100% Moringa diet had higher litter size, litter weight at birth and litter weight at weaning than those receiving 100% Centrosema. Similarly, significant enhancement in litter weight and size at birth of NZW rabbit bucks fed red algae compared to the control group were detected by Ali and Ghazal (2013). Recently, Zeng *et al*. (2019) also stated that dietary *Moringa oleifera* leaf (MOL) was able to improve litter size, litter birth weight, and litter survival of weaning mice. In general, the reproductive efficacy of rabbits depends on semen quality of bucks, the physiological status of the does, and the environmental aspects (Theau-Clement and Roustan, 1992).

Table 1. Effect of dietary *Spirulina platensis* supplementation on the productive performance traits of rabbit does during gestation and suckling periods

Traits	*Spirulina platensis* (g/kg diet)			Pooled ± SEM	Significant
	control	0.3	0.6		
Initial body weight (g)	3287.5	3248.8	3285.0	83.20	NS
Final body weight (g)	3598.8	2441.3	3331.3	90.72	NS
Weight gain (g)	311.3^{a}	192.5^{b}	46.30^{c}	36.58	0.05
Feed intake (kg/doe/65 days)	16.43^{a}	14.25^{b}	13.68^{b}	14.02	0.05
Feed intake (g/doe/day)	252.8^{a}	219.3^{b}	215.1^{b}	6.11	0.05
Total milk yield (kg/doe)	3.38	3.47	3.37	0.03	NS

a,b,c :Mean in the same row bearing different superscripts are significantly different (P ≤ 0.05). NS= non- significant

Table 2. Effect of dietary *Spirulina platensis* supplementation on productive performance traits of kids

Traits	*Spirulina platensis* (g/kg diet)			Pooled ± SEM	Significant
	control	0.3	0.6		
Litter size and weight at birth					
Litter size (cm) at birth	7.50	6.75	6.50	0.23	NS
Litter weight (g) at birth	355.5	311.75	339.15	14.88	NS
Weight (g) / kid at birth	47.39	45.95	52.14	1.53	NS
Litter size (cm) at 35 days	7.00	6.25	6.25	0.20	NS
Viability (%)	93.75	92.71	96.43	2.06	NS
Performance traits of kids					
Weight (g) / kid at 35 days	681.04	680.74	687.14	5.69	NS
Weight gain (g) / kid	633.64	634.69	635.00	5.77	NS
Daily weight gain (g) / kid / day	18.10	18.14	18.14	0.17	NS
Feed conversion ratio	3.45	3.38	3.27	0.06	NS

NS= non-significant

In the present study, addition of SP significantly increased of all semen quality parameters of adult rabbits compared to SP-untreated group (Table 3). The positive effects of dietary SP on semen quality parameters of adult Red Ballady rabbits in the present study was recognized in boars (Kistanova *et al*., 2009), and on sperm motility and semen concentration in rats (El-Desoky *et al*., 2013; Bashandy *et al*., 2016). Additionally, Fouda and Ismail (2017) also concluded that dietary addition of 700 mg SP / diet for 5 weeks significantly improved all tested semen quality parameters of adult NZW rabbit bucks, and fertility of doe rabbits compared to those in the untreated group. Generally, these improvement in semen quality measurements of treated adult rabbits in the present study or others may be due to the antioxidant components of SP (Gumbo and Nesamvuni, 2017), which SP has avoided the cell damage through containing enzymatic and non-enzymatic antioxidant defense system, as well as protected the cellular components against the harmful or stress factors (El-Tohamy *et al*., 2012). Moreover, dietary SP contains β-carotene and other strong antioxidative phytochemicals (kaempferol, quercetin, rutin, and caffeoylquinic acids), essential antioxidative micronutrients (selenium and zinc as explained) and antioxidative vitamins (C, E, and A), that have regulative effects in fertility performance (Ramadan *et al*., 2008).

Sperm abnormalities have long been associated with male infertility and sterility in most species (Saacke, 2001). An important discovery in the present study is that SP-fed groups had significantly lower sperm abnormality rates than the control group (Table 3). In a recent study, Zeng *et al*. (2019) also reported that mice fed-MOL had lower sperm abnormality rates than untreated group. In this respect, the exact mechanism for the decrease in the frequency of abnormal sperm is not clear. It was suggested that lower sperm abnormality resulted from lower chromosome abnormality and less minor alterations in testicular DNA and point mutation.

Table 3. Effect of dietary *Spirulina platensis* supplementation on semen quality parameters of rabbits

Traits	*Spirulina platensis* (g/kg diet)			Pooled ± SEM	Significant
	control	0.3	0.6		
Volume (mL)	0.77^{c}	0.83^{b}	0.90^{a}	0.02	0.05
Motility (%)	65.43^{c}	70.37^{b}	76.80^{a}	1.69	0.05
Live sperm (%)	76.57^{ab}	79.23^{ab}	84.07^{a}	1.25	0.05
Dead sperm (%)	23.43	20.77	15.93	1.25	0.05
Concentration ($\times 10^{9}$)	1.68^{c}	1.79^{b}	1.94^{a}	0.04	0.05

a,b,c: Mean in the same row bearing different superscripts are significantly different (P ≤ 0.05). NS= non- significant

Regarding the tested serum biochemical parameters, rabbits fed 0.6g SP / kg diet significantly ($P \leq 0.05$) improves the lipids profiles compared to SP-untreated group or tested low level of SP (0.3 g / kg diet). Where, dietary SP at level 0.6 g / kg diet significantly ($P \leq 0.05$) decreased serum total cholesterol, and triglycerides, as well as increased ($P \leq 0.05$) HDL among other experimental groups (Table 4). Meanwhile, no significant differences ($P \geq 0.05$) of other serum biochemical parameters were detected among all treatments (Table 4). In this context, SP is considerable a rich source of lipids and consequently essential fatty acids and lipid-soluble antioxidants (Ramadan *et al*., 2008). Likewise, they suggested that it recovered lipids may be suitable for commercial exploitation as a source of lipids for food use,

as well as the tocopherols at the level estimated may be of nutritional importance in the application of this blue-green microalga. The lipid extract from SP shows antioxidant and antimicrobial activity, hence, SP lipid extracts presented a promising potential as an accessible and safe alternative to synthetic antioxidants and antimicrobials (Gumbo and Nesamvuni, 2017).

Albumin and globulin are the main components of plasma protein, which are typically monitored as Al/Gl ratio (Russell and Roussel, 2007). Albumin concentrations are indicative of long-term dietary protein intake (Sargison and Scott, 2010). Thus, serum total protein, globulin, and albumin are all directly related to protein metabolism and their concentrations in both supplemented and control animals fell within the normal range (Table 4). Normality was also evident in the electrolyte concentrations of Ca, P, Mg, Na and K indicating that mineral metabolism was not negatively impacted by dietary SP supplementation. Similarly, with the obtained findings herein Heidarpour *et al.* (2011) didn't find out any tendency for changing of plasma proteins in growing calves compared to the control group depending on the different content of SP in the ration. Additionally, in the current study the activity of the tested liver function enzymes (AST and ALT) of SP-treated groups are within the normal range compare to the SP-untreated group, where SP can be associated with improved the rabbits' liver health. Other researchers have confirmed this association by linking SP consumption with improved liver status (Colla *et al*., 2008; Ismail *et al*., 2009).

Generally, the obtained findings in the present study regarding the serum biochemical parameters demonstrated that dietary SP had no harmful effect on adult rabbits' reproductive physiology performance. Similarly, with the current findings no negative effects on the reproductive performance of mice fed-MOL have been reported by Zeng *et al*. (2019). These positively effects of dietary SP on serum biochemistry traits of treated rabbits in the current study due to its known as a powerful antioxidant in herbal supplements, as well as its contents phycocyanin, tocopherols, beta carotene and vitamin C are in progress of growth and health status of experimental animals (Karkos *et al*., 2011; Abdel-Daim *et al*., 2013; Seyidoglu *et al*., 2019).

The present findings revealed that 0.6g or 0.3g SP / kg diet, respectively achieved insignificant ($P \geq 0.05$) increased of economic efficiency and relative economic efficiency parameters compared to SP-untreated group (Table 5). This superiority of dietary SP for improved the economic efficiency (EE, %) parameters than SP-untreated group may be related with the positively effects of SP on the productive traits Tables (1 and 2), and physiological reproductive performance parameters Tables (3 and 4), as reflected of increase EE and relative EE compared to the control group (Table 5), which consequently led to increase the profitability. In this respect, Ghazal *et al.* (2016) reported that the reproductive performance of rabbits plays a significant role for increasing the profitability of rabbits breeding.

Table 4. Effect of dietary *Spirulina platensis* supplementation on serum biochemical parameters of rabbits does

Traits	*Spirulina platensis* (g/kg diet)			Pooled ± SEM	Significant
	control	0.3	0.6		
Total protein (g/dL)	5.90	5.67	5.68	0.73	NS
Albumin (g/dL)	3.04	3.68	3.38	0.17	NS
Globulin (g/dL)	2.86	1.99	2.30	0.22	NS
Al / Gl ratio	1.07	2.38	1.58	0.30	NS
Total cholesterol (mg/dL)	50.67^{a}	41.34^{b}	42.75^{b}	1.47	0.05
Triglycerides (mg/dL)	91.35^{a}	97.48^{a}	58.50^{b}	5.97	0.05
HDL (mg/dL)	29.98^{ab}	24.15^{b}	33.53^{a}	1.76	0.05
LDL (mg/dL)	7.49	6.88	6.28	0.48	NS
AST (U/L)	50.45	48.48	62.68	4.45	NS
ALT (U/L)	39.75	31.18	27.38	6.09	NS

a,b: Mean in the same row bearing different superscripts are significantly different ($P \leq 0.05$). NS= non- significant; Al / Gl ratio= Albumin / globulin ratio; HDL= High density lipoprotein; LDL= low density lipoprotein; ALT= alanine aminotransferase; and AST= aspartate transaminase.

Table 5. Effect of dietary *Spirulina platensis* supplementation on economic efficiency of rabbits does

Traits	*Spirulina platensis* (g/kg diet)		
	control	0.3	0.6
Feed intake (kg / doe / 65 days)	16.43	14.25	13.68
Price/kg feed (LE) [1]	4.68	4.78	4.89
Total feed cost / rabbit (LE)	67.90	68.20	66.87
Litter weight (kg) at birth	0.355	0.312	0.339
Total weight gain (kg) / litter	4.41	3.94	3.96
Price of kg body weight (LE)	40.00	40.00	40.00
Total return (LE)	176.47	157.68	158.18
Net return (LE)	99.57	89.48	91.31
Economic efficiency (EE, %) [2]	129.50	130.42	136.98
Relative EE	100.00	106.50	106.12

[1]Price/ kg feed by LE = the price of one kg feed by Egyptian pound and the price of one kg Spirulina 700 LE;
[2]Economic efficiency (%) = (Net return / Total feed cost) × 100

CONCLUSION

According to the attained findings herein, it could be concluded that the beneficial addition of *S. platensis*, especially at the tested level 0.6g SP / kg diet for improving the productive, and reproductive performance of adult Red Balady rabbits, which consequently led to achieve the highest profitability for farmers rearing this local species. Furthermore, other investigations into SP's active components, their mechanisms of action, and related biological pathways are essentially needed to increase our information, and applicable consequences in the sustainable animal production into the predictable future.

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تأثير الإضافة الغذائية لطحلب السبيرولينا فى علائق أمهات الأرانب على الكفاءة التناسلية والإقتصادية

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سبيرولينا بلاتينسيس هو مسحوق الطحالب الخضراء المجهرية التي يتم إنتاجها واستهلاكها بصورة متكررة في جميع أنحاء العالم، إلى جانب إفريقيا أيضًا بسبب خصائصها الغذائية والعلاجية. لذلك أجريت هذه الدراسة لتقييم تأثير الإضافة الغذائية للسبيرولينا بلاتينسيس على قياسات الكفاءة التناسلية والإقتصادية للأرانب الحمراء البلدي الناضجة لمدة ٦٥ يوم. أوضحت النتائج المتحصل عليها أن إضافة ٠.٦ جرام سبيرولينا بلاتينسيس / كجم علف أدت إلى زيادة غير معنوية في قياسات الأداء الإنتاجي للأرانب الناضجة وصغارها، والقياسات البيوكيميائية في السيرم ومعايير الكفاءة الاقتصادية. بينما أدى هذا المستوى من السبيرولينا بلاتينسيس إلى تحسن معنوي فى كل قياسات جودة السائل المنوي التي تم اختبارها وصورة الدهون في السيرم للأرانب الناضجة مقارنة مع تلك المغذاه على المستوى الأقل (٠.٣ جرام سبيرولينا بلاتينسيس / كجم علف) أو المجموعة غير المعاملة بطحلب سبيرولينا بلاتينسيس. وبالتالي يمكن التوصية بإن إضافة ٠.٦ جرام سبيرولينا بلاتينسيس / كجم علف يمكن أن تحسن من الأداء التناسلي والإنتاجي، والتي أدت بشكل متتابع إلى زيادة الكفاءة الاقتصادية لتربية الأرانب الحمراء البلدي الناضجة.