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Effect of *Spirulina platensis* Supplementation in Growing Rabbit's Diet on Productive Performance and Economic Efficiency

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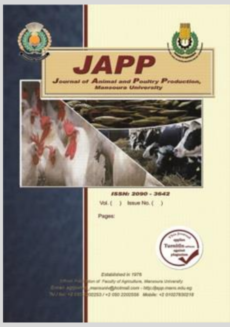

ABSTRACT

The current study carried out to assessment the effect of addition of low levels 0.3 and 0.6 g/kg diet of *Spirulina platensis* (SP) on the productive performance and economic efficiency parameters of growing Red Balady rabbits for 8 weeks. A total of 36 growing rabbits (6-week old) were randomly assigned into three dietary experimental treatments (12 rabbits of each), that was conducted from 6 to 14 weeks of age. The obtained results revealed that addition of SP to the diet of growing rabbits at level 0.6 g/kg diet led to significantly increased of growth performance parameters and improved feed conversion ratio, as well as led to insignificantly enhanced of carcass traits, and hematological and economic efficiency parameters compared to those fed the low level (0.3 g/kg diet) or those fed free SP diet. Thus, it could be concluded that the useful addition of SP for improving the productive and economic performance of growing Red Balady rabbits, especially at the tested level 0.6 g SP/kg diet.

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

INTRODUCTION

In recent decades, the development of rabbit production is the influences by different factors such as management, health, nutrition, environment, genetics, and other convinced technologies (Trocino *et al*., 2019). The production of rabbits considered as an important source of animal protein in Africa (Amaefule *et al*., 2005). Additionally, rabbit's farms paly a very imperative role in the national economics of North African countries (Ayyat *et al*., 2018). However, the high cost of feeds is the main problem in marketable production of rabbits, which represents about 65 to 75% of the total cost of rabbit's production. Reducing the high costs of feed for producing rabbits could be attained by using inexpensive non-conventional feedstuffs or by addition of some natural feed additives to the rabbit's diet (Risam *et al*., 2005).There is a potentially trend of getting the ecological and safety feed additive, which effect on the high biological value of feed (Gamko and Ufimcev, 2011; Heidarpour *et al*., 2011; Peiretti and Meineri, 2011).

Microalgal incorporation in animal feed could not only benefit of animal performance, but it also can add value to the end products, hence stimulating human health (Lum *et al*., 2013). *Spirulina platensis* (SP) is a blue-green microalgae, which commercially cultivated around the world, mainly as a human food supplement due to its potential properties as antioxidant, hepato protective, antiviral, antiallergenic and immunomodulator activities (Khan *et al*., 2005), as well as for animal feed, due to its high protein content up to 60% (Richmond, 1990). Additionally, Holman and Malau-Aduli (2013) reported that microalgae species, including SP considered as an appropriate dietary supplement for different farm animals due to their highly contents of vitamins (especially Vitamin B12 and provitamin A) and minerals; essential fatty acids (Belay *et al*., 1996); carotenoids, chlorophyll, and sterols (Henrikson, 1997). Thus, Bonos *et al*. (2016) stated that SP could be a promising functional component in broiler nutrition. SP has been intensively assessed as an effective feed additive on productive performance of different experimental animals such as sheep (Holman and Malau-Aduli, 2013); dairy cow (Christaki *et al*., 2012; Otto and Malau-Aduli, 2017); pigs (Grinstead *et al*., 2000; Nedeva *et al*., 2014); poultry (Bonos *et al*., 2016) and fish (Promya and Chitmanat, 2011; El-Sheekh *et al*., 2014), rabbits does (Meineri *et al*., 2009; Ragab *et al*., 2019), but few studies for growing rabbits (Peiretti and Meineri, 2008 & 2011; Gerencser *et al*., 2014). Consequently, the current research carried out to estimate the effect of addition of low levels of blue–green algae, *S. platensis* on the productive performance and economic efficiency parameters of growing Red Balady rabbits for 8 weeks.

MATERIALS AND METHODS

This study was conducted at El-Serw Poultry Research Station, Animal and Poultry Research Institute (APRI), Agriculture Research Center, Ministry of Agriculture, Egypt. Growing Red Balady rabbits obtained from APRI, Ministry of Agriculture, Giza, Egypt. Thirty-six rabbits (n = 36) six-week old were randomly assigned into three dietary experimental treatments (12 rabbits in each), that was conducted from 6 to 14 weeks of age. At the beginning of the experiment, rabbits were weighed and assigned to 3 treatments based on body weight (BW), where mean BW were similar for rabbits on all treatments with an average initial live BW of 736.4g/rabbit. Each treatment had four replicates (3 rabbits in each). The rabbits in each replicate kept in grower-galvanized cages and

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fed their respective experimental diets (Table 1). The cages are equipped with feeders and drinkers.

The experimental diets:

The experimental basal diet (BD) was obtained from APRI, Ministry of Agriculture, Giza, Egypt. The ingredients and the nutrients composition of BD are presented in Table 1. The calculated analysis of BD was used based on feed composition tables for rabbits' feedstuffs according to De Blas and Wiseman (2010) and Villamide *et al.* (2010), as well as the requirements of digestible energy (DE kcal/kg diet) and crude protein (%) were calculated according to FEDNA (2013).

The dried powder SP algae used in the present study was produced by National Research Center (NRC), Dokki, Giza, Egypt. The different levels of SP are carefully added to the experimental BD during the mixed of its ingredients. The experimental treatments were planned as follow: T_1: Rabbits fed free SP supplemented BD (as a control treatment), T_2: Rabbits fed supplemented BD with 0.3g SP/kg diet, T_3: Rabbits fed supplemented BD with 0.6g SP/kg diet. All rabbits fed the experimental BD *ad libitum*.

Growth performance traits:

Live body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were determined every week. However, the dead rabbits daily recorded to estimate the viability rate. The performance index (PI, %) was calculated on a group basis;

$$\mathbf{PI}\ (\%) = \frac{\mathbf{Final\ live\ BW\ (kg)}}{\mathbf{FCR}} \times \mathbf{100}$$ (North, 1981).

Table 1. Composition and calculated analysis of the basal diet

Ingredient	%
Barley grain	24.60
Alfalfa hay	31.00
Soy bean meal (44% CP)	13.25
Wheat bran	28.00
Di-calcium phosphate	1.60
Limestone	0.95
Sodium chloride	0.30
Minerals-vitamins premix [1]	0.30
Total	100
Calculated analysis [2] (% on dry matter basis)	
Crude protein (CP, %)	17.08
Digestible energy (DE, kcal/kg)	2416
Crude fiber (CF, %)	12.55
Ether extract (EE, %)	2.20
Calcium (%)	1.20
Total Phosphorus (%)	0.76
Lysine (%)	0.84
Methionine (%)	0.23
Price (LE/kg) [3]	4.68

[(1)] One kilogram of minerals–vitamins premix contains; Vitamin A, 150,000 UI; Vitamin E, 100 mg; Vitamin K3, 21mg; Vitamin B1, 10 mg; VitaminB2, 40mg; Vitamin B6, 15mg; Pantothenic acid, 100 mg; Vitamin B12, 0.1mg; Niacin, 200 mg; Folic acid, 10mg; Biotin, 0.5mg; Choline chloride, 5000 mg; Fe, 0.3mg; Mn, 600 mg; Cu, 50 mg; Co, 2 mg; Se, 1mg; and Zn, 450mg; [(2)] Calculated analysis according to feed composition tables for rabbits feedstuffs used by De Blas and Wiseman (2010); [(3)] Price of one kg (LE / kg) for different ingredients: Barley grain, 4.6.; Alfalfa hay, 2.8.; Soybean meal, 8.0.; Wheat bran, 2.1.; Di-calcium, 10.8; limestone, 0.20; Premix, 60.0; Sodium chloride, 0.50 and kg of *S. platensis* powder 300 (LE).

Carcass and hematological traits:

At the end of the experiment, three rabbits (n = 3) were randomly taken from each treatment, fasted for 12 hrs, weighed and slaughtered to estimate some of carcass traits. Carcass parts were presented as a percent of live BW, which included carcass, heart, liver, giblets, kidney, abdominal fat gastrointestinal tract and cecum.

Blood samples were collected from three rabbits (n = 3) per treatment in vial tubes containing EDTA as an anticoagulant agent to determine the hematological parameters including, red blood cells (RBCs, $\times 10^6/mm^3$), hemoglobin (Hb, g/dL), white blood cells (WBCs, $\times 10^3/mm^3$), lymphocytes (L, %), neutrophils (N, %), N/L ratio, monocytes (%) and eosinophils (%).

Economic efficiency:

At the end of the experiment, economic efficiency for weight gain was expressed as rabbit-production throught the study and calculated using the following equations;

$$\mathbf{Economic\ efficiency}\ (\%) = \frac{\mathbf{Net\ return\ (LE)}}{\mathbf{Total\ feed\ cost\ (LE)}} \times \mathbf{100}$$

Where;

$$\mathbf{Net\ return\ (LE) = Total\ return\ (LE) - The\ cost\ of\ feeding\ (LE)}$$

Statistical analysis:

All data were statistically analyzed using General Linear Model (GLM) procedure of the SPSS (2008) program, where a one-way design was used. Differences between means among treatments were subjected to Duncan´s Multiple Range-test (Duncan, 1955). The following model was used to study the effect of different levels of SP on investigated parameters as follows:

$$\mathbf{Yij = \mu + Tj + e_{ij}}$$

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

RESULTS AND DISCUSSION

Growth performance parameters:

SP can be used as an effective growth promoter for rabbits without any adverse effects (Peiretti and Meineri, 2008). The growth performance parameters of growing rabbits fed diet supplemented with or without SP in different intervals period are presented in Table 2. The obtained results showed that the growth performance parameters (BW, BWG, and PI) increased ($P \leq 0.05$) by feeding 0.6 g SP/kg diet at the end of the experiment, as well as significantly improved have been detected of FCR by feeding of SP at the end of the experiment. Inversely, FI of growing rabbits fed SP significantly decreased at the end of the experiment. Meanwhile, nonsignificant ($P \geq 0.05$) differences were detected of viability (%) among all treatments. The potential explanation for the improved performance at the end of the experiment with no response in other intervals was the duration of SP feeding, which may be enhanced the immune responses (Grinstead *et al*., 2000).

Similarly, with the current findings addition of SP increased growth performance parameters 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). More recently, Jin *et al*. (2020) stated that SP powder has an effective feed supplement for improved the growth performance. Moreover, Grinstead *et al*. (2000) showed slight improvements of growth performance and feed efficiency of weanling pigs fed diet supplemented with 0.2, 0.5 and 2% SP. Additionally, El-Sabagh *et al*. (2014) found that dietary SP had valuable effects on final BW, and FCR of fattening lambs. Inversely, Gerenscer *et al*. (2014) reported that SP and thyme did not improve growth rate of the rabbits. Other studies also did not observe better rabbit growth performance when diets were supplemented with SP at 0.5% (Colla *et al*., 2008), 1% (Peiretti and Meineri, 2009), 3% (Dalle Zotte *et al*., 2013) or 5, 10 and 15% (Peiretti and Meineri, 2008) levels. In this respect, Khanna *et al*. (2016) also noted that SP had no significant effect on the body measurements of weaned New Zealand White rabbits. Considering the opposing results between the current findings and others may be related with the nutritional and functional properties of SP used in these studies. Where, SP used in the present study was grown in fresh water, not in sea water as is the common commercial SP presented in the market, as well as the experimental conditions may be responsible.

Table 2. Effect of dietary *Spirulina platensis* supplementation on growth performance traits of growing rabbits from 6 to 14 weeks of age

Traits	*Spirulina platensis* (g/kg diet)			Pooled	Significant
	control	0.3	0.6	SEM (±)	
	Body weight (BW, g / rabbit)				
6	735.0	739.2	735.0	10.01	NS
8	1107.5	1068.3	1139.6	68.02	NS
10	1375.0	1343.3	1440.8	23.3	NS
12	1686.7[ab]	1597.9[b]	1754.2[a]	28.3	*
14	1866.4[ab]	1840.1[b]	1998.9[a]	27.10	*
	Body weight gain (g/ rabbit/ day)				
6-8	26.6	23.5	28.9	1.25	NS
8-10	19.1	19.6	21.5	1.67	NS
10-12	22.3	18.2	22.4	1.23	NS
12-14	12.8	17.3	17.5	1.0	NS
6-14	20.20[ab]	19.66[b]	22.57[a]	0.56	*
	Feed intake (g / rabbit/ day)				
6-8	62.01[a]	40.99[c]	46.69[b]	2.74	*
8-10	84.14[b]	87.20[a]	87.83[a]	0.62	*
10-12	82.21	83.33	81.78	1.21	NS
12-14	92.16	103.46	102.91	2.49	NS
6-14	80.13	78.75	79.80	0.82	NS
	Feed conversion ratio (FCR, g feed / g BW gain)				
6-8	2.36[a]	1.78[b]	1.65[b]	0.12	*
8-10	4.94	4.75	4.41	0.45	NS
10-12	3.75	4.93	3.71	0.31	NS
6-12	7.35	6.28	5.93	0.40	NS
6-14	3.98[ab]	4.02[a]	3.55[b]	0.97	*
	Performance index (Final BW (kg) / FCR) × 100				
6-14	47.08[b]	46.05[b]	56.64[a]	1.91	*
	Viability (%)				
6-14	91.7	91.7	91.7	4.35	NS

a,b,c: Mean in the same row bearing different superscripts are significantly different ($P \leq 0.05$). NS= non-significant; SEM= Standard error of mean.

Carcass traits:

Results in Table 3 showed the carcass traits of growing rabbits fed diet supplemented with or without SP. No significant ($P \geq 0.05$) differences were detected of all carcass traits of growing rabbits fed SP compared to the control group. As in the present study, no substantial effect on the carcass weight also was detected in rabbit, as reported by Mahmoud *et al*. (2017) when including SP to replace soybean with percentage of 20, 40 and 60% in the diets of rabbits. In contrast, treatment with SP was reported to increase carcass percentage and ready-to-cook yields of broiler chicks in the studies of Raju *et al*. (2004), Kaoud (2012) and Holman and Malau-Aduli (2013).

Moreover, Bonos *et al*. (2016) showed that SP supplementation (5 g/kg) could improve the meat quality of broiler. Several studies reported that feeding SP increased carcass percentage of broiler chicks (Kaoud, 2012; Mariey *et al*., 2014) and Japanese quails (Jamil *et al*., 2015). Inversely, the present findings did not show any significant effect of SP on carcass traits of growing rabbits. These differences between the present findings and others may be related with the animal age, type, dietary levels of SP, experimental period and management.

Table 3. Effect of dietary *Spirulina platensis* supplementation on carcass traits (% of live BW) of growing rabbits

Traits	*Spirulina platensis* (g/kg diet)			Pooled SEM	Significant
	Control	0.3	0.6	(±)	
Live body weight	1988.3	1906.7	1938.3	27.46	NS
Carcass	65.0	66.0	63.8	0.67	NS
Heart	0.21	0.21	0.25	0.01	NS
Liver	3.0	3.0	3.7	0.22	NS
Edible parts	3.2	3.2	3.95	0.24	NS
Spleen	0.08	0.07	0.10	0.02	NS
Kidney	1.0	1.0	1.0	0.00	NS
GIT	18.4	13.8	19.8	1.35	NS
Cecum	4.3	4.9	5.8	0.32	NS
Abdominal fat	0.99	0.91	1.18	0.10	NS
Dressing	68.4	69.6	67.7	0.60	NS

GIT= Gastrointestinal tract; NS= non-significant; SEM= Standard error of mean.

Hematological measurements:

As in the case of carcass traits of experimental rabbits in the present study (Table 3), no significant ($P \geq 0.05$) improvement were also detected of all tested hematological parameters of growing rabbits treated with SP compared to the control group (Table 4). As in the current hematological findings, also Kambou *et al*. (2015) reported that the mean WBCs, lymphocytes and neutrophils, despite being heterogeneous, are not significantly different within each parameter on the one hand and do not differ significantly from the values found by other authors on the other hand (Aboh *et al*., 2012; Bléyéré *et al*., 2013). These values show that in a homogeneous population of rabbits, the number of WBCs is not a fixed value but varies within a certain range compatible with the normal life of rabbits (Abba *et al*., 2014). In a recent study, Sugiharto *et al*. (2018) reported that feeding SP resulted in lower values of RBCs, and Hb of broilers. Inversely, with the obtained results herein other authors found that that SP stimulates the immune system with the front-line proliferation of WBCs. This immunostimulant is dose-dependent. It activates macrophages and natural killer (NK) cells. It induces the production of antibodies, and also activates T and B cells (Mathew *et al*., 1995). It has immune modulating effects by increasing the production of cytokines (interleukin-4 (IL-4), interferon-γ (IFN-γ), interleukin-2 (IL-2) and NK cells (Barry *et al*., 2014). Moreover, Simsek *et al*. (2007) recognized that 300 mg SP/kg diet increase RBCs count and Hb concentration in rats, as well as RBCs and WBCs counts were also increased of *Clarias gariepinus* fish fed 3% and 5% SP (Promya and Chitmanat, 2011). The authors stated that these positive effects of dietary SP are correlated with its stimulating effect on the stem cell activity of bone marrow.

SP is a rich source in polysaccharides, which may function as prebiotics (Beheshtipour *et al*., 2013; de Jesus

Raposo *et al*., 2016), thus the increase number of lymphocytes in the present study indicated by other hematological parameters herein also is due to the presence of the antigen, which stimulated the transformation of B cells into plasma cells, and antibody producing cells (Artur *et al*., 1989). Furthermore, the immunostimulatory effect of spirulina is due to its composition in γ-linolenic acid (GLA), vitamins and various minerals, phycocyanin (Falquet and Hurni, 2006), and sulfolipids (Kiet and Durand-Chastel, 2006). In relations of immunity, earlier studies by Qureshi *et al*. (1996) and Raju *et al*. (2004) reported that feeding SP enhanced the immune responses of chicks. Moreover, Lokapirnasari *et al*. (2016) recently showed that treatment with SP increased the number of WBCs of broiler chicks. In this respect also, Farag *et al*. (2016) pointed out that the antimicrobial and immunomodulatory capacities, as well as antioxidant potential seemed to be responsible for the health promoting effect of SP on poultry.

Economic efficiency parameters:

From economical point of view, rabbit fed SP led to insignificant ($P \geq 0.05$) enhancement of total return, net return and economic efficiency compared to the control group fed the basal diet free of SP (Table 5).

Table 4. Effect of dietary Spirulina platensis supplementation on hematological parameters of growing rabbits

Traits	*Spirulina platensis* (g/kg diet)			Pooled SEM	Significant
	control	0.3	0.6	(±)	
Hemoglobin (g / dL)	11.7	10.2	10.9	0.42	NS
RBCs ($\times 10^6$)	3.5	3.8	3.5	0.17	NS
WBCs ($\times 10^3$)	4.0	4.0	5.9	0.33	NS
Neutrophils (N, %)	62.3	57.3	67.3	2.63	NS
Lymphocytes (L, %)	31.3	39.0	24.7	3.32	NS
N / L ratio	2.3	1.6	2.8	0.28	NS
Monocytes (%)	0.0	0.67	1.7	0.40	NS
Eosinophils (%)	6.3	3.7	6.3	1.22	NS

NS= non-significant; SEM= Standard error of mean.

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

Traits	*Spirulina platensis* (g/kg diet)			Pooled SEM	Significant
	control	0.3	0.6	(±)	
Feed intake/rabbit (kg)	4.81	4.72	4.79		
Price/kg feed (LE)[1]	4.68	4.89	5.03		
Total feed cost/rabbit	22.50	23.10	24.08		
Total weight gain/rabbit	1.13	1.10	1.26		
Price kg of body weight	35.0	35.0	35.0		
Total return	39.60	38.53	44.24		
Net return	17.56	15.43	20.15		
Economic efficiency (%)[2]	75.87	66.93	83.83	3.83	NS

[1]Price/kg feed (LE) = The price of one kg feed and the price of one kg Spirulina powder 300 LE; [2]Economic efficiency (%) = (Net return / total feed cost) × 100; NS= non-significant; SEM= Standard error of mean.

These improvement in economic efficiency parameters were closely related with the significantly increased of growth performance parameters of rabbit fed 0.6g SP/kg diet (Table 2), or insignificant ($P \geq 0.05$) improved of carcass traits (Table 3) or hematological parameters (Table 4) compared to the control group. The success of commercial large-scale production of microalgae depends on many factors, and one of these is the development of cost-effective large-scale culture systems for the algae and the development of these has been, and continues to be, a gradual process (Borowitzka, 1999). However, SP a cheaper feed additive and / or ingredient than others of animal origin (FAO, 2008).

CONCLUSION

Based in the currently obtained findings, it could be concluded that the useful addition of SP for Red Balady growing rabbits, especially at the tested level 0.6g SP/kg diet. Nevertheless, many results contradict other findings and together present an inconsistent trend of SP's usefulness as a promising animal feed. Therefore, further attempts with SP are needed to clarify its potential used in a large scale of the different animal production systems.

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تأثير الإضافة الغذائية لطحلب السبيرولينا فى علائق الأرانب النامية على الأداء الإنتاجي والكفاءة الإقتصادية

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أُجريت هذه الدراسة لمعرفة تأثير الإضافة الغذائية لمستويات منخفضة 0.3 و0.6 جم سبيرولينا بلاتينسيس / كجم علف على الأداء الإنتاجي وقياسات الكفاءة الاقتصادية للأرانب البلدي الحمراء النامية لمدة 8 أسابيع. تم توزيع 36 أرنب يبلغ من العمر 6 أسابيع بشكل عشوائي إلى ثلاث مجموعات تجريبية غذائية (12 أرانب لكل منها)، التي استمرت من 6 إلى 14 أسبوعًا من العمر. كشفت النتائج التي تم الحصول عليها أن إضافة سبيرولينا بلاتينسيس إلى نظام غذائي للأرانب النامية عند المستوى 0.6 جم / كجم علف أدى إلى زيادة معنوية في معايير كفاءة النمو وتحسين معنوى فى معدل التحويل الغذائى للأرانب ، وكذلك أدى إلى زيادة غير معنوية في صفات الذبيحة ، والقياسات الهيماتولوجية والكفاءة الإقتصادية مقارنة بتلك التي تغذت على المستوى المنخفض (0.3 جم / كجم علف) أو تلك التي تتغذى على العليقة الخالية من سبيرولينا بلاتينسيس (كمجموعة ضابطة) وبالتالي ، يمكن أن نخلص إلى أهمية الإضافة الغذائية المفيدة للسبيرولينا بلاتينسيس لتحسين الأداء الإنتاجى والإقتصادي للأرانب البلدي الحمراء النامية ، خاصة عند المستوى المختبر 0.6 جم / كجم علف.