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EFFECT OF USING DIFFERENT LEVELS OF *Spirulina platensis* ON GROWTH PERFORMANCE OF MARINE SHRIMP *Litopenaeus vannamei*

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SUMMARY

This study was designed to evaluate the effect of replacement of fish meal with a marine microalgae species spirulina (*Spirulina platensis*) on Pacific white shrimp *Litopenaeus vannamei* post larvae for 90 days. Spirulina was used with four levels (5, 10, 15 and 20). Experiment was carried out in 15 tanks with water volume 150 L. Each treatment had 3 replicates, each tank contained 50 Post-larvae of 0.02 g body weight. Shrimp were fed the experimental diets containing about 40% protein twice daily at 14% from body weight (initial weight) and readjusted gradually to 5% at the end of the experiment. Growth performance of shrimp was recorded biweekly. It was found that the best treatment of growth performance, feed utilization and economic evaluation under these experimental conditions was the treatment in which fish meal was replaced by 10% spirulina.

Keywords: *L. vannamei*, *Spirulina platensis*, growth performance, feed utilization and economic evaluation.

INTRODUCTION

Pacific white shrimp, *Litopenaeus vannamei*, is an economically important farm-raised shrimp because of its great economic value, rapid growth rate and tolerance of a big range of temperatures and salinities (Huang *et al*., 2015). In 2010, *Litopenaeus vannamei* accounted for 71.8% of world production of all farmed marine shrimp species (FAO, 2012). To increase the growing market for cultured shrimp, the demand for improved feeds has created a need for high-quality protein sources (Tacon & Forster, 2000). Fish meal is considered as primary ingredient in marine shrimp diets because of its balanced amount of essential amino acids, fatty acids, minerals, vitamins and palatability (Suárez *et al*., 2009). Commercial shrimp feed formulations generally include between 25% and 50% fish meal, which considered the primary and most expensive ingredient (Gonzalez-Rodriguez & Abdo de la Parra, 2004). The increasing demand for fish meal and overexploitation of fish stocks, has spurred a search for sustainable and cheaper protein ingredients to reduce or eliminate the use of fish meal in aquaculture diets (Kiron *et al*., 2012).

Microalgae are known as a potential source of food and energy due to their photosynthetic efficiency and high nutritional value. The high protein contents of different microalgae species are considered as promising substitutes for fish meal protein or as a valuable additive in aqua feeds (Ju *et al*., 2012). In addition, microalgae are the rich source of proteins, vitamins, fatty acids and minerals (Radhakrishnan *et al*., 2014), they may possibly be an ideal alternative ingredient for sustainable aquaculture feeds. In terms of the amino acid profile, almost all microalgae compare positivly with that of other food proteins (Becker, 2007).

Among many types of microalgae that considered for feed complement in fish and shrimp culture until now, *Spirulina platensis* is known as one of the richest sources of protein, vitamins, minerals, essential amino acids, fatty acids and antioxidant pigments, like carotenoids (Radhakrishnan *et al*., 2014). Spirulina protein has a balanced composition of amino acids and concentrations of methionine, tryptophan and other amino acids similarity to these of casein. It can be used as partial supplementation or complete replacement for protein in aqua-feeds and is a cheaper feed ingredient than another animal origin (Habib *et al*., 2008).

The aim of the present study was to evaluate the effect of replacement of fish meal with a marine microalgae species *S. platensis* on growth performance, feed utilization and economical evaluation, in Pacific white shrimp *L. vannamei* post larvae.

MATERIALS AND METHODS

The white leg shrimp *L. vannamei* post-larvae were obtained from a commercial shrimp hatchery (Berket Ghalioun, Kafr Al-Sheikh, Egypt). Shrimps were transported in double –layered polythene bags that were oxygenated. When the shrimp arrived at the laboratory, they were moved into the acclimation tanks filled with seawater (salinity, 32ppt). Before start the experiment, shrimps were acclimated to laboratory condition for two weeks and were fed twice daily with a commercial diet (Skretting: 38% crude protein, 8% crude fat, and 5.9 crude fiber with 3980 Kcal of energy).

The experiment was carried out in 15 tanks with water volume 150 L. Each treatment had 3 replicates, each tank contained 50 Post-larvae of 0.02 g body weight. Tanks were filled with seawater after filtered by plankton net (50µm) to prevent the entry of un wanted materials and suspended particles into the tanks and was diluted with fresh water to make a salinity (32 ppt). All tanks were supplied with 3 air stone-hoses type of diffuser system which is fitted to air-blower (220 w). Aeration was provided 24 hours throughout the experiment. All tanks were always covered with plastic sheet to reduce escapes of shrimp. water was exchanged once a week.

After two weeks, all tanks were stocked with shrimp post-larvae. Shrimp were fed the experimental diets (as shown in Table 1) twice daily at 14% from body weight (initial weight) and decreasing gradually to 5% at the end of the experiment. Each treatment's daily feeding ration was calculated and adjusted by estimating the biweekly sampled mean biomass.

Table (1): Composition and proximate analysis of the experimental diets (% on DM basis).

Ingredient	Treatments				
	Control	T1 (5%)	T2 (10%)	T3 (15%)	T4 (20%)
fish meal (70%) protein	34.00	30.00	27.00	23.00	20.00
Soybean meal (44%) protein	34.00	34.00	34.00	34.00	34.00
Commercial Spirulina	00.00	4.00	7.00	11.00	14.00
Yellow corn	23.00	23.00	23.00	23.00	23.00
Sun flour oil	6.00	6.00	6.00	6.00	6.00
Mineral mixture[1]	2.00	2.00	2.00	2.00	2.00
Vitamin mixture[2]	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100
Proximate analysis					
Protein	41.39	40.00	39.55	39.35	39.89
Lipids	10.32	10.31	10.32	10.36	10.35
Ash	7.35	7.31	7.32	7.34	7.36
Fibers	3.16	3.24	3.36	3.44	3.54
Mositure	23.71	23.69	23.43	23.81	23.80
NFE[3]	37.78	39.14	39.45	39.51	38.86
Gross energy (Kcal/100g)[4]	486.65	484.29	483.12	482.62	482.90

[1]*Each Kg mineral mixture premix contained Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.*
[2]*Each Kg vitamin contained Vitamin A, 4.8 million IU, D3, 0.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin,20 mg.*
[3]*Nitrogen Free Extract = 100 – (%Protein + %Fat + %Fiber + %Ash).*
[4]*Gross Energy based on protein (5.65 Kcal/g), fat (9.45 Kcal/g) and carbohydrate (4.11Kcal/g). According to (NRC, 2011)*

The experimental diets were prepared by weighing of each component individually and mixing the mineral, vitamins and additives with corn. Then, this mixture was added to the components together with oil. Water was added to the mixture until became suitable for making granules. This mixture was passed through CBM granule machine with 2mm diameter. The pellets were dried at room temperature and kept frozen until experimental start.

Spirulina (*Arthrospira platensis*) used in present study was obtained from local market (Alhlw, Co. for biological production-Zagazik, Egypt). The chemical analysis of Spirulina (*Arthrospira platensis*) was shown in Table (2).

Table (2): The Chemical analysis of Spirulina (*Arthrospira platensis*).

Component	% (Mean ± SD)
protein	57.79 ± 1.53
Carbohydrates	14.60 ± 0.60
Ash	12.05 ± 0.31
Lipids	9.33 ± 0.16
Moisture	6.98 ± 0.05
Fibers	6.23 ± 0.25

Growth performance parameters:

Shrimp weight (g) was measured at the initial of the experiment and biweekly by collected randomize number of shrimp from each tank and weighted in an analytical digital balance and then returned back to their tanks during the experiment. Shrimp Weight gain (WG), Specific growth rate (SGR) and Survival rate% (SR) were calculated according to the following equations:

- Weight gain (WG) = Final body weight (g) - Initial body weight (g).
- Specific growth rate % (SGR) = [(ln FBW - ln IBW) /day of experiment] ×100
- Survival rate % (SR) = (Final number of shrimp / Initial number of shrimp) ×100.

Feed utilization parameters:

Feed utilization parameters were calculated according to the following equations:

- Feed Conversion Ratio (FCR)= Total feed consumption/ weight gain.
- Feed Efficiency (FE) %= (Final weight – initial weight) / feed consumed ×100.
- Protein Efficiency Ratio (PER)= body weight gain (g)/ protein intake (g)

Statistical analysis:

All data of variables measured were analyzed by two-way ANOVA. The ANOVA was performed by using the SAS v9.0.0 (SAS, 2004) program. The ANOVA was followed by Duncan's test (Duncan, 1955) at P<0.05 level of significance.

Economical evaluation:

The cost of feed to raise unit biomass of shrimp was estimated by a simple economic analysis. The estimation was based on local retail sale market price of all the dietary ingredients at the time of the study.

- Cost /kg diet (LE) = Cost per Kg diet L.E.
- Consumed feed to produce 1kg shrimp (kg) = Feed intake per shrimp per period/ final weight per shrimp Kg/Kg.
- Feed cost per kg fresh shrimp (LE) = Step 1× step 2.
- Relative % of feed cost/ kg shrimp = Respective figures for step 3/ highest figure in this step.
- Feed cost /1Kg gain (LE) = Feed intake per Kg gain × step 1.
- Relative % of feed cost of Kg gain = Respective figures for step 5/ highest figure in this step.

RESULTS AND DISCUSSION

Growth performance and feed utilization:

Table (3) presents the effect of different dietary replacement of *spirulina platensis* protein levels on growth performance and feed utilization in experimental tanks of *L. vannamei* . After the 90 days feeding trial, mean weight gain of the shrimp fed with diet T1 and T2 were differed significantly (P<0.05) than control diets. The spirulina diets with the replacement of fish meal at 5 and 10% showed higher weight gain; 8.55±0.08g for T1 and 8.63±0.72g for T2. The 10% treatment (T2) had significantly (P<0.05) the best feed efficiency (FE) and protein efficiency ratio (PER) compared with the rest of experimental groups.

Table (3): Effect of using *spirulina platensis* protein levels on growth performance and feed utilization (Mean±SD) in experimental tanks of *L. vannamei* for 90 days.

Parameter	Treatment				
	Control	T1 5%	T2 10%	T3 15%	T4 20%
IBW (g)	0.02	0.02	0.02	0.02	0.02
FBW (g)	7.86 ± 0.71^{c}	8.57 ± 0.08^{a}	8.65 ± 0.92^{a}	8.33 ± 1.7^{b}	7.32 ± 1.58^{c}
WG (g)	7.84 ± 0.71^{c}	8.55 ± 0.08^{a}	8.63 ± 0.72^{a}	8.31 ± 1.6^{b}	7.31 ± 1.18^{c}
SGR (%/day)	6.93 ± 0.67^{b}	7.02 ± 0.69^{a}	7.03 ± 0.67^{a}	6.99 ± 0.69^{b}	6.85 ± 0.41^{b}
FI (g feed/shrimp)	10.42 ± 0.72^{c}	11.83 ± 0.54^{a}	10.18 ± 0.24^{c}	11.73 ± 0.93^{b}	10.16 ± 0.24^{c}
FCR	1.33 ± 0.19^{b}	1.38 ± 0.07^{a}	1.18 ± 0.09^{c}	1.41 ± 0.23^{a}	1.39 ± 0.12^{a}
PER	1.82 ± 0.21^{b}	1.81 ± 0.35^{b}	2.14 ± 0.23^{a}	1.80 ± 0.24^{b}	1.80 ± 0.12^{b}
FE (%)	75.27 ± 2.3^{b}	72.26 ± 2.51^{b}	84.81 ± 1.89^{a}	70.87 ± 1.35^{c}	71.92 ± 1.23^{c}
SR (%)	92.67 ± 3.56^{b}	94.67 ± 4.65^{a}	94.67 ± 6.42^{a}	92.00 ± 3.49^{b}	90.67 ± 3.7^{c}

Data are presented as means ±SD. Values in the same row with different superscript letters are significantly different (P< 0.05).

In this study, best growth rates and most efficient FCR were achieved at T2. Growth enhancement effect of spirulina is because of its role in nutrient digestibility and its high contents of several nutrients, like vitamins and minerals (Abdel-Tawwab and Ahmad, 2009). On the other hand, negative effects of high dietary inclusion levels of spirulina on fish growth can be resulted from reduced phosphorous availability and decreased feed palatability (Olvera-Novoa *et al*., 1998). The variations in spirulina effects on fish growth performance are attributed to different nutrient content of spirulina species used in the studies (Nandeesha *et al*., 1998). Nandeesha *et al*. (2001) found that fish meal (FM) can be completely replaced with spirulina in diets for rohu carp (*Labeo rohita*) and even significantly higher growth can be obtained compared to the use of FM as the sole protein source, whereas no significant effect was observed on growth performance of catla (*Catla catla*) by the same spirulina supplemented diets. Such as differences in growth response of *L. rohita* and *C. catla* to dietary spirulina clearly show that the growth response of fishes to spirulina is likely to be species-specific. The other significant factor that affects the results of spirulina administration is the composition of experimental diets in which spirulina is combined (Takeuchi *et al*., 2002).

James *et al*. (2006) observed that 8% of spirulina diet showed higher food consuming rate in *Xiphophorus helleri*. *Rhabdosargus sarba* fingerlings fed with 32% spirulina feed had higher feed intake (El-Sayed, 1994). The study is in agreement with the report by Nakagawa and Gomez-Diaz (1995) who found that the diet with 5 – 20% supplementation of spirulina meal improved the *Macrobrachium rosenbergii* survival and growth. The results showed improvement of fish growth by replacing 5% -10 % FM protein with spirulina, while higher substitution levels could not provide further enhancement. Tongsiri *et al*. (2010) observed that replacement of 5% FM with spirulina showed the best growth performance of *P. gigas*, but higher replacement levels lowered the fish weight gain. Olvera-Novoa *et al*. (1998) reported that spirulina can replace up to 20% of FM protein in diets for *O. mossambicus*, but reduced growth and feed utilization were showed at higher replacement levels. Güroy *et al*. (2012) also observed better growth in yellow tail cichlids, with an increase in weight of between 12% and 17% following the inclusion of 2.5% and 10% *Spirulina*, respectively. In an even better scenario, Adel *et al*. (2016) observed that sturgeon juveniles fed with 10% *Spirulina* showed a growth increase of 57% and an improvement in the FCR of 21%. Another important study by Kohal *et al*. (2018) reported that the red cherry shrimp showed a dramatic improvement in growth from 70.8 mg to 114.6 mg following the addition of 10% *Spirulina* to the diet, which represents a gain of 63%, an improvement in the FCR of 15%, and a survival increase from 25.7% on the control diet to 81.3% on the 10% *Spirulina* diet. Partial substitute of fish meal with *S. platensis* has showed promising growth in juvenile *L. vannamei* (Hanel *et al.*, 2007). The admissible level of spirulina meal as the dietary inclusion to replace the fishmeal was about 25% with no harmful effect on the growth of shrimp *L. vannamei*, and up to 50% of replacement could not affects the feed intake (Sá and Nunes, 2011). Many such examples explain that if Spirulina is used at low levels in fish diets could get not only health benefits but also economic benefits by improving the FCR.

Economical Evaluation:

Calculations of economic efficiency of the tested diets based on the cost of feed, costs of one Kg gain in weight and its ratio with the control group are shown in Table (4)**.** T2 have the lowest feed cost per kg fresh shrimp (18.59 LE), relative % of feed cost / kg shrimp (85%), feed cost /1Kg gain (18.59 LE) and relative % of feed cost of Kg gain (85%).

Table (4): Economic analysis of *Litopenaeus vannamei* supplemented with different levels of Spirulina for 90 days.

Item	Treatment				
	Control	T1 5%	T2 10%	T3 15%	T4 20%
Cost /kg diet (LE)	16.37	15.93	15.75	15.31	14.93
Consumed feed to produce 1kg shrimp (kg)	1.33	1.38	1.18	1.41	1.39
Feed cost per kg fresh shrimp (LE)	21.77	21.98	18.59	21.59	20.75
Relative % of feed cost/kg shrimp	99	100	85	98	94
Consumed feed to produce 1Kg gain (Kg)	1.33	1.38	1.18	1.41	1.39
Feed cost /1Kg gain (LE)	21.77	21.98	18.59	21.59	20.75
Relative % of feed cost of Kg gain	99	100	85	98	94

CONCLUSION

It can be concluded that the diet in which fish meal was replaced with 10% spirulina was the best in terms of growth performance, feed utilization and economic evaluation under these experimental conditions.

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تأثير استخدام مستويات مختلفة من طحلب الاسبيرولينا على اداء نمو الجمبرى البحرى الفانمى

شيماء على حسن زيدان[1] ، عبد الحميد صلاح عيد [1] ، مرفت على محمد [1] و زكى زكى شعراوى [2]

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تم اجراء هذة الدراسة لتقييم تأثير استبدال مسحوق السمك بأحد انواع الطحالب البحرية طحلب الاسبيرولينا على يرقات الجمبرى الفانمى لمدة 90 يوم.تم استخدام اربعة مستويات من طحلب الاسبيرولينا (5،10،15،20%). تم اجراء التجربة فى 15 تانك سعة 150 لتر ماء. احتوت كل معاملة على 3 مكررات وكان عدد الجمبرى فى كل خزان50 . *قبل التوزيع تم وزن الجمبرى فكان الوزن الابتدائى له (متوسط ،0.02 جرام).* تم تغذية الجمبري بعلائق تجريبية تحتوي على حوالى 40٪ بروتين مرتين يومياً وبنسبة 14٪ من وزن الجسم (الوزن الأولي) وتم تعديله تدريجياً إلى 5٪ في نهاية التجربة.تم تسجيل معدلات نمو الجمبرى كل اسبوعين. وقد وجد ان افضل معاملة من حيث أداء النمو ،الاستفادة الغذائية والتقييم الاقتصادى تحت هذه الظروف التجريبية هى المعاملة رقم 2 التى تم فيها استبدال مسحوق السمك بنسبة 10% طحلب الاسبيرولينا.