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Growth Response of the Freshwater Prawn, *Macrobrachium rosenbergii* (De Man), to Diets Having Different Levels of Biogen®

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Abstract: The present study was conducted to investigate the impact of adding probiotics(Biogen®) in the diet of prawn (*Macrobrachium rosenbergii)* during the post larval stage of growth.Two hundred and fifty post larvae prawn (Mean weight of 0.01±0.002 g) were divided into five experimental groups each with two replicates. The experiment was conducted for 12 weeks. Experimental diets were identical in all aspects except for variation in the probiotics ratio. Control diet had no Biogen®, diet I (contained 1% Biogen®), diet II (2% Biogen®), diet III (3% Biogen®) and diet IV(4% Biogen®). Generally, growth performance and survival of the probiotic fed groups were significantly higher ($P<0.05$) than the control group. Significantly higher growth ($P<0.05$), for final body weight (FBW), specific growth rate (SGR) and improving in normalized biomass index (NBI) were recorded in groups of prawn fed diets II and III. In addition, the food conversion ratio (FCR), protein efficiency ratio (PER) in treatments receiving Biogen® were significantly better ($P<0.05$) than those fed the control diet. Concerning the influence of the Biogen® on the proximate composition of carcass, no significantly differences ($p>0.05$) were observed among different treatments in carcass moisture and ash content. While protein content showed the highest value for prawn fed both diet II and diet III, but the lowest value of protein was observed with diet IV. No significant differences were observed in lipid content among groups of prawn fed diet I, IV and those fed control diet, while the best and lowest values of lipid carcass were recorded for those received diet II and III. In conclusion, the additions of Biogen® in the diets (2 or 3%) improves and enhance the growth performance of *Macrobrachium rosenbergii* larvae.

Key words: Probiotic · Growth · Feed utilization · *Macrobrachium rosenbergii*

INTRODUCTION

Probiotics are beneficial microorganisms that protect the host from diseases, [1] defined probiotics as " live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance". Microbes play very important and critical roles in aquaculture systems, both at hatchery and grow-out levels, because water quality and disease control are directly affected by microbial activity [2]. The range of probiotics examined for use in aquaculture has encompassed both Gram-negative and Gram- positive bacteria, yeast and unicellular algae. In particular probiotics have been reported to be successful with a wide range of invertebrates [3-5]. Most probiotics are supplied as live supplements in diets, which has the ability to survive passage through the intestinal tract [1]. Several mechanisms have been suggested as modes of action for probiotic bacteria. The competitive exclusion, based on the removal of pathogen by the beneficial population has been regarded as important by many authors [1, 6, 8]. Some studies have attributed the enhancement of animal growth to the nutritional benefits of probiotic bacteria, such as vitamin production, a viability of minerals and trace elements and production of important digestive enzyme [7]. Also, [8] reported that the bacteria, *Vibrio alginolyticus* may have characteristics capable of conferring some of protection against disease in shrimp hatcheries. The use of lactic acid bacteria (LAB) as probiotics [9] and non-specific immunostmulants [10,11] has been proposed in addition to the effort to improve water quality [12, 13] and nutrition [14] as a means to increase larval survival and aquaculture out put. Recently there has been great interest in the use of LAB

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and their metabolic products as potential probiotics in aquaculture to improve population growth in rotifer cultures [15-17], their nutritional value for turbot larvae [18] and thus larval survival [19]. They have also been used in the disinfection treatment of *Artemia nauplii* [20], as growth promoters of *Oreochromis niloticus* [21]. Also the addition of probiotics as a food supplement to Xenic culture of *Crassostrea giyas* larvae was found to consistently enhance the growth of the Oyster larvae [22]. Though, probiotics have been shown to be effective in a wide range of species for the promotion of growth, enhanced nutrition, immunity and survival rate. Some studies were conducted on the use of probiotics in the diets of the fresh water prawn *Macrobrachium rosenbergii* [23 - 26]. So the present study was, therefore, taken up with the objective of supplementing of probiotics in the diet of *M. rosenbergii* and asses their growth performance and its body composition.

MATERIALS AND METHODS

Experimental Conditions: The experiment was conducted for 12 weeks at Invertebrate Laboratory (Barrage Fish Farm, National Institute of Oceanography and Fisheries Cairo, Egypt). The experimental prawns were obtained from Marute Fish Farm Company, Alexandria and were divided into five experimental groups each with two replicates following a completely randomized design in 10 uniform size glass aquaria (30 L capacity). The details of the experimental diets are as follow:

The control diet, whereas, no Biogen®) added and four other test diets included Biogen ® at 1, 2, 3 and 4% levels. The fish meal, soybean meal, wheat bran and yellow corn were purchased from the Islamic Company (APICO), Dokki- El-Giza Egypt. Biogen®) was obtained from El-zahar Veterinary Trading Company (Exclusive Agent of the manufacture chain way Corporation, Taiwan). The Biogen® contained: Allicin (not less than 0.247 M mole/g), *Bacillus subtillis* not than $6x10^7$ cfu/g and High Unit Hydrolytic Enzyme (not less than 3690 U/g). Five isonitrogenous (35% crude protein) and isocaloric (4.4 Kcal/g). The diets were processed by bending the dry ingredients into a homogenous mixture and then passing the mixed feed through a laboratory pellet mill (California Pellet Mill Co., San Francisco, CA, USA). The experimental diets and its chemical composition are shown in Table (1). Feed was given at 10% of the initial body weight of larvae and it offered twice daily at 9.00 and 14-00 hours.

Table 1: Composition and proximate analysis of the experimental diets/ 100 g fed to *Macrobrachium rosenbergii*

	Diets				
Ingredients	Control	I	II	III	IV
Fish meal	30	30	30	30	30
Soybean meal	30	30	30	30	30
Yellow corn	20	20	20	20	20
Wheat bran	15	14	13	12	11
Vitmin & Mineral premix	2	2	2	2	2
Corn oil	3	3	3	3	3
Biogen %	0	1	2	3	4
Total	100	100	100	100	100
Dry matter	85.85	84.96	84.07	83.18	82.73
Crude protein	35.4	35.19	35.03	34.87	34.7
Crude lipid	6.69	6.65	6.61	6.57	6.53
Crude fiber	4.38	4.28	4.18	4.08	3.98
Ash	9.51	9.46	9.41	9.36	9.30
NFE*	44.02	44.42	44.77	45.12	45.49
Gross energy (Kcal/Kg)**	4437.32	4438.12	4439.72	4441.31	4443.15
Met. Energy(Kcal/Kg)****	3106.12	3106.68	3107.8	3108.92	3110.21

* Nitrogen free extract concluded by difference

** Gross energy content (Kcal/Kg) calculated according to [27] using following calorific values: 5.64, 9.44 and 4.11 Kcal/g whole body of protein, fat and carbohydrate, respectively.

*** Metabolizable energy was calculated from gross energy as 70% reported by [28].

Rearing: Twenty five larvae of *M. rosenbergii* of uniform size (0.01 ±0.002 g) were kept in each aquarium. The total volume of water in each aquarium was maintained 20 L throughout the experimental period. Aeration was continuously provided to all aquaria by using air stone and oxygen bump. The larvae were acclimatized for one week to formulated feed before the starting the experiment. Aquaria were cleaned on alternate days and about 30% of the water was replaced with freshwater dechlorenated tap water every two days.

Growth Measurements: The total length (cm), body weight(g), of larvae were measured biweekly and specific growth rate (SGR), normalized biomass index (NBI), total feed consume (TFC), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated According to Sweilum [29] as follow:

SGR = (lin Final Wt – Lin initial Wt) x100/ Days

NBI = Final Wt x Prawn number – Initial Wt. x number/ 100

TFC = Average Wt. x number of prawn x feeding days x 10 /100

FCR = Feed consumed (g) x number of prawn / weight gain (g).

PER = Weight gain (g) x number of prawn / protein intake.

Larvae of prawn were counted at the end of the experiment and compared with the initial stock to calculate survival.

Biochemical Analysis: The proximate composition of feed and tissue of prawn were analyzed,for example, crude protein, lipid, ash, crude fiber and nitrogen-free extract according to Standard Methods [30]. Similarly tissues of prawn were analyzed at the end of experiment.

Water Quality Parameters: Temperature, pH, dissolved oxygen, total alkalinity, nitrite and phosphate were measured every week using Standard Methodology [31].

Statistical Analysis: Data were statistically processed for one-way analysis of variance (ANOVA) to find out any significant differences among the experimental groups and the comparison between two treatments were done using Duncan,s multiple range test (DMRT) according to Snedecor and. Cochran [32].

RESULTS

Physico-Chemical Parameters of Water: Table 2 shows the means values ±SE of water quality parameters throughout the experimental period. All the water quality were within the acceptable range for freshwater prawn.

Growth Performance Analysis: Table 3. indicates the growth performance of the freshwater prawn fed different test diets, it was found that prawn fed diets containing Biogen® exhibited greater growth than those fed the control diet and the highest significant values ($P<0.05$) of FBW and SGR and NBI, were found for prawn fed diet ll during the first 6 weeks. With the beginning of the 8^{TH} week prawn fed diet III showed the highest significantly values ($P<0.05$) until the end of experiment. While groups of prawn fed on control diet represented the lowest value ($P>0.05$).

In addition, FCR and PER of prawn fed on diets II and III supplemented with 2 and 3% Biogen®, were significantly better ($P<0.05$) than those fed the other test diets (Table 4).

Table 2: Water quality of the experimental aquaria throughout the experimental period (Mean±SE)

	Diets				
Items	Control	I	II	III	IV
Water temperature °C	28±0.65	28±0.69	28±0.58	28±0.60	29±0.44
Dissoved oxygen (mg/L)	5.4±0.34	5±0.38	5.2±0.27	5.4±0.25	5.3±0.27
pH	7.5±0.04	7.5±0.12	7.6±0.12	7.8±0.14	7.8±0.07
Total alkalinity (mg CaCO3/L)	200±6.76	210±6.71	218±6.59	210±6.62	220±6.98
Nitrite (NO2, mg/L)	0.006±0.0004	0.005±0.0002	0.005±0.0004	0.006±0.0004	0.007±0.0004
Po4 (mg/L)	0.79±0.03	0.85±0.05	0.88±0.06	0.91±0.06	0.80±0.04
Photoperiod	Natural	Natural	Natural	Natural	Natural

Table 3: Growth parameters of the freshwater prawn fed different levels of Biogen® for 12 weeks

	Weeks																	
	2			4			6			8			10			12		
Treatments	FBW	SGR	NBI	FBW	SGR	NBI	FBW	SGR	NBI	FBW	SGR	NBI	FBW	SGR	NBI	FBW	SGR	NBI
Control	0.02^{b}	4.62^{b}	100	0.062^{b}	6.08^{c}	210	0.111^{b}	5.35^{b}	79	0.126^{b}	4.22^{b}	13.5	0.192^{c}	3.94^{b}	52.38	0.25^{d}	3.58^{c}	30.21
I	0.03^{b}	7.32^{c}	200	0.09^{c}	7.32^{b}	200	0.105^{b}	5.23^{b}	16.67	0.138^{b}	4.37^{b}	31.43	0.229^{c}	4.17^{b}	65.94	0.34^{c}	3.92^{c}	48.47
II	0.052^{a}	10.999^{a}	420	0.146^{a}	8.94^{a}	180	0.22^{a}	6.87^{a}	50.68	0.340^{a}	5.88^{a}	54.55	0.420^{b}	4.98^{a}	23.53	0.64^{b}	4.62^{b}	52.38
III	0.04^{ab}	9.24^{a}	300	0.115^{a}	8.14^{a}	187.5	0.181^{a}	6.44^{a}	57.39	0.337^{a}	5.86^{a}	86.19	0.641^{a}	5.55^{a}	90.21	1.09^{a}	5.21^{a}	70.05
IV	0.028^{b}	6.86^{c}	180	0.09^{c}	7.32^{b}	221.43	0.149^{b}	6.00^{a}	65.56	0.215^{c}	5.11^{a}	44.29	0.322^{b}	4.89^{b}	82.33	0.47^{c}	4.28^{b}	19.89

FBW= Final body weight SGR= Specific growth rate NBI= Normalized biomass index

Means with different superscripts in the same columns are significantly different at least at $P<0.05$

Table 4: Feeding efficiency of freshwater prawn fed different levels of Biogen® for 12 weeks

	Weeks																	
	2			4			6			8			10			12		
Treatments	TFC	FCR	PER	TFC	FCR	PER	TFC	FCR	PER	TFC	FCR	PER	TFC	FCR	PER	TFC	FCR	PER
Control	0.0006^{a}	1.36^{a}	2.07^{d}	0.001^{a}	0.59^{b}	4.75^{b}	0.003^{b}	1.75^{c}	1.61^{a}	0.005^{ab}	4.10^{a}	0.25^{d}	0.005^{b}	2.71^{c}	1.04^{b}	0.006^{b}	3.69^{c}	0.76^{b}
I	0.0006^{a}	0.73^{b}	3.89^{b}	0.001^{a}	0.83^{a}	3.41^{c}	0.004^{b}	5.62^{a}	0.36^{b}	0.004^{b}	3.67^{a}	0.77^{c}	0.005^{b}	1.67^{b}	1.71^{a}	0.009^{b}	2.70^{b}	1.05^{b}
II	0.0006^{a}	0.29^{c}	9.99^{a}	0.003^{a}	0.68^{c}	4.21^{b}	0.008^{a}	2.30^{b}	1.24^{a}	0.012^{a}	2.50^{b}	1.14^{b}	0.016^{a}	5.86^{a}	0.54^{c}	0.019^{a}	2.27^{b}	1.26^{a}
III	0.0006^{a}	0.42^{d}	6.88^{c}	0.002^{a}	0.59^{b}	4.84^{b}	0.006^{a}	2.16^{b}	1.32^{a}	0.009^{a}	1.37^{c}	2.09^{a}	0.017^{a}	1.39^{b}	2.05^{a}	0.03^{c}	1.71^{d}	1.67^{a}
IV	0.0006^{a}	0.775^{b}	4.32^{b}	0.001^{a}	0.40^{d}	7.15^{a}	0.004^{b}	1.99^{c}	1.45^{a}	0.006^{a}	2.84^{b}	1.01^{b}	0.008^{c}	1.507^{b}	1.91^{a}	0.014^{b}	6.19^{a}	0.47^{c}

TFC= Total feed consumed (Kg)

FCR= Feed conversion ratio

PER= Protein efficiency ratio

Means with different superscripts in the same columns are significantly different at least at P<0.05

Table 5: Survival performance of freshwater prawn fed different levels of Biogen® for 12 weeks

	Biogen® %														
	Control			I			II			III			IV		
Period															
(Weeks)	NLP	NSP	TSR	NLP	NSP	TSR	NLP	NSP	TSR	NLP	NSP	TSR	NLP	NSP	TSR
2	6	44	88	9	41	82	-	50	100	2	48	96	7	43	86
4	4	40	80	1	40	80	3	47	94	4	45	90	3	40	80
6	5	35	70	6	34	68	-	47	94	3	42	84	6	34	68
8	5	30	60	1	33	66	7	40	80	-	42	84	2	32	64
10	2	28	56	-	33	66	2	38	76	2	40	80	2	30	60
12	-	28	56	3	30	60	-	38	76	1	39	78	1	29	58

NLP= Number of losses prawn

NSP= Number of survival prawn

TSR= Total survival rate

Table 6: Proximate composition of the final prawn carcasses at the end of experiment of different test diets

	Different test diets				
Parameters	Control	I	II	III	IV
Protein	63.1±3.51^{b}	64.5±3.90^{b}	67.1±2.50^{a}	65.5±2.90^{a}	61.6±2.77^{b}
Lipid	9.55±0.951^{a}	9.2±0.82^{a}	7.35±0.259^{b}	7.75±0.435^{b}	9.89±0.85^{a}
Ash	19.1±0.245^{a}	19.5±0.35^{a}	20.2±0.540^{a}	19.6±0.455^{a}	18.2±0.522^{a}
Moisture	77.9±0.792^{a}	75.8±1.55^{a}	77.3±1.71^{a}	76.3±2.78^{a}	77.8±1.92^{a}

Results are a dry matter basis and are based upon the means of three pooled samples ± SE.

Means with different superscripts in the same row are significantly different at least at P<0.05.

Table 5. shows the survival performance of prawn larvae fed different test diets, it was found that the best and highest survival rate were observed for group of prawn fed on diet II and III which represented by 76 and 78%, respectively.

Concerning the influence of different dietary probiotic levels on chemical proximate analysis of carcass, table 6 shows no significant differences in carcass moisture and ash contents. While the highest significant (P<0.05) values of protein content were

observed on group of prawn fed on diets II and III and its lowest value with diet IV. Differences were observed in carcass lipid content with significantly higher values ($P<0.05$) for both groups of prawn fed control diet, prawn fed diet I and those fed on diet IV, while prawn fed diet II and III, represented the best values.

DISCUSSION

The presence of the probiotic significantly improved shrimp survival in most treatments. Because administration of the probiotic significantly changed the proportion of *Bacillus* bacteria in the gut flora, the increase survival by shrimp may be due to exclusion of other bacteria (especially harmful bacteria), particularly in the larval and early postlarvae stage where the *Bacillus* bacteria were dominant. In *Penaeus, monodon*, *Bacillus,* used as a probiotic was able to colonize both the culture water and shrimp digestive tract, *Bacillus* also was able to replace *vibria* spp. In the gut of shrimp, thereby increasing shrimp survival [33]. *Bacillus* are able to out-complete other bacteria for nutrients and space and can exclude other bacteria thought the production of antibiotic [9,34].

In the present study, all probiotic- supplemented diets resulted in higher growth in prawn than the control diet, suggesting that the addition of probiotics enhance the growth performance and feed utilization. *Bacillus subtilis* have been shown to produce digestive enzymes such as amylase, protease and lipase which enrich the concentration of intestinal digestive enzymes [35]. The bacteria could also have improved digestive activity via synthesis of vitamins and cofactors or via enzymatic improvement [6]. [36] demonstrated a significant growth increase in shrimp inoculated with *Bacillus* sp. Whilst [37] found that enhanced growth was generally obtained in shrimp fed diets with *B. subtilis* inclusion. The observed increase in specific activities of the digestive enzymes in probiotic treatment may have led to enhanced digestion and increased absorption of food, which in turn contributed to the improved survival and growth in *Fennerpenaeus indicus,* including improved feed conversion ratio (FCR) and specific growth rate (SGR) [5]. On the contrary, [38, 39] found that treatment of *Penaeus mondon* and *Litopenaeus vannamei* with commercial *Bacillus* probiotic did not significantly increase ($P>0.05$) either survival or growth this may be due to the differences between species.

Biogen ® supplementation to diets resulted in reduced FCR and improved PER.. [40] found similar effects of Biogen® supplementation on feed utilization by Nile tilapia, [41] observed a significant improvement in FCR, FER and PER of shrimp larvae when fed with *L. plantarumade* bio encapsulated *Artemia*. Similar observation were also reported [25] when feeding *Probiotic* L. *cermoris* at $8.5x10^{11}$ CFUg-1 diet to postlarvae of *Macrobrachium rosenbergii.*

From the above experiment it may be concluded that a significant growth and FCR were recorded when probiotic (Biogen®) were fed to larvae of *M. rosenbergii* through supplementation diets. From a nutritional point of view and in agreement with the previous data [42], recommended the use of the probiotic Biogen as a feed additive for Nile tilapia to stimulate productive growth performance and nutrient utilization.

Further research is still needed to detect the mode of action of probiotic on *M. rosenbergii* digestibility and its effect on immune response and stress resistance. However our finding should be confirmed in outdoor, earthen pond trials before they are applied commercially.

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