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Basic Biochemical Constituents and Profiles of Amino Acids in the Post Larvae of *Macrobrachium rosenbergii* Fed with *Spirulina* and Yeast Enriched *Artemia*

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Abstract

This study was aimed to assess the growth performance of *Macrobrachium rosenbergii* post larvae fed with *Spirulina* and yeast enriched *Artemia* for 60 days. The length and body weight in prawns fed with enriched *Artemia* were increased significantly ($P<0.05$) However, *Spirulina* enriched *Artemia* fed prawns produced better growth than that of the yeast enriched *Artemia* fed prawns. *Spirulina* enriched *Artemia* acquired more level of total protein, amino acids and lipid than yeast enriched *Artemia*. While, yeast enriched *Artemia* acquired higher level of total carbohydrate than that of the *Spirulina* enriched *Artemia*. The similar trends in the content of biochemical constituents were also recorded in prawns fed with enriched *Artemia*. The content of essential amino acids, such as phenylalanine, leucine, tyrosine, isoleucine, tryptophan, methionine, valine, threonine, arginine, histidine and lysine were found to be higher in prawns fed with *Spirulina* enriched *Artemia* than that of prawns fed with yeast enriched *Artemia*. *Spirulina* has produced better growth in *M. rosenbergii* than that of yeast. However, yeast as well produced appreciable growth when compared with control. Therefore, both *Spirulina* and yeast can be taken as supplementary materials in feed management practices.

Keywords: Prawn; *Artemia*; *Spirulina*; Yeast; Protein; Amino acid; Lipid; Carbohydrate.

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1. Introduction

In India, the cultivable species of freshwater prawns are *Macrobrachium rosenbergii* and *Macrobrachium malcolmsonii*. Among these two the former has gained attention than the latter because of its little larger abdominal portion. *M. rosenbergii* is an omnivorous scavenger, and feed on variety of foods of animal and vegetable origin. Feeding is one of the most important functions of an organism since all other activities start from the food energy consumed by it. Traditional live feeds like zooplankton and oligocheate worms

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play a very important role in the nutrition of freshwater prawn culture. Live food supplies all the necessary nutrients for the development and can contribute with exogenous digestive enzymes that aid in digestion [1, 2]. In crustacean food intake regulates larval development, the rate of molt cycle duration and the growth rate at ecdysis [3, 4, 5-9]. Among live feeds used in aquaculture the brine shrimp, *Artemia* has received considerable attention, largely for feeding the late larval and post larval stage crustacean [10-12]. It has been reported that *Artemia* biomass used as a supplement to or a replacement for polycheates in *Litopenaeus vannamei* maturation diets [13]. A*rtemia* nauplii have also been supplemented with inert food for the culture of *M. rosenbergii* [14, 15]. *Artemia* an omnivore and feed on protozoa, micro-algae, yeast and bacteria.

Algae contribute to increase food utilization, growth, carcass quality, stress tolerance and disease resistance as they contain good protein source [16, 17]. Supplementation of micro algae has been reported to restrict the addition of various micro capsules in feed formulations [18]. *Spirulina* is considered as rich source of protein, vitamins, minerals, essential amino acids and fatty acids (γ-linolenic acid (GLA) and antioxidant pigments, carotenoids and perform immune modulator function [19, 20]. Yeasts are excellent source of protein, essential amino acid, vitamin B-complex and folic acid, and therefore, it is an alternative protein source to fish meal [21-23]. As *Artemia* is ideal food for the growth of crustacean larvae and post larvae, and in the view of the nutritional status of *Spirulina* and yeast, the present study was aimed to assess the growth performance of *M. rosenbergii* post larvae fed with *Spirulina* and yeast enriched *Artemia*. This is also to promote the healthy and sustainable inland aquaculture practices of freshwater prawns and to encourage on farm feed management. In order to assess the quality of enriched feed, the contents of basic biochemical constituents, such as total protein, amino acids, carbohydrate and lipid were analyzed in the enriched feeds and the prawns fed with these feeds. Further, the profiles of amino acids were also analyzed.

2. Materials and Methods

The post larvae (PL-10) of the freshwater prawn, *M. rosenbergii* were procured from Rosen Fisheries, Thrissur, Kerala, India. They were transported in oxygenated polythene bags and acclimatized to laboratory condition in 1000 L cement tank (6'x3'x3') for two weeks using ground water. The prawns were fed with boiled egg albumin and zooplankton, such as *rotifers*, *daphnia*, and *Artemia* nauplii alternatively. Water was adequately renewed daily. At the same time, the faecal matter and unfed feed were removed. The medium was adequately aerated. The ground water had these physicochemical parameters: pH, 7; total dissolved solids, 0.9 g L^{-1}; dissolved oxygen, 7.2 mg L^{-1}; BOD, 30.0 mg L^{-1}; COD, 125.0 mg L^{-1}; ammonia, 0.028 mg L^{-1}. Similarly the mother water (the hatchery from where the PL was procured) had the following physicochemical characteristics: pH, 6.8; total dissolved solids, 1.2 g L^{-1}; dissolved oxygen, 6.5 mg L^{-1}; BOD, 42.0 mg L^{-1}; COD, 140.0 mg L^{-1}; ammonia, 1.20 mg L^{-1}. The physicochemical parameters were estimated following the method of APHA [24].

The brine shrimp, *Artemia parthenogenetica* cysts were purchased from Aqua world, Paris Corner, Chennai, India. The cysts equivalent to 50 g kg^{-1} body weight of the prawn was taken and hydrated in L^{-1} of purified artificial saltwater (prepared from artificial sea salt powder 35.0 g L^{-1}, pH of 6.5). The hydrated cysts were de-capsulated in 0.5 g L^{-1} sodium hypochloride solution and deactivated in 0.1 N HCl. The deactivated cysts were again hydrated in saltwater. After 12-15 hours, the cysts burst and brownish orange coloured nauplii come out. *Artemia* nauplii (48 hr old) were collected and enriched with *Spirulina platensis* and yeast (baker's yeast). The *Artemia* nauplii were fed with 5 g L^{-1} *Spirulina* and yeast separately for 6 hr in salt water medium. To ensure oxygenation and to keep the microorganism in suspension mild aeration was provided. The enriched *Artemia* nauplii was collected and thoroughly washed with freshwater and fed to the prawns two times per day for 60 days. The content of biochemical constituents, such as total protein [25], amino acid [26], carbohydrate [27] and lipid [28] were measured in both un-enriched and enriched *Artemia*. About 100 nauplii were pooled to constitute a single observation of a parameter and three such pooled observations were made for each parameter (100x3=300x4=1200).

The PL of *M. rosenbergii* ranged from 1.2-1.5 cm in length and 0.03-0.05 g of body weight were taken and divided into three groups. Each group consisted of 90 individuals, housed in a aquarium of 45 L capacity. They were allowed to acclimatize for a week and maintained as described previously. From each group 10 prawns were randomly taken and pooled together (10x3=30). After measuring the initial morphometric data they were reintroduced into the aquaria. For estimation of content of initial biochemical constituents, such as total protein, amino acid, carbohydrate and lipid, tissues from 30 prawns in each group were pooled separately and taken for analyses of biochemical parameters. Thus, three such observations were made for each parameter. The remaining individuals (60 prawns) in each group were equally transferred to three aquaria of 15 L capacity and taken for experimental feeding trial. Of which one group served as control and fed with un-enriched *Artemia*. Another two groups were fed with *Spirulina* and yeast enriched *Artemia* respectively for a period of 60 days. Thus, the study was conducted in triplicate. The water medium was renewed daily by siphoning method causing minimum disturbance to the prawns. On the final day, the final morphometric data and estimation of final concentrations of total protein, amino acid, carbohydrate and lipid were estimated. HPTLC profile of amino acids was also done on the final day of the feeding schedule [29]. At least four animals from each aquarium were sacrificed and pooled to constitute a single observation. Thus, three such observations were made on each parameter (4x5=20x3=60). All the data were analyzed statistically by adopting Student't' test and one way ANOVA [30] using the IBM software, SPSS, version- 13.0. The post Hoc tests (Scheffe and Duncan) were also conducted.

3. Results

The initial body length and weight of the PL was recorded to 1.2 cm and 0.04 g respectively. After 60 days of feeding the final length and weight of PL was found to elevate in experimental groups when compared with control. However, the final length

and weight was found to maximum in PL fed with *Spirulina* enriched *Artemia* followed by *yeast* enriched *Artemia* (Table 1). These differences were found to statistically significant (P<0.05).

Table 1. Morphometric data of *M. rosenbergii* post larvae fed with *Spirulina* and yeast enriched *Artemia.*

Parameters		Control	Experiment		F-value
		(Un-enriched *Artemia)*	*Spirulina* (enriched *Artemia)*	Yeast (enriched *Artemia)*	
Average body length (cm)	Initial	1.22 ± 0.11	1.22 ± 0.11	1.22 ± 0.11	--
	Final	2.76 ± 0.14	3.93 ± 0.16	3.45 ± 0.12	52.23
Average body mass (gm)	Initial	0.044 ± 0.005	0.044 ± 0.005	0.044 ± 0.005	--
	Final	0.182 ± 0.007	0.346 ± 0.012	0.279 ± 0.009	223.32
Survival ratio		99%	98%	98%	--

Each value is mean ± SD of thirty individual observations.
The mean difference (length: -1.17, -0.69; 1.17, 0.48; 0.69, -0.48; weight: -0.16, -0.09; 0.16, 0.06; 0.09, -0.06) is significant at P<0.05 (Post Hoc Test: Scheffe).

In feeds, the proximate composition of biochemical constituents was recorded in the order of total protein > total amino acids > total lipid > total carbohydrate (Table 2).

Table 2. Concentrations of biochemical constituents in un-enriched and enriched *Artemia.*

Experimental state	Feed type	Concentrations of biochemical constituents (mg g^{-1}), wet wt. basis			
		Protein	Amino acid	Carbohydrate	Lipid
Control	Un-enriched *Artemia*	18.74 ± 1.90	15.97 ± 1.60	7.85 ± 0.75	13.83 ± 1.15
Experiment	*Spirulina* enriched *Artemia*	38.25 ± 2.60	37.13 ± 2.25	16.20 ± 1.80	18.28 ± 2.20
	Yeast enriched *Artemia*	31.70 ± 2.55	28.53 ± 2.10	17.35 ± 1.85	17.72 ± 1.80
	F-value	52.58	84.70	33.48	5.62
Mean difference (Post Hoc Test: Scheffe)		-19.51, -12.96; 19.41, 6.55; 12.96, -6.55	-21.16, -12.56; 21.16, 8.60; 12.56, -8.60	-8.35, -9.50; 8.35, -1.15; 9.50, 1.15	-4.45, -3.89; 4.45, 0.56; 3.89, -0.56

Each value is mean ± SD of three individual observations.
The mean difference in each parameter is significant at P<0.05.

Artemia enriched with *Spirulina* was acquired more level of protein, amino acid and lipid than yeast enriched *Artemia*. In contrast yeast enriched *Artemia* was acquired more level of carbohydrate than that of the *Spirulina* enriched *Artemia*. The nutritive quantity of feeds was in the order of *Spirulina* enriched *Artemia* > yeast enriched *Artemia* > un-enriched *Artemia*. Therefore, *Artemia* enriched with *Spirulina* acquired more nutrients than that of *Artemia* enriched with yeast (Table 2). These differences were found to statistically significant (P<0.05).

The initial level of biochemical constituents in PL was recorded in the order of total protein > total amino acids > total lipid > total carbohydrate (Table 3). On final day, the content of total protein, amino acids and lipid was found to higher (greater than one fold) in experimental groups when compared with control (Table 3). However, levels of these biochemical constituents were higher in PL fed with *Spirulina* enriched *Artemia* than that of the PL fed with yeast enriched *Artemia* (Table 3). In contrast, the concentration of total carbohydrate was found to higher in PL fed with yeast enriched *Artemia* than that of the PL fed with *Spirulina* enriched *Artemia* (Table 3). These differences were found to statistically significant (P<0.05).

Table 3. Biochemical constituents in the post larvae of *M. rosenbergii* fed with *Spirulina* and yeast enriched *Artemia.*

Experimental State		Concentrations of biochemical constituents (mg g^{-1}), wet wt. basis			
		Protein	Amino acid	Carbohydrate	Lipid
Control (PL fed with un-enriched *Artemia)*	Initial	41.20 ± 2.48	32.74 ± 2.12	7.85 ± 0.90	6.60 ± 0.65
	Final	51.50 ± 3.64	50.79 ± 3.59	9.43 ± 1.88	13.82 ± 1.21
Exptl. (PL fed with enriched *Artemia*)	*Spirulina*	116.58 ± 9.18	106.19 ± 9.62	11.73 ± 1.41	17.75 ± 2.07
	Yeast	103.46 ± 8.10	87.76 ± 9.14	12.42 ± 1.31	16.95 ± 1.91
F-value		65.35	37.90	3.04	4.13
Mean difference (Post Hoc Test: Scheffe)		-65.08, -51.96; 65.08, 13.12; 51.96, -13.12	-55.40, -36.97; 55.40, 18.43; 36.97, -18.43	-2.30, -2.99; 2.30, -0.69; 2.99, 0.69	-3.93, -3.13; 3.93, 0.80; 3.13, -0.80

Each value is mean ± SD of three individual observations.
The mean difference in each parameter is significant at P<0.05.

There are eighteen amino acids have been deducted through HPTLC analysis (Table 4). Among these phenylalanine, leucine, tyrosine, isoleucine, tryptophan, methionine, valine, threonine, arginine, histidine, lysine are essential amino acids, and, alanine, cysteine, proline, glutamic acid, serine, aspartic acid, glutamine and glycine are non-essential amino acids. In this study, generally, the content of essential amino acids was found to elevate in experimental groups when compared with control. However, the elevation was maximum in the PL fed with *Spirulina* enriched *Artemia* than that of the PL fed with yeast enriched *Artemia* (Table 4). The differences recorded in the level of amino

acids between different groups of prawns were found to be statistically significant (P<0.05).

Table 4. HPTLC profiles of amino acids in *M. rosenbergii* post larvae fed with *Spirulina* and yeast enriched *Artemia.*

Amino Acid	Concentration of amino acid (μg g^{-1}), dry wt. basis			F-value	Mean difference		
	Control	Experiment			Control	Experiment	
	PL fed with un-enriched *Artemia*	PL fed with *Spirulina* enriched *Artemia*	PL fed with yeast enriched *Artemia*			*Spirulina*	Yeast
Phenylalanine*	22.16 ± 1.25	31.85 ± 1.73	26.66 ± 1.76	456.96	-43.30, -4.50	43.30, 38.80	4.50, -38.80
Leucine*	22.50 ± 1.86	65.46 ± 2.55	51.83 ± 2.76	246.72	-42.96, -29.33	42.96, 13.63	29.33, 13.63
Tyrosine* & Isoleucine*	52.86 ± 1.90	74.01 ± 3.02	66.23 ± 2.97	47.78	-21.15, -13.37	21.15, 7.78	13.37, -7.78
Tryptophan*	25.06 ± 1.10	73.76 ± 2.61	66.06 ± 2.30	4.42	-48.70, -21.00	48.70, 27.70	21.00, -27.70
Methionine*	24.06 ± 1.90	75.73 ± 2.80	66.66 ± 2.76	409.21	-51.67, -42.60	51.67, 9.07	42.60, -9.07
Valine*	53.08 ± 2.10	75.34 ± 2.91	66.20 ± 2.01	66.60	-22.26, -13.12	22.26, 9.14	13.12, -9.14
Alanine	24.36 ± 2.70	31.30 ± 1.85	27.83 ± 1.65	8.06	-6.94, -3.47	6.94, 3.47	3.47, -3.47
Threonine*	23.00 ± 1.10	30.50 ± 1.32	25.00 ± 1.50	26.09	-7.50, -2.00	7.50, 5.50	2.00, -5.50
Cysteine	22.66 ± 1.76	30.56 ± 1.60	25.16 ± 1.40	19.26	-7.90, -2.50	7.90, 5.40	2.50, -5.40
Proline	25.10 ± 1.20	29.16 ± 1.04	25.23 ± 1.75	8.58	-4.06, -0.13	4.06, 3.93	0.13, -3.93
Glutamic acid	26.23 ± 1.87	29.01 ± 2.00	26.60 ± 1.60	2.03	-2.78, -0.37	2.78, 2.41	0.37, -2.41
Arginine*	25.96 ± 1.05	28.36 ± 1.59	26.00 ± 1.00	3.67	-2.40, -0.04	2.40, 2.36	0.04, -2.36
Serine	26.13 ± 1.32	28.53 ± 1.50	27.00 ± 1.75	1.88	-2.40, -0.87	2.40, 1.53	0.87, -1.53
Aspartic acid	24.96 ± 1.60	28.56 ± 1.70	25.76 ± 1.53	4.12	-3.60, -0.80	3.60, 2.80	0.80, -2.80
Histidine*	9.13 ± 0.80	13.41 ± 0.74	11.26 ± 0.51	28.47	-4.28, -2.13	4.28, 2.15	2.13, -2.15
Glutamine	57.73 ± 2.92	76.00 ± 2.20	70.16 ± 2.76	38.22	-18.47, -12.63	18.47, 5.84	12.63, -5.84
Lysine*	56.36 ± 2.46	75.40 ± 2.60	70.00 ± 2.50	45.46	-19.04, -13.64	19.04, 5.40	13.64, -5.40
Glycine	25.46 ± 1.44	28.50 ± 1.50	27.90 ± 1.85	3.01	-3.04, -2.44	3.04, 0.60	2.44, -0.60

*Essential amino acids
Each value is mean ± SD of three individual observations.
The mean difference in each amino acid is significant at P<0.05.

4. Discussion

Protein is essential for growth and development as it provides the body with energy and is needed for the production of hormones, antibodies, enzymes and tissues. The gross dietary protein requirement is influenced directly by the amino acid composition of the diet [31]. Carbohydrate provides immediate energy and increases protein sparing effect on growth [32]. Total lipid including cholesterol is also essential for growth and survival of crustacean [33].

It has been reported that arginine, alanine and glycine are stimulated ingestion in sea bream larvae [34]. Tryptophan plays an important role in the brain as a precursor of the

neurotransmitter, serotonin which has a major effect on the feeding behaviour of animals [35]. In the present study, levels of these amino acids in experimental groups, particularly the PL fed with *Spirulina* enriched *Artemia* was increased over control. This suggests feeding of PL was higher on *Spirulina* enriched *Artemia*. Histidine is involved in many metabolic functions including production of histamines, which take part in allergic and inflammatory reactions. It plays an important role in maintaining the osmoregulation and energy production [36]. Methionine and lysine are generally critical in feed formulation [37]. In the present study, levels of these amino acids in experimental groups, particularly in PL fed with *Spirulina* enriched *Artemia* was higher. This suggests that the specific functions governed by these amino acids were performed well in the PL fed with *Spirulina* enriched *Artemia*.

Artemia nauplii employed as larval feed because of their optimal nutrition and energy value and are nutritionally enriched with forage, such as algal cells and yeast in order to improve the survival and growth of prawns [38]. *Spirulina* has no cellulosic cell wall, and therefore, the entire cell content can be easily digested and absorbed by the predator [39]. Algae contribute to an increase in protein assimilation and feed utilization [16]. It has been reported that supplementation of *Spirulina* resulted in increased food utilization, protein efficiency ratio, carcass quality and growth of cultured organisms [40-43]. Baker's yeast also offers promising possibilities as a substitute for algal live feeds in aquaculture. Yeast provides nutrients and enhancing the flavor of food. It has excellent source of protein and essential amino acids [44].

In the present study, it is suggested that *Spirulina* is a better supplementary material than yeast since *Spirulina* enrichment *Artemia* has produced better growth of *M. rosenbergii* than that of yeast enriched *Artemia.* However, yeast enriched *Artemia* also produced favourable result on growth of *M. rosenbergii* when compared with un-enriched *Artemia*. The results indicate the fact that *Artemia* was acquired nutrients present in *Spirulina* and yeast, which in turn transferred to the prawns. In fact commercially available whole powders of *Spirulina* and yeast has significantly improved the nutritional quality of *Artemia*, which in turn produced appreciable growth on *M. rosenbergii*. Therefore, *Spirulina* and yeast can be utilized as enrichment/supplementary materials in feed management practices of freshwater prawn culture.

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