ISSN 0975-6299 Vol 3/Issue 2/April – June 2012

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE BIO CHEMISTRY

NUTRITIONAL INDICES AND BIOCHEMICAL CONSTITUENTS IN THE PRAWN *MACROBRACHIUM MALCOLMSONII* FED WITH FORMULATED FEEDS

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ABSTRACT

This study was conducted to assess and categorize the feeds formulated with three different combinations of cereals and pulses (black gram and maize; Bengal gram and barley; red gram and rice) along with coconut oilcake and soya meal for sustainable maintenance of *Macrobrachium malcolmsonii* post larvae (PL). Two types of feeds in each combination (type-A: 25% pulse + 25% cereal; type-B: 40% pulse + 10% cereal) were prepared along with equal proportion of coconut oilcake and soya meal (20% each). Tapioca flour (5%) and egg albumin (4%) were used as binding agents. 1% of vitamin B-complex with vitamin-C was also mixed. These feeds were fed to *M. malcolmsonii* PL for a period of 45 days. Commercially available standard Scampi feed was served as control. The overall influence of these feeds on survival, growth and concentrations of total protein, amino acid, carbohydrate and lipid of PL were found to be significantly higher ($P<0.225$) in scampi feed followed by the feeds rich in pulse (type-B) and the feeds with equal proportion of pulse and cereal (type-A). Among three combinations of pulses and cereals the feed formulated with black gram and maize showed the best overall performance, followed by Bengal gram and barley, and red gram and rice. Therefore, on-farm feed formulation can be made with these commodities of pulses and cereals for sustainable culture of *Macrobrachium*.

KEY WORDS

Macrobrachium malcolmsonii, cereals, pulses, growth, protein

INTRODUCTION

Crustaceans including prawns constitute nutritious delicacy for human consumption and, hence, there is a universal stress on the need for their culture fishery (Lee and Wickins, 1992). The global annual production of freshwater prawns (excluding crayfish and crabs) in 2003 was about 280,000 tons, of which China produced some 180,000 tons, followed by India and Thailand with some 35,000 tons each (FAO, 2007). Next to the giant freshwater prawn *Macrobrachium rosenbergii* (de Man), the monsoon river prawn *Macrobrachium malcolmsonii* (H. Milne Edwards) is a potential species for culture. It is a compatible species for polyculture along with Indian major carps and Chinese carps, which may yield 400 kg prawns and 3000 kg carps/ha/year (Kanuajia and Mohanty, 1996; Kutty *et al*., 2000; Radheysham., 2009). In a semi intensive system with a stocking density of 30,000-40,000 prawns/ ha, it has been reported that the production was achieved in the range of 500-1000 kg/ ha/ year (Radheysham, 2009). However, the commercial farming of this species is still in infancy due to non-availability of adequate quantities of seeds in the wild and the viable hatchery and culture technology.

In south India, the economically important *M. malcolmsonii* is found in abundance and constitutes a major capture fishery in the Cauvery River (Bhavan *et al*., 2008; Bhavan *et al*., 2010a). Present day culture of *Macrobrachium* depends on commercially available artificial feeds, which are highly expensive and are not always affordable to small farmers. Therefore, a well-balanced nutritional and economic diet is required. The increasing pressure on global fish meal stocks has generated interest on uses of plant products (Gimenez *et al*., 2009).

Well balanced low cost feed with essential dietary nutrients can be formulated to attain desirable results. The expensive protein sources can be limited by including adequate levels of carbohydrate and lipid, which also enhances the protein sparing effect on growth (Bhavan *et al*., 2010b; Bhavan *et al*., 2011a; Rebecca and Bhavan, 2011) and decreases the accumulation of nitrogen waste in culture ponds (Raj *et al*., 2008). Soybean meal is one of the most promising alternatives because of its availability, reasonable price, high digestibility and amino acid profile (Floreto *et al*., 2000; Hasanuzzaman *et al*., 2009). Most grains exerted some functional nutritional properties on growth (Thomas and Vander Poel, 2001; He *et al*., 2002; Glencross *et al*., 2005; Pavasovic *et al*., 2007; Jimoh and Aroyehun, 2011; Bilguven and Barıs, 2011; Jaferian and Fayazi, 2011). There are many other plant materials available to check their suitability for protein and carbohydrate sources.

In the present study, nutritional requirement for the growth of *M. malcolmsonii* PL was fully relied on basal ingredients, such as pulses (black gram, Bengal gram and red gram), and cereals (maize, barley and rice), coconut oilcake and soya meal without inclusion of fish meal and plant oils. This work was conducted to assess the growth potential of these pulses and cereals used in three different combinations (black gram + maize; Bengal gram + barley; red gram + rice) on *M. malcolmsonii* PL.

MATERIALS AND METHODS

The post larvae of *M. malcolmsonii* were collected from Lower Anicut of the Cauvery River (Kumbakonam, Tanjavoor District, Tamilnadu, India). They were transported to the laboratory in well-oxygenated polythene bags and acclimatized in a large cement tank (1000 L capacity) for two weeks in ground water (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}). During acclimatization they were fed *ad libitum* with boiled egg albumin, beef liver, commercially available scampi feed and rice alternatively twice a day, 2/3rd of water medium was renewed daily by siphoning method causing minimum disturbance to the prawns. The unfed feed, excreta, and exuvia if any were removed. The water medium was adequately aerated.

Three different combinations of pulses and cereals (black gram and maize; Bengal gram and barley; red gram and rice) were taken in two different ratios (pulse: 25% & 40%; cereal: 25% & 10%) along with coconut oilcake (20%) and soy meal (20%) as basal ingredients. The egg albumin (4 ml) and tapioca flour (5%) were used as binders. A mixture of these ingredients except egg albumin was stream cooked for 15 minutes and cooled. 1% vitamin B-complex with vitamin-C was also mixed. A semi solid paste was prepared and then made to form pellets (3-5 mm) using a manual pelletizer. These pellets were sun dried for 48 hrs. Water stability of these feeds was assessed and the leaching was not exceeded 10% loss of dry matter in eight hour duration. Exactly 1 g pellet in triplicate was taken in a glass beaker and soaked in known quantity of water for eight hours. After soaking, the samples were drained on pre-washed Whatman filter paper and dried in an oven at 40°C for 24 h.

In this study, eight groups of 60 PL each (length: 0.80±0.02 cm; body mass: 0.083±0.021 g) was taken. Each group was equally divided and housed in three aquaria of 20 L capacity (three aquaria represents triplicate). One group served as initial control. After initial morphometric measurement from randomly taken ten individuals the entire PL in this group were sacrificed to estimate the initial proximate composition of total protein (Lowry *et al*., 1951), amino acid (Moore and Stein, 1948), carbohydrate (Roe, 1955), and lipid (Folch *et al*., 1957) in the muscle tissue of pooled sample in triplicate. The remaining seven groups were subjected to feeding trial for a period of 45 days. One group served as control and remaining six groups were served as experiments. The control group was fed with standard Scampi feed and the experimental groups were fed with six different feeds formulated by three combinations of pulses and cereals along with other ingredients (Table 1). On the 45th day of feeding trial, final morphometric data including survival rate were taken. The nutritional indices, such as weight gain, biomass index, specific growth rate and condition factor were calculated by adopting these formulae.

Table 1

Formulated feeds and their protein, carbohydrate and lipid concentrations

Basal Ingredients	Feed -A (pulse-25% + cereal-25%) + (COK-20% + SM-20%) + (EA-4% + tapioca-5%)			Feed –B (pulse-40% + cereal-10%) + (COK-20% + SM-20%) + (EA-4% + tapioca-5%)		
	Protein %	**Carbohydrate %**	**Lipid %**	**Protein %**	**Carbohydrate %**	**Lipid %**
Black gram + Maize	6.0+2.75=8.75	15.0+16.5=31.5	0.25+1.0=1.25	9.6+1.1=10.7	24+6.6=30.6	0.4+0.4=0.8
COK + SM	9.4+11.8=21.2	4.0+2.0=6.0	1.6+2.8=4.4	9.4+11.8=21.2	4.0+2.0=6.0	1.6+2.8=4.4
EA + Tapioca	0.4+0.0=0.4	0.0+4.0=4	0.0+0.0=0.0	0.4+0.0=0.4	0.0+4.0=4.0	0.0+0.0=0.0
Total	*30.3*	*41.5*	*5.65*	*32.3*	*40.6*	*5.2*
Bengal gram + Barley	5.25+2.75=8.0	15+17.5=32.5	1.5+0.25 =1.75	8.4+1.1=9.5	24+7.0=31.0	2.4+0.07=2.47
COK + SM	9.4+11.8=21.2	4.0+2.0=6.0	1.6+2.8=4.4	9.4+11.8=21.2	4.0+2.0=6.0	1.6+2.8=4.4
EA + Tapioca	0.4+0.0=0.4	0.0+4.0=4	0.0+0.0=0.0	0.4+0.0=0.4	0.0+4.0=4.0	0.0+0.0=0.0
Total	*29.6*	*42.5*	*6.15*	*31.1*	*41.0*	*6.87*

Red gram + Rice	5.5+1.75=7.25	14.5+19.5=34.0	0.5+0.0=0.50	8.8+0.7=9.5	23.2+7.8=31.0	0.8+0.0=0.8
COK + SM	9.4+11.8=21.2	4.0+2.0=6.0	1.6+2.8=4.4	9.4+11.8=21.2	4.0+2.0=6.0	1.6+2.8=4.4
EA + Tapioca	0.4+0.0=0.4	0.0+4.0=4	0.0+0.0=0.0	0.4+0.0=0.4	0.0+4.0=4.0	0.0+0.0=0.0
Total	*28.85*	*44.0*	*4.9*	*31.1*	*41.0*	*5.2*

COK, coconut oilcake; SM, soya meal; EA, egg albumin
Protein: 28.85-30.3 (Feed – A); 31.1-32.3 (Feed – B); Overall protein: 29-32%
Carbohydrate: 41.5- 44.0 (Feed – A); 40.6-41.0 (Feed – B); Overall carbohydrate: 41-44%
Lipid: 4.9-6.15 (Feed – A); 5.2-6.87 (Feed – B); Overall lipid: 5-7%

$$\text{Survival Rate (SR)} = \frac{\text{No. of live prawns}}{\text{No. of prawns introduced}} \times 100$$

Weight Gain (WG) = Final weight (g) – Initial weight (g)

$$\text{Biomass Index (BI)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

$$\text{Specific Growth Rate (SGR)} = \frac{\textit{log}\text{ of Final weight (g)} - \textit{log}\text{ of Initial weight (g)}}{\text{No. of days}} \times 100$$

$$\text{Condition Factor (CF)} = \frac{\text{Final weight (g)}}{\text{Final length}^3\text{ (cm)}} \times 100$$

The final proximate composition of total protein (Lowry *et al*., 1951), amino acid (Moore and Stein, 1948), carbohydrate (Roe, 1955), and lipid (Folch *et al*., 1957) in the muscle tissue of pooled sample were estimated in triplicate. Fifteen prawns from each aquarium were sacrificed (15x3=45 from each group) for estimating the proximate composition of total protein, amino acid, carbohydrate, and lipid. From each aquarium tissue from a group of three PL was pooled to constitute as single observation of each parameter. Thus, three such observations were made from each group for each parameter (3x5=15x3=45). The data obtained in control and experiments were subjected to statistical analysis by adopting paired samples t-test using SPSS, version-13.0 of IBM software.

RESULTS AND DISCUSSION

MORPHOMETRY AND NUTRITIONAL INDICES

Table 2 represents the morphometric data and nutritional indices in *M. malcolmsonii* PL fed with formulated feeds prepared from three different combinations of pulses and cereals. The initial body length and weight of PL were recorded to be 0.80±0.02 cm and 0.083±0.021g respectively. The final weight was found to be increased in both control and experimental feeds fed PL when compared with respective initial weight. The survival rate was found to be higher in formulated feeds (95±2 - 95±3) when compared with control (94±2). However, no difference was seen in survival rate among the formulated feeds. The weight gain/ biomass index was found to be higher in the scampi feed (control) fed PL followed by feeds prepared with higher pulses ratio (feed-B), and feeds with equal proportions of cereals and pulses (feed-A) in all combinations. Among three combinations of pulses and cereals used, the feed prepared with black gram and maize showed the highest weight gain/ biomass increase followed by Bengal gram and barley, and red gram and rice when compared with control (P<0.225). The similar trend was naturally observed in the case of specific growth rate (P<0.189). The condition factor was just reverse to weight gain/ biomass index, and specific growth rate as this was the least in PL fed with feed formulated by using black gram and maize, less in Bengal gram and barley, and low in red gram and rice. However, control feed showed very least condition factor, which reflects its superior quality when compared with experimental feeds (P<0.047). Therefore, the condition factor was in the order of control < black gram and maize < Bengal gram and barley < red gram and rice.

Table 2
Morphometric data and nutritional indices of M. malcolmsonii PL fed with formulated feeds

Parameters		Prepared Feeds	Control (Scampi feed)	Feed - A (pulse-25% + cereal-25% +COK-20% + SM-20% + EA-4% + tapioca-5%)	Feed - B (pulse-40% + cereal-10% +COK-20% + SM-20% + EA-4% + tapioca-5%)
Morphometric Data	Initial length (cm)	Control (Scampi feed)	0.80±0.02	0.80±0.02	0.80±0.02
	Final length (cm)	Black gram + Maize	2.06±0.15	1.83±0.11 (0.01)	1.96±0.15 (*)
		Bengal gram + Barley	2.06±0.15	1.76±0.12 (0.003)	1.91±0.13 (0.006)
		Red gram + Rice	2.06±0.15	1.62±0.11 (0.003)	1.83±0.12 (0.006)
	Initial weight (g)	Control (Scampi feed)	0.083±0.021	0.083±0.021	0.083±0.021
	Final weight (g)	Black gram+ Maize	0.147±0.013	0.130±0.010 (0.01)	0.143±0.017 (0.225)
		Bengal gram + Barley	0.147±0.013	0.120±0.010 (0.004)	0.130±0.020 (0.052)
		Red gram + Rice	0.147±0.013	0.116±0.015 (0.001)	0.123±0.025 (0.074)
	Survival rate (%)	Black gram + Maize	94±2	95±2 (0.225)	95±3 (*)
		Red gram + Rice	94±2	95±2 (0.225)	95±3 (*)
		Bengal gram + Barley	94±2	95±3 (*)	95±2 (0.225)
Nutritional Indices	Weight gain (g)	Black gram + Maize	0.063±0.001	0.046±0.005 (0.018)	0.060±0.005 (0.324)
		Bengal gram + Barley	0.063±0.001	0.036±0.005 (0.007)	0.045±0.007 (0.035)
		Red gram + Rice	0.063±0.001	0.033±0.006 (0.009)	0.040±0.010 (0.047)
	Biomass Index (%)	Black gram + Maize	78.2±15.4	54.6±12.7 (0.004)	73.2±11.5 (0.157)
		Bengal gram + Barley	78.2±15.4	44.6±4.6 (0.033)	56.5±6.2 (0.055)
		Red gram + Rice	78.2±15.4	38.5±11.7 (0.003)	45.3±6.0 (0.026)
	Specific growth rate	Black gram + Maize	0.561±0.094	0.450±0.064 (0.023)	0.527±0.064 (0.189)
		Bengal gram + Barley	0.561±0.094	0.360±0.094 (*)	0.431±0.038 (0.057)

	Red gram + Rice	0.561±0.094	0.329±0.070 (0.004)	0.375±0.027 (0.041)
Condition factor	Black gram + Maize	1.67±0.24	2.28±0.14 (0.009)	1.89±0.22 (0.003)
	Bengal gram + Barley	1.67±0.24	2.33±0.40 (0.019)	1.99±0.20 (0.005)
	Red gram + Rice	1.67±0.24	2.80±0.14 (0.003)	2.08±0.08 (0.047)

COK, coconut oilcake; SM, soya meal; EA, egg albumin
Each value is mean ± SD of triplicate observations.
Significance (P<) of paired samples t-test are given in parentheses (* the correlation and 't' cannot be computed because the SE of the difference is '0').

As far as freshwater prawn culture is concerned there are many factors relates the growth and feeding activity (New and Valenti, 2000), which include a functional digestive system to efficiently utilize the nutrients present in the food offered (Anderson and De Silva, 2003) and the physiological conditions, and the rearing environment (Lee and Lawrence, 1997). Growth of prawn is normally very fast during the early life and slows down during adult; the survival rates are also very high during the early life and fell subsequently (Miller, 1971). In the present study, in formulated feeds, particularly black gram and maize, the overall performance of growth and survival were found to be almost equal to that of control (Scampi feed). This observation is supported by our previous studies on *Macrobrachium* and revealed that feeds formulated with cereals, pulses, groundnut oilcake, and feeds incorporated with vegetable wastes, and herbals have yielded sustainable growth (Bhavan *et al*., 2010b, c 2011a,b; Rebecca and Bhavan, 2011).

BIOCHEMICAL CONSTITUENTS

The content of total protein was found to be higher in Scampi feed (control) followed by feed type-B and type-A in all combinations of pulses and cereals (Table 3). Among three combinations of pulses and cereals used the content total protein was higher in black gram and maize followed by Bengal gram and barley, and red gram and rice. Therefore, the content of total protein in PL fed with feed type-B of black gram and maize was nearest to control, the Scampi feed fed PL (P< 0.002). The similar trend was recorded in the case of total amino acid (P<0.002). Generally, the content of total carbohydrate was found to be significantly higher (P<0.018) in PL fed with formulated feeds when compared with control. However, there was no much difference observed between feed type-A and type-B of all combinations of pulses and cereals used feed fed PL (Table 3). The content of total lipid was found to be significantly lower (P<0.056) in PL fed with formulated feeds when compared with control except feed type-B of Bengal gram and barley. The decrease in total lipid was a maximum in red gram and rice combination fed PL (Table 3).

Table 3
Concentrations of biochemical constituents in M. malcolmsonii PL fed with formulated feeds

Parameters (mg g^{-1} wet wt.)	Prepared Feeds	Initial	Control (Scampi feed)	Type - A (pulse-25% + cereal-25% + COK-20% + SM-20% + EA-4% + tapioca-5%)	Type - B (pulse-40% +cereal-10% + COK-20% + SM-20% + EA-4% + tapioca-5%)
Protein	Black gram + Maize	25.20±2.14	56.55±3.28	50.26±3.65 (0.001)	51.40±3.32 (0.000)
	Bengal gram + Barley	25.20±2.14	56.55±3.28	47.24±3.55 (0.000)	50.43±3.75 (0.002)
	Red gram +	25.20±2.14	56.55±3.28	43.30±3.80	45.32±3.50

	Rice			(0.001)	(0.000)
Amino Acid	Black gram + Maize	15.00 ±2.0	30.25±2.25	25.30±2.25 (*)	26.52±2.52 (0.002)
	Bengal gram + Barley	15.00 ±2.0	30.25±2.25	22.16±2.22 (0.000)	23.42±2.25 (*)
	Red gram + Rice	15.00 ±2.0	30.25±2.25	20.24±2.20 (0.000)	22.56±2.46 (0.001)
Carbohydrate	Black gram + Maize	10.50±1.25	23.26±2.48	24.02±2.44 (0.001)	24.28±2.24 (0.018)
	Bengal gram + Barley	10.50±1.25	23.26±2.48	24.06±2.48 (*)	25.06±2.12 (0.013)
	Red gram + Rice	10.50±1.25	23.26±2.48	25.02±2.48 (*)	25.00±2.25 (0.006)
Lipid	Black gram + Maize	0.20±0.01	1.60±0.12	1.40±0.14 (0.003)	1.45±0.17 (0.035)
	Bengal gram + Barley	0.20±0.01	1.60±0.12	1.46±0.18 (0.056)	1.65±0.15 (0.102)
	Red gram + Rice	0.20±0.01	1.60±0.12	1.22±0.17 (0.006)	1.25±0.12 (*)

GOK, groundnut oilcake; SM, soya meal; EA, egg albumin
Each value is mean ± SD of triplicate observations.
Significance (P<) of paired samples t-test are given in parentheses (* the correlation and t cannot be computed because the SE of the difference is '0').

Nutrition is regarded as a key factor controlling survival and growth of organisms. One of the major requirements of prawn culture is the transformation of dietary protein in the form of amino acids into tissue protein for maintenance of normal body function and growth. Therefore, protein constitutes the most expensive ingredients in artificial feed. The protein requirements in animals are influenced by various factors, such as body size, water temperature, feeding rate, availability and quality of protein, and over all digestible energy content (Watanabe, 1988). In general, larvae and juveniles have greater protein requirement than adults (Teshima and Kanazawa, 1984) because the former usually have faster growth rates and higher metabolic rates. Feed protein level up to 57% is recommended for suitable growth of *Penaeid* shrimps (Shiau, 1998; Kureshy and Davis, 2002). In the case of *M. rosenbergii* it is about 30-45% (Rangacharyulu, 1999). In the present study, the formulated feeds contained 28.8-32.3% protein (Table 1) and *M. malcolmsonii* PL was efficiently utilized total protein available in formulated feeds (Table 1), particularly with feed type-B of black gram and maize (32.3%) as almost equal growth performance was recorded in this feed when compared with control, the Scampi feed (Tables 1-3).

Carbohydrate is inexpensive source of energy supplying nutrient and serve as precursors for the dispensable amino acids and metabolic intermediates necessary for growth (NRC, 1993). The requirement of carbohydrate for *M. rosenbergii* has been reported to be 25-35% as optimum in the diet (Raj, 1993). As for as *M. malcolmsonii* is concerned, there are no data is available pertaining to this. Therefore, in this study, it is suggested that the crude carbohydrate available (40.6-44%) with formulated feeds enhanced protein sparing towards growth rather than maintenance (Tables 1-3). Lipids provide a source of indispensable nutrients as they are important in maintaining structural and physiological integrity of cellular membranes, serve as an alternate source of energy and act as carriers of certain non fat nutrients notably the fat soluble vitamins like A, D, E and K (Gillbert and O'Conner, 1983; New, 1986). The dietary lipid level as low as 5% can be provided the sufficient levels of essential fatty acids. The optimum level of dietary lipid required for crustacean generally ranged from 2 to 10% (Deshimaru *et al.,* 1979; Ponat *et al.*, 1980; D'Abramo, 1997). In the present study, the calculated total crude lipid content was optimum (4.9 – 6.8 %) in formulated feeds (Table 1).

CONCLUSION

In this study, *M. malcolmsonii* was used dietary carbohydrate as a major energy source since its availability was plenty. Therefore, proteins sparing by lipid is not considered to be crucial. However, its role is vital as essential nutrient. Since the levels of both carbohydrate and lipid were optimum in formulated feeds, the available protein was fully utilized for growth rather than maintenance. The protein sparing mechanisms on growth was worked well in the feed formulated with black gram and maize followed by Bengal gram and barley, and red gram and rice. Therefore, black gram and maize can be included directly in feed preparation for on farm feed management of *Macrobrachium*. However, the other combinations as well can be utilized with supplementation of some additional quality feed materials. This needs further study.

ACKNOWLEDGEMENT

The University Grants Commission, Government of India, New Delhi is gratefully acknowledged for the financial support provided. The first author is expressing his sincere thanks to P. Selvi, P. Mohana, and S. Ragajeevitha of 2008-2009 batch project students for initiating this work.

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