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BIOFORTIFICATION IN *MACROBRACHIUM ROSENBERGII* USING CRUDE EXTRACTS FROM *CURCUMA LONGA*: ASSESSMENT VIA GROWTH AND BIOCHEMICAL INDICES.

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ABSTRACT

The all-round nutritional status of aquaculture candidate can be augured by the application of medicinal herbs. In the present work nutritional status of freshwater prawn, *Macrobrachium rosenbergii* was assessed subsequent to certain concentrations of *Curcuma longa* oral supplementation. Terminations were affected on 30th and 60th days for experimental analysis of growth, feed utilization were done. Biochemical parameters such as protein, glycogen and lipid of muscle, gill and gut tissues were assessed as follows at 30th and 60th days of the experimental period. From the experiments it can be concluded that the *Curcuma longa* (25 mg/kg of feed) with daily administration has better growth promotion and feed utilization efficacy in *M.rosenbergii.*

KEYWORDS: Medicinal herbs, *Macrobrachium rosenbergii*, *Curcuma longa*, oral supplementation, feed utilization and lipid.

INTRODUCTION

Growth of fish is dependent mainly on the feed quality and optimum ingredient concentration. A complete diet requires nutrients such as protein, carbohydrates, fats, vitamins, and minerals etc, which is necessary for the optimal growth and health of the prawn (New, 2008).Freshwater prawns are omnivorous and mainly bottom feeders, feeds on plants, microalgae, organic matter, insects, molluscs etc. When comes to intensive and semi – intensive culture of *M.rosenbergii*, the prepared diet must satisfy availability of all these ingredients (Louis *et.al.*, 2000). For preparing effective feed for *M.rosenbergii*, the knowledge of nutrient requirement for each stage must be known **(**Tayamen, 2001). There were many feed additives which increase the growth of aquaculture candidate especially prawn (Pavadi *et.al.*, 2012) Antibiotics or antibacterial are a type of antimicrobial used in the treatment and prevention of bacterial infection. They may either kill or inhibit the growth of bacteria (Cabello, 2006). Continuous use will lead to residual accumulation, drug resistance and immunosuppression. Chloramphenicol can cause fatal aplastic anaemia and Nitrofurans are classified as carcinogen. Presently the importance of medicinal plants as feed additive call for great attention in aquaculture sector. Several plant products have antimicrobial, anti- stress, immune stimulants and growth promotion properties which will influence the growth of fish and shrimp larviculture (Citarasu *et.al.*, 2002). Venkateshwarlu *et.al.*, 2009 have reviewed plants traditionally used in fish harvest and angling potential feed attracts in aquaculture. Bhavan *et.al.*, 2014, tested the effectiveness of medicinal plants such as *Syzygium cumini*, *Phyllanthus emblica, Azadirachta indica* and *Ricinus communis* on growth promotion in early juveniles of *M.malcolmsonii*. These medicinal plants have acted as appetizer and significantly enhanced the activities of digestive enzymes (protease, amylase and lipase). In another study by Bhavan *et.al.*, (2013) they reported the effect of three medicinal plants on the growth promotion in *M.rosenbergii*, (such as *Myristica fragrans, Glycyrrhiza glaba* and *A.infectoria).* Plants such as *Ocimum sanctum* and *Withania somnifera* were also effective as nutritional supplements in *M.rosenbergii* larvae. All nutritional indices and energy utilization and biochemical status ($p<0.05$) were high in medicinal herb incorporated diets. (Jasmine *et.al.*, 2012). *Curcuma longa* feed additives in *M.rosenbergii* increased feed consumption (Salini *et.al.*, 2014). The present study was undertaken to evaluate the overall effect of herbal extract oral supplementation in *Macrobrachium rosenbergii*. Medicinal plants *Curcuma longa* was selected owing to its strong credentials reporting growth promotion.

MATERIALS AND METHODS

Collection and maintenance of *Macrobrachium rosenbergii:* Giant freshwater prawn, *Macrobrachium rosenbergii* is taken as the experimental candidate. The PL (PL-30) of *M. rosenbergii* were purchased from

Government prawn hatchery of ADAK (Agency for Development of Aquaculture (ADAK), Varkala, Kerala and acclimated in the laboratory for two weeks before the experimentation. During acclimatization they were fed *ad libitum* with control diet and egg albumin. Rationing of feed was twice daily. After rearing, prawns with approximate size of (12.00 ± 0.8 mg) were taken for feed supplementation experiment.

The PLs of *Macrobrachium rosenbergii* were reared in laboratory under optimum salinity, pH and temperature .Prawns were fed with medicated feed and control feed prepared in the laboratory. Three sets of experiments were carried out in glass tanks of 100 L capacity and proper aeration was provided. 50% of water was renewed daily during removal of waste feed and feacal matter. In each sets, 20 numbers of PLs were checked and fed at 10% of their body weight/day. Prawns were assessed at the end of the one and two months of treatments for growth, feed utilization and biochemical indices.

The medicinal plant *Curcuma longa* (rhizome) were identified and procured from Pankajakasthuri Research Centre, Kattapada, Trivandrum. The rhizomes were washed thoroughly, shade dried at room temperature till constant weight was recorded. Powdered in a grinder and then stored in an air tight container for further use. The extraction was done by following the procedure of Cooper and Gunn, (2005). The extraction was performed by a cold maceration process for seven days with mild agitation on a shaker. 100 grams of powdered plant parts were successively extracted with 85 % absolute methanol and then subjected to vaccum filtration. The filtrate was then collected and the solvent evaporated using rotary vacuum evaporator (Buchi SMP, Switzerland). The dried residue obtained after evaporation were carefully collected in screw capped tubes and stored at -20 ^{0}C until use.

After the extraction procedure the methanolic extract of the medicinal herbs were subjected to LD_{50} assay to ascertain effective sub-lethal physiologic dosage for further studies. Different concentrations range of the extract were given to the post larvae. The concentration at which 50% mortality occurs within 96 hrs was recorded, and concentrations below 1/10th of that concentration is used for further studies.

All these ingredients "Table.1" were steamed at 90 ^{0}C-100 ^{0}C for 5 minutes. After cooling fish oil (30 mg/kg), vitamin mineral mixture (20 mg/kg) also added .Feed mixture was immediately squeezed through a hand pelletizer with 3mm diameter mesh size. The pellets obtained were sun dried till a constant weight was recorded too ascertain total dryness. Collected and kept in air tight bottles for further use.

The methanolic extracts with 6 different concentrations was mixed thoroughly prior to pelletization as follows.

Feed composition

Test Diet 1 (TD 1): 12.5 mg of *C.longa* extract per kg of basal feed.

Test Diet 3 (TD 3): 25 mg of *C.longa* extract per kg of basal feed.

Test Diet 5 (TD 5): 30 mg of *C.longa* extract per kg of basal feed.

Test diet 2(TD 2), 4 (TD 4) and 6 (TD 6) have same concentrations of *C. longa* as Test diet 1, 3 and 5 respectively. Only the feeding schedule is different.

Table .1. Ingredients of experimental feed.

Ingredients	Quantity (mg/kg)
Fish Meal	300
Prawn Head	50
Squid Meal waste	50
Squilla	50
Soyabean Meal	250
Wheat Flour	250
Fish Oil	30
Vitamin Mineral Mixture*	20

Vitamin mineral mixture contains : The ingredients as: Chloecalciferol (vitamin D3)- 1000 mg, Thiamine mononitrate-10 mg, Riboflavin-10 mg, Pyridoxine hydrochloride -3 mg, Cyanocobalamine-15 mcg, Nicotinamide 100mg, Calcium panthothenate -16.30 mg, Ascorbic acid-150 mg, α- Tocopherylacetate -250mg, biotin usp-0.25 mg, tribasic calcium phosphate-129 mg, magnesium oxidelight-60 mg, dried ferrous sulphate -32.04 mg, manganese sulphate mono hydrate -2.03 mg , total phosphate in preparation-25.80mg, copper sulphate pentahydrate -3.39 mg, zinc sulphate -2.20 mg, sodium molybdate dehydrate -0.25 mg , sodium borate -0.88 mg.

Feeding experiments.

The acclimated prawns were randomly divided into 12 groups (n-20), one control group. Test Diet 1 , Test Diet 3 , Test Diet 5 were given twice daily. Test Diet 2 , Test Diet 4 , Test Diet 6 , were given twice a week only ,while on the other days they were fed the basal feed. Experiments ran in triplicates. Daily monitoring was done and mortality was recorded. Terminations were affected on 30th and 60th days for experimental analysis of growth, feed utilization and biochemical analysis. Proximate parameters such as protein (Lowry *et.al.*, 1951), lipid (Folch, *et.al.*, 1957), moisture and ash were (APHA, 2005) calculated. All parameters were checked on the percentage of dry matter basis. NFE% (carbohydrate) = 100-(% moisture+% crude protein+% crude lipid+ % ash+% fibre).

Data were statistically analyzed, mean and standard deviation were found .The data were subjected to one way ANOVA using SPSS version 16. In all test, $P<0.05$ was considered as significant.

RESULTS.

Table 2. Proximate analysis of experimental and control feeds

Parameters	TD1	TD3	TD5	control
Crude protein (%)	40	40.01	42.07	38.01
Crude lipid (%)	7.4	8.01	7.5	6
Carbohydrate (%)	33.02	32.04	29.36	28
Moisture (%)	1.02	1.06	1.05	2.03
Ash (%)	8.33	8.03	8.06	8.03

% on Dry matter basis

Biogrowth parameters were checked on 30th and 60th days of the experiments ("Table.3" and "Table."). Following 30 days of *C.longa* supplementation diets, highest weight gain of *M.rosenbergii* is observed in TD 3 fed prawns (1.16 ±0.00 g), lowest were observed in TD 2 fed prawns (0.02 ±0.01 g). On 60th day PLs fed TD 3 (2.10 ±0.00g), recorded highest weight gain and lowest was seen in control prawns (0.47 ±0.01g). Following 30 days of *C.longa* incorporated diets, better length gain is recorded in prawns fed TD 3(2.64 ±0.02cm) and least observed in control prawns (0.27 ±0.01 cm). On 60th day highest length gain was obtained in prawns fed TD 3 (3.49 ±0.01cm) and lowest in prawns fed TD 6 (0.9 ±0.03 cm).Following 30 days of *C.longa* supplementation diets, least FCR were observed in prawns fed TD 3(2.23 ±0.01).Highest FCR were obtained in TD 5(7.00 ±0.02) fed prawns. In the experiment it is concluded that the diets are statistically significant at(P>0.05).On 60th day lowest FCR was observed in TD 3 fed prawns (3.78 ±0.02) supplementation group/highest observed in TD 1(7.03 ±0.00).Following 30 days *C.longa* incorporated diets highest SFC was obtained in prawns fed TD 3(2.31 ±0.01) and least in prawns fed TD 5 and prawns fed TD 6 (0.19 ±0.03 and 0.19 ±0.01 respectively). On 60th day maximum SGR was recorded in prawns fed TD 3(1.57 ±0.02), and minimum in control prawns (0.50 ±0.02). 30 and 60 days of *C.longa* supplementation failed to reveal diets any marked variation in PER. On 30th day prawns fed TD 3, TD 4 and TD 5 diets showed comparatively slight increase in PER than other and lower than control prawns (0.01 ±0.03). On 60 days TD 3(0.05±0.02) fed prawns had higher PER compared to others. So the experiment id statistically significant at (P>0.05).

Table.3.Biogrowth parameters of *M.rosenbergii* reared for 30 days days on herbal supplementation diets .

Diets	Weight gain(g)	Length gain(cm)	Feed conversion ratio	Specific growth rate	Protein efficiency ratio
Test Diet 1	0.45 ±0.01	1.95 ±0.01	3.29 ±0.00	1.63 ± 0.01	0.01±0.01
Test Diet 2	0.02 ±0.01	0.33 ±0.01	6.00±0.02	1.08 ±0.01	0.02 ±0.02
Test Diet 3	1.16 ±0.00	2.64±0.02	2.23±0.01	2.31±0.01	0.03±0.02
Test Diet 4	1.00±0.01	1.99±0.02	3.00±0.01	2.00±0.01	0.03±0.02
Test Diet 5	0.34±0.02	0.84±0.01	7.00± 0.02	0.19± 0.02	0.03±0.01
Test Diet 6	1.07 ±0.03	0.65±0.02	5.00±0.01	0.19±0.03	0.02±0.02
Control Diet	0.25 ±0.01	0.27±0.01	2.30±0.00	1.09±0.01	0.01±0.03

Table.4. Biogrowth parameters of *M.rosenbergii* reared for 60 days days on herbal supplementation diets.

Diets	Weight gain(g)	Length gain(cm)	Feed conversion ratio	Specific growth rate	Protein efficiency ratio
Test Diet 1	0.66 ± 0.02	2.42 ±0.01	7.03± 0.00	1.04 ±0.01	0.02 ±0.01
Test Diet 2	1.21 ±0.01	1.70 ±0.02	4.99 ±0.01	0.37 ±0.01	0.02 ±0.02
Test Diet 3	2.10 ±0.00	3.49 ±0.01	3.78 ±0.02	1.57 ±0.02	0.02 ±0.03
Test Diet 4	1.79 ±0.01	1.99 ±0.00	3.80 ±0.03	0.06 ±0.03	0.05 ±0.02
Test Diet 5	1.87 ±0.02	2.84 ±0.01	6.01 ±0.01	0.78 ±0.02	0.02 ±0.01
Test Diet 6	0.65 ±0.04	0.90 ±0.03	7.00 ±0.02	0.62 ±0.01	0.03 ±0.02
Control Diet	0.47 ±0.01	1.94 ± 0.02	7.01 ±0.01	0.50 ±0.02	0.02 ±0.01

Table .5. ANOVA of Biogrowth parameters of *M.rosenbergii* reared on herbal supplementation diets at 30 and 60 days.

	Source of Variation	SS	df	MS	F	P-value	F crit
30 days.	Between Groups	69.69039	4	17.4226	16.2828	3.42	2.689628
	Within Groups	32.1	30	1.07			
	Total	101.7904	34				
60 days	Between Groups	136.9084	4	34.2271	47.99742	1.28	2.689628
	Within Groups	21.39309	30	0.713103			
	Total	158.3015	34				

The muscle protein is high in prawns fed with TD 1(12.94 ± 0.02) mg % and low obtained in Control prawns (8.32 ± 0.02) mg % ("Figure.1"). Gill protein content is high in prawns fed with TD 1(11.10 ± 0.10) mg % and low in control prawns (4.32 ± 0.02) mg %. In the case of gut high protein content was obtained in TD 3 fed prawns (12.43 ± 0.52) mg %, low in TD 6 fed prawns (6.33 ± 0.03) mg%. All the observations except TD2 and TD3, significantly ($p<0.05$) increased than the control diet. On 60th day Prawns fed with TD 3 had high muscle protein content (48.52 ± 0.60 mg %) and low at control prawns (14.82 ± 0.06 mg %). High gill protein concentrations were obtained at TD 3 fed prawns (22.07 ± 1.02 mg %) low obtained in TD 6 fed prawns (10.10± 0.01 mg%). Protein concentration of gut was observed and recorded high in TD 3 fed prawns (27.83 ± 1.05 mg %), low in TD6 fed prawns (12.83 ± 0.02 mg %).

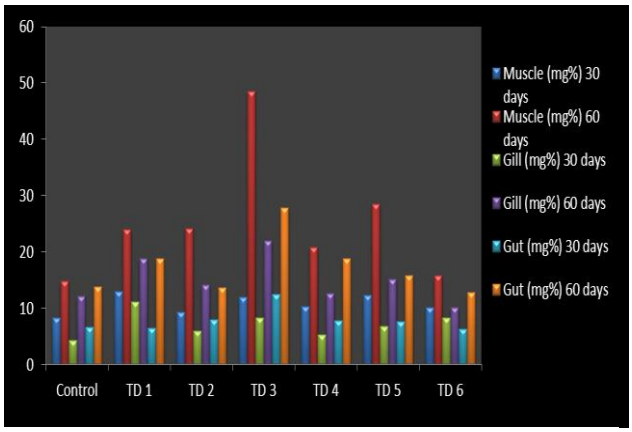

Figure.1. Protein concentrations at selected sites of *M.rosenbergii* supplemented with *C.longa* incorporated diet for 30 days and 60 days.

In *C.longa* 30 days supplementation elevated muscle glycogen were observed in TD 3 fed prawns (0.85 ± 0.02) mg %, low were recorded at control prawns (0.42 ± 0.02) mg % ("Figure.2"). Glycogen content of gill was high at TD 3 fed prawns (0.14 ± 0.1) mg % treatment. Low in control prawns (0.05 ± 0.02) mg%. On 30 days control diet fed prawns have (0.13 ± 0.06 mg %) highest amount of gut glycogen, lowest obtained in TD1, TD3, TD4 fed prawns (0.02 ± 0.01 mg%). All treatments are significantly increased ($p<0.05$) from control group. On 60 days highest concentration of muscle were obtained in TD 6 fed prawns (1.02 ± 0.05) mg %, low recorded in control prawns (0.75± 0.02).On 60 days of experiment TD 3 fed prawns (0.16 ± 0.01 mg %) had elevated gill glycogen level. Lowest was observed in TD 5 fed prawns (0.03 ± 0.01 mg%). On 60 days of experiment TD 5 fed prawns (0.09 ± 0.01 mg%) has increased gut glycogen concentration.TD 4 fed prawns (0.02 ± 0.02 mg%) had least gut glycogen concentration. All treatments have glycogen concentration significantly increased ($p<0.05$) than control treatment.

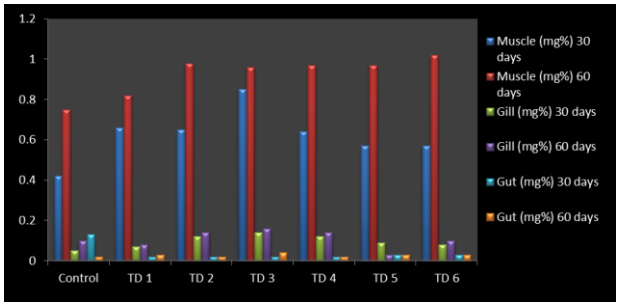

Figure.2. Glycogen concentrations at selected sites of *M.rosenbergii* supplemented with *C.longa* incorporated diets for 30 days and 60 days.

In *C.longa*, On 30 days of experiment high muscle lipid concentration observed TD 5 fed prawns (2.87 ± 0.03 mg%), least observed in control prawns (0.97 ± 0.02 mg%) ("Figure.3"). TD 2 fed prawns (1.25 ± 0.02 mg%) had highest gill lipid content, lowest obtained in TD 6 fed prawns (0.01 ± 0.01 mg%). Gut lipid content of TD 9 fed prawns (0.27 ± 0.01 mg%) had high lipd concentration compared to other treatments. TD 6 (0.03 ± 0.02 mg%) have least. In *C.longa*, on 30 days lipid concentrations of all treatments are significantly increased ($p<0.05$) from control diet.On 60 days TD 1 fed prawns (3.43 ± 0.03 mg%) had increased muscle lipid concentration. Lowest observed in control prawns (1.20 ± 0.03 mg%). Increased gill lipid content was obtained in TD 5 fed prawns (8.83 ± 0.03 mg%), least lipid content is recorded in control prawn (1.20 ± 0.01 mg%). TD 7 fed prawns (1.81 ± 0.04 mg%) showed highest gut lipid concentration, least observed in TD 6 fed prawns (0.07 ± 0.07) mg%.TD1 and TD2 fed prawns were significantly ($p<0.05$)decreased than the control prawns. All other groups have significantly ($p<0.05$) increased lipid concentrations when compared to control prawns.

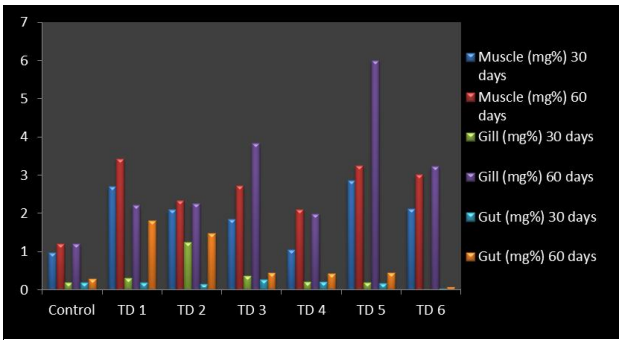

Figure.3. Lipid concentrations at selected sites of *M.rosenbergii* supplemented with *C.longa* incorporated diet for 30 days and 60 days.

DISCUSSION

When aquaculture production becomes more intensive, the incidence of infectious diseases has increased and as a result of it, significant economic losses have been incurred. The present study discusses about the advantages of herbal plants such as *C.longa* on the nutritive aspects of *Macrobrachium rosenbergii*. *C.longa* was administered to the *M.rosenbegii* at selected

concentrations. Both daily and twice a week administrations were carried out. The activity was checked at 30days and 60 days of the experiments. On assessing the growth promotion of *C.longa* with 25mg/Kg with daily administration shown better result than other concentrations. In both cases the twice a week administration did not shown any marked improvements. PER of *C.longa*, both at 30^{th} and 60^{th} days were done not shown any significant results.Work by Poongodi (2012) on *Macrobrachium rosenbergii* revealed the growth promotion properties of turmeric, ginger and garlic. Medicinal plants such as *Myristica fragrans, Glycyrrhiza glaba* and *Quercus infectoria* have effects on growth promotion in the post larvae of the prawn, *Macrobrachium rosenbergii* (Bhavan.e*t.al.*, 2013).Medicinal plants such as *Centella asiatica* has improved the nutritional and biochemical status of *Macrobrachium rosenbergii*(Salini. *et.al.*, 2014). Works by Jasmine (2012) and Jayasree et.al., 2016(a) revealed the growth promotion and antimicrobial properties of *Withania somnifera* and *Ocimum sanctum* in aquaculture and Triphala respectively. Present study also evaluated the biochemical status such as protein, glycogen and lipid content of muscle, gill and gut of prawns fed with *C.longa* . Protein content of *C.longa* (Test Diet 3) got better concentration .But in the case of glycogen content of muscle, gill and gut of prawns with were not got marked improvement . In lipid concentrations of muscle, gill and gut treated with *C.longa* have better concentration. According to Rebecca *et.al.*, (2014) plants such as *Allium sativum, Zingiber officinale* and *Curcuma longa* have improved the biochemical and antioxidant status of *M.rosenbergii*. Abdelwahab *et.al.*, 2012 examined the influence of black cumin seeds (*Nigella sativa*) and turmeric (*Curcuma longa* Linn.) mixture on performance. Bhavan *et.al.*, 2014. Checked the effectiveness of *Papaver somniferum*, *Elettaria cardamomum*, *Foeniculum vulgare* and *Syzygium aromaticum* on growth promotion biochemical status improvements in *Macrobrachium malcolmsonii* early juveniles. This suggests enhanced food consumption and assimilation, which leads to elevation of total protein, carbohydrate and lipid were also found to be significantly elevated in these spices.

CONCLUSION

From the experiments it can be concluded that the *Curcuma longa* (25 mg/kg of feed) with daily administration has better growth promotion and feed utilization efficacy in *M.rosenbergii.* Daily administration of experimental feeds were found effective than the twice a week administration. So the above plants under study cab be recommend for better health managements in *M.rosenbergii.*

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