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Functional Feed Efficacy of Curcumin Derivatives in *Macrobrachium Rosenbergii*

Salini .M .P[1], A. Akhila Thomas[2]

[1,2]Fatima Mata National College,Fisheries Biotechnology Unit, Dept.of Zoology, Kollam

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 Curcuma longa oral supplementation. Three main bioactive ingredients selected were Curcumin, Demethoxy curcumin and Bisdemethoxy curcumin. subsequent to 60 days of feed supplementation morphometric analysis, nutritional indices and feed utilization parameters were assessed. The prawns were challenged with Vibrio harveyi on the 61st days, antioxidant assays were also evaluated. Relative percentage survival (RPS) was also calculated. From the experiment it was clear that Curcumin incorporated diet shown excellent nutritional status comparative to Demethoxy curcumin and Bisdemethoxy curcumin.***

Keywords: Curcumin, Demethoxy curcumin , Bisdemethoxy curcumin, *Macrobrachium rosenbergii*

1. Introduction

Plant products influence various activities such as antistress, growth promotion, immunostimulation, aphrodisiac antimicrobial properties etc in culture organisms. The active principles that evoke these are of as alkaloids, flavanoids pigments, phenolics, terpenoids, steroids and essential oils [21].These are considered as "functional additives". Most of the herbs used as feed additives in aquaculture acts as appetite stimulators and growth promoters [31],[27]. Traditional medicinal plants such as Garlic [25],[8] *Zingiber officinalis* [10],[3] *Curcuma longa* [4],[18] *Ricinus communis* [6] and Triphala [12]-[13] are extensively studied in the field of aquaculture. Black Tiger Shrimp *Penaeus monodon* showed better resistance against *V. harveyi when fortified with turmeric exract.* Phenoloxidase activity of shrimps fed at 25 and 50 mg/kg feed of turmeric extract was significantly ($P<0.05$) higher than the control. All turmeric extract-treated shrimps showed higher bactericidal activity than the control [18].*Curcuma longa* application in aquaculture reported enhanced growth of *Poecilia reticulate* and increased serum bacterial activity in *Oreochromis niloticus* [28].Curcuminoids refer to the main chemical substances, namely curcumin, demethoxycurcumin, and bis-demethoxycurcumin. These have been reported to exhibit several biological activities in animal and human clinical studies [29]. Curcumin, a polyphenolic compound derived from dietary spice turmeric, possesses diverse pharmacologic effects including anti-inflammatory, antioxidant, antiproliferative and antiangiogenic activities [23]. Enhanced bioavailability of curcumin in the near future is likely to bring this promising natural product to the forefront of therapeutic agents for treatment of human disease [1]. *Centella asiatica* is a nutrient rich herb which is reported to increase the growth and feed utilization parameters of *M.rosenbergii*.

Oxidative stress is caused by an imbalance of oxidants and antioxidants in favor of the former, and is capable of inflicting injury on membrane lipids, proteins and nucleic acids. For the protection of cells against reactive oxygen species (ROS), organisms have evolved a highly sophisticated and complex antioxidant protection system [24].Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPX), Glutathione S- trasferase (GST), etc are the major antioxidant enzymes and their activities are interrelated [7]. Lipid peroxidation refers to the oxidative degradation of lipids.AST and ALT activities are usually used as general indicators of the functioning of invertebrate hepatopancreas. It is found to be the active site responsible for the major metabolic events, including enzyme secretion, absorption and storage of nutrients, molting and vitellogenesis. Lactate dehydrogenase(LDH) is found in all most all organisms. In the case of specific LDHs , all are tetramers of about 140 kDa. In substrate affinity and pyruvate inhibition, most invertebrate LDHs are intermediate between the muscle and heart types of the vertebrates.Herbs-derived antioxidants such as tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavonols, catechins, anthocyanins and proanthocyanins could delay or prevent the onset of degenerative diseases because of their redox properties, which allow them to act as hydrogen donors, reducing agents, hydroxyl radicals or superoxide radical scavengers and may lead to increase immune factors, thus indirectly raising fish resistance to various stresses Thus the application of plant extracts with antioxidant property will improve overall total antioxidant status of the organisms [11].

In the present study we evaluated the various growth parameters of the feeds incorporated with active principles such as curcumin, demethoxycurcumin, bisdemethoxycurcumin also evaluated the influence of these on the antioxidant enzyme profiles of *Vibrio harveyi* infected *Macrobrachium rosenbergii*.

2. Materials and Methods

Post larvae of *Macrobrahium rosenbergii* (PL20) *was* purchased from ADAK (Agency for Development of Aquaculture (ADAK), Varkala, Kerala, India**.** Acclimated in laboratory under optimum salinity, pH and temperature. During acclimatization they were fed *ad libitum* with control diet and egg albumin. *M.rosenbergii* of size 10.20 ± 0.20 g was taken for experiment. 60 prawns were selected, grouped into 4 categories. Each treatment ran in triplicates. Each

treatment was fed with consent medicated feed. The experiments were conducted for a period of 60 days. Morphometric analysis(Length gain(%),Weight gain(%), Biomass Index(BI),Condition Factor(CF)),Nutritional Indices(Survival (%), Relative Growth Rate(RGR) ,Absolute Growth Rate(AGR), Specific Growth Rate (SGR,%/day) ,Average Daily growth Rate(ADGR g/days), Feed Efficiency (FE),Gross Conversion Efficiency(GCE)) and Feed utilization parameters(Feeding Rate (FR), Mean Absorption (MA), Absorption Rate (AR), Mean Conversion(MC), Conversion rate (CR),NH3 Excretion Rate (ER), Metabolic Rate (MR)) checked. After rearing for a period of 60 days the prawn were challenged with 30 µL of 10^7 CFU bacterial suspensions between 3rd and 4th abdominal segments. Relative percentage survival (RPS) were noted and tabulated. After 72 hours the prawns were sacrificed and haemolymph and tissues such as muscle, digestive gland (hepatopancresas) and gut were removed, washed thoroughly in ice-cold physiological saline, wiped and respective tissue extracts were prepared. Subsequently the corresponding tissues were prepared for antioxidant enzyme . AST and ALT activities and LDH were determined spectrophotometrically at 340 nm using a UV 2000 spectrophotometer (Hitachi) at 37 ^{0} C. using the Erba Manrhelm according to manufacturer's instructions.Other antioxidant assays performed were Super Oxide Dismutase(SOD),Catalase (CAT),Glutathione peroxidase (GPX), Glutathione –S- Transferse (GST),Lipid peroxidation (MDA)[7].

Feed was prepared by using the ingredients listed in the Table.1.The main bioactive principles used in the present study was Curcumin, Demethoxy curcumin(DMC) and Bisdemethoxy curcumin(BDMC) from *Curcuma longa.* From our standardization studies(Salini *et.al.*,2014) it was conclusive that 25 mg/Kg of *Curcuma longa* at daily administration showed promising results than other concentrations. Taking it as a standard and also from phytochemical analysis, the concentrations of Curcumin, Demethoxy curcumin (DMC), Bisdemethoxy curcumin (BDMC) were determined. Feed modifications are as follows.

Test Diet 1 (TD 1) : 0.13 mg of Curcumin per kg of basal feed.
Test Diet 2 (TD 2): 0.057 mg of Demethoxy curcumin per kg of basal feed.
Test Diet 3 (TD 3): 0.105mg of Bisdemethoxy curcumin per kg of basal feed.
Control : Basal feed.

Table 1: Ingredients of experimental feed.(FAO, 1978).

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

The proximate compositions such as protein[16], lipid[9], moisture, ash , fibre and dry matter [2] were also calculated. Gross energy content [14], Metabolizable energy [15] and ME/DE coefficient [22] were also calculated. Data were statistically analyzed for mean and standard deviation. The data were subjected to One- way analysis of variance (ANOVA) using SPSS version 16.0. One- way ANOVA was used to test whether differences between groups existed between parameters. In all test, $P<0.05$ was considered as significant.

3. Results

Table 2: Proximate analysis of feeds

	Crude protein (%)	Crude lipid (%)	Moisture (%)	Dry matter (%)	Ash (%)	Fibre (%)	NFE (%)	Gross energy (Kj/g)	ME:DE Coefficient (Kj/g)	Metabolizable energy (%)
TD1	40.10	7.54	1.00	99.00	8.20	0.065	43.10	16.80	99.46	15.01
TD 2	39.86	7.00	6.5	93.5	8.70	0.003	37.94	15.78	99.42	14.13
TD 3	39.76	6.80	9.00	91.00	8.60	0.002	35.84	15.38	99.47	13.65
Control	39.06	6.98	8.00	92.00	8.45	0.003	37.51	15.56	99.45	13.93

Highest length gain is observed in samples fed with TD 1 (2.50±0.01 cm). Least length gain was observed in control diet (1.30±0.01 cm). In the case of weight gain also same mode of improvement was observed as in length gain**.** TD 1 fed prawns got significantly higher BI (28.03±2.62) than others. Least BI is in control prawns (2.34±1.17). Highest condition factor was in prawns fed with TD 1 (1.78±0.05).Least was obtained in control prawns (0.45±0.49)(figure.1)..

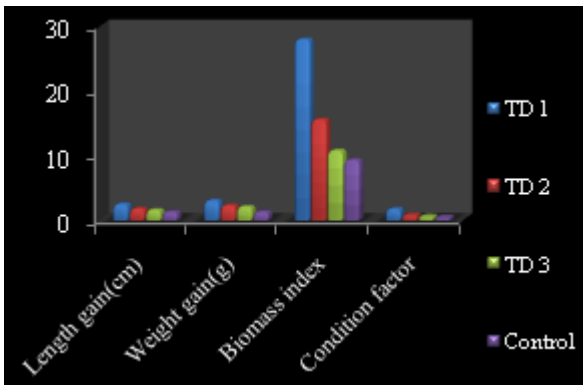

Figure 1: Morphometric analysis of prawns supplemented with the bioactive principles from *C.longa*.

Survival rate is high in prawns fed with TD 1 (50). In prawns fed TD 2 had low survival rate than others (25±0.12).

Relative growth rate (RGR). TD 1 fed groups had comparatively higher RGR values (16.01±0.92). Control prawns had least RGR (2.01±0.01). Higher AGR values obtained in prawns fed TD 1 (0.04±0.02). TD 1 fed prawns had higher SGR (3.90±0.30).In control prawns the SGR is very low(0.33±0.200).The average daily growth rate is high in TD 1 fed prawn (0.05±0.02).In control groups the Average daily growth rate is relatively less (0.003±0.00). Feeding efficiency is significant in the treated groups ($p<0.05$). TD 2 fed prawns had relatively high feed efficiency (22.58±1.94) values. Control group had low FE values (1.86±0.25).GCE of prawns fed with TD 1 had highest GCE (60.89±3.43). TD 3 fed prawns had very low GCE (8.78±1.33) compared with the other treatments (figure.2).

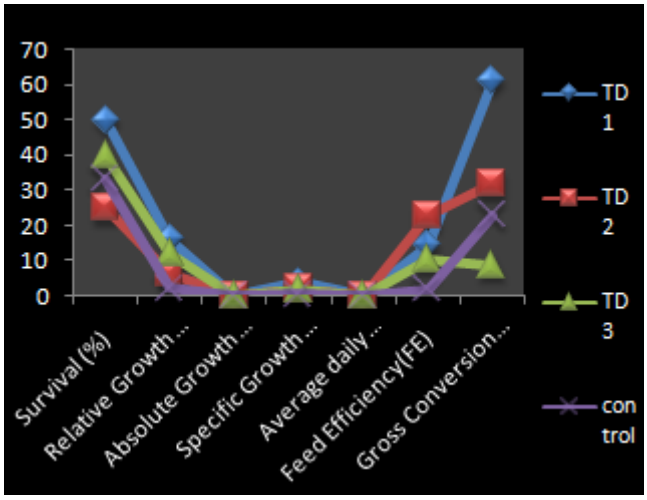

Figure 2: Nutritional Indices of prawns supplemented with the bioactive phytonutrients from *C.longa*

The feeding rate is related to the food consumption per day and weight gain. The prawns fed with TD 1 fed prawns (Figure.2.) had relatively high feeding rate (6.34±.036). Control prawns had less feeding rate compared to the other treatments (6.00± 0.01).Mean Absorption is related to the mean food consumption per day and food excreted as faeces. The prawns fed with TD 1 had high (4.04±0.09) mean absorption.. Control prawns had least mean absorption rate compared to the other groups (1.05±0.155).Except in prawns fed TD 3 (0.14±0.04), all other treatments had relatively similar absorption rate. From this TD 1 fed prawns (0.39±0.53) had relatively highest absorption rate. Mean conversion was high in prawns fed with in TD 1 (3.36±0.15). Control prawns (0.82±0.22) had least mean conversion. The highest conversion rate is obtained in prawns fed TD 1 (0.30±0.02) and least value obtained in TD 5 fed prawns (0.13±0.02).Excretion rate is the mean ammonia excretion per day in relation to initial live weight of the prawn. Prawns fed with TD 1 had high ammonia excretion rate than other treatments (0.61±0.05).Least excretion rate was obtained in control (0.16±0.05) prawns. TD 1 fed prawns had high metabolic rate (4.11±0.21) than others. Control prawns (0.40±0.57) had least metabolic rate(figure.3).

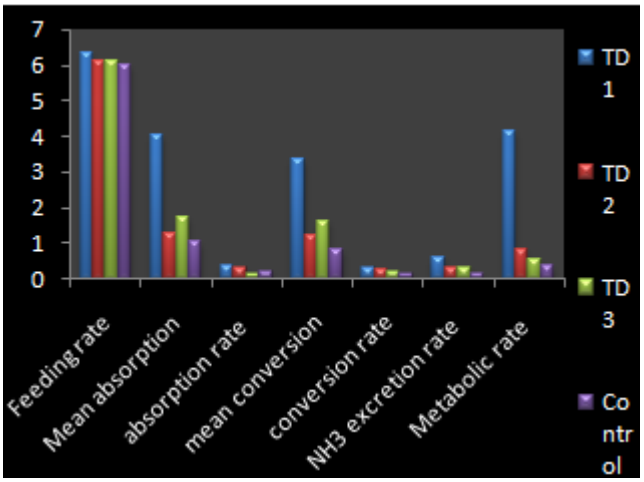

Figure 3: Feed utilization parameters of prawns supplemented with the bioactive phytonutrients from *C.longa*.

Haemolymph of prawns fed with TD 1 (0.05±0.01 IU/L) reported lower GOT activity. Least GOT activity was observed in prawns fed control diet (0.07±0.01 IU/L). The Glutamate pyruvate transaminase (GPT) activity of all treated groups were comparatively lower than that of control. Haemolymph of TD 2 (0.06±0.00 IU/L), TD 3 (0.06±0.01 IU/L) and control (0.06 ± 0.01 IU/L) had higher GPT activity. Least GPT activity was in TD 1 supplementation with (0.05±0.00 IU/L).The Lactate dehydrogenase (LDH) activity was higher in control diet (0.58±0.04 IU/L) . Least activity was observed in TD 1 fed prawns (0.40 ±0.01 IU/L). Sites such as muscle, digestive gland and gut were analyzed for the detection of catalase enzyme activity(table.3).

Table 3: Antioxidant enzymes of haemolymph of *M.rosenbergii* 72 hours post infection with *V.harveyi*.

	GOT (IU/L)	GPT (IU/L)	LDH(IU/L)
TD 1	0.05±0.01	0.05±0.00	0.40±0.01
TD 2	0.06±0.01	0.06±0.01	0.53±0.01
TD 3	0.06±0.01	0.06±0.00	0.52±0.04
CONTROL	0.07±0.01	0.06±0.00	0.58±0.01

In muscle tissue, highest CAT activity was observed in TD 1 fed prawns (26.56±0.02 µmoles /mg protein), least activity was observed on control prawns (21.36±0.50 µmoles /mg protein). The digestive gland of prawns supplemented with TD 1 (38.36±0.15 µmoles /mg protein) had higher catalase activity and least in control prawns being (24.36±0.30 µmoles /mg protein).The gut catalse activity was higher in TD 1 fed prawns (28.33±0.20 µmoles /mg protein), least was observed in control prawns (20.00±0.58 µmoles /mg protein)(figure.4).

TD 1 fed prawns (12.60±0.30 µmol H_2O_2 /min/mg protein) expressed higher SOD activity at the muscle. Lower activity was obtained in the control prawns (7.36 ±0.19 µmol H_2O_2 /min/mg protein).Digestive gland TD 1 fed prawns (18.36 ±0.18 µmol H_2O_2 /min/mg protein) had maximum SOD activity. Least was observed in control prawns (10.00 ±0.69 µmol H_2O_2 /min/mg protein). SOD activity at the gut tissue when analyzed, revealed TD 1 fed prawns and TD 3 fed prawns had comparable similar SOD activities 14.00±0.12 µmol H_2O_2 /min/mg protein and 13.89±0.03 µmol H_2O_2

/min/mg protein respectively, least activity is in the group fed with control diet 6.39±0.08 µmol H_2O_2 /min/mg protein(figure.4).

The TD 1 fed prawns expressed the highest muscle glutathione peroxidase activity (8.90±0.30 µmol H_2O_2 /min/mg protein). Both control and TD 3 fed prawns having values being 8.36±0.03 µmol H_2O_2 /min/mg protein and 8.36±0.02 µmol H_2O_2 /min/mg protein respectively. In the three selective sites, digestive gland showed enhanced enzyme activity. The digestive gland of TD 1 fed prawns expressed maximum enzyme activity (10.23±0.09 µmol H2O2 /min/mg protein). Control prawns recorded least enzyme activity 9.23±0.04 µmol H_2O_2 /min/mg protein. TD 2 fed prawns 9.89±0.05 µmol H_2O_2 /min/mg protein had higher gut GPX activity .Least were observed in test diet 1 9.02±0.01 µmol H_2O_2 /min/mg protein, test diet 3 9.00±0.01µmol H_2O_2 /min/mg protein and control diet 9.00±0.01µmol H_2O_2 /min/mg protein(figure.4).

TD 3 fed prawns had higher muscle GST activity (7.96±0.02 µmoles/mg protein) least was observed in control prawns 6.23±0.02 µmoles/mg protein. On digestive gland maximum GST activity was observed in the TD 2 fed prawns (9.78±0.02 µmoles/mg protein).Control prawns showed least enzyme activity (8.23±0.03 µmoles/mg protein). In the case of gut, GST activity , higher enzyme activity was observed in TD 1 fed prawns (9.65±0.03 µmoles/mg protein), least was observed at TD 3 fed prawns (6.02±0.012 µmoles/mg protein) (figure.4). Lower lipid peroxidation was observed in the muscle tissue of *M.rosenbergii* supplemented with TD 1 (0.78±0.02 nmol MDA/ mg protein).Higher activity was expressed in *M.rosenbergii* supplemented with control diet (1.86±0.03 nmol MDA/ mg protein).Lipid peroxidation values of digestive glands revealed TD 1 and 2 supplemented *M.rosenbergii* showed least enzyme activity (1.02±0.01 nmol MDA/ mg protein). Higher activity was observed in control prawns 2.58±0.06 nmol MDA/ mg protein. TD 1 incorporated *M.rosenbergii* had lower enzyme activity .98±0.02 nmol MDA/ mg protein). Control prawn had higher lipid peroxidation rate 2.15±0.01 nmol MDA/ mg protein(figure.4).

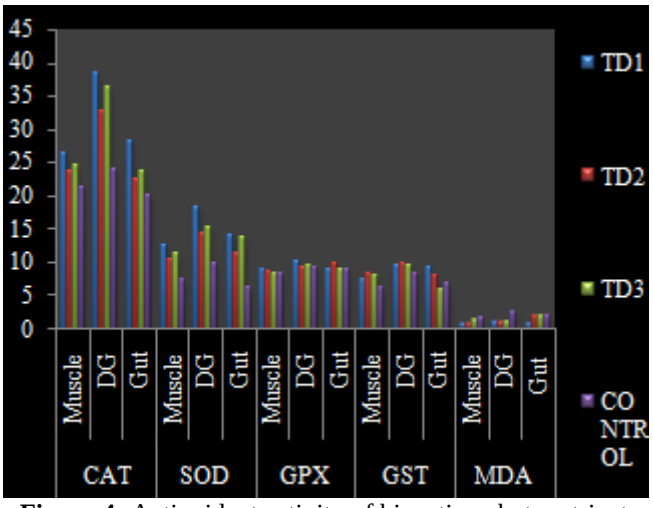

Figure 4: Antioxidant activity of bioactive phytonutrient supplemented *M.rosenbergii* at 72 hours of post infection with *V.harveyi* (*DG-Digestive gland).

Relative percentage of survival rates were checked after 72 hours. Relative percentage of survival was found higher in prawns fed with TD 1 , (0.50) and lower was observed in TD 3 fed prawns (0.00)(Table.5.)

Table 5: Relative percentage survival of *M.rosenbergii* fed with bioactive phytonutrient supplementation and infected with *Vibrio harveyi* (10^7 cfu/ ml).

Treatments	Relative percentage survival (RPS).
TD 1	0.50
TD 2	0.40
TD 3	0.00
Control	0.00

4. Discussion

There are numerous works documenting the effects of medicinal herbs as feed additives and growth stimulator [18] Application of crude extracts of *C.longa* on *Macrobrachoium rosenbergii* was also effective [25],[18]. Effect of curcumin supplementation in aquaculture feeds is gaining momentum [4],[19]-[20].Curcumin is the major content in *C.longa*, responsible for the color and most of its pharmacological effects. Demethoxy curcumin and bisdemethoxy curcumin was also the leading active ingredients with medicinal properties. In morphometric analysis curcumin treated groups have shown significant improvement. From the results it was clear that Curcumin had the growth promotion property as reflected by biogrowth patamerters.Crude extracts from various parts of *Azadirachta indica*, *Curcuma longa*, *C. zedoaria*, and *Callotropis gigentia* was given to *Barbonymus gonionotus* showed wide range of promising effects. *Aeromonas hydrophila*, a bacterial pathogen with different doses were injected intramuscularly in *B.gonionotus* and the effect of *C.longa* supplementation was encouraging[32]. *C.longa* served as a good source of non-enzymatic antioxidants and enhanced the survival of the freshwater prawns. Excretion rate was higher in treated groups, this can be correlated with increased food consumption, lead to increased metabolic rate and all other metabolic parameters. Curcumin treated groups have shown good biogrowth. [25] evaluated the growth promoting potential of garlic, ginger, Turmeric and fenugreek on the freshwater prawn *Macrobrachium rosenbergii*. The metabolic rate was higher in curcumin supplemented diets. This can be correlated with improved growth.In the present study , the partitioning of ingested energy was recorded in terms of feeding rate, mean absorption, absorption rate, mean conversion, conversion rate, ammonia excretion rate, metabolic rate($p<0.05$).

The activity of GOT, GPT and LDH in treated groups were significantly decreased than the control group after the infection.SOD activity was higher in muscles of prawns supplemented with Curcumin and Demethoxy curcumin compared to the control prawns. CAT activity was higher in Curcumin and Bisdemethoxy curcumin supplemented prawns. Lower values were obtained in Demethoxycurcumin. In lipid peroxidation, the least activity was observed in Curcumin fed prawns.All antioxidant enzymes of digestive glands were higher. SOD activity of Curcumin fed prawns showed higher SOD activity, compared to control diet. CAT activity was highest in

Curcumin fed prawns .compared to control prawns. Control prawns had higher lipid peroxidation.Curcumin fed prawns showed higher CAT activity compared control prawns. Gut tissue of Curcumin and Bisdemethoxy curcumin supplementation groups showed higher SOD activity with respect control prawns. GPX activity of all treatment were more less similar, but Demethoxycurcumin fed prawns showed higher GPX activity. Gut tissue of Curcmin fed prawns showed least lipid peroxidation and control highest .Control prawns showed higher lipid peroxidation activity.There are many studies which reveal the effectiveness of medicinal plants in *M.rosenbergii.* The addition of antioxidant rich feed additives will increase the total antioxidant enzyme activity of the prawns such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX).

In the present study the bioactive phytonutrient supplemented *M.rosenbergii* were found to have increased antioxidant enzyme activity. But in the case of toxicity makers such as GOT.GPT and LDH was found to have decreased during the treatments. The phytonutrient supplemented *M.rosenbergii* showed increased antioxidant activity when challenged with *V.harveyi.* Curcumin I, curcumin II (monodemethoxycurcumin) and curcumin III (bisdemethoxycurcumin) from *Curcuma longa* were assayed for their cytotoxicity, antioxidant and anti-inflammatory activities [26].[28] studied the enzymatic and nonenzymatic activities in *Oreochromis mossambicus* larvae fed with commercial herbal enriched *Artemia nauplii*. The level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) decreased when the *Penaeus monodon* juvenile were supplemented with astaxanthin followed by *Vibrio damsela* challenge but could alter the shrimp's antioxidant defense capability and hepatopancreatic enzymes.

Form the present study, in most of the case hepatopancreas expressed improvised enzymatic profile than gut and muscle. This may due to the fact that hepatopancreas acts as key site with high metabolic rate and key site for xenobiotic detoxification .Lactate dehydrogenase(LDH), Glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminase (GPT) and lipid peroxidation (MDA) is known as toxicity markers . Their decrease in concentration and the increase in antioxidant status denotes the effects of medicinal plants. The seeds of medicinal plants, *Syzygium cumini, Phylanthus emblica, Azadirachta indica* and *Ricinus communis* was evaluated for growth promotion in *Macrobrachium Malcolmsonii* early Juveniles [6].The activities of enzymatic antioxidants, super oxide dismutase and catalase, and lipid peroxidation were not altered significantly.

To conclude oral supplementation from *C.longa* bioactive phytonutrient improved the feed intake and assimilation in *M.rosenbergii*. Among the five supplementation groups Curcumin incorporated diet expressed the excellent growth promotion properties. Coupled with improvised health status as revelaed by *V.harveyi* challenge.In the case of toxicity markers, GOT, GPT and LDH was found decreased in bioactive principles incorporated diets. Curcumin, Demethoxycurcumin and Bisdemethoxy curcumin supplementation the activity was at moderate levels. In the case of SOD and CAT the Curcumin supplemented diets showed higher activity than others. In all other groups the activity fell below the Curcumin enriched prawns and was moderate. Lipid peroxidation was lower in Curcumin enriched prawns. Thus the secondary metabolites from *C.longa* with promising credentials can be used in aquaculture to biopotentiate the culture species, *M.rosenbergii* leading to sustainable, organic aquaculture strategy.

References

[1] Anand Preetha, Ajaikumar, B. Kunnumakkara., A.Robert, A. Newman., and B. Bharat.B, Aggarwal. "Bioavailability of Curcumin: Problems and Promises". Molecular pharmaceuticals,4(6),pp.807–818, 2007.(Journal style).
[2] APHA, Standard Methods for the examination of water and waste water,20th Edition, American Public Health Association, Washington, D.C, 2005.(Book style).
[3] Arulvasu Chinnasamy, Kamaraj Mania, Devakumar Chandhirasekara, Durai Prabhua, Shanmugam Sivagnanamb,"Effect of dietary administration of *zingiber officinale* on growth, survival and immune response of Indian major carp, *catla catla* (ham.)," International Journal of Pharmacy and Pharmaceutical Sciences,5 (2),pp.174-179.2013,(journal style).
[4] T. Behara , P. Swain , S.K. Sahoo, D. Mohapatra and B.K Das, "Immunostimulatory effects of curcumin in fish, *Labeo rohita*", Indian Journal of natural products and resources, 2(2),pp.184-188,2010.(Journal style).
[5] T. Behara, P. Swain, P. Sahoo, S.K. Mohapatra and B.K Das, " Immunostimulatory effects of curcumin in fish, *Labeo rohita",*Indian Journal of natural products and resources,2(2),pp.184-188,2010. (Journal style).
[6] S. Bhavan Saravana, V. Mohammedsiddiq, T. Srinivasan, Muralisankar, M. Manickam , "Effects of Seeds of Medicinal Plants, *Syzygium Cumini, Phylanthus Emblica, Azadirachta Indica* and *Ricinus Communis* on Growth Promotion in *Macrobrachium Malcolmsonii* Early Juveniles", International Journal of Research Studies in Biosciences (IJRSB),2(11), pp. 95-106.2014. (Journal style).
[7] R.M Bruce, Santodonato Neal, M.W,"Summary review of the health effects associated with phenol",Taxicol.Indust.Health,3,pp. 535-568,1975. (Journal style).
[8] G. Chelladurai, J. Venil, Mohanraj and R. Nagarajan, "Effect of herbal extracts supplemented diets on Non Specific Immunity and Resistance to *Aeromonas hydrophila* in Indian cat fish (*Mystus montanus*)". Journal of Zoological and Bioscience Research,1(1),pp.10-14, 2014. (Journal style).
[9] J. Folch , M. Lees, and G.H. Sloane Stanley."A simple method for isolation and purification of lipids from animal tissues", J. Biol. Chem, 226,pp.497-308,1957. (Journal style).
[10] Haghighi Masoud and Sharif Rohani Mostafa,"The effects of powdered ginger (*Zingiber officinale*) on the haematological and immunological parameters of rainbow trout *Oncorhynchus mykiss",* JMPHTR,1,pp.8-12,2013. (Journal style).

[11] A. Hamid, O.O Aiyelaagbe,L.M.Usman1, O.M. Ameen and A. Lawal," Antioxidants: Its medicinal and pharmacological applications", African Journal of Pure and Applied Chemistry,4(8), pp.142-151,2010. (Journal style).
[12] S.Jayasree, S.A. Usha, Akhila Thomas," Immunomodulatory effect of Triphala biofortification on *Oreochromis mossambicus"*. International Journal of Science and Resaerch(IJSR),5(8),pp.659-662.2016(a).(Journal style).
[13] S.Jayasree, S.A. Usha, Akhila Thomas, "Growth promotion effect of Triphala biofortification on *Oreochromis mossambicus"*. International Journal of Science and Resaerch(IJSR),5(2),pp. 1126-1130.2016 (b). (Journal style).
[14] M. Jobling," A Short review and critique of methodology used in fish growth and nutritional studies",J.Fish.Biol, 23,pp.685-703.1983.(Journal style).
[15] P.G.Lee and A.L.Lawrence, Digestibility. In: D'Abramo, L/R/, Conklin, D.E., Akiyama, D.E. (Eds.), Crustacean Nutrition. World Aquaculture Society, Baton Rouge, LA, USA, pp. 194-260.1997.(Conference style).
[16] O.H. Lowry,V.J. Rosenbrough, A.L. Faro, and R.J.Radell,"Protein measurement with Folin-Phenol reagent", J. Biol. Chem,193,pp. 265-276,1951. (Journal style).
[17] M.E.Mahfouz, "Ameliorative Effect of Curcumin on Aflatoxin B1Induced Changes in Liver Gene Expression of *Oreochromis niloticus"*. Molecular Biology.49 (2),pp.275–286,2015. (Journal style).
[18] H.L.Malar Vidhya and Charles P.Maria,"Effect of Turmeric *Curcuma Longa* Linn. extract on immunity and Resistance to *Vibrio Harveyi* In Black tiger shrimp *Penaeus Monodon*, International Journal of Research in Zoology, 3(2),pp.21-26,2013. (Journal style).
[19] M. Manju, T.G. Sherin, K.N. Rajasekharan, O.V.Oommen,"Curcumin analogue inhibits lipid peroxidation in a freshwater teleost, *Anabas testudineus* an in vitro and in vivo study", Fish Physiology and Biochemistry,35,413-420,2009.(Journal style).
[20] M.Manju,T.G. Sherin,K.N. Rajeesha, P.Sreejith, K.N.Rajasekharan,O.V.Oommen," Curcumin and its derivatives prevent hepatocyte lipid peroxidation in *Anabas testudineus",* Journal of. Fish Biology.,73,pp.1-13,2008. (Journal style).
[21] Murthy Shankar and B.R.Kiran," Review on usage of medicinal plants in fish diseases", Int J Pharm Bio Sci,4(3),pp.975 – 986,2013. (Journal style).
[22] J.Noblet, X.S. Shi and S. Dubois,"Metabolic utilization of dietary energy and nutrients for maintenance energy requirements in sows: basis for a net energy system". Br. J. Nutr,70,pp. 407-419,1993. (Journal style).
[23] A.M. Osho and L.Lajide,"Prescreening evaluation of some plant extracts used in ethno- veterinary practices as anti trypanosomal agents", Journal of Medicinal Plants Research,6(11),pp.2056-2060,2012. (Journal style).
[24] Pham-Huy Lien Ai., Hua He., Chuong Pham-HuyFree,"Radicals, Antioxidants in Disease and Health", Int J Biomed Sci,4 (2),pp.89,2010. (Journal style).
[25] Poongodi,"Growth promoting potentional of garlic, ginger, turmeric and fenugreek on the fresh water prawn *M.rosenbergii",*Internationl journal of Pharma and biosciences,3(4)(B),pp.914-926,2012.(Journal style).
[26] R.S. Ramsewak, D.L. DeWitt,M.G. Nair,"Cytotoxicity, antioxidant and anti-inflammatory activities of curcumins I-III from Curcuma longa", Phytomedicine,7(4),pp.303-308,2000. (Journal style).
[27] Ramudu Kurva Raghu and Dash Gadhadar,"A review on herbal drugs against harmful pathogens in aquaculture", American journal of Drug Delivery and development,5(3),pp.56-59,2013. (Journal style).
[28] Rebecca Anne, Bhavan P Saravana, Radhakrishnan S,"*Allium sativum*-, *Zingiber offi cinale*- and *Curcuma longa*-induced digestive and antioxidant enzyme activities in *Macrobrachium rosenbergii* post larvae",3(1),pp.22-27,2014. (Journal style).
[29] Rohman Abdul,"Analysis of curcuminoids in food and pharmaceutical products",International Food Research Journal,19(1),19-27,2012. (Journal style).
[30] M.P. Salini, Jasmine Anand, A. Akhila Thomas.2014. *"In vivo* antimicrobial efficacy and biochemical turn over in *Macrobrachium rosenbergii* supplemented with *Curcuma longa"*. In Proceedings of National Seminar on Impact of Climate Change on Fish and Fisheries (ICCFF).Post Graduate and Research Department of Zoology. Sree Narayana College, Cherthala, Alapuzha, Kerala.26th and 27th June, pp.24-28,2014. (Conference style).
[31] A. Syahidah, C. R. Saad,H.M Daud, Y.M. Abdelhadi," Status and potential of herbal applications in aquaculture: A review". Iranian Journal of Fisheries Sciences. 14(1), pp.27-44,2015. (Journal style).
[32] Tareq-Uz-Zaman, Md. Bazlur Rashid Chowdhury-Md. Rasheduzzaman Khan Dipu," Isolation of bacterial fish pathogen *Aeromonas hydrophila* and therapeutic effects of medicinal plants on its invasion", Journal of Fisheries,2 (1),pp.76-79,2014. (Journal style).

Author Profile

Salini. M. P received her B.Sc and M.Sc degrees from Sree Narayana College for Women Kollam and Sree Narayana College Kollam respectively under Kerala University. During 2008 she got her M.Phil from Dept. of Environmental Sciences, Kayavattom, Kerala. Her PhD was from Kerala University under the guidance of Dr.A.Akhila Thomas, Dept. of Zoology, Fatima Mata National college, Kollam from University of Kerala. Now she is working as Govt.Guest lecturer in Sree Narayana College for Women Kollam.