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Research Article

EFFECT OF CURCUMA LONGA ENRICHED MESOCYLOPS THERMOCYCLOPOIDES ON FRESH WATER FISH, *CYPRINUS CARPIO*

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ABSTRACT

The Indian major carp, the Cyprinus carpio, is an important commercial fish in India. Most of the Indian farmers are culturing Cyprinus carpio in the farm. C. carpio culturing in the farm are more susceptible to diseases. Hence the present investigation was focused on disease on C. carpio. Natural immuno-stimulants are biocompatible, biodegradable, cost effective and safe for the environment and eco-friendly. There has been growing importance in the immune-stimulating functions of plants in aquaculture. Fish were divided into four groups being fed for 45 days with 0.3, 0.6 and 0.9gm with add-on commercial diet as the control. After the Groups fed with copepods mediate treated with Cyprinus carpio using by different concentration at 0.3gm, 0.6gm and 0.9gm when compare to the high dose, were differential leukocyte counts in C. carpio Neutrophils,Lymphocytes, Monocytes, Esnophils and basnophils analysis was showed a highly significant difference compared to controls . The effect of turmeric Curcuma longa on Cyprinus carpio was studied.

Keywords: Curcuma longa, Cyprinus carpio, Mesocylops thermocyclopoides, Aquaculture.

INTRODUCTION

Recreational fisheries science has made great strides in understanding how various factors influence the hatchability of fish[1]. However, the most common approach[2] involves holding fish in captivity (e.g., pens, cages, tanks) to assess mortality. The impacts of climate change on coastal communities around the world include effects on both humans and human uses of the environment. In addition, climate change is interacting with other anthropogenic impacts, such as pollution and habitat destruction that are currently negatively affecting the marine environment[3]. About 80% of the total ornamental fish trade is rooted from wild catch and is contributed by this region of India via Kolkata Airport[4] Mahseer fish[5] and Indian fresh water fish. Here, we employed the combined approach of morpho-taxonomy and nutritional analysis as a reliable species C. carpio growth and development. Among the infectious diseases, bacterial fish diseases are reported to infest to most of the cultivable as well as wild fish species. There are 40 - 60 bacterial fish pathogens found to be involved in fish diseases.

The present paper is to encourage the application of natural products to improve the immune system of fisheries, achieve greater weight gains and feed conversions and thereby increase production and consequently economic gains for

producers. Although numerous studies have been conducted on administration of pre-biotics in aquaculture, no information is available on the effects of pre-biotics on growth performance, carcass composition and digestive enzymes activities in early life stages of common carp, Cyprinus carpio[6] .

The water column Copepods are distributed in various kinds of environments; play an important role in aquatic food webs, as natural prey items of fish larvae, at which typically making up 50 percent or more of their stomach contents[7,8,9]. They are an important food source for planktivorous fish and fish larvae in general[10]. A major determinant of successful intensification of aquaculture is feed.

Turmeric, a derivative of the plant (Curcuma longa Linnaeus, 1758) is a spice commonly used in Middle East and Asia as an herbal remedy[11]. Several studies found turmeric to have anticarcinogenic, antioxidant, antiinflammatory, anti-allergy, anti-mutagenic, immune-modulatory properties[12] and[13] antiviral and antibacterial activities[14] and antiproliferative properties. Turmeric extract and curcumin have also been used widely as a hepatoprotective agent. A variety of medicinal herbs are known to stimulate phagocyte cells including ginger, garlic, curcumin and turmeric (Curcuma longa), etc,[15] . Curcuma (Curcuma longa L.) contains phenolic compounds (curcuminoid pigments) in its rhizomes that are responsible for the functional properties of the plant such as antioxidant[16,17,18,19,20] . Antimicrobial, anti-inflammatory and anticancer activities. A recent study reported that ethanol and hexane turmeric extract showed inhibitory effects against 13 bacteria, including V. alginolyticus, isolated from shrimp and chicken[21] Hence, in the present study revealed that copepod enhanced with turmeric powder and feed on common crop, C. carpio to enhance the growth and nutritional value.

MATERIALS AND METHODS

The cyclopses were collected from Muthanna lake at Coimbatore, India. The Collection was done during early morning hour (6 to 8^{0}C), because maximum numbers of Cyclops were available only during morning hour. The Cyclops was collected by using 100 mm mesh size plankton net. Collected cyclops was isolated based on the methodology of[22] .The selected live planktons *Mesocyclops thermocyclopoides* were carefully isolated by using dissection microscope. At the time many undesired organisms seen along with plankton were carefully removed using a fine tip Pasteur pipette. *Mesocyclops thermocyclopoides* were kept in the separate culture plate and this culture was maintained in a lab condition. The analysis of Cyclops species was completed within 24 hour after the collection. Nauplii stage of *M. thermocyclopoides* (42 hours) were collected and filtered for the enrichment purpose.

Collection of turmeric powder (*Curcuma longa*) and enrichment

C. longa was collected from Erode district directly from the farmers then it was sundried for 72 hours and powdered using cylco mixer. *C. longa* powder (sieved by 20 micron sieve) was used in different concentration for the enrichment of *M. thermocyclopoides*. Different concentration of enriched *M. thermocyclopoides* were fed with different groups (EXP-I 0.3g/l, EXP-II 0.6g/l, EXP-III 0.9 g/l and control as without enrichment of Cyclops) of fish larva.

Growth analysis and Nutritional analysis

The growth parameters were calculated by using the following formula according to reference no. 23 and 24.

Biochemical Composition

Protein, lipid and carbohydrates were determined by the following methods. The basic procedures followed were for protein[25] lipids[26] and carbohydrates[27]. Fatty Acid Analysis was done using Gas Chromatography described by reference no. 28.

Erythrocyte counting (RBC) in fish blood cells

RBC cells were counted using neubauer counting chamber is an apparatus used to estimate the total RBC blood cells.

Leukocyte Count

Leukocytes were counted by the method of reference no. 29 using haemocytometer. Counting is done with a microscopic under low power and knowing the volume of fluid examine and the dilution of the blood, the number of WBC per cubic millimeter in undiluted Whole blood is calculated.

Differential leukocyte count

The white blood cell differential count determines the number of each type of white blood cell, present in the blood. It can be expressed as a percentage (relative numbers of each type of WBC in relationship to the total WBC).

The dried and stained WBC film was examined without a coverslip under compound microscope. For different

leucocyte counts the area was choose where the morphology of the cells is clearly visible. Differential count was made by moving the slide in area including the central and peripheral of the smear. A total of 100 cells counted in which every white cell seen recorded in a table under the following heading: Neutrophil, Basophil, Eosinophil, Monocyte and Lymphocyte.

Estimation of Haemoglobin

Sahel's Haemometer (Haemoglobinometer) was used. The acid haematin method in which hemoglobin was converted into acid haematin by diluted hydrochloric acid and the brownish yellow colour was matched with the standard in the comparator.

Fatty acid analysis using Gas chromotography

To a known volume of lipid sample (0.1 ml), 1ml of saponification reagent was added and kept in screw-capped vial. The sample was mixed thoroughly with a vortex mixture and boiled for 30 minutes. To the sample 2 ml of methylation reagent was added, mixed thoroughly and allowed to boil in a water bath for 20 minutes at 80°C. After cooling to room temperature 1.25 ml of extraction solvent was added and shaken vigorously. The aqueous lower phase was discarded. 3 ml of base wash was added to the sample and mixed well for 5 minutes. The extracted methylated fatty acid (Organic upper phase) was added to the GC vial. Two micro liter of sample was injected and analyzed using Chemito8610 Gas chromatography, with flame ionization detector. The chromotogram was taken for calculation.

RESULTS

In the present study, C. carpio were fed with Mesocyclops thermocyclopoides enriched with C. longa. The experiment was conducted for 45 days, after the end of the experimental period the following parameters were analyzed and recorded.

Growth and Nutritional indices

The length, Weight gain, Specific growth rates, Food conversion ratio and specific growth rate were observed. The maximum length, weight gains were observed in C. carpio fed with group III experimental organisms (0.9 mg/l). Table 1 and Table 2.

Biochemical Analysis: The Biochemical composition was observed in all experimental fishes. The assay for the C. *carpio* fed with live feed Mesocyclops enriched with C*urcuma longa*. Protein content was very high in 30th day 0.3mg/L (46.61 ± 0.988µg/mg) 0.6mg/L (48.0667± 0.960) 0.9mg/L (48.4567± 0.661) and very low content in control (44.3197 ±1.248µg/mg) Table 3. Carbohydrate content was high in 30th day 0.3mg/L (14.5667±0.569µg/mg) 0.6mg/L (15.2667±0.551) 0.9mg/L(15.5633± 0.574) and very low content in control (13.297 ± 0.187µg/mg). Lipid content was very high in 30th day 0.3mg/L (7.62 ± 0.325 µg/mg) 0.6mg/L (8.67 ± 0.632µg/mg) 0.9mg/L (8.63 ± 0.393µg/mg) and less content level in control (7.96 ± 0.043µg/mg) Table 3.

Heamatological parameters

Red Blood Cells (RBC)

The RBC's on 15th day was found to be increase in 0.9mg/L to 2.88x106. At the end of the experiment, the highest numbers of RBC counts were observed in 0.9mg/L Table 4.

White Blood Cells (WBC)

The number of WBC cells was found to be increase in 0.9mg/L to 1.9 x104 of 15th day sample. At the end of the experiment, the lowest numbers of WBC counts were observed on 45th day 1.42x104 in 0.3 mg/L sample concentration Table 4.

Leukocyte differentiation

The differential leukocyte (%) counts in C. carpio were Neutrophils (%) in control- 51% Ex- 1: 44% Ex- 2: 41% Ex-3: 40%. Lymphocytes (%) in control 35% Ex 1: 32% Ex 2: 30% Ex 3: 30%. Monocyctes (%) in control 7% Ex 1: 3% Ex 2 :1% Ex 3 :2% . Esnophils (%) in control 3% Ex 1: 2% Ex 2 :1% Ex 3: 1% .basnophils (%) in control 1% Ex 1: 0% Ex 2: 1% Ex 3: 0% Table 5.

Haemoglobin

The haemoglobin content (% in 100cmm-3) was found to be high in 15th day of 0.3 mg/L concentration fed groups which is 7.09±1.62. The percentage of haemoglobin was found to be 7.1 ±1.52 in 0.3mg/L in 30th day. The percentage of haemoglobin level was found to be high (8.09 ±1.43) in 0.9mg/L fed groups on 45th day.

Fatty Acid Analysis Using Gas Chromotography

Fatty acids profile of C. carpio fed with Mesocyclops and Curcuma long were present in The level of different fatty acids were Table 6: DHA 4.6, EPA 11.5, Omega 3 fatty acids 17.4, Palmitic acid 6.4, Mystric acid 4, Stearic acid

Table 1: Morphometric data of *Curcuma longa* enriched *Mesocyclops thermocyclopoids* fed with *Cyprinus carpio* supplemented diets.

Diets (mg/l)	Length (cm)	LG (cm)	Weight (g)	WG (g)
Control	8.55±0.03^{d}	4.75±0.02^{d}	4.32±0.01^{d}	1.12±0.01^{d}
0.3	10.26±0.02^{c}	6.46±0.03^{c}	5.76±0.02^{c}	2.56±0.02^{c}
0.6	11.26±0.01^{b}	7.46±0.01^{b}	7.20±0.03^{b}	4.00±0.02^{b}
0.9	13.68±0.03^{a}	9.88±0.02^{a}	8.64±0.02^{a}	5.44±0.01^{a}

Each value is mean ± standard deviation of three individual observations. Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at P<0.05 (one-way ANOVA and subsequent post hoc multiple comparison with DMRT.
LG- Length Gain; WG -Weight Gain

Table 2: Nutritional indices of Curcuma longa enriched Mesocyclops thermocyclopoids fed with Cyprinus carpio supplemented diets.

Diets(mg/l)	SR (%)	SGR (%)	FCR (g)	PER (g)
Control	83.00±1.00^{d}	0.31±0.01^{d}	0.17±0.02^{a}	1.6±0.2^{c}
0.3	86.00±2.00^{c}	0.57±0.03^{c}	0.13±0.02^{b}	2.2±0.4^{b}
0.6	93.00±4.00^{b}	0.77±0.02^{b}	0.12±0.01^{c}	2.9±0.2^{a}
0.9	96.00±3.00^{a}	0.95±0.01^{a}	0.11±0.02^{d}	3.3±0.3^{a}

Each value is mean ± standard deviation of three individual observations.Initial length and weight were 3.80±0.10cm and 3.20±0.1respectively.Mean values within the same row sharing different alphabetical letter superscripts are statistically significant at P<0.05 (one-way ANOVA and subsequent post hoc multiple comparison with DMRT.
SR-Survival Rate; SGR- Specific Growth Rate; FCR- Food Conversion Ratio; PER- Protein Efficiency Ratio

Table 3: Estimation of Protein, Carbohydrate, Lipid in C. *carpio* fed with live feed Mesocyclops enriched with *Curcuma longa* (µg/mg).

Enrichments	Expt. groups	Protein	Carbohydrate	Lipid
Control	25.7033± 0.531	44.3197 ±1.248	13.297 ± 0.187	7.96 ± 0.043
0.3mg/L	27.8833±0.105	46.61 ± 0.988	14.5667±0.569	7.62 ± 0.325
0.6mg/L	28.3267±0.574	48.0667± 0.960	15.2667±0.551	8.67 ± 0.632
0.9mg/L	30.63 ± 0.539	48.4567± 0.661	15.5633± 0.574	8.63 ± 0.393

Table 4: Red blood cells 106 (RBC) and White blood cells104 mm-3 (WBC) counts in *Cyprinuscarpio* fish fed with different concentration of C. *longa* powder enriched with Cyclops.

Conc.	RBC (10^6 cmm^{-3}) count / days			WBC (10^4cmm^{-3}) count /days		
	15th	30th	45th	15th	30th	45th
Control	2.45 x10^6	2.41 x10^6	2.52 x10^6	1.074x10^4	1.0452x10^4	1.0761x1^4
0.3	2.66 x10^6	2.51 x10^6	2.73 x10^6	1.8372x10^4	1.8525x10^4	1.4258x1^4
0.6	2.82 x10^6	2.9 x10^6	2.83 x10^6	1.9031x10^4	1.9503x10^4	1.9943x1^4
0.9	2.88 x10^6	2.93 x10^6	2.82 x10^6	1.9906x10^4	1.9770x10^4	1.9211x1^4

Table 5: Differential leukocyte (%) count in C. *carpio* fed with different concentration of C.*longa* emulsion enriched Cyclopes.

S.NO	Control	Experiment 1	Experiment 2	Experiment 3
NEUTROPHILS	51	44	41	40
LYMPHOCYTES	35	32	30	30
MONOCYTES	7	3	1	2
ESNOPHILS	3	2	1	1
BASNOPHILS	1	0	1	0

Table 6: Fatty acids profile of *Cyprinus carpio* fed with live feed *Mesocyclops thermocyclopoidae* enriched with *Curcuma longa*.

Fatty acids	Control	0.3mg/L	0.6mg/L	0.9mg/L	Total fatty acids
DHA	2.3	-	1.2	1.1	4.6
EPA	7.2	-	4.3	-	11.5
Omega 3 fatty acids	4.4	4.4	4.3	4.3	17.4
Palmitic acids	1.4	3.6	1.4	-	6.4
Mystric acids	0.5	3.5	-	-	4
Stearic acids	6.0	4.5	4.5	5.5	20.5
Lauric acids	6.8	4.7	5.3	2.8	19.6
Behanic acids	16.9	3.7	5.5	-	26.1
Linolenic acids	6.5	-	17.8	4.3	28.6
Eiocosenoic acid	4.0	-	-	-	4.0
Oleic acids	-	31.0	5.8	25.5	62.3
Arachidic acid	-	8.0	-	6.1	14.1
Linoleic acid	-	-	17.8	18.8	36.6
Myristric acid	-	-	-	0.5	0.5
Lingnoceric acid	-	0.4	-	-	0.4

Plate 1

Plate 2(a)

Plate 2(b)

Plate 1: Experimental fish *Cyprinus carpio*

Plate 2: Collection sites of C. *carpio* and M. *Thermocyclopoides*; (a) Bhavani sagar dam, (b) Muthannan lake

Plate 3: C. *longa* enriched *M. Thermocyclopoides*

Plate 4: C. *longa* raw material and powder

20.5, Lauric acid 19.6, Behanic acid 26.1, Linoleic acid 28.6, Eiocosenoic acid 4.0, Oleic acid 62.3, Arachidic acid 14.1, Linoleic acid 36.6, Myristric acid 0.5, Lingnoceric acid 0.4% of total fatty acids, respectively (Table 6).

DISCUSSION

The source of the turmeric plant and the extraction process are key factors that determine the curcuminoid content. The amount of curcuminoids indicated the degree of bactericidal activity and immuno-stimulant effects in the tested animals.[30] As per report the production of disease-resistant fry of Indian major carp, Catla catla by spawn treatment with turmeric, neem leaves and garlic powder. There have been very few studies on turmeric with regard to enhancing immunity in invertebrates[31]. In this present study, concentrated turmeric powder enrichment with M. thermocyclopoides showed considerable increased in length and weight of C. *carpio*. Different concentration of enriched M. thermocyclopoides were fed with different groups (EXP-I 0.3g/l, EXP-II 0.6g/l, EXP-III 0.9 g/l and control as without enrichment of Cyclops) of fish larva. Similarly, nutritional values are significantly increased in different concentration and it was concentration at 0.6g/L increased 93% in SR values compared to control 83% without enriched turmeric concentration with M. thermocyclopoids fed on C. *carpio*.

Medicinal plants have been used as antibiotic and chemical alternatives. For example,[32] has shown that rainbow trout fed with diets containing aqueous extracts of mistletoe (Viscum album), nettle (Urtica dioica), and ginger (Zingiber officinale) exhibited significant non-specific immune responses. Since all medicinal plants are able to stimulate only nonspecific immune responses, vaccines might be a better way to prevent the deadly diseases. These plants could be used as additive though in order to create more effective for immunity in fish growth and development.

Prior to understanding that innate immune response of copepods, several assumptions are necessary on the relationship between copepods and detrimental or pathogenic bacteria. First, many pathogenic and nonpathogenic bacteria are known to be strongly associated with copepods that are likely microorganism mediators as its trophic position in marine food chains is prominent. The Muthannan lake provides an example of zooplankton composition and species-level differences that may influence vibrio dynamics. This species occur commonly in estuaries throughout i.e., India, Bangladesh, Southeast Asia, and Peru.

Differences in WBC cell counts, and increased granulocytes coupled with reduced monocyte numbers in blood circulation have been observed in C. carpio-treated carp after 30 days of feeding. These differential effects on leukocyte populations lead to effects on cellular immune responses. Immuno-stimulants have the competence to promote the nonspecific resistance of fish before infection of pathogens. In recent decades, many substances have been shown to

enhance the non specific immunity of fish and the route of their administration has differential effects on the immune system.

Erythrocytes are reliable indicator of stress and RBCs transport Heamoglobin that in turn transports O_2, and the amount of O_2 received by tissues depends on the maturity of RBCs and Amount of Hb[33,34,35]. Thus highly significant increase in RBC and Hb levels in 0.9g/L diet fed fishes is a response to tolerate stress or on the other hand is a measure to maintain general health. Leucocytic Parameters Highly significant increase in total leucocyte counts were observed in both 0.6 and 0.9g/L diet fed fishes over 0.3 g/L diet fed fishes. In Differential leucocyte counts, in all the three experimental groups it was observed that the Lymphocyte counts were higher followed by Neutrophil, Monocyte, Eosinophil and Basophil. The Lymphocytes, Neutrophils and Monocytes exhibited an increasing trend in both 0.6 and 0.9g/L diet fed fishes; however the lymphocyte increase was significant in both 0.6 & 0.9g/L diet fed fishes and monocyte increase was significant only in the 0.9g/L diet fed fishes, whereas insignificant increase was observed in neutrophil counts when compared to infected fishes fed with 0.3g/L diet. On the other hand Eosinophil and Basophil counts exhibited highly significant decrease in both 0.6 & 0.9g/L diet fed fishes than their control counterpart. Similarly, Also observed increase in WBC in common carp fed with feed incorporated with plants like *Inula helenium, Tussilago farfaro, Brassica nigra, Echinacea purpurea & Chelidoniume majus* and infected with *Aeromonas hydrophila*.[36]. Reported that the WBC & RBC counts were higher in *Labeo rohita* fingerlings fed with *Magnifera indica* kernel.

The increase in serum protein content might be in part due to an increase in the WBC, which is a major source of serum protein production such as lysozyme, complement factors and bactericidal peptides[37] and [38] reported that serum proteins include various humoral elements of the non-specific immune system and increase in serum total protein, globulin and albumin are likely to be a result of the enhancement of the non-specific immune response of fishes. Serum albumin not only maintains osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues but also acts as plasma carrier protein to transport steroid hormones, hemin, fatty acids and also compounds like drugs [39]. In the present study increased in total protein levels in 0.6 and 0.9g/L diet fed fishes in the experiment may be an indication to increased levels of non-specific immunity and may facilitate the transport of more humoral compounds in the blood.

Fish feeding with the diet containing copepod blend carotenoids food at a higher level resulted in increased serum bactericidal activity. Increase in serum bactericidal activity is indicative of the involvement of various humoral factors in innate and/or adaptive immunities for effective protection of the host from infection. Serum bactericidal activity is due to other humoral responses such as respiratory burst activity and serum lysozyme activity and is one of the key killing mechanisms of clearing bacteria in teleost [40].

Immune response in fish [41] reported that incorporation of carotenoid containing vegetable powder from tomato and sweet pepper in the diets of rainbow trout improved growth performance and immune system[42] .Suggested that b-carotene can be used a immunostimulant to alleviate the suppression effect resulted from immune depressive stressful condition in farmed Nile tilapia. Hence, the present study concluded that turmeric containing flovonoid, carotenoid can effectively be used as immunostimulants in aquaculture of carps and in addition with copepod feed enhanced the fish growth and totally thus combination boost the various immune defense parameters and also provided protection against pathogen.

CONCLUSION

Copepods play very important and diverse roles in freshwater aquaculture operations. Copepods that serve as food for small fish. Thus turmeric powder usually having antiseptic, antifungal and antibacterial activity. Hence, in this present study C. *carpio* (fish) fed on copepods enriched turmeric concentration may potential enhancer for the growth and development of fish and protect for diseases.

Conflict of interest: No conflict of interest.

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