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Formulation of Fish Feed Using Medicinal Herb *Curcuma Amada* and Its Biochemical and Haematological Changes in *Labeo Rohita*

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ABSTRACT

A characteristic feature of fish is the wide physiological range of blood parameters and also the large individual variations. The aim of this study was to evaluate the haematological profile and biochemical profile of fish *Labeo rohita*. The blood parameters viz., total WBC and RBC count, Hb, MCV, MCH and MCHC values were analyzed using standard methods. The differences found in this study can be attributed to the feeding behaviour, life style and adaptation of the different fish species to the habitat in which they dwell. *Curcuma amada* Roxb is commonly known as mango ginger. It is a perennial, rhizomatous, aromatic herb belonging to the family Zingiberaceae. The ranges of serum biochemistry vary from species to species and can be influenced by many biotic and abiotic factors such as water temperature, seasonal pattern, food, age and sex of the fish. The present study deals with the use of medicinal plant *Curcuma amada* in fish diet to study the biochemical and haematological profile on *Labeo rohita* a common fish. The plant was extracted and processed for decoction method. The extract obtained was then mixed with 0.5 kg salt (NaCl) and 1.0 kg fish feed powder in the ratio 10:1. Required amount of sterilized water was mixed to make it paste. Haematological and biochemical studies were done after administering the extracts for 30days. Based on the results it is appropriate to conclude that the plant extract of *Curcuma amada* incorporated in fish diet may improve the biochemical and haematological profile in blood of fish *Labeo rohita*. It was observed that the herbal diet fed fishes exhibited significant increase in RBC, WBC, serum protein and globulin and also maintained hepatics disorder enzymes (ALT, AST, ALP) which may be considered as a sign of improvement in health status. It may be due to the presence active components i.e. carbohydrates, proteins, glycosides, alkaloids, flavanoids). Providing quality fish feed became a prime aim of every aquaculturist. Though fish feed devours around 50% of the production cost, yet it plays the pivotal role in the production and yield outcome.

Keywords: *Labeo rohita, Curcuma amada* , Zingiberaceae, Haematological, Biochemical

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INTRODUCTION

Increases in human populations have trigger production of freshwater fish for human consumption hence creates demand for high quality fish feeds. Feeds expense is the major variable operating cost during production of fish[1]; accordingly, considerable activity has been utilized to focus on quantity and nature of feed stuffs to accomplish optimum performance of fish. The demand for fish meal is not just increasing but the supply of it is also dwindling with stagnating marine catches and alternative use of it for livestock and human consumption[2]. So, the quest for possible alternative protein sources to replace (complete/partial) fish meal in the feed became paramount[3]. As animal protein sources are mostly expensive and not easily available, plant sources are considered to be one possible alternative that can be used in fish feed without compromising the nutritional quality of the feed[4]. Moreover, use of cheaper and locally available plant sources to substitute the expensive fish meals would mean reduction in the production cost and thereby enhance the profit[5]. *Curcuma amada* is a unique spice having morphological resemblance with ginger (Zingiber officinale) but imparts a raw mango (Mangifera indica) flavour. The genus name Curcuma was coined by Linnaeus in 1753 in his Species Plantarum. The word probably derives from the Arabic word 'kurkum', which means yellow colour[6,7]. *Curcuma amada* Roxb. is commonly known as mango ginger. It is a perennial, rhizomatous, aromatic herb belonging to the family Zingiberaceae. This family is composed of 70–80 species of rhizomatous annualor perennial herbs[8]. The genus originated in the Indo-Malayan region, and is widely distributed in the tropics of Asia to Africa and Australia[9]. At the rhizome nodes scaly leaves are arranged circularly

giving the appearance of growth rings with scars on the surface. The rhizomes are branched, and the branching is sympodial. The rhizomes emulate a raw mango flavour and taste pungent. The geographical distribution of this genus ranges from India to Thailand, Indo-China, Malaysia, Indonesia and northern Australia. *C. amada* is found the wild in parts of West Bengal, and is cultivated in Gujarat, Uttar Pradesh, Kerala, Karnataka, Tamil Nadu and the north-eastern states. The rhizome is rich in essential oils, and more than 130 chemical constituents with biomedical significance have been isolated from it [10]. Ayurveda and Unani medicinal systems have given much importance to mango ginger as an appetizer, alexteric, antipyretic, aphrodisiac, diuretic, emollient, expectorant and laxative and to cure biliousness, itching, skin diseases, bronchitis, asthma, hiccough and inflammation due to injuries[11]. Fish is highly nutritive and rich source of animal proteins. For the improvement of fisheries and to achieve maximum yields from resources of fresh water, it is necessary to provide artificial feed, by which fish grows rapidly and attains maximum weight in shortest possible time. Among commonly used feed ingredients, fish meal is considered to be the best ingredients, due to its compatibility with the protein requirement of fish[12]. Replacement of fish meal with cheaper ingredients of plant origin in fish feed is necessary because of rising cost and uncertain availability of fish meal. Inclusion of plant rich components with high level of carbohydrates, alkaloids, flavanoids, saponins etc. in formulation of fish feed is preferred as it is cost effective. According to *Rumsey* (1993) [13], increased use of plant protein supplements in fish feed can reduce the cost of fish meal. The research has focused on utilizing less expensive and readily available resources to replace fish meal, without reducing the nutritional quality of feed[14].

MATERIALS AND METHODS

Collection of fish

The live specimens of fresh water collection of fish *Labeo rohita* will be collected from local fisherman of Bhopal and acclimated for 7 days in lab conditions. Live fishes (irrespective of sex and almost medium size group) were taken and brought to the laboratory. Fishes would acclimatize into dechlorinated water for 30 days and treated with 0.01% of potassium permagnate ($KMnO_4$) solution to avoid dermal infection. They were fed dried pellet feed every day and water was renewed an alternate days, leaving no fecal matter, unconsumed food or dead fish in aquaria. Water in all aquaria replaced by freshwater at every 24h.The fishes were examined for any bruise, lesions or external infections.

Preparation of fish feed containing herbs

The feed containing herbs for therapy was prepared according to the method described by [15] with minor modification. 2 kg powder of herb *Curcuma amada* was extracted and processed for decoction method. The extract obtained was then mixed with 0.5 kg salt (NaCl) and 1.0 kg SABINCO fish feed powder in the ratio 10:1. Required amount of sterilized water was mixed to make it dough. Then pellet feed was prepared from this dough using feed preparation hand machine and air dried at room temperature. The dried fish feeds were stored in airtight container.

Experimental treatments

Fish would divide into 4 groups of 12 fishes each and would be kept in glass aquaria; each containing 20 liters stored dechlorinated tap water.

In the present study, Group 1 was treated with standard pellet feed and Group 2 was treated with herbal extract *Curcuma amada* along with standard pellet feed.

Haematological study[16]

Blood samples were collected on 30th day. During sampling, care was taken to minimize stress in the netted fish and fish remaining in the tank. All fishes were netted within 20 seconds. Blood samples were collected from the caudal tail vessels with 21 or 23 gauze needles and 1 or 3 cc syringe before ventilator response was noticeably depressed. To prevent repeated sampling, fish were not returned to the same tanks after blood collection.

Biochemical study[17]

Blood was collected as described for biochemical analysis in dry and sterilized centrifuge tubes by cutting the caudal peduncle of living fish with a sharp sterilized dry knife. Before cutting the caudal peduncle the fish was blotted with blotting paper to avoid haemolysis. The blood was allowed to clot for 10 minutes and centrifuged at 3000 rpm for 20 minutes. The serum was carefully taken out with the help of pipette and stored at 4^0C. All the tests were performed within 72 hours.

RESULTS

Blood biochemical parameters with significant variations were observed for the concentrations of glucose, protein, cholesterol and urea. The ranges of serum biochemistry vary from species to species and can be influenced by many biotic and abiotic factors such as water temperature, seasonal pattern, food, age and sex of the fish. An increased blood glucose and protein level was recorded in fishes; this may probably be due to an increased depletion of liver glycogen[18]. The significant variations were observed in biochemical markers were shown in (Figure 1-3) Knowledge of the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology. The analysis of blood indices has proven to be a valuable approach for analysing the health status of farmed animals as these indices provides reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting. Blood biochemistry parameters can be also used to detect the health of fish[19]. Blood glucose level may vary according to season and water temperature, and glucose level in fish decreased with age and size[20]. Also, changes in blood glucose have been suggested as useful general indicator of stress[21] reported that blood glucose appeared to be a sensitive indicator of environmental stress in fish. The changes observed in *Labeo rohita* after administration of *Curcuma amada* in their feed was shown in Figure 4

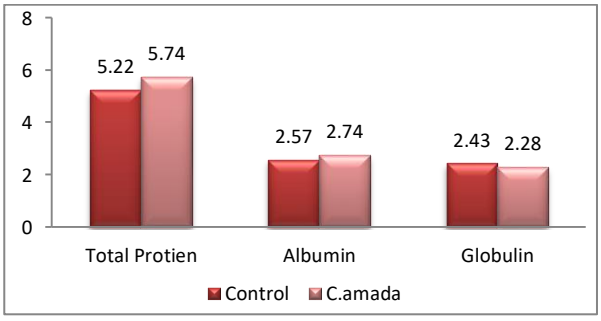

Figure: 1 Variation in Total protein, albumin and globulin in control and after treatment with *C. amada*

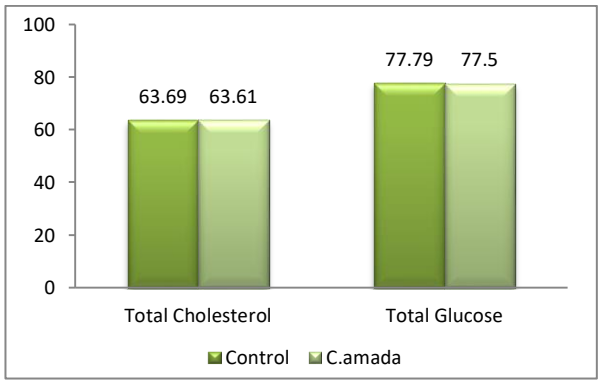

Figure: 2 Variation in Total cholesterol and total glucose in control and after treatment with *C. amada*

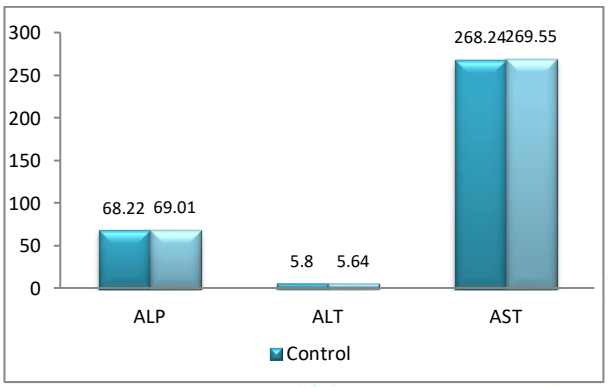

Figure: 3 Variation in Liver enzymes assay in control and after treatment with *C. amada*

The high erythrocyte number was associated with fast movement, predaceous nature and high activity, with streamlined body (Rambhaskar and Srinivasa Rao 1986). However, significant differences in the values of erythrocyte volumes between the groups were observed in this study, suggesting that in the intensive estuary environments, the elevated RBC counts and HB concentration are a response to the higher metabolic demand and have no impact on erythrocyte volume. The increased number of RBC indicates oxygen demand in the tropical region to meet the higher oxygen requirement at higher metabolic rates (Engel and Davis 1964).

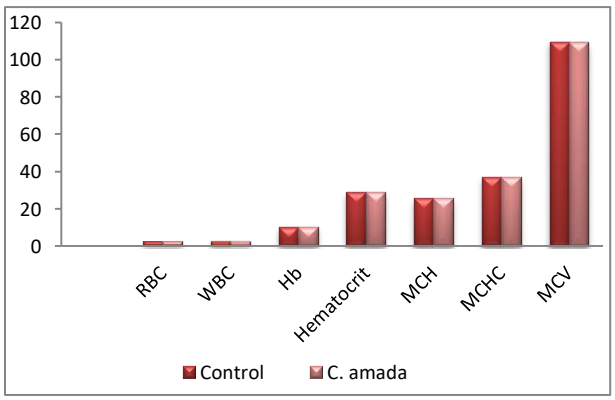

Figure 4: Blood serum biochemical parameters of *Labeo rohita*

DISCUSSION

The haematological and biochemical characteristics of some fish species have been investigated with the aim of establishing normal blood values and ranges with respect to sex, age, size and environmental and physiological conditions[22-24]. Also, comparative studies on blood parameters of fish have been carried out to determine the systematic relationship among certain species. Carnivorous fish species show an impaired ability to clear excesses in blood glucose levels[25] and therefore have been traditionally considered as relatively glucose-intolerant species[26]. However, plasma glucose levels fluctuate[27]and glucose is also essential for brain function[28] suggesting the existence of a gluco-sensing system in fish. In support of this hypothesis, our results showed levels of glucose to be highest in carnivorous fish and lowest in mullet (herbivore). Blood lactate concentrations obtained for the four species show that this parameter was higher in more active fish, such as sea bass and mullet, compared to less active species. High-speed movements require large increases in muscular activity; therefore, when this exceeds the ability of the circulatory system to transport oxygen to the active tissues, anaerobic metabolism supplements the aerobic metabolism [29,30] The capacity for anaerobic energy production has been estimated from decreases in substrate reserves and product accumulation with the conversion of glucose to lactate followed by stimulation of pyruvate metabolism and oxygen debt[31, 32] . *Panigrahi and Misra, (1978)* observed reductions in haemoglobin percentage and red blood cell (RBC) count of the fish Anabas scandens treated with mercury[33]. Decrease in haemoglobin, RBC count and Hct was observed in fish *Tinca tinca* exposed to mercuric chloride and lead[34]. White blood cells play a major role in the defence mechanism of the fish and consist of granulocytes, monocytes, lymphocytes and thrombocytes. Granulocytes and monocytes function as phagocytes to salvage debris from injured tissue and lymphocytes produce antibodies[35,36]. Elevations in serum total and conjugated bilirubin are attributable to liver and/or biliary tract disease[37] while elevations in ALT and AST are usually due to disruption of hepatic cells as a result of necrosis or altered membrane permeability and elevations in ALP is often elevated due to cholestasis [38].

CONCLUSIONS

Fish meal is one of the most expensive ingredients in prepared fish diets. Fish nutritionists have tried to use less expensive plant protein sources to partially or totally replace fish meal. Of all the plant protein feedstuff, inclusion of *curcuma amada* extract is considered to be the most nutritious and is consist of phytochemicals which play a major role in fish diets. Determination of palatability of a feed ingredient is an important criterion in the evaluation of that ingredient for fish. The growth of fish depends upon the ingredients and its percentage in the formulated feed. The digestibility of a particular feed ingredient reflects in growth of fish. Digestibility depends upon various factors like nature, dietary component, and type of nutrient and level of inclusion. The present study revealed that plants have an important role when use mixing with fish feed to control health issue in edible fishes. The choice of proper nutrient diet for fish using herbs improves the quality of diet as well as improving the growth rate.

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