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Effect of mangosteen (*Garcinia mangostana* L.) extracts as a feed additive on growth and hematological parameters of African catfish (*Clarias gariepinus*) fingerlings

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Abstract. – *Aim:* **The efficacy of dietary inclusion of various parts of mangosteen (*Garcinia mangostana* L.) extract on growth and hematological parameters of African catfish (*Clarias gariepinus*) fingerlings were investigated.**

Material and Methods: **Ninety six fingerlings were randomly distributed into each of ten 20L plastic aquaria. Each treatment groups consists of three replicates. Four experimental diets were prepared: controls, leaf extract, shoot extract and rind extract. The 0.5% of various parts (leaf, rind and shoot extract) of mangosteen were included in the experimental diets at the expense of 0.5% rice bran. The fingerlings were fed with experimental diets at satiation for five weeks.**

Results: **The results showed that no significant difference on growth, specific growth rate, average daily growth, feed conversion ratio and condition factors of the dietary inclusion of the different mangosteen extract treatment when compared to control groups except leaf extract. Significantly higher red blood cells (RBC) count and white blood cells (WBC) count were recorded in fish fed with shoot extract incorporated diet fed groups than the other groups. However, no significant impact on hemoglobin content between the experimental groups and control groups.**

Conclusions: **Based on the results of the present study, it is suggested that feeding fish with 0.5 % of mangosteen extracts for 35 days has no adverse effect on growth and enhanced the hematological parameters of African catfish fingerlings.**

Key Words:

Mangosteen extract, African catfish (*Clarias gariepinus*), Hematological.

Introduction

Aquaculture fish production has been increased significantly over the past few decades which has led to intensive fish culture practices where stressors like over crowding, transport, handling, size grading and poor water quality are common problems[1]. It has been widely demonstrated that farmed fish are more susceptible to various pathogenic microbes in intensive farming. In order to improve health conditions in the rearing of aquatic organisms, several alternatives such as improved husbandry, nutrition, and water quality; optimal stocking density; and use of vaccines, probiotics[2] and immunostimulants[3] have been proposed. The enhancement of the immune system of fish is considered the most promising method of preventing fish diseases in aquaculture. This enhancement can be achieved with application of vaccines, which enhance the specific immune response of the fish and are considered to be the most effective agents. Further the use of antibiotics and chemotherapeutics to combat fish diseases has several drawbacks including the risk of generating resistant pathogens, bioaccumulation and environmental pollution. The available commercial vaccines are expensive for fish farmers and are specific against particular pathogens[4]. In contrast to vaccines, immunostimulants enhance the non-specific immune response of fish[5,3]. The major components of the innate immune system are macrophages, monocytes, granulocytes and humoral elements, like lysozyme[6,7]. Several biological and synthetic compounds have been shown to enhance non specific immune system of cultivated fish[3,8-16].

Mangosteen, (*Garcinia mangostana* L.) belongs to the family Guttiferae, is one of the most widely recognized tropical fruits and has universal appeal because of its quality in colour, shape and flavour. Mangosteen is known as the "Queen of fruits" and is widely distributed throughout

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India, Myanmar, Malaysia, Philippines, Sri Lanka, and Thailand[17]. The pericarp (peel, rind, and hull) and the ripe fruit of mangosteen have been used as a traditional medicine for the treatment of abdominal pain, diarrhea, dysentery, wound infection, suppuration, and chronic ulcer[18]. Xanthones, terpenoids and sugars have been reported from the different parts (pericarp, whole fruit, bark, and leaves) of mangosteen and some of them have shown a variety of biological activities[17-19]. Further it also possesses several pharmacological properties including antitumor[20], antibacterial[21,22], antifungal[23], anti-inflammatory[24], antioxidant[25-26], antiplasmodial[27], and cytotoxic activities[18,28,]. Immunostimulating activity of mangosteen is not yet been explored. Hence the present study was aimed to evaluate the effects of dietary inclusion of various parts of mangosteen extract on growth response and hematological parameters of African catfish (*Clarias gariepinus*) fingerlings.

Materials and Methods

Experimental Fish and Maintenance

Fingerlings of African catfish (average weight 2.0± 0.2 g) were obtained from a private fish farm, Sungai Petani, Kedah Darul Aman, Malaysia. The fish were transported in a polythene bag with sufficient aeration to the AIMST University animal house. They were carefully transferred to circular cement tanks (400 L) and left undisturbed overnight. The fish was acclimatized under aerated conditions for a period of 10 days at 28 ± 1.5°C. Fish were fed with a formulated catfish pelleted diet at *ad libitum* twice daily during the acclimatization period.

Preparation of Plant Extract and Fish Feed

The plant material *Garcinia mangostana* leaves, shoots and fruit rinds were collected from Kuala Ketil farm, Sungai Petani, Malaysia. Different parts were washed thoroughly with distilled water and dried separately under the sunlight and ground into powder. Fifty g each respective source of powdered plant material was soaked in 250 ml of methanol for 24hrs. The slurry obtained was left in a clean, sterile glass container and shaken vigorously to allow for proper extraction. After filtering of the extract through Whatman No. 1 paper, the residue was re-extracted and then filtered. Filtrates were combined and concentrated using a rotary evaporator at 40ºC. Four experimental diets were prepared: control, leaves, shoot and rind. Percentage inclusions of ingredients of the experimental diets are given in Table I. Fish meal, dehulled soybean were used as the protein sources. Wheat flour and fish oil was used as the carbohydrate and lipid sources, respectively. The 0.5% of various sources (leaves, shoot and rind) of mangosteen were included in the experimental diets at the expense of 0.5% rice bran. The mangosteen free feed was used as a control diet. The dry ingredients were mixed thoroughly with water for 10 minutes. The resulting dough was pelleted, dried at room temperature for 48 hours and then stored in airtight containers until fed. All experimental diets satisfied the nutrient requirements for growth of African catfish fingerlings[29].

Table I. The composition of ingredients (for 100 g feed) in basal and experimental diets with desired crude protein and lipid.

Ingredients	Control (T1)	Leaf extract (T2)	Shoot extract (T3)	Rind extract (T4)
Fish meal	45.7	45.7	45.7	45.7
Soyabean meal	16.6	16.6	16.6	16.6
Rice bran	11.5	11.0	11.0	11.0
CMC binder	2.0	2.0	2.0	2.0
Wheat flour	21.0	21.0	21.0	21.0
Vitamin mix	2.0	2.0	2.0	2.0
Fish oil	1.2	1.2	1.2	1.2
Mangosteen extract	–	0.5	0.5	0.5
Total	100	100	100	100

Composition of protein: 35.3%; composition of lipid: 7.6%.

Experimental Design and Feeding Trail

Ninety six African catfish fingerlings were randomly divided into four groups (T1, T2, T3 and T4). Each group of 24 fingerlings was again divided into three equal subgroups. The fish were then introduced into twelve 20L capacity circular plastic troughs. Each aquarium was supplied with aeration by aquarium aerators. Group T1 was fed with basal diet and treated as the control. The remaining groups were fed with dietary inclusion of leaf extract (Group T2), shoot extract (Group T3) and rind extract (Group T4) for 35 days. The fish were fed with twice daily at 09.00 hours and 17.00 hours. Mean weight gain (MWG), specific growth rate (SGR), average daily growth rate (ADG) and feed conversion ratio (FCR) were estimated for both control and experimental groups. The following formula was used to calculate the growth parameters.

MWG = (Mean final weight – Mean initial weight);

SGR (%/day) = $100 *[(\ln W_1 - \ln W_0)/t]$, where W_0 and W_1 are average initial and final body weights, respectively, and t is time (days);

ADG (g/day) = Growth/Experimental duration

FCR = Food consumed (g)/Weight gain (g);

CF (g/cm^3) = Weight (g)/[Length (cm)]^3.

Water (50%) was renewed daily and water quality was monitored throughout the experimental days at weekly intervals. Temperature was 28.7 ± 0.05°C, pH 6.6 ± 0.05, dissolved oxygen concentration 4.76 ± 0.59 mg/L. After 35 days of post feeding, blood samples were collected from fish in each subgroup and examined for the following hematological parameters: WBC, RBC and hemoglobin.

Collection of Blood and Determination of Hematological Parameters

After 35 days of post feeding, the feed was withheld for 24 hours prior to collection of blood from fish. Five fish from each subgroup were randomly selected for blood collection. Heparin was used as an anticoagulant. Blood was collected from the caudal vein with a 1 ml plastic syringe ringed with heparin and stored at 4ºC. The blood was then transferred immediately to a test tube containing heparin solution and shaken gently. The blood was used for determination of RBC, WBC count and hemoglobin. Total erythrocyte count (RBC) was performed following the method of Hendricks[30] using a haemocytometer where a total leukocyte count (WBC) was determined following the method of Shaw[31]. The hemoglobin content was determined by using Sahli's method as described by Schalm et al[32].

Statistical Analysis

The effects of the different sources of mangosteen extract as feed additive on growth, hematological parameters were analysed using one-way analysis of variance (ANOVA) and significant differences among treatment means were compared using Duncan's multiple range test (DMRT) using SPSS version 11 (Duncan, 1955). Significance was tested at 5% level.

Results

Growth Performance of African Catfish Fingerlings

The growth response (weight gain, average daily growth rate and specific growth rate) feed conversion ratio of African catfish, *Clarias gariepinus* fingerlings fed with experimental diets containing various sources of mangosteen for 5 weeks are presented in Table II. No mortalities were noticed in the mangosteen sources incorporated diet fed groups and control groups throughout the experimental period. The best weight gain (9.97 ± 1.21) of African catfish fed with dietary inclusion of shoot extract was significantly ($P<0.05$) higher than that of fish fed with diet containing leaf extract but was not significantly ($P>0.05$) different from that fish fed with control diet and diet containing rind extract (Table II). The dietary inclusion of mangosteen extract had no significant ($P>0.05$) impact on specific growth rate of *Clarias gariepinus* fingerlings among the mangosteen extract fed groups and control group. The best FCR (1.16 ± 0.11) was observed in fish fed with diet containing shoot extract. No significant difference ($P>0.05$) was observed between catfish fed with dietary inclusion of shoot extract, rind extract and the control group (Table II). The highest ADG was observed in fish fed with diet containing shoot extract when compared to other treatments except leaf extract incorporated diet fed groups. No significant difference was noticed for initial condition factors of finger-

Table II. Growth performance and feed utilization of fingerlings of African catfish, *Clarias gariepinus* fed experimental diets containing various sources of mangosteen extract for 5 weeks.

Treatments	Control (T1)	Leaf extract (T2)	Shoot extract (T3)	Rind extract (T4)
Initial weight (g)	3.23 ± 0.06^{a}	2.97 ± 0.12^{a}	3.00 ± 0.10^{a}	3.10 ± 0.20^{a}
Final weight (g)	12.03 ± 1.50^{a}	10.53 ± 0.15^{b}	12.97 ± 1.14^{a}	12.67 ± 1.16^{a}
Weight gain (g)	9.77 ± 1.45^{ab}	7.60 ± 0.17^{b}	9.97 ± 1.21^{a}	9.57 ± 1.17^{ab}
SGR (%/day)	3.96 ± 0.31^{a}	3.62 ± 0.09^{a}	4.17 ± 0.33^{a}	4.03 ± 0.31^{a}
ADG (g/day)	0.28 ± 0.04^{a}	0.21 ± 0.01^{b}	0.28 ± 0.04^{a}	0.27 ± 0.03^{a}
Initial CF (g/cm^3)	0.48 ± 0.05^{a}	0.44 ± 0.03^{a}	0.46 ± 0.01^{a}	0.47 ± 0.01^{a}
Final CF (g/cm^3)	1.10 ± 0.10^{a}	0.96 ± 0.04^{a}	1.08 ± 0.07^{a}	1.06 ± 0.11^{a}
FCR	1.19 ± 0.08^{a}	1.42 ± 0.12^{b}	1.16 ± 0.11^{a}	1.26 ± 0.04^{a}

Values are means±SD of three replicates. Values within column with different superscript are significantly different at $P<0.05$.

lings in all the dietary treatments used in the present study. After 35 days of feeding trial, the increased condition factor was noticed in different sources of mangosteen fed groups and control group than initial condition factor of fish. However no significant difference ($P>0.05$) was noticed among the dietary treatments after 35 days of feeding (Table II).

Hematological Parameters

The hematological parameters (RBC, WBC and hemoglobin) of fish fed with various sources of mangosteen extract and control groups are shown in Table III. The higher red blood cells count (12.07 ± 0.55) was recorded in shoot extract fed groups followed by (9.91 ± 1.65) in rind extract and (6.90 ± 1.04) in leaf extract fed groups. Significantly elevated RBC count was noticed in all the mangosteen extract fed groups than control group ($P<0.05$). Significantly highest white blood cell count (9.72 ± 0.11) was recorded in shoot extract fed fish and low WBC (3.40 ± 0.06) was noticed in control diet fed fish. Similar to RBC significantly elevated WBC count was noticed in all the mangosteen extract fed groups than control group ($P<0.05$). None significant impact ($P>0.05$) on hemoglobin content was observed in all the dietary treatment groups.

Discussion

The use of natural immunostimulants in aquaculture has been increasing rapidly for the prevention of diseases and also to avoid the indiscriminate use of hazardous antibiotics[3,33]. Plant based immunostimulants are biocompatible, biodegradable and safe for the environment and human health. Hence the present study investigated the effect of different parts of mangosteen extract as an immunostimulant in African catfish fingerlings. Several plants and natural compounds were tested for their growth promoting activities in aquatic animals[34-36]. To best of our knowledge, no work has been reported using mangosteen extract as feed additive and immunostimulatory substance in aquaculture. Survival of the fish was not signifi-

Table III. Effect of mangosteen extracts as feed additive on red blood cell (RBC) count, white blood cell (WBC) count and hemoglobin (Hb) content of African catfish fingerlings.

Treatments	RBC (million/µl)	WBC (million/µl)	Haemoglobin (grams %)
Control	3.65 ± 0.65^{d}	3.40 ± 0.06^{d}	3.19 ± 0.65^{a}
Leaf extract	6.90 ± 1.04^{c}	5.91 ± 0.17^{c}	3.07 ± 0.76^{a}
Shoot extract	12.07 ± 0.55^{a}	9.72 ± 0.11^{a}	3.77 ± 0.58^{a}
Rind extract	9.91 ± 1.65^{b}	9.23 ± 0.63^{b}	4.00 ± 1.20^{a}

Values are (means of triplicates ± SD) in the same column sharing a common superscript are not significantly different ($P<0.05$).

cantly (P>0.05) affected by the experimental diets. Similarly no difference was noticed with regard to SGR of fish fed with experimental diets. Sahu et al[37] reported that the dietary intake of mango kernel had no significant (P>0.05) impact on specific growth rate of *Labeo rohita juveniles* within the treated groups and control group. However, weight gain and FCR of African catfish fed with experimental diet containing shoot and rind extract of mangosteen and control diets were higher than those of fish fed with leaf extract. This results partially agreeing with the study results of Lin et al[38] which showed that the body weight of rats fed a diet containing 2.5% green tea leaves was decreased 10-18% compared to that of rats fed the control diet without supplementation of green tea for 27 and 63 weeks. The dietary inclusion of mangosteen extract had no much impact on growth response, survival and feed conversion efficiency of African catfish fingerlings compared to the control group. Cho et al[39] found that the weight gain of the juvenile olive flounder, *Paralichthys olivaceus* fed the control diet or the experimental diet containing green tea extracts had no significant difference (P>0.05). Unlike this study, however, dietary inclusion of oriental and Chinese herbs or algae improved the growth performance of fish[40-46]. Dietary inclusion of the byproduct of *Bupleurum falcatum* improved the growth of broilers[47]. In fisheries science, the condition factor is used in order to compare the "condition" and "fatness" or well being of fish. It has been hypothesized that heavier fish of a particular length are in a better physiological condition[48]. Condition factor is also a useful index for the monitoring of feeding intensity, age, and growth rates in fish[49]. It is strongly influenced by both biotic and abiotic environmental conditions and can be used as an index to assess the status of the aquatic ecosystem in which fish live. In the present study increased condition factor was noticed in all the dietary treatment groups when compared to initial condition factor of fish. Condition factor of African catfish fed the diet containing extracts of mangosteen and control diet might have provided optimal nutrients for fish and also fish were fed with satiation twice daily and probably produced heavier fish than the initial fish.

The erythrocyte count was higher in all the sources of mangosteen extract fed groups when compared to control group. The erythrocyte count increased with the administration of mangosteen, which might indicate an immunostimulant effect[50]. This study results is similar to the observation of Sahu et al[37], wherein the dietary intake of mango kernel by *Labeo rohita juveniles* had significantly (P>0.05) increased RBC in all the treatment groups as compared with control group. The findings also conform to those by Duncan and Klesius[51], who reported that the number of erythrocytes was significantly (P>0.05) greater in channel catfish fed with a diet containing b-glucan. Leucocytes play an important role in non specific or innate immunity and their count can be considered as an indicator of the health status of fish. This result is supported by Choudhury et al[52], that the total leukocyte count of the *Labeo rohita juveniles* fed with ribonucleic acid and chitin was significantly (P<0.05) higher than the control fish. With regard to hemoglobin content, no difference was noticed among the mangosteen extract fed groups and control group. The hemoglobin content in the blood and oxygen consumption increases when fishes are under stress conditions. Under such conditions there will be an increase in release of immature RBCs from the haemopoietic organs, which in turn elevate hemoglobin concentration in blood[53]. In the present study, the hemoglobin content was not significantly different from the control, which indicates the fish was not under stress. This results also supported by Choudhury et al[52] who found that the dietary ribonucleic acid and chitin had no significant (P>0.05) effect on hemoglobin content in *Labeo rohita* fingerlings.

It is evident from the present study that mangosteen could enhance hematological parameters of fish after incorporation in feed at a dose of 5 g/kg feed. It suggests that inclusion of mangosteen in the diet would improve the non-specific immune response parameters of fish and prevent bacterial infections in culture systems. This work is a preliminary study conducted for the first time, provides a new perspective for the use of mangosteen waste (rind, shoot, and leaf) as a dietary supplement added to fish feed to enhance disease resistance. Further purification of the active compounds and their evaluation may substantially improve quality as well as their usage in aquaculture.

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