



Effects of Dietary Turmeric (*Curcuma longa*) Supplementation on Growth Performance and Hematological Parameters of Green Terror (*Andinoacara rivulatus*)

Mooraki N.^{1*}; Batmany Y.¹; Zoriehzahra S.J.²; Kakoolaki Sh.²

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Abstract

Body shape, coloration, and health status are among the most important factors influencing the market value of ornamental fish. Herbal feed additives have attracted considerable attention as natural growth promoters and immunostimulants in aquaculture. This study evaluated the effects of dietary turmeric (*Curcuma longa*) powder on growth performance, feed utilization, survival, and hematological parameters of Green Terror (*Andinoacara rivulatus*). A total of 144 fish with an initial mean weight of 1.53 ± 0.22 g were randomly distributed among four dietary treatments, including a control diet and three experimental diets supplemented with 0.1%, 0.2%, and 0.3% turmeric powder. All diets were formulated to be isocaloric and isonitrogenous and were fed for 100 days. Fish were measured at 20-day intervals, and hematological analyses were conducted at the end of the experiment. Fish fed the diet containing 0.3% turmeric (T3) exhibited the highest values for growth performance, feed conversion ratio, condition factor, and survival rate; however, these differences were not statistically significant compared with the control group ($p > 0.05$). Red blood cell count (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), and mean corpuscular hemoglobin concentration (MCHC) increased in the turmeric-fed groups, although the differences were not significant ($p > 0.05$). In contrast, white blood cell count (WBC) was significantly higher in the T3 group than in the other treatments ($p < 0.05$). Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) tended to decrease in fish receiving turmeric supplementation, but no significant differences were observed ($p > 0.05$). These findings suggest that dietary turmeric supplementation at 0.3% may enhance certain hematological responses, particularly leukocyte production, while having no significant effect on growth performance in Green Terror under the conditions of the present study.

Keywords: Green Terror, *Andinoacara rivulatus*, turmeric, *Curcuma longa*, hematological parameters, immunostimulant, growth performance, ornamental fish

1-Department of Fisheries Science, College of Marine Science and Technology, North Tehran Branch, Islamic Azad University (IAU), Tehran, Iran.

2-Iranian Fisheries Science Research Institute, Agricultural Research and Education Organization, Tehran, IRAN

*Corresponding author's Email: n_mooraki@iau-tnb.ac.ir

Introduction

The importance of ornamental fish trade is based on producing healthy, appealing specimens in a given period of time. In this regard, as it has been proved for cultivating food fish species, specific attention should be paid to feed formulation and nutritional aspects of ornamental fish in order to reduce the feed conversion ratio, increase growth performance and survival rate and also producing attractive colorful fish, in which all the factors will ultimately affect the economic aspects; consequently, many research projects have been conducted concerning the nutritional requirements, evaluating the possibility of applying herbal and microbial additives; among which herbal additives has a special importance in feed formulation. Turmeric (*Curcuma longa*) belongs to Zingiberaceae family, possessed rhizomes, which been used as an additive with pharmaceutical characteristics and widely distributed in warm areas of Asia (i.e. India, Pakistan, Indonesia, southern part of China), Africa, and south America. It has been extensively exported from India, Indonesia and China. The turmeric powder contains of 6.3% protein, 5.1 % lipid, 3.5% mineral, 69.4% carbohydrate, 13.1% moisture and 5% curcuminoids, 50-60% of which are a mixture of curcumin (diferuloylmethane), monodesmethoxy curcumin and bis desmethoxy curcumin (Chattopadhyay *et al.*, 2004; Innocent, 2008; Sivagurunatan *et al.*, 2011). The anti-inflammatory, cholesterol reducing, and anti-oxidation characteristics of Turmeric have been

reported by Chattopadhyay *et al.* (2004). Moreover, the anti-carcinoma and antibacterial and antifungal functions of this herbal additive have been expressed through previous researches (Banerjee and Nigam 1987; Apisariyakul *et al.*, 1995; Kumar *et al.*, 2001). Turmeric affected liver and stimulates the secretion of bile, Lipase, Maltase, Amylase, Trypsin and also stimulates activity of the pancreas, and protecting the RBCs (Bhavani shankar *et al.*, 1979; Platel and Srinivason 1996; Platel and Srinivason 2000; Srinivasan, 2005). The effect of Turmeric has been evaluated on Immune stimulatory response of fish *Labeo rohita* as an effective compound (Behera *et al.*, 2011). Manju *et al.* (2011) Reported that curcumin had a protective effect on Bloch tissue and increase the growth performance. In this regard, the consumption of turmeric by fantail guppy (*Poecilia reticulata*) caused the reduction of FCR and improvement of growth performance (Mukherjee *et al.*, 2009). Sand Goby (*Oxyeleotris marmoratus*) also showed a positive reaction to consumption of turmeric powder by an increment in Amylase, Lipase, Trypsin and Chemotrypsin secretions (Rojtinnakon *et al.*, 2012). The effectiveness of turmeric powder on Immunity response of *Labeo rohita* to *Aeromonas hydrophila* and white shrimp (*Litopenaeus vannamei boone*) has been evaluated by Sahu *et al.* (2008), Vanichkul *et al.* (2010) and Lawhavit *et al.* (2011), respectively. Moreover El-Bahr and Saad (2008) evaluate the effect of turmeric on hematological and immunological parameters of *Mugil*

cephalus vaccinated with *Aeromans hydrophila* bacterin; this synergistic effect were also investigated on Japanese flounder (Ji *et al.*, 2007) and Nile Tilapia fingerlings (El-Maksoud *et al.*, 2002) fed by diet supplemented with turmeric.

The aim of the present study was to evaluate the effect of turmeric powder as an additive on the growth performance, feed conversion ratio, survival rate and the changes of hematological parameters of the fresh water ornamental fish, Green Terror with high demand in the market.

Materials and methods

One hundred fortyfour specimens of Green Terror fingerlings with mean initial weights and lengths of 1.53 ± 0.22 (g) and 42.9 ± 1.43 mm, respectively were obtained from a local center of ornamental fish located in the studied area Tehran- Iran. The specimens transported by use of aerated plastic bags and being divided randomly equally into 12 aquarium ($55 \times 40 \times 50$ cm) (12 fish in each aquarium). During the trial water temperature and dissolved oxygen controlled by use of a central heater and pump. To control the water quality, temperature, dissolved oxygen, ammonia, hardness and pH were measured daily (Table 1).

After 14 days of adaptation, the initial weight and length of fish were measured and the 12 aquariums were divided into four groups, including three Treatments and one control; each group introduced as three replicates. During the 100 days

of cultivation, all groups were fed three times a day (6, 12, 18) based on two percent of their biomass. The turmeric powder was added to dry food, which being formulated by Win Feed 2.8 (Table 2), at levels of T₁ (% 0.1), T₂ (%0.2) and T₃ (%0.3) and C (%0) dry food. The turmeric powder added to the main mixture of the food with rest of additives and then the prepared dough was pelleted through a 1mm die of meat grinder and the pellets oven dried for 8 hours at 60°C, bagged separated and frozen at -20 C° until use. Proximate analyses of feed were determined following the method provided in AOAC (1995) (Table 3).

Samplings

For evaluating the growth and feeding performance of the specimens in the four groups, biometry was done once every 20 days during the 100 days of cultivation. The weight was measured by use of a digital balance (bearing 0. 01g) and the lengths were obtained by use of the caliper, after anesthetizing fish with MS222. Regards to the result of biometry the growth and feeding performance measured by calculating the below equations.

$$\text{Weight gain (g/ fish}^{-1}\text{)} = W_F \text{ (g)} - W_I \text{ (g)}$$

$$W_F = \text{Final mean body weight (g)}$$

$$W_I = \text{Initial mean body weight (g)}$$

$$\text{Length gain (mm)} = L_F \text{ (mm)} - L_I \text{ (mm)}$$

$$L_F = \text{Final mean body length (mm)}$$

$$L_I = \text{Initial mean body length (mm)}$$

Table1: Daily average of physic-chemical parameters of water during the cultivation period.

Hardness(mg/l ⁻¹)	Ammonia(mg/l ⁻¹)	pH	Dissolved oxygen(mg/l ⁻¹)	Temperature (C°)
193±2.5	0.02	6.5 ±0.5	7.3 ±0.5	27±1

Table 2: Basic diet formulated by Win Feed 2.8, supplemented by different levels of turmeric powder.

Ingredients (%)	Treatment			
	T ₁	T ₂	T ₃	C
Fish meal	47.8	47.8	47.8	47.8
Yeast powder	11.1	11.1	11.1	11.1
Wheat gluten	8.8	8.8	8.8	8.8
Wheat flour	7.1	7.1	7.1	7.1
Wheat bran	6.5	6.5	6.5	6.5
Dicalcium phosphate	1	1	1	1
Sunflower oil	1.6	1.6	1.6	1.6
Antioxidant	0.1	0.1	0.1	0.1
Binder	5.8	5.8	5.8	5.8
Mineral premix	2	2	2	2
Vitamin premix	2.5	2.5	2.5	2.5
Mild inhibitor	0/6	0/6	0/6	0/6
Astaxanthin	3	3	3	3
DL- methionine	1	1	1	1
Lysine	1	1	1	1
Turmeric	0.1	0.2	0.3	0

Table 3: Approximate analyses of the basic diet used to feed (*Andinocara rivulatus*).

Properties (%)	Composition
Dry Matter	93.24
Protein	44.01
Lipid	10.73
Fiber	2.63
Ash	9.17
Carbohydrate Ca/	7.52
Pratio	1/2
Energy	340.25Kcal

Body weight increase (%) = $(BW_F - BW_I) / BW_I \times 100$

BWF = Final mean body weight

BW_I = Initial mean body weight

Condition factor = $(\frac{W}{L^3}) \times 100$

W= Weight (g)

L= Length (Cm)

Specific growth rate for Length (%days⁻¹) = $(\ln L_F - \ln L_I) / t \times 100$

t= the number of days in the feeding period

Specific growth rate for Weigh (%days⁻¹) = $(\ln w_F - \ln w_I) / t \times 100$

Feed conversation ratio (g/g⁻¹) = $F / (W_I - W_F)$

F= Feed intake

W_F = Final mean body weight (g)

W_I =Initial mean body weight (g)

Survival rate =Final number of fish individuals×100 /initial number

Blood samples were taken at the end of the trial through anesthetized nine specimens randomly selected from each group by using MS222 and then

dissecting the peduncles. Blood indices, i.e. MCH, MCHC, and MCV were calculated as bellow (FAO, 2003). Red and white blood cells diluted by using Natt and Herrick's stain solution and counted by use of Neubaur hemocytometer. Hemoglobin samples were used for measured by use of the spectrophotometer (Drobkin, 1945). Heparinized samples were used for measuring hematocrit.

$$\text{MCV } (\mu\text{m}^3) = [\text{Hct}/ \text{RBC (million)}] \times 10$$

$$\text{MCH (pg cell}^{-1}\text{)} = [\text{Hb}/ \text{RBC (million)}] \times 10$$

$$\text{MCHC (g dl}^{-1}\text{)} = (\text{Hb}/ \text{Hct}) \times 100$$

Statistical analysis

The obtained data with normal distribution analyzed through One- way ANOVA analysis and being compared

through post hoc Bonferroni test at $p < 0.05$ confidence.

Results

Growth and feeding performance

Growth and feeding performance and survival rate of *Andinocara rivulatus* fed with different levels of turmeric powder presented in Table 4.

Among the treatments, fish consumed feed contain 0.2 and 0.3 percent turmeric powder showed a higher weight gain but not significantly different from the control group ($p > 0.05$). In terms of length gain, condition factor, SGR the same trends were observed with no significant difference ($p > 0.05$). The T₂ and T₃ also showed the best FCR among the other groups but did not show a significant different ($p > 0.05$) (Table 4).

Table 4: Growth performance of the reared *Andinocara rivulatus* after 100 days of consuming diets supplemented with turmeric powder.

Parameters	Treatment T ₁	T ₂	T ₃	C
Weight gain (g/ fish ⁻¹)	3.72±0.63 ^a	3.89±0.81 ^a	3.72±0.65 ^a	3.42±0.26 ^a
Body weight increase (%)	224.88±45.5 ^a	267.30±77.16 ^a	270.49±57.72 ^a	223.35±47.65 ^a
Length gain (mm)	2.16±0.73 ^a	2.30±0.65 ^a	2.55±0.22 ^a	2.01±18 ^a
Condition factor (%)	2.13±0.55 ^a	1.85±0.29 ^a	1.66±0.15 ^a	1.88±0.12 ^a
Specific growth rate _(w) (%days ⁻¹)	1.18±0.15 ^a	1.29±0.20 ^a	1.30±0.15 ^a	1.16±0.15 ^a
Specific growth rate _(L) (%days ⁻¹)	0.41±0.12 ^a	0.42±0.11 ^a	0.47±0.03 ^a	0.38±0.02 ^a
Feed conversation ratio (g/g ⁻¹)	1.60±0.20 ^a	1.50±0.19 ^a	1.50±0.22 ^a	1.61±0.25 ^a
Survival rate (%)	91.66±8.34 ^a	91.66±8.34 ^a	88.88±4.81 ^a	88.88±4.81 ^a

T₁= diet supplemented with %0.1 turmeric powder, T₂= diet supplemented with %0.2 turmeric powder, T₃= diet supplemented with %0.3 turmeric powder and C= control group. Values are expressed as (means± standard deviation); significant differences ($p < 0.05$) among the experimental groups are indicated by different superscript letters.

Hematological parameters

Table 5 shows the hematological parameters belong to the four examined groups. As it is obvious, the highest counts of RBC was observed in fish fed diet contains % 0.3 (T₃) turmeric powder but did not have a significant difference

with other groups ($p > 0.05$). The same trend was also present for hematocrit, hemoglobin and MCHC, but the highest value for MCH was reported for the control group and T₃, respectively. Regards to the results the highest MCV value was observed in fish fed with

control diet, T₁, and T₂ respectively with no significant difference between the four groups ($p>0.05$). The highest counts for WBC measured for T₃ which was

significantly differ from T₂, T₁, and also control group ($p<0.05$).

Table 5: Blood indices of the treated *Andinocara rivulatus* after 100 days of consuming diets supplemented with turmeric powder.

Treatment	T ₁	T ₂	T ₃	C
parameters				
RBC (10 ⁶ μL)	1.78±0.37 ^a	1.88±0.18 ^a	1.97±0.09 ^a	1.69±0.16 ^a
Hematocrit (%)	19.42±0.75 ^a	17.85±1.42 ^a	19.59±1.02 ^a	18.98±1.80 ^a
Hemoglobin(g/dL ⁻¹)	7.29±0.34 ^a	7.10±1.94 ^a	8±1.74 ^a	7.30±0.31 ^a
MCHC((g dL ⁻¹)	37.53±0.40 ^a	37.30±7.69 ^a	40/61±6.67 ^a	38.59 ± 2.21 ^a
MCH(pg cell ⁻¹)	41.82±6.17 ^a	37.35±7.14 ^a	40.40±6.86 ^a	43.37±3.06 ^a
MCV(μm ³)	111.54±17.45 ^a	100.38±1.98 ^a	99.42±0.54 ^a	112.62±10.67 ^a
WBC(103μL)	12.82±1.63 ^b	13.60±1.43 ^b	15.60±2.57 ^a	9.82±1.35 ^c

T₁= diet supplemented with %0.1 turmeric powder, T₂= diet supplemented with %0.2 turmeric powder, T₃= diet supplemented with %0.3 turmeric powder and C= control group. Values are expressed as (means± standard deviation); significant differences ($p<0.05$) among the experimental groups are indicated by different superscript letters.

Discussion

Regards to the results of the present study, the use of 0.3 percent turmeric powder as an herbal additive in the diet of Green Terror has a relative positive effect on growth performance and FCR, however this positive effect was not verified significantly. The obtained results are in line with Mahmoud *et al.* (2014) which reported that turmeric supplementation (0.5%) did not significantly improve growth performance of *Oreochromis niloticus*, but significantly affected feed consumption ratio in treated fish. On the other hand FCR and SGR increased in *Labeo rohita* fingerlings fed different dosage of turmeric (0.1, 0.5, 1.0, and 5g/kg feed) for 60 days, reported by Sahu *et al.* (2008). El-Bahr and Saad (2008) applied a mixture of black cumin seed and turmeric as additives in the diet (5g Kg⁻¹ diet) of *Mugil cephalus* for six weeks, which results in growth

improvement of the specimens; but applying the same mixture for Asian sea bass (*Lates calcarifer*) resulted in relatively weak promotion of growth performance, while the feed efficiency utilization remained unchanged (Abdelwahab and El-Bahr, 2012). Applying ethanolic extract of turmeric for nine weeks in feed of white shrimp did not affect FCR and SR significantly, but when the amount of ethanolic extraction increased to 15g Kg⁻¹, the body weight gain of white shrimp was observed (Lawhavinit *et al.*, 2011).

Rojtinnakorn *et al.* (2012) investigated the effect of adding turmeric extract to the feed of sand Goby (*Oxyeletoris marmoratus*) on the activity of gastrointestinal enzymes, including Amylase, Lipase, Trypsin and Chymotrypsin. Sand Goby fries were fed by diets containing 0.03, 0.05, 0.1 and 0.5 percent turmeric powder for seven days equal to 3 percent of biomass. The

results showed that 0.3 percent turmeric powder had a significant effect on the enzyme's increment, which was the baseline for dosage determination in the present study. Therefore, by enzyme increment, the food digestion and absorption will be improved. Moreover as it is said by Osawa *et al.* (1995) the antioxidant activity of turmeric could also stimulates protein synthetize through enzymatic systems. This is in accordance to Pransin (2006) who reported that goldfish fed turmeric supplemented diets, had highest acid protease, alkaline protease and lipase activity, enhanced growth rate and yellow pigmentation. In the present study the amount of enzymes did not consider, but it seems that applying turmeric powder can affect the quality and quantity of digested and absorbed feeds based on growth indices measurement. Mukherjee *et al.* (2009) investigated the effect of turmeric powder on growth performance and body color of guppy. In this study 0.03, 0.06, 0.09, 0.1 and 0.2 percent of turmeric powder were added to the basal diet, and the results revealed that fish fed with diet contain %0.09 turmeric powder had a better growth performance compared to other groups ($p < 0.05$). Different amount of applied TP as an additive to the basal diets in various studies is related to species-specific expedience. Manju *et al.* (2011) investigated the protective effect of curcumin on the tissue of *Anabas testudineus*; in this study 0.5 and 1 percent of curcumin were added to diet contain 40 percent protein for duration of approximately three weeks. The analysis

revealed that in long term duration, both concentrations (0.5 and 1 percent) caused a significant protein increment in the tissue, which ultimately result in better growth performance. The results verify the previous explanations that turmeric could affect the digestibility and absorption which ultimately affect the growth performance and carcass analysis. Lawhainit *et al.* (2011) evaluated the effect of turmeric concentration opposed to *Vibrio* disease in *Litopenaeus vannamei*, growth performance and immune system of the specimens were also traced. The results revealed that adding 7.5 and 15 g/kg of ethanol turmeric extract to the basal diet caused a better growth performance compared with other groups. It could be concluded that relative improvement of growth performance and reduction of FCR in *Andinocara rivulatus* fed with turmeric powder maybe due to the increment of enzymatic activities, including Amylase, Lipase, Trypsin and Chymotrypsin which may also result in protein concentration increment of the carcass. Further studies are needed to consider the effectiveness of turmeric powder as an additive with special reference to the duration of consumption, dosage, method of administration, along with identifying the best size of fish for being treated.

The results of evaluating the effect of adding turmeric powder to the diet of *Andinocara rivulatus* on hematological parameters revealed that RBC counted, hemoglobin density, hematocrit, MCV, MCH and MCHC did not altered significantly among the treatments, but

the fish fed with 0.3 percent turmeric powder had a higher RBC counts, hemoglobin density and hematocrit compared with the control group but MCV index was lower than the control group. The reduction of MCV result to reduction of RBCs volume in blood vessel and reduce the possibility of blood coagulation. Moreover, the reduction of RBC volume is a sign of no inflammation, which all are positive changes in the physiology of the circulatory system (Jha *et al.*, 2007). The increment of RBC counts, hemoglobin density and hematocrit maybe cause of an increment in circulatory system activity of fish which may have positive effect on growth performance of the treated specimens. Nevertheless, in the present study the amount of MCH and MCHC did not affect by turmeric consumption, regard to the increase of RBC count, hemoglobin density and hematocrit, the constant ratio of MCH and MCHC showed that treated fish were all healthy. In consistence with the obtained results, the increment of total erythrocyte count, hemoglobin, hematocrit, and MCHC were not significant compare to the control group of *Cirrhinus mrigala*, which did not fed by supplemented diet contain two grams of turmeric powder; moreover MCV was also decrease not significantly in treated groups compare to the control (Sivagurunathan *et al.*, 2011). The WBC count increased significantly in all treatments fed with diet containing turmeric compared with the control group. It seems that treated fish had a more potential to challenge with disease

as WBCs are the main source of lysozyme synthesis and phagocytic activity. It is obvious that cellular and humoral immunity depends on WBCs; however, it should be mentioned that challenge with infectious disease does not depend only on leukocytes. Thus it could be mention that adding turmeric to the diet of *Andinocara rivulatus* causes a potent immune system compared to control group. Abdelwahab and El-Bahr (2012) were also reported that adding a mixture of cumin seed and turmeric to the diet of Asian sea bass (*Lates calcarifer*) did not affect hematological parameters. On the other hand Sahu *et al.* (2008) studied the effect of adding turmeric powder to the diet of *Labeo rohita* on enzymatic profile and immunological characteristics; specimens fed with diets containing 0.01, 0.05, 0.1 and 0.5 percent supplement for 60 days at a level of %4 biomass. The results revealed that many of the immunity activities, including lysozyme activity, producing anion superoxide were significantly higher compared to control group and the best result gain for the group fed with diet contains 0.1 percent turmeric. Vanichkul *et al.* (2010) were also shown that applying 0.0025 percent turmeric to diet of *Litopenaeus vannamei* increases the resistant against pathogenic *Vibrio*. Malar and Charles (2013) showed better resistance of *Penaeus monodon* against *V. harveyi* through feeding with diet containing turmeric extract (25 mg/kg feed). Furthermore Behera *et al.* (2011) determined that injecting 1.5 and 15, 150 µg curcumin to *Labeo vonito* with initial

weight of 30-40 g after 7 to 42 days caused in potent activity of immune system. Moreover, El-Bahr and Saad (2008) showed that adding turmeric to diet of *Mugil cephalus* vaccinated with *Aeromonas hydrophila* could be positively affecting the hematological parameters.

As a conclusion the results of the present study reveal that applying turmeric powder at the level of 0.3 percent of the basal diet could not alter the growth indices significantly but through increasing white blood cells can develop and increase fish resistance toward disease and by reducing the amount of red blood cells (MCV) caused in quicker movement of blood, which prevents red blood cell sedimentation as well as blood coagulation. Considering the fact that various factors (i.e. duration of consumption, applied dose, method of administration, use of direct herbal additive or extracted form) affect the reaction of treated organism in general and particular aspects, this field demands further researches.

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