Journal of Aquaculture and Fish Health Vol. 12(2) - June 2023
P-ISSN: 2301-7309 / E-ISSN: 2528-0864
doi : 10.20473/jafh.v12i2.31675

Substitution of *Hibiscus sabdariffa* with *Curcuma longa* in the Diets of *Clarias gariepinus* and the Effects on the Growth, Nutrient, and Hematobiochemistry

Uchechukwu Dennis Enyidi[1,2*] and I. Ayogu[1]
[1]Department of Biotechnology and Applied Biology, Godfrey Okoye University Thinkers Corner Emene, Enugu State 400001, Nigeria
[2]Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Agriculture Umudike Umuahia, Abia State 440101, Nigeria

*Correspondence :
enyidiuche@yahoo.com

Received : 2021-11-24
Accepted : 2022-10-14

Keywords :
Antioxidants, Cholesterol, Phytogenics, Specific growth rate

Abstract

Phytogenic feed additives are plausible alternatives to antibiotics and serve as growth promoters in aquafeed. This research aims at finding the effects of varying inclusions of natural antioxidants like hibiscus (*Hibiscus sabdariffa* L.) and turmeric (*Curcuma longa*) on the growth, hematology, and nutrient utilization of *Clarias gariepinus* after 60 days of feeding. This research used six feeds labeled as F1- F6 that varied in the composition of *C. longa* and *H. sabdariffa*. The weight (g) inclusions of *C. longa* and *H. sabdariffa* were as follows, F1 0:250, F2 50:200, F3 100:15, F4 150:100, F5 200:50 and F6 250:0. Juvenile African catfish stocked at 9 catfish per 15L aquaria per 3 replicate aquariums (27/treatment feed) were fed for 60d. The catfish fed with F1 (250g *H. sabdariffa*) had the best specific growth rate of 5.76 ± 0.04 % day^{-1}, lowest feed conversion ratio of 1.01 ± 0.01, weight gain of 31.65 ± 0.13 g, and protein efficiency ratio of 0.96 ± 0.07. The growth of African catfish increased with the increasing inclusion of hibiscus supplements. Hematobiochemical parameters ALT, AST was better for catfish fed high hibiscus supplements. We noticed that hibiscus inclusion was inversely proportional to cholesterol and total triglyceride levels of fish. The deposit of adipose tissues in the catfish was higher, with increasing inclusion of hibiscus than turmeric supplement.

INTRODUCTION

The high cost of aquaculture feed in Nigeria has led to the search for alternative feed ingredients that can enhance the growth and immunity of African catfish. Several non-conventional feedstuffs have been tried in the diets of *Clarias gariepinus* example, hibiscus, and turmeric. Hibiscus is a proteinous flowering plant whose derivatives have been used for various purposes in catfish feed (Fagbenro, 2005; Adewole, 2014; Iheanacho *et al*., 2017). Turmeric also known as *Curcuma longa*, a perennial plant and rhizomatous herbaceous Zingiberaceae (Chan *et al*., 2009). Turmeric is a phytogenic supplement that possesses polyphenolic and hydrophobic anti-inflammatory and immune-modulatory actions on fish (Abdel-Tawab and Abbas, 2016; Zheng *et al*., 2018; Enyidi and Orji, 2020).

Cite this document as Enyidi, U.D. and Ayogu, I., 2023. Substitution of *Hibiscus sabdariffa* with *Curcuma longa* in the Diets of *Clarias gariepinus* and the Effects on the Growth, Nutrient, and Hematobiochemistry. *Journal of Aquaculture and Fish Health*, *12*(2), pp.269-280.

Hibiscus sabdariffa is an ornamental plant belonging to Malvaceae. Hibiscus is also known as "roselle" and has a good content of vitamin C and several antioxidants (Robert, 2005; Qi *et al*., 2005; Cisse *et al*., 2009; Singh *et al.,* 2017). Hibiscus seed meal has been used in replacing 60% of soybean meal in the diets of African catfish, without any deleterious effects no adverse effects on growth (Fagbenro, 2005). In recent research, there were no harmful effects of hibiscus and *Zingiber officinale* (ginger) inclusion in the diets, in the blood serum of *C. gariepinus* (Abdel-Tawwab and Abbass, 2016). In similar research on tilapia, El Mesallamy *et al*. (2016) noted that hibiscus calyx inclusion in tilapia diets enhanced growth and increased tilapia's immunity to *Aeromonas hydrophila.* Similarly, Enyidi and Orji (2020) pointed out that the inclusion of turmeric supplements in the diets of Nile tilapia enhanced growth and increased immunity when challenged with a consortium of bacteria. Turmeric has been reported to improve digestion and metabolic activities (El Mesallamy *et al*., 2016). Different parts of the hibiscus have been used in feed manufacturing. The effects of hibiscus and turmeric may be due to their vitamins, antioxidants, and protein content, and lipids.

This research aims at finding the effects of varying inclusions of natural antioxidants like hibiscus (*H. sabdariffa* L.) and turmeric (*C. longa*) on the growth, hematology, and nutrient utilization of *C. gariepinus* after 60 days of feeding.

METHODOLOGY

Place and Time

This research was conducted at the wet laboratories of Godfrey Okoye University Thinkers Corner, Emene Enugu Enugu State Nigeria. The work took place between June 2018 and January 2019. The analytical works were carried out at the hematology department laboratory of University of Nigeria Teaching Hospital Ituku-Ozala Enugu State Nigeria and nutritional analysis at the National Root Crop Research Institute, Umudike Umuahia, Abia State Nigeria. Other analysis took place at Godfrey Okoye University's central laboratories.

Research Materials

The feed production experiment was conducted with locally constructed grinders, mixers, and pelletizers. The industrial feed mill equipment was made at Uchetch Industry at Tinker Industrial Market Enugu. We also utilized a locally fabricated electric oven equipped with a thermostat. Average dissolved oxygen was measured with YSI oxygen meter model 550A (YSI Inc. Yellow Springs, Ohio, USA). Ammonia was measured with an ammonia test kit (Tetra Merke, Melle, Germany). Water pH was with Hannah pH meter. Alkalinity and Nitrate content were measured with HANNA water testing kit. Photoperiods were observed with (HD 9221 lux meter, Delta OHM, Padua, Italy), Kjedahl.

Research Design

This was designed as a completely randomized experiment (CRD). There were three replicates per treatment feed. A total of six feeds were made, and they varied in the inclusion of turmeric: hibiscus as follows, Feed 1 (F1) 0 : 250, Feed 2 (F2) 50 : 200, Feed 3 (F3) 100 : 150, Feed 4 (F4) 150 : 100, Feed 5 (F5) 200 : 50 and Feed 6 (F6) 250 : 0. Feed 6 was a control diet equally.

Work Procedure

Phytogenic Supplements Preparation

The hibiscus calyxes were obtained from a dealer at the Enugu relief market. The calyxes were carefully picked while removing unwanted materials. The calyx was rinsed with clean water and allowed to dry in an oven at 40 $^{\circ}$C. The hibiscus was autoclaved at 70 $^{\circ}$C for 10 mins and then allowed to cool. The hibiscus was dried

Cite this document as Enyidi, U.D. and Ayogu, I., 2023. Substitution of *Hibiscus sabdariffa* with *Curcuma longa* in the Diets of *Clarias gariepinus* and the Effects on the Growth, Nutrient, and Hematobiochemistry. *Journal of Aquaculture and Fish Health*, *12*(2), pp.269-280.

and ground to dust and stored in a black nylon bag and kept at -20° C till used.

Fresh turmeric was purchased from the National root crop research institute Umudike. The turmeric was sorted to remove unwanted parts and stones. The root was then washed in running tap water and sliced into 3 mm bits. The sliced turmeric was then dried in an oven at 40 °C to a constant weight of 8 % wet weight. The dried turmeric was ground to dust and stored in a cold room freezer at -20 °C.

Feed Preparation

The protein supplements and basal ingredient contents of the experimental diets were the same for all feed types. The diets were made to be isoproteinous and isolipidic. The energy content of the diets was also similar for all feeds. Appropriate weights of ingredients as stipulated in the proximate composition table (Table 1) were mixed. Mixing of ingredients utilized a locally fabricated electric mixer. The dry ingredients were mixed with approximately 250 ml of water and then preconditioned at 100 °C for 25 minutes. Vitamin C and vitamin premix were added to the mixed dough. The dough was then pelletized using a 2 mm die, fitted to a locally fabricated feed pelleting machine manufactured at Enugu industrial manufacturing market Tinker Enugu. The pelleted feeds were dried in an air chamber at 40 °C for 24 hours. Dried pellets were stored in black nylon bags and kept in a freezer at -20 °C till used. Proximate compositions of the dried feed pellets were analyzed.

Table 1. The composition table of treatment feeds varies in inclusion levels of turmeric and hibiscus meal, used to feed African catfish *Clarias gariepinus* (all weights in g).

Composition	F1	F2	F3	F4	F5	F6
Turmeric	0	50	100	150	200	250
Hibiscus	250	200	150	100	50	0
Fishmeal	300	300	300	300	300	300
Soybean Meal	100	100	100	100	100	100
Bambara Meal	150	150	150	150	150	150
Swine entrails	50	50	50	50	500	500
Bentonite	40	40	40	40	40	40
Vitamin C	20	20	20	20	20	20
Oleic oil	30	30	30	30	30	30
Vit. premix [a]	20	20	20	20	20	20
Lysine	20	20	20	20	20	20
Methionine	20	20	20	20	20	20
Totals	1000	1000	1000	1000	1000	1000
Proximate composition						
% Crude protein	32.69	32.42	32.67	33.47	33.82	33.83
% Lipids	10.42	10.83	11.27	11.54	12.66	13.03
% Carbohydrate	26.49	25.98	28.03	24.83	20.14	22.48
%Crude fiber	6.77	6.95	8.31	8.48	8.77	8.96
%Ash	10.79	11.2	8.34	10.31	12.27	10.6
%Moisture	12.84	12.62	11.38	11.37	12.34	11.1
Dry matter (g)	87.16	87.38	88.62	88.63	87.66	86.9
Phytic acid (mg g^{-1})	0.46	0.46	0.35	0.43	0.49	0.53

[a]Vitamin premix : The following vitamins were added to supply the following Kg^{-1}diet: cholecalciferol, 1300 IU; all-race-α-tocopheryl acetate, 140 IU; menadione sodium bisulfite, 12 mg; thiamin HCL, 8 mg; riboflavin, 16 mg; calcium d-pantothenate, 17 mg; biotin, 0.2 mg; folic acid, 5 mg; vitamin B12, 0.02, niacin, 40 mg; pyridoxine HCl, 16 mg; ascorbic acid (Stay C), 80 mg. Magnesium phosphate, 5000 mg, potassium carbonate, 400 mg, manganous sulfate, 10; ferrous sulfate, 5 mg; zinc sulfate, 80 mg.

Cite this document as Enyidi, U.D. and Ayogu, I., 2023. Substitution of *Hibiscus sabdariffa* with *Curcuma longa* in the Diets of *Clarias gariepinus* and the Effects on the Growth, Nutrient, and Hematobiochemistry. *Journal of Aquaculture and Fish Health*, *12*(2), pp.269-280.

Experimental Setup, Feeding of Fish, and Protocols

African catfish of average weight 3.0 ± 0.75 g were purchased from a commercial fish farm in Enugu, Nigeria. The catfish was transported to the wet laboratory of Godfrey Okoye University Emene Enugu. Nine African catfish fingerlings were stocked in three replicate aquaria per treatment feed. Each of the aquaria had 15 L filtered and aerated water. There were 18 aquariums in all. The fish were acclimated for seven days. During the period of acclimation, the catfish were fed 32 % protein commercial feed. The aquaria were aerated and supplied with water at a constant flow rate of (c. 0.6 L min^{-1}). The temperature of the aerated water was 30.0 ± 1.5 °C. The culture water was tap water supplied by the University waterworks.

The tanks were subjected to a photoperiod of L12:D12, and the light intensity was 8 lux (HD 9221 lux meter, Delta OHM, Padua, Italy). The aquariums were cleaned by gentle washing every morning before feeding time. Care was taken not to stress fish, and three-quarters of the water was removed and replaced daily. The catfish were hand-fed between 08.00 AM and 05.00 PM. The catfish were fed *ad libitum* three times daily, morning, afternoon, and evening. The method used in feeding was by initially weighing a portion of the feed on a plate. From the weighed feed, feeding was done *ad libitum*. The plate from where feeding was going on is reweighed, and the difference is recorded as feed given. United feed was collected, dried to constant weight, and deducted. The catfish were collectively weighed every two weeks. On the day of weighing, the catfish were not fed for 9 hours before weighing to enhance gut clearance. After 60 days, five fish were removed from each treatment feed tank and killed by a sharp blow on the head. The length (to 0.1 cm) and weight (to 0.1 g) of the fish were recorded individually. The liver and visceral fat of the sample fish were removed and weighed (to 0.01 g). Adipose tissue deposits were removed and weighed. The sampled fish were frozen at -20 °C.

Chemical Analysis

Analysis of the crude protein contents of the fish and feeds was carried out by the Kjeldahl method. The nitrogen content of the samples was analyzed, and this was used in calculating the protein. The calculations were as follows: crude protein as % N x 6.25. The % crude protein contents are listed in Table 1. The lipids content of the feed and the fish muscles were measured by modified chloroform: methanol as described by Enyidi (2012). Chloroform : methanol ratios of 2 : 1. Total lipid was estimated as the weight difference in non-chloroform : methanol extracted and chloroform : methanol extracted muscle samples of feeds feed and fish, was taken from two fishes per tank and measured by freeze-drying muscle samples to a constant weight. Muscle samples were taken below the dorsal fin and between the pectoral and caudal fins, excluding the skin. Ash content was calculated by burning a known amount of muscle sample of the catfish in a muffle furnace for 24 hours at 550 °C. The phytochemical components of the feeds were analyzed. The phytochemicals analyzed were alkaloids, saponins, flavonoids, and tannins. The % phytochemical constituents of the feeds are listed in Table 2.

Table 2. Phytochemical analysis of feeds varying in hibiscus and turmeric used in feeding *C. gariepinus* for 60 days.

Phytochemicals	F1	F2	F3	F4	F5	F6
Flavonoid mg kg^{-1}	0.22±0.08	0.26±0.07	0.28±0.55	0.29±0.02	0.3±0.04	0.36±0.01
Alkaloids mg kg^{-1}	0.44±0.06	0.58±0.06	0.74±.05	0.81±0.06	0.88±0.07	0.95±0.06
Tannin mg kg^{-1}	2.91±0.03	2.71±0.01	2.41±0.07	2.82±0.09	2.840.01	2.96±0.03
Saponin mg kg^{-1}	0.53±0.01	0.59±0.03	0.57±0.08	0.65±0.02	0.74±0.04	0.85±0.01

Cite this document as Enyidi, U.D. and Ayogu, I., 2023. Substitution of *Hibiscus sabdariffa* with *Curcuma longa* in the Diets of *Clarias gariepinus* and the Effects on the Growth, Nutrient, and Hematobiochemistry. *Journal of Aquaculture and Fish Health*, *12*(2), pp.269-280.

Physiochemical Analysis of Water

All the physiochemical parameters were measured in the morning before feeding and fortnightly. The culture water dissolved oxygen content was measured with the YSI oxygen meter model 550 A (YSI Inc. Yellow Springs, Ohio, USA). The pH value of the water was measured with HANNA pH meters. The ammonia content of the water was also measured fortnightly with an ammonia test kit (Tetra Merke, Melle, Germany). Similarly, Nitrate content was measured with HANNA water testing kit, same test kit was used in measuring the dissolved phosphorous content of the water.

Hematobiochemical Parameters

The hematobiochemical parameters were carried out to analyze the effects of the diets and the supplements on the fish. The sample fishes were 3 per treatment, and the experiment was conducted in three replicate aquariums per treatment feed. A total of three fish were randomly selected from each treatment feed tank. The fish blood was collected from the caudal vein by using sterilized syringes. The blood was centrifuged at 3,800 x g for 5 min, and then samples of blood serum were separated and collected. The serum was stored at −70 °C for the analyses of aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, total protein (TP), total cholesterol (TC), and triglycerides (TG). These biochemical parameters were measured, using a chemical analyzer (Fuji DRI-CHM 3500 i, Fuji Photo Film, Tokyo, Japan).

Data Analysis

The catfish specific growth rate (SGR) was calculated as follows:

$$SGR = 100 \times \frac{(\text{Ln W2} - \text{Ln W1})}{t}$$

Where:
SGR = specific growth rate (% day^{-1})
W1 = initial average weight (g)
W2 = final average weight (g)
t = periods of the experiment (days)

Feed conversion ratio (FCR) was calculated as:

$$FCR = \frac{\text{feed fed (g)}}{\text{weight gain (g)}}$$

Protein efficiency ratio (PER) was calculated as:

$$PER = \frac{\text{FCR} \times \text{\% feed protein}}{\text{\% catfish protein}}$$

Daily feed intake (DFI) was calculated as:

DFI = intake (g) – rejected food per day

One-way ANOVA was used for testing possible differences in treatment means and Fishers least significant differences ($FLSD_{.05}$) test was used for the separation of means and detection of the least significant difference.

RESULTS AND DISCUSSION

Hematobiochemical Parameters

The hematobiochemical analysis results of catfish showed that alanine aminotransferase (ALT), also known as glutamic-pyruvic transferase (GPT), and aspartate aminotransferase (AST) were significantly ($P < 0.05$) lower for fish fed high hibiscus diets compared to turmeric (Table 4). The AST of the fish provided feed F1 was 8.8 μ^{-L}, but it was 26.1 μ^{-L} for the fish fed the reciprocal diet feed F5. The AST of the fish was reduced as the hibiscus supplements in the diet were reduced. Conversely, AST was increasing as the quantity of turmeric increased. Similarly, ALT was 2.3 μ^{-L} for the catfish fed feed F1 while it was 12.8 μ^{-L} for the reciprocal diet F5. The ALT values of the fish were inversely proportional to the increasing percentage of hibiscus supplements. The result is also in agreement with Enyidi and Orji (2020), who noted that the inclusion of turmeric in diets of *O. niloticus* resulted in no liver damage and a fast growth rate.

The serum protein of the catfish was not significantly different ($P > 0.05$), irrespective of treatment feed (Table 4). The total cholesterol level of the catfish was constantly diminishing, with the increasing inclusion of hibiscus supplement (Table 4). There was a negative correlation between the inclusion of hibiscus and the increase in total cholesterol of the catfish

Cite this document as Enyidi, U.D. and Ayogu, I., 2023. Substitution of *Hibiscus sabdariffa* with *Curcuma longa* in the Diets of *Clarias gariepinus* and the Effects on the Growth, Nutrient, and Hematobiochemistry. *Journal of Aquaculture and Fish Health*, *12*(2), pp.269-280.

(Fig. 2), R^2 0.95. The catfish fed feed F1 had total cholesterol of 99.3 mg^{-dL}, significantly different from all other treatments ($P<0.05$). The total cholesterol level of the catfish fed feeds F2, F3, and F4 were as follows, 110.3 mg^{-dL}, 123 mg^{-dL}, and 129.8 mg^{-dL}, respectively. There were no serious differences ($P>0.05$) between the total cholesterol level of catfish fed with F4 and F5. The highest cholesterol level was analyzed for catfish fed feed F6 140.2 mg^{-dL}. The blood glucose level was noted to be the lowest.

Total triglycerides were highest for the catfish fed feed F1 235.6 mg^{-dL} but decreased with hibiscus meal inclusion in the diet. The catfish fed feed F6 had the lowest content of total triglycerides, 215.4 mg^{-dL} (Table 4). It seems that the combination of conditioned gut health and improved feed conversion ratio and nutrient utilization. This is similar to the findings of Rico *et al*. (2013), Qi *et al*. (2005), and Enyidi and Orji (2020).

The high lipid content of the catfish was not inclusive of cholesterol. The total cholesterol content of the fish was constantly reduced with increasing hibiscus supplements (Fig 3). Cholesterol aids the growth of fish (Guerra-Olvera and Viana, 2015; Xu *et al*., 2018). Fish have been known to synthesize cholesterol (Deng *et al*., 2010), and fish oil is high in cholesterol (Moreau *et al*., 2002). The substitution of fish oil with vegetable oil can result in reduced cholesterol (Guerra-Olvera and Viana, 2015). Hibiscus enhances the utilization of cholesterol for high SGR and weight gain more than turmeric. The high SGR and weight gain we noted in this work could be due to the anti-cholesterol activities of hibiscus calyx. Administering the dried calyx of *H. sabdariffa* significantly decreased serum cholesterol, triglycerides, and LDL levels. Calyx of *H. sabdariffa* possesses both antioxidant effects against LDL oxidation and hypolipidemic, but the underlining processes are not yet transparent (Hirunpanich *et al*., 2006).

There was no adverse effect of the feed on the fish. The liver of the catfish does not seem to be adversely affected based on the ALT and aspirate AST levels.

Table 3. Hematobiochemical characteristics of African catfish fed diets varying in inclusion levels of hibiscus and turmeric.

Parameters	F1	F2	F3	F4	F5	F6
AST (μ L^{-1})	26.1^{a}	22.1^{b}	18.9^{c}	15.4^{d}	12.1^{d}	8.8^{e}
ALT (μ L^{-1})	12.8^{a}	8.3^{b}	7.0^{b}	6.2^{bc}	4.1^{c}	2.3^{d}
Glucose (mg dL^{-1})	180.3^{d}	193.0^{a}	185.0^{c}	183.2^{c}	189.1^{b}	185.3^{c}
Total protein mg dL^{-1}	6.6^{ns}	6.4^{ns}	6.2^{ns}	6.0^{ns}	6.02^{ns}	6.1^{ns}
Total cholesterol mg dL^{-1}	99.3^{a}	110.3^{ab}	123.4^{c}	129.8^{d}	132.0^{d}	140.2^{e}
Total glycerides mg dL^{-1}	235.6^{a}	231.5^{b}	220.7^{a}	220.8^{b}	218.6^{b}	215.4^{bc}

Note: AST= Aspartate aminotransferase, ALT = Alanine aminotransferase, TP (g dL^{-1}) = Total protein, TC (mgdL^{-1}) = Total cholesterol, TG (mg dL^{-1}) = Triglycerides

Growth and Feed Utilization Parameters

The fingerlings of African catfish quickly accepted the experimental diets. The catfish fed feed F1 had an SGR of 5.75 ± 0.04 % $day^{-1,}$ and this was significantly higher than all other SGR of catfishes fed other feeds from F2-F6 ($P < 0.05$). The catfish fed feed F2 had an SGR of 5.06 ± 0.05 % day^{-1}. There were no significant differences between the SGR of catfish fed feed F2 and those provided feed F3, 5.00 ± 0.15 % day^{-1} ($P > 0.05$). The catfish SGR was reduced by reducing the inclusion of hibiscus in the catfish diets (Table 5). The SGR % day^{-1} of the catfish fed hibiscus supplemented diets was higher than the control diet F6.

Cite this document as Enyidi, U.D. and Ayogu, I., 2023. Substitution of *Hibiscus sabdariffa* with *Curcuma longa* in the Diets of *Clarias gariepinus* and the Effects on the Growth, Nutrient, and Hematobiochemistry. *Journal of Aquaculture and Fish Health*, *12*(2), pp.269-280.

Table 4. Growth performances of *C. gariepinus* fingerlings fed with diets varying in the inclusion of hibiscus and turmeric for 60 days.

Feed	In Wt (g)	Final Wt (g)	Wt gain(g)	SGR (%)	FCR	PER	Protein%	DFIg
F1	27.0±0.07	58.65±0.-4[a]	31.65±0.13[a]	5.76±0.04[a]	1.01±0.01[a]	0.96±0.07[a]	32.69	32
F2	23.0±0.03	43.93±0.12[c]	20.93±0.05[b]	5.06±0.05[b]	1.52±0.05[b]	0.64±0.12[b]	32.42	32
F3	33.24±0.12	53.36±0.20[b]	20.12±0.12[c]	5.00±0.15[c]	1.78±0.02[c]	0.62±0.07[b]	32.67	36
F4	33.36±0.11	39.1±0.07[d]	5.74±0.07[d]	2.91±0.78[d]	2.61±0.01[d]	0.24±0.13[d]	33.47	15
F5	26.61±0.09	31.97±0.04[e]	5.37±0.04[d]	2.80±0.45[d]	2.79±0.05[d]	0.15±0.09[e]	33.82	15
F6	22.12±0.55	31.45±0.01[e]	9.33±0.09[e]	3.72±0.08[e]	1.71±0.04[c]	0.27±0.05[d]	33.83	16

Where: Wt is in g, SGR is % day^{-1}, crude protein is in %, feed intake is in g day^{-1}, protein is fed protein.

There were different effects of the inclusion of hibiscus and turmeric on the growth rate of the catfish. The results of the inclusion levels of the hibiscus on the SGR of the fish showed a positive correlation (Fig. 1), $R^2 = 0.68$. Conversely, the SGR of the catfish was reduced with increases in the inclusion of turmeric supplements (Fig. 2), $R^2 = 0.68$. The catfish grew fast with the experimental diet. The growth rate of the fish shows that the inclusion of hibiscus and turmeric supplements was beneficial to African catfish. The effects of hibiscus in this research are in line with Fagbenro (2005) and Adewole (2014), who noted that African catfish *C. gariepinus* grew well when hibiscus was added to their feed. The faster growth rate of the catfish fed with diets containing high hibiscus meals could be due to the high lipid deposit on the fish and the high total glyceraldehyde. It seems that the antioxidative effects of the feed were energy saving for the catfish, therefore diverting energy for growth.

The growth performances of catfish fed with F1 to F3 indicates that catfish grew better at higher hibiscus inclusion than turmeric. Similarly, our result agrees with Sodamola *et al*. (2016), who stated that the addition of 7.5% turmeric in the diets of *C. gariepinus* increased the growth rate. The growth rate of *C. gariepinus* has been reported to be increased by the dietary inclusion of *Telfairia occidentalis* leaf meal (Ochokwu *et al*., 2020).

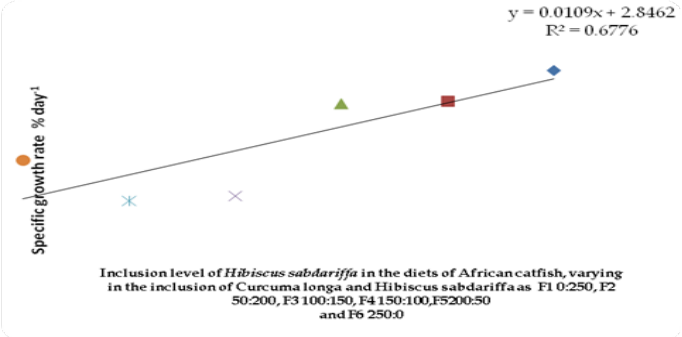

Figure 1. The effects of increasing inclusion levels of *H. sabdarifa* on the SGR of *C. gariepinus* provided 6 feeds varying in inclusions of hibiscus and turmeric supplements.

Cite this document as Enyidi, U.D. and Ayogu, I., 2023. Substitution of *Hibiscus sabdariffa* with *Curcuma longa* in the Diets of *Clarias gariepinus* and the Effects on the Growth, Nutrient, and Hematobiochemistry. *Journal of Aquaculture and Fish Health*, *12*(2), pp.269-280.

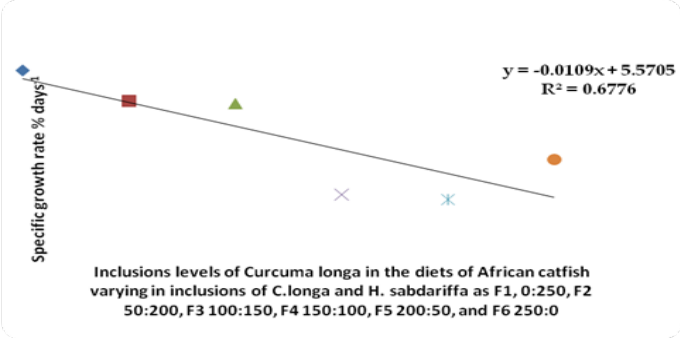

Figure 2. The effects of increasing inclusion levels of *C. longa* on the SGR of *C. gariepinus* provided 6 feeds varying in inclusions of hibiscus and turmeric supplements.

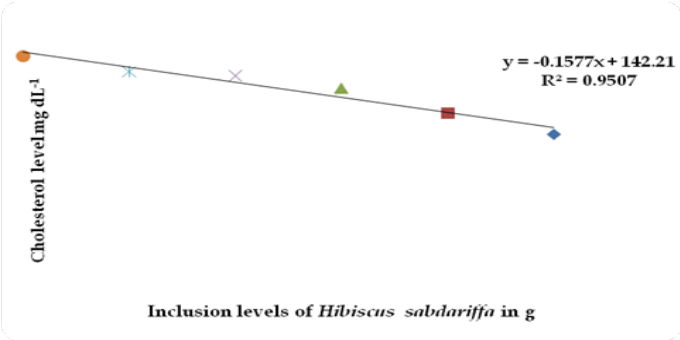

Figure 3. The effects of inclusion levels of hibiscus supplement on the cholesterol level of *C. gariepinus* fed for 60 d with 6 types of feed varying in *Curcuma longa* and *H. sabdariffa* supplements as follows: F1 0:250, F2 50:200, F3 100:15, F4 150:100, F5 200:50 and F6 250:0.

Catfish fed feed F1 had the lowest FCR of 1.01±0.01. The FCR of feed F1 was significantly lower than all treatment feeds ($P<0.05$). The FCR of catfish fed feed F1 was especially ($P<0.05$) much lower than those of catfish fed F2 (1.52 ± 0.05), and also those fed feed F3, 1.78 ± 0.02. There were, however, no significant differences between the FCR of catfish fed with F3 and the control feed F6, 1.71 ± 0.04. Generally, the FCR of the catfish was reduced by reducing the inclusion of hibiscus meals in the diets (Table 5). Hibiscus inclusion in the diets affected the weight gain of the fish. Catfish fed high hibiscus meals gained more weight than those that received less. The weight gains of feeds, F1, F2, and F3, were 31.65 ± 0.13 g, 20.93 ± 0.05 g, and 20.12 ± 0.12 g, respectively (Table 5).

The weight increases we noted in this experiment supported, Al-Sultan and Gameel (2004) and Rahmadani *et al.* (2016) who stated that adding turmeric to the diets of *C. gariepinus* did not result in any liver damage. Moreover, Dewi *et al.* (2020) stated that turmeric powder included in catfish diets at a dose of 480 mg/100 g increases the body weight, egg vitellogenin deposition, and gonad development. The better growth of fish in our experiment could be because hibiscus is antioxidant while turmeric is a phytogenic antibiotic growth promoter. The protein efficiency ratio (PER) of the catfish was highest for those catfish fed with feeds having a higher inclusion rate of hibiscus than turmeric (Table 5). There were, however, no significant differences ($P<0.05$) between the daily feed intake of catfish-fed feed containing a high percentage of hibiscus meals (Table 5). Similarly, the DFI of the catfish was similar for those fed with high turmeric supplemented ($P>0.05$).

Cite this document as Enyidi, U.D. and Ayogu, I., 2023. Substitution of *Hibiscus sabdariffa* with *Curcuma longa* in the Diets of *Clarias gariepinus* and the Effects on the Growth, Nutrient, and Hematobiochemistry. *Journal of Aquaculture and Fish Health*, *12*(2), pp.269-280.

Physiochemical Analysis of Water

The physiochemical parameters of the culture water are recorded in Table 3. The culture water dissolved oxygen content ranged from 7.0 ± 0.05 mg L^{-1} to 8.4 ± 0.06 mg L^{-1}. The pH ranged from 5.04±0.05 to 5.52 ± 0.06mg L^{-1}. The ammonia content of the water was from (1.0 ± 0.06 mg L^{-1} to 1.5 ± 0.08 mg L^{-1}). Nitrate content ranged from 2 ± 0.04 mg L^{-1} to 5.0 ± 0.01 mg l^{-1}. The dissolved phosphorous content of the water ranged from 2.56 ± 0.12 mg L^{-1} to 3.95 ± 0.77 mg L^{-1} (Table 6).

Table 5. Physiochemical parameters of culture water used in rearing *C. gariepinus* fed diets varying in the inclusion of hibiscus and turmeric for 60 days.

Parameters	F1	F2	F3	F4	F5	F6	Reference
pH	5.4±0.02	5.5±0.06	5.4± 0.01	5.1 ±07	5.0±0.05	5.2±0.02	5.12[1]
Turbidity	100 ± 0.6	100±0.09	100 ±0.02	100 ±0.07	100±0.05	100±0.07	100[1]
DO (mg L^{-1})	7.0±0.05	7.8±0.07	8.4 ±0.06	7.5 ±0.02	8.1±0.01	7.5±0.14	7-8[2]
Ammonia (mg L^{-1})	1.3±0.04	1.0 ±0.06	1.5±0.08	1.2 ±0.06	1.02±0.08	1.4±.05	8.8[3]
Nitrate (mg L^{-1})	3.0±0.12	2±0.04	5.±0.01	2.0 ±0.07	3.4 ±0.04	5.0±0.01	200[4]
Phosphorus (mg L^{-1})	3.95±0.77	2.99±0.03	2.8 ±0.12	2.56±0.12	3.00±0.08	3.89±0.04	5.8[5]

Note: [1]Enyidi and Agi (2016), [2]Zidni *et al*. (2019), [3]Eding and Kamstra (2001), [4]Bovendeur *et al*. (1987), [5]Ariffin *et al*. (2019).

The recorded data showed that the physiochemical parameters of the culture water were within acceptable values and in line with Hussain *et al*. (2014), Ariffin *et al*. (2019), and Zidni *et al*. (2019) who had similar water quality. The water quality could have supported the growth rate of the fish especially as the water was changed daily.

CONCLUSION

Hibiscus and turmeric meal supplement support the fast growth rate of *C. gariepinus*. Weight gain increased with increasing inclusion of hibiscus meal than turmeric—hibiscus supplement lower FCR, total triglycerides, and cholesterol.

ACKNOWLEDGEMENT

We are grateful to Mr. Agbakwuru of the College of Veterinary Medicine Michael Okpara University for his help in laboratory analysis. We are also thankful to the Pathology Department University of Nigeria Teaching Hospital for hematological analysis and interpretation.

REFERENCES

Abdel-Tawwab, M. and Abbass, F.E., 2016. Turmeric powder, *Curcuma longa* L., in common carp, *Cyprinus carpio* L., diets: Growth performance, innate immunity, and challenge against pathogenic *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society*, *48*(2), pp.303-312. https://doi.org/10.1111/jwas.12349

Adewole, A.M., 2014. Effects of roselle as dietary additive on growth performance and production economy of *Clarias gariepinus*. *Journal of Emerging Trends in Engineering and Applied Sciences*, *5*(7), pp.1-8. https://hdl.handle.net/10520/EJC156945

Al-Sultan, S.I. and Gameel, A.A., 2004., Histopathological changes in the livers of broiler chicken supplemented with turmeric (*Curcuma longa*). *International Journal Poultry Science*, *3*(5), pp.333-336. https://doi.org/10.3923/ijps.2004.333.336

Ariffin, F.D., Halim, A.A., Hanafiah, M.M., Awang, N., Othman, M.S., Azman, S.A.A. and Bakri, N.S.M., 2019. The effects of African Catfish, *Clarias gariepinus* pond farm's effluent on water quality of Kesang River in Malacca, Malaysia. *Applied Ecology and Environmental Research*, *17*,

Cite this document as Enyidi, U.D. and Ayogu, I., 2023. Substitution of *Hibiscus sabdariffa* with *Curcuma longa* in the Diets of *Clarias gariepinus* and the Effects on the Growth, Nutrient, and Hematobiochemistry. *Journal of Aquaculture and Fish Health*, *12*(2), pp.269-280.

pp.1531-1545. https://doi.org/10.15666/aeer/1702_15311545

Bovendeur, J., Eding, E.H. and Henken, A.M., 1987. Design and performance of a water recirculation system for high-density culture of the African catfish, *Clarias gariepinus* (Burchell 1822). *Aquaculture*, *63*(1-4), pp.329–353. https://doi.org/10.1016/0044-8486(87)90083-4

Chan, E.W.C., Lim, Y.Y., Wong, S.K., Lim, K.K., Tan, S.P., Lianto, F.S. and Yong, M.Y., 2009. Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. Food Chemistry, *113*(1), pp.166–172. https://doi.org/10.1016/j.foodchem.2008.07.090

Cisse, M., Dornier, M., Sakho, M., Ndiaye, A., Reynes, M. and Sock, O., 2009. The bissap (*Hibiscus sabdariffa* L.): composition and principal uses. *Fruits*, *64*, pp.179-193. https://doi.org/10.1051/fruits/2009013

Deng, J., Mai, K., Ai, Q., Zhang, W., Wang, X., Tan, B., Xu, W., Liufu, Z. and Ma, H., 2010. Interactive effects of dietary cholesterol and protein sources on growth performance and cholesterol metabolism of Japanese flounder (*Paralichthys olivaceus*). *Aquaculture Nutrition*, *16*(4), pp.419-429. https://doi.org/10.1111/j.1365-2095.2009.00681.x

Dewi, C.D., Manalu, W., Ekastuti, D.R. and Sudrajat, A.O., 2020. The role of turmeric (*Curcuma longa*) powder in improving liver function to increase vitellogenin synthesis and deposition in the oocytes of catfish (*Pangasianodon hypophthalmus*). *Jordan Journal of Biological Sciences*, *13*(3), pp.357-362. https://jjbs.hu.edu.jo/files/vol13/n3/Paper%20Number%2014.pdf

Eding, E. and Kamstra, A., 2001. Design and performance of recirculation systems for European eel *Anguilla anguilla* and African catfish *Clarias gariepinus*. *In Proceedings of the AES Workshop, Orlando, FL, USA*, pp.18–28.

El Mesallamy, A.M.D., Ahmad, M.H., Souleman, A.M.A., El Morsy, A.T. and El-Naby, A.S.A., 2016. Effects of roselle calyx (*Hibiscus sabdariffa* L.)-supplemented diets on growth and disease (*Aeromonas hydrophila*) resistance in Nile tilapia (*Oreochromis niloticus* L.). *Egypt Pharmaceutical Journal*, *15*(2), pp.78-87. https://doi.org/10.4103/1687-4315.190403

Enyidi, U.D., 2012. Production of feeds for African catfish *Clarias gariepinus* using plant proteins. *Jyväskylä Studies in Biological Sciences*, *251*, pp.1456-9701. https://jyx.jyu.fi/bitstream/handle/123456789/40445/1/978-951-39-4925-9.pdf

Enyidi, U.D. and Agi, P.I., 2016. Analyses of pH, dissolved oxygen, and turbidity of aquatic ecosystems at Bori, Gokana, Okirika, Tai and Eleme areas of rivers State Nigeria. *Fundamental Journal of Applied Sciences*, *1*(1), pp.1-18. https://frdint.com/analyses_of_ph_dissolved_oxygen_and.pdf

Enyidi, U.D. and Orji, C.P., 2020. Effects of *Curcuma longa* on growth and biochemical variables of *Oreochromis niloticus* challenged with a consortium of bacteria. *Journal of Aquatic Sciences*, *35*(2), pp.229-244. https://doi.org/10.431 4/jas.v35i2.25

Fagbenro, O.A., 2005. Soybean meal substitution with rosella (*Hibiscus sabdariffa* L.) seed meal in dry practical feed diets for fingerlings of the African catfish, *Clarias gariepinus* (Burchell 1822). *Journal of Animal and Veterinary Advances*, *4*(4), pp.473-477. https://medwelljournals.com/abstract/?doi=javaa.2005.473.477

Guerra-Olvera, F.M. and Viana, M.T., 2015. Effect of dietary cholesterol content on growth and its accumula-

Cite this document as Enyidi, U.D. and Ayogu, I., 2023. Substitution of *Hibiscus sabdariffa* with *Curcuma longa* in the Diets of *Clarias gariepinus* and the Effects on the Growth, Nutrient, and Hematobiochemistry. *Journal of Aquaculture and Fish Health*, *12*(2), pp.269-280.

tion in liver and muscle tissues of juvenile yellowtail kingfish (*Seriola lalandi*). *Ciencias Marinas*, *41*(2), pp.143–156. https://doi.org/10.7773/cm.v41i2.2514

Hirunpanich, V., Utaipat, A., Morales, N.P., Bunyapraphatsara, N., Sato, H., Herunsale, A. and Suthisisang, C., 2006. Hypocholesterolemic and antioxidant effects of aqueous extracts from the dried calyx of *Hibiscus sabdariffa* L. in hypercholesterolemic rats. *Journal of Ethnopharmacology*, *103*(2), pp.252-260. https://doi.org/10.1016/j.jep.2005.08.033

Hussain, T., Verma, A.K., Tiwari, V.K., Prakash, C., Rathore, G., Shete, A.P. and Saharan, N., 2014. Effect of water flow rates on growth of *Cyprinus carpio* var. *koi* (*Cyprinus carpio* L., 1758) and spinach plant in aquaponic system. *Aquaculture International*, *23*, pp.369–384. https://doi.org/10.1007/s10499-014-9821-3

Iheanacho, S.C., Ogunji, J.O., Ogueji, E.O., Nwuba, L.A., Nnatuanya, I.O., Ochang, S.N., Mbah, C.E., Usman, I.B. and Haruna, M., 2017. Comparative assessment of ampicillin antibiotic and ginger (*Zingiber officinale*) effects on growth, haematology and biochemical enzymes of *Clarias gariepinus* juvenile. *Journal of Pharmacognosy and Phytochemistry*, *6*(3), pp.761-767. https://www.phytojournal.com/archives/2017/vol6issue3/PartL/6-3-147-191.pdf

Moreau, R.A., Whitaker, B.D. and Hicks, K.B., 2002. Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. *Progress in Lipid Research*, *41*(6), pp.457-500. https://doi.org/10.1016/S0163-7827(02) 00006-1

Ochokwu, I.J., Umaru, J. and Saidu, K.A., 2020. Growth and nutrient utilization of *Clarias gariepinus* fingerlings (Burchell, 1822) fed *Telfairia occidentalis* leaf meal as feed additive. *International Journal of Fisheries and Aquaculture Research*, *6*(2), pp.1-12. https://doi.org/10.33997/j.afs.2018.31.1.002

Qi, Y., Chin, K.L., Malekian, F., Berhane, M. and Gager, J., 2005. Biological characteristics, nutritional and medicinal value of roselle, *Hibiscus sabdariffa*. *CIRCULAR-Urban forestry natural resources and environment*, *604*, pp.1-2. https://www.suagcenter.com/assets/suag/Sustainable_Agricultural_Systems/PDFs/hibiscus.pdf

Rahmadani, T.B.C., Jusadi, D., Setiawati, M. and Hastuti, Y.P., 2020. The evaluation of turmeric (*Curcuma longa*) supplementation within feed as an antioxidant towards growth performance of catfish *Clarias gariepinus* Burchell 1822 in zero water exchange condition. *Jurnal Iktiologi Indonesia*, *20*(2), pp.105-115. https://doi.org/10.32491/jii.v20i2.518

Rico, A., Phu, T.M., Satapornvanit, K., Min, J., Shahabuddin, A.M., Henriksson, P.J.G., Francis, J.M., Little, D.C., Dalsgaard, A. and Van den Brink, P.J., 2013. Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. *Aquaculture*, *412–413*, pp.231-243. doi.org/10.1 016/j.aquaculture.2013.07.028

Robert, S.M., 2005. *Roselle production*. Botanical description.

Singh, P., Khan, M. and Hailemariam, H., 2017. Nutritional and health importance of *Hibiscus sabdariffa*: a review and indication for research needs. *Journal of Nutritional Health & Food Eng*ineering, *6*(5), pp.125-128. https://doi.org/10.15406/jnhfe.2017.06.00212

Sodamola, M.O., Jimoh, W.A., Adejola, Y.A., Akinbola, D.D., Olanrewaju, A. and Apiakason, E., 2016. Effect of

Cite this document as Enyidi, U.D. and Ayogu, I., 2023. Substitution of *Hibiscus sabdariffa* with *Curcuma longa* in the Diets of *Clarias gariepinus* and the Effects on the Growth, Nutrient, and Hematobiochemistry. *Journal of Aquaculture and Fish Health*, *12*(2), pp.269-280.

turmeric (*Curcuma longa*) root powder (TRP) on the growth performance, hematology and serum biochemistry of African Catfish (*Clarias gariepinus*). *Academia Journal of Agricultural Research*, *4*(9), pp.593-596. https://doi.org/10.15413/ajar.2016.0312

Xu, C., Li, E., Xu, Z., Su, Y., Lu, M., Qin, J.G., Chen, L. and Wang, X., 2018. Growth and stress axis responses to dietary cholesterol in Nile Tilapia (*Oreochromis niloticus*) in brackish water. *Frontiers in Physiology*, *9*, p.254. https://doi.org/10.3389/fphys.2018.00254

Zheng, J., Cheng, J., Zheng, S., Feng, Q. and Xiao, X., 2018. Curcumin, a polyphenolic curcuminoid with its protective effects and molecular mechanisms in diabetes and diabetic cardiomyopathy. *Frontiers in Pharmacology*, *9*, 472. https://doi.org/10.3389/fphar.2018.00472

Zidni, I., Iskandar, Buwono, I.D. and Mahargyani, B.P., 2019. Water quality in the cultivation of catfish (*Clarias gariepinus*) and Nile Tilapia (*Oreochromis niloticus*) in the aquaponic biofloc system. *Asian Journal of Fisheries and Aquatic Research*, *4*(2), pp.1-6. https://doi.org/10.9734/ajfar/2019/v4i230048

Cite this document as Enyidi, U.D. and Ayogu, I., 2023. Substitution of *Hibiscus sabdariffa* with *Curcuma longa* in the Diets of *Clarias gariepinus* and the Effects on the Growth, Nutrient, and Hematobiochemistry. *Journal of Aquaculture and Fish Health*, *12*(2), pp.269-280.