GROWTH PERFORMANCE AND HAEMATO-BIOCHEMICAL RESPONSE OF *CLARIAS GARIEPINUS* JUVENILES FED *HIBISCUS SABDARIFFA* LEAF SUPPLEMENT REARED IN HAPA-IN-POND SYSTEM

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Abstract

The growth performance and haemato-biochemical response of *Clarias gariepinus* juveniles fed *Hibiscus sabdariffa* leaf supplement reared in hapa-in-pond system was evaluated. 150 Catfish juveniles were acclimatized for two weeks and were weighed and stocked into 15 hapas-in-pond system at a density of 10 (ten) fish per hapa with three replicates per treatment. Five isonitrogenous diets were formulated and supplemented by 0.5, 1.0, 1.5 and 2.0 g of *H. sabdariffa* leaf powder / 100 g feed respectively (designated as HSB 2, HSB 3, HSB 4 and HSB 5). The juveniles were fed 5% body weight on two equal proportions per day for 60 days. The growth data were collected bi-weekly and was evaluated using weight gain (g); specific growth rate; and feed conversion ratio (FCR) as indices. The haematological and biochemical indices were evaluated. Highest values were observed in weight gain and specific growth rate in HSB 5 at 2.0g/100g inclusion level of *H. sabdariffa* leaf powder while HSB 3 revealed that fish fed 1.0g/100g inclusion level of *H. sabdariffa* leaf powder showed the highest food conversion ratio (FCR) as compared to the control. Albumin and Glucose showed highest values in HSB 5 at 2.0g/100g inclusion level of *H. sabdariffa* leaf powder while Total protein was higher in HSB 2 at 1.0g/100g inclusion level of *H. sabdariffa* leaf powder. The result from the study revealed that *H. sabdariffa* leaf powder enhanced the growth performance and haemato-biochemical parameters in *C.gariepinus* juveniles reared in hapa-in-pond system and should be encouraged in fish production.

Keywords: Haematology, Biochemical, *Clarias gariepinus*,*Hibiscus sabdariffa*, hapa-in-pond system.

Introduction

The African catfish *C.gariepinus* is a major cultivated fish of high commercial value in Nigeria and is ideal for captive breeding (Adesulu and Syndeham, 2007) but many limitations are associated with fry production and the development of better broodstock management techniques is crucial for improvement of fry yield and system efficiency. Development of fish seeds production has been identified as a rational way of augmenting the dwindling fish supply from the capture fisheries (Dada and Fagbenro, 2008). Roselle (*Hibiscus sabdariffa*) is an annual dicotyledonous, erect, herbaceous tropical plant. The plant is cultivated majorly in the northern part of Nigeria as edible vegetable and considered to be medicinal (Ijeomah *et al.,* 2012). The chemical constituents of the flower include the flavonoids, gossypetine and sabdaretine (Pietta, 2000). Roselle is reported to be diuretic, digestive, antiseptic, sedative, purgative, emollient, demulcent and astringent (Adewole, 2014). The calyces have many medicinal applications in curing kidney stone, pyrexia, liver damage, hypertension and leukemia (Abu-Tarboush *et al.,* 1997; Estrella *et al.,* 2000). The development of low-cost feed is imperative in order to witness expansion and profitability of aquaculture enterprise in Nigeria and other developing countries (Fagbenro *et al.,* 2003). Natural materials such as medicinal plants could be widely accepted as feed additives to enhance feed utilization and aquaculture productive performance and sustainability (Levic *et al.,* 2008).

Materials and Methods

Study Site

The study was carried out in the Teaching and Research farm of the Department of Fisheries Technology, Federal College of Agriculture, Akure, Ondo State, Nigeria.

Study Fish

One hundred and fifty (150) *C. gariepinus* juveniles were used for the experiment. They were transported in a 50L gallon filled with water to the Research farm of the Department of Fisheries Technology, Federal College of Agriculture, Akure, Ondo State, Nigeria. The fish were acclimated and distributed into hapa nets in pond (1 m × 1 m × 0.6 m), filled with water and acclimatized to the experimental conditions for 2 weeks, during which they were fed the test diets. During acclimatization, the average body weight and length of the fish were measured using sensitive scale and meter rule respectively.

Collection, Preparation and Processing of Roselle flower (*Hibiscus sabdariffa*)

The Roselle leaves were purchased from Oja-Oba Market located at Akure, Ondo State, Nigeria. It was ground into powder, using electric blender, sieved, and stored in an air tight container until use.

Experimental Design

Five isonitrogenous diets were formulated from practical ingredients where the control basal diet were without the *H. sabdariffa* leaf powder (HSB 1) and the other diets were supplemented by

0.5, 1.0, 1.5 and 2.0 g *H. sabdariffa* leaf powder / 100 g feed respectively (designated as HSB 2, HSB 3, HSB 4 and HSB 5). The experimental diets were formulated to contain almost 40% crude protein. All dietary ingredients were weighed with a weighing top load. The ingredients were milled to a 1.8 mm particle size. Ingredients including vitamin premix and *H. sabdariffa* leaf powder were thoroughly mixed to obtain a homogenous mass, cassava starch was added as a binder. The resultant mash was then pressed without steam. The pellets were dried and stored in a refrigerator until the start of the experiment.

Experimental Procedure

The *Clarias gariepinus* juveniles of an average weight of 32.31 ±1.52 were stocked into 15 hapas-in-pond system (1 × 1 × 0.6 m) at a density of 10 (ten) fish per hapa with three replicates per treatment. The diets were fed to the juveniles at a daily rate of 5% body weight (BW), twice a day (09:00 and 16:00 h) for 60days. Fish were weighed collectively at bi-weekly intervals, their average weights were recorded and the daily amount of feed for each hapa-in-pond was readjusted accordingly. The hapa nets and ponds were washed weekly, and about 50% of the culture water was replaced with fresh well water. Water quality parameters including dissolved oxygen, pH and temperature were monitored weekly.

Water Quality Parameters

Water quality parameters such as temperature, pH, and dissolved oxygen concentration were monitored weekly during the period of the study using mercury-in-glass thermometer, pH meter (Hanna H198106 model) and dissolved oxygen meter (JPP- 607 model) as described by APHA (2005).

Growth Parameters

Weight measurement of the fish was obtained at the end of the experiment and the following growth parameters were determined according to the formula of Iheanacho *et al.* (2018): Mean weight gain (MWG); Specific growth rate (SGR); Food conversion ratio (FCR);

Mean weight gain (MWG) = final weight - initial weight

$$\text{Specific growth rate (SGR)} = \frac{(\text{Ln mean final weight} - \text{Ln mean initial weight})}{\text{Time (days)}} \times 100$$

Ln = Natural logarithm

$$\text{Food conversion ratio (FCR)} = \frac{\text{Weight of food fed (Dry gram weight)}}{\text{Weight gain of fish (Wet gram weight)}}$$

Haemato-Biochemical Analysis

Three fish per hapa-in-pond were sampled for blood collection at the end of the experiment. Blood was collected from the caudal vein into an EDTA lithium tubes. Blood samples were immediately transported to the haematology laboratory. The blood was analyzed to determine the packed cell value (PCV) with micro haematocrit using heparinised capillary tube (25 mm), while

red blood cell (RBC), white blood cell (WBC) counts, haemoglobin (Hb) concentration. Biochemical examination of the catfish juveniles was performed. The body surface were cleaned and blotted dry with adsorbent paper. Blood samples were collected from the caudal vein using disposable 3-c syringes and 21-gauge needles, and were transferred into vacuette tubes containing K2EDTA solution as an anticoagulant for determination. Total protein, Albumin and Glucose was determined.

Statistical Analysis

Data obtained from the experiment were subjected to one-way analysis of variance (ANOVA), using Statistical Package for Social Science (SPSS 2006, version 22). Duncan multiple range test (DMRT) was used to compare the differences between means at $p<0.05$. Data were presented as mean ± SE.

Results and Discussion

Water quality parameters

The water quality parameters measured varied as follows: temperature; 26.15 to 26.30^{0}C, dissolved oxygen; 6.21 - 6.85 mg/l, and hydrogen ion concentration (pH); 8.9 – 9.5, for the experiment.

Table 1: Ingredient Composition (g/100g) of the Experimental diets fed to experimental fish

	Experimental Diets				
Ingredients	**HSB 1**	**HSB 2**	**HSB 3**	**HSB 4**	**HSB 5**
Fishmeal (65% cp)	25	25	25	25	25
Soy bean (45% cp)	40	40	40	40	40
Yellow Maize	15	15	15	15	15
Blood meal (85% cp)	5	5	5	5	5
Fish oil	4	4	4	4	4
Vegetable Oil	6	5.5	5	4.5	4
Vit premix	3	3	3	3	3
Binder	2	2	2	2	2
H. sabdariffa leaf powder	0.5	1.0	1.5	2.0	

Mineral-vitamin premix**- An Animal Care Optimix Aqua product for catfish, containing the following per 5kg of premix: A = 20,000,000 I.U, D3 – 2,000,000 I.U, E – 200,000 mg, K3 = 10,000 mg, B2 = 12,000 mg, B12 = 9mg, B1 = 6,000 mg, B6 = 11,000 mg, C = 50,000 mg, folic acid = 2,000 mg, Niacin = 80,000 mg, Calpan = 25,000 mg, Biotin = 100 mg, x Zinc = 30,000 mg, Copper = 5,000 mg, Iron = 30,000 mg, Manganese = 50,000 mg, Iodine = 1,000 mg, Selenium = 100 mg, antioxidant = 125,000 mg.

Table 2: Proximate composition (% Dry matter) of experimental diets

	Experimental diets				
Parameters	**HSB1**	**HSB 2**	**HSB 3**	**HSB 4**	**HSB 5**
Crude protein	40.27±0.02[a]	40.35±0.01[a]	40.38±0.03[a]	41.31±0.02[b]	41.48±0.02[b]
Crude lipid	11.63±0.03[a]	11.75±0.03[a]	12.31±0.01[b]	12.38±0.01[b]	12.43±0.01[b]
Crude fibre	3.25±0.04[a]	3.32±0.03[a]	3.47±0.03[a]	4.12±0.01[b]	4.24±0.04[b]
Ash	10.02±0.03[a]	9.20±0.08[b]	9.01±0.06[b]	10.00±0.01[a]	10.01±0.03[a]

Mean on the same row without superscript are not significantly different (p>0.05) from each other.

Table 3. Growth response of *C. gariepinus* juvenile fed *H. sabdariffa* supplemented diets

Parameter	**HSB 1**	**HSB 2**	**HSB 3**	**HSB 4**	**HSB 5**
Initial weight (g)	32.46±0.20[a]	32.27±0.52[a]	32.08±0.57[a]	35.28±0.28[a]	32.34±0.11[a]
Final weight (g)	61.52±1.43[ab]	52.01±1.21[b]	61.95±2.45[ab]	53.72±2.38[ab]	65.53±0.34[a]
Weight gain (g)	29.06±1.25[ab]	19.74±1.38[b]	29.87±5.07[ab]	18.44±2.10[ab]	33.19±0.43[a]
FCR	1.01±0.12b	1.16±0.17a	1.21±1.19a	1.09±0.26ab	0.87±0.42b

Values in the same rows with the same alphabet superscript are not significantly different (p>0.05).

Table 4. Haemato-biocemical data of *C. gariepinus* fed *H. sabdariffa* supplemented diets

Parameter	HSB 1	HSB 2	HSB 3	HSB 4	HSB 5
PCV (%)	30.23±2.61[ab]	25.00±3.15[ab]	25.37±0.68[b]	27.01±3.26[ab]	33.23±0.66[a]
HB (g.dL-1)	8.63±0.92[a]	7.65±1.23[a]	7.31±0.65[a]	8.25±1.03[a]	10.58±0.67[a]
RBC (10^{12} L)	3.53±0.12[ab]	3.05±0.33[b]	3.26±0.08[ab]	3.39±0.45[ab]	4.02±0.04[a]
WBC(10^{9} L)	4.20±0.25[b]	6.40±0.28[a]	5.69±0.16[a]	6.21±0.28[a]	6.37±0.34[a]
Total protein (g/dl)	4.78±0.64[b]	6.13±0.10[a]	5.12±1.05[d]	5.23±1.56[e]	5.20±0.06[c]
Albumin (g/dl)	1.25±0.71[b]	0.57±0.22[d]	0.65±0.45[c]	1.16±0.04[a]	1.32±0.25[d]
Glucose (g/dl)	63.50±4.32[a]	82.61±12.00[c]	87.55±12.18[b]	92.37±14.22[e]	110.27±11.05[d]

PCV, Packed cell value; Hb, haemoglobin; RBC, red blood cell; WBC, Values in the same rows with the same alphabet superscript are not significantly different (p>0.05).

Results and Discussion

The results in Table 2 revealed the proximate composition of the experimental diets where there were higher percentages of crude protein (41.48±0.02), crude lipid (12.43±0.01), crude fibre (4.24±0.04) and ash (10.01±0.03) in HSB 5 fed diets containing 2.0g /100g of *H. sabdariffa* leaf powder. The increase across diets could be attributed to the inclusion level of *H. sabdariffa* leaf

powder in the experimental diets. Table 3 reveals the growth response of *C. gariepinus* juvenile fed *H. sabdariffa* supplemented diets. According to Reverter *et al.* (2014), medicinal plants have been reported to be an essential growth promoter in fish nutrition. Highest values were observed in weight gain in HSB 5 (33.19±0.43) at 2.0g/100g inclusion level of *H. sabdariffa* leaf powder as compared to the control (29.06±1.25). The findings revealed the increase in the final weight of the *C.gariepinus* juveniles across the treatments. The specific growth rate (SGR) were observed to be highest in HSB 5 (3.11±0.21) with the fish fed 2.0g/100g inclusion level of *H. sabdariffa* leaf powder and lowest in HSB 2 (2.53±0.01) with the fish fed 0.5g/100g inclusion level of *H. sabdariffa* leaf powder whereby HSB 3(1.21±1.19) revealed that fish fed 1.0g/100g inclusion level of *H. sabdariffa* leaf powder showed the highest food conversion ratio (FCR) as compared to the control. Adewole (2014) reported significant increases ($p<0.05$) in growth parameters (final weight, weight gain, specific rate and relative growth rate) in *C. gariepinus* fed roselle supplemented diets when compared with the control. Buddington *et al.* (1997) reported that carnivorous and omnivorous fish take longer time to digest plant protein-based diets, hence the significant differential growth recorded. The present study revealed that there were significant changes in the haematological and biochemical parameters in the *C. gariepinus* juveniles used in the study as shown in Table 4. PCV, HB and RBC values were highest in HSB 5 at 2.0g/100g inclusion level of *H. sabdariffa* leaf powder with 33.23±0.66, 10.58±0.67, 4.02±0.04 values respectively. WBC had the highest value on HSB 2 (6.40±0.28) at 0.5g/100g inclusion level of *H. sabdariffa* leaf powder. Total protein was higher in HSB 2 (6.13±0.10) as compared to the control, while Albumin and Glucose showed highest values in HSB 5 at 2.0g/100g inclusion level of *H. sabdariffa* leaf powder with 1.32±0.25 and 110.27±11.05 values respectively. This revealed that *H. sabdariffa* leaf powder enhanced the haemato-biochemical parameters in *C.gariepinus* juveniles.

Conclusion

The present study revealed that *H. sabdariffa* leaf powder enhance the growth, haematology and biochemical indices of *C. gariepinus* juveniles at 2.0g/100g inclusion level reared in hapa-in-pond system. This medicinal plant should be encouraged by fish farmers as it is cost effective in order to reduce the cost of feed in fish production.

References

Abu-Tarboush H.M, Ahmed S.A.B., Alkahtani H.A. (1997). Some nutritional properties of karkade (*Hibiscus sabdariffa*) seed products. *Cereal Chem.* 74:352-355

Adesulu, E.A. and Syndeham, D.H., (2007). The Freshwater Fishes and Fisheries of Nigeria, Macmillian Publishing Company, Ibadan, Nigeria. pp49.

Adewole A.M. (2014). Effects of roselle as dietary additive on growth and production economy of *Clarias gariepinus*. *J. Emerg. Trends Eng. Appl. Sci.* 5(7):1-8.jeteas.

Buddington RK, Krogdahl A and Bakke-McKellep AM. (1997). The intest ines of carnivorous fish structure and functions and the relations withdiet. *Acta Physiol. Scand. Suppl.,* 161: 67-80.

Dada A.A. and Fagbenro O.A. (2008): Catfish fingerlings production in Nigeria. *Proceedings of the 4th Annual Conference of School of Agriculture and Agricultural Technology,* Federal University of Technology, Akure. P. 107-110.

Estrella M.C.P, Mantaring J.B.V, David GZ (2000). A double blind randomized, controlled trial on the use of malunggay (*Moringa oleifera*) for augmentation of the volume of the breast milk among nonnursing mothers of preterm infants. *Philippine J. Ped.* 49:306.

Fagbenro OA, Akande TT, Fapounda OO. (2003). Use of Roselle (*Hibiscus Sabdariffa*) seed meal as a soybean meal replacer in practical diets for fingerlings of African Catfish (*Clarias gariepinus*). *P. 3rd Int. Conf., Cotonou*, Benin, 10-14 November, 2003, pp. 73-79.

Ijeomah A.U, Ugwuona F.U, Abdullah H, (2012). Phytochemical composition and antioxidant properties of *Hibiscus sabdariffa* and *Moringa oleifera*, *Nigerian J. Agric. Environ*. 8(1):10-16.

Levic J.G, Sinisia M.G, Djuragic O, Slavica S. (2008). Herbs, organic acids as alternative to antibioticgrowth promoter. *Arch. Zootechn.* 11:15-1

Reverter M, Bontemps N, Lecchini D, Banaigs B, Sasal P (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current statue and future perspectives. Aquaculture 433:50-61.