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Effects of herbal plants (*Zingiber officinale* and *Hibiscus sabdariffa*) as dietary additives on serum biochemistry and some metabolites in *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

Objective: To evaluate the effect of *Zingiber officinale* (ginger) and *Hibiscus sabdariffa* (roselle) as dietary supplements on serum biochemistry and some metabolites (urea and creatinine) in *Clarias gariepinus* (*C. gariepinus*).

Methods: A total of 150 *C. gariepinus* juveniles [initial weight (35.41 ± 1.53) g] were randomly assigned to five test diets. Ginger and roselle were added to diets as additives at 2% and 4% respectively while control diet contained none of the substances. Blood samples were collected from treatment groups for biochemical analysis after 70 days feeding trial.

Results: Significant changes ($P < 0.05$) were observed in serum enzymes (alkaline phosphatase, alanine amino transferase and aspartate amino transferase) activities. Significant increase ($P < 0.05$) in serum total protein was noticed in treated fish when compared to control. Relative changes in urea and creatinine levels were observed in fish fed with roselle based diets while insignificant changes ($P > 0.05$) were seen in ginger treated fish.

Conclusions: The present study revealed that ginger and roselle added to the diets as additives had no adverse effect on the examined serum biochemistry in *C. gariepinus*.

1. Introduction

The use of herbal plants for different purposes predates medical industrialisation[1]. Evolution of red technology phased out the use of herbs for medication and nutrition both in human and animals[2,3]. It was reported that plant medicine became a renewed area of interest due to the side effects and high cost of synthetic drugs[4]. Herbs are valuable for the prevention and control of fish diseases in aquaculture, enhancing growth, humoral and cellular response in both specific and non-specific ways[3,5-7]. Herbal plants are

antimicrobial, antifungal and immunostimulants, comprising groups of biological and chemical compounds that enhance non-specific defense mechanisms in animals[8]. It was reported that natural plant products are of various activities such as anti-stress, growth promotion, appetite stimulation, immune-stimulation, aphrodisiac and antimicrobial properties due to the active principles such as alkaloids, phenolics, terpenoids, flavonoids pigments, essential oils and steroids. Herbal plants have been found to be of great economic importance and are useful in the area of medicine, pharmacology and in both human and animal nutrition[2].

The application of dietary medicinal herbs as immune-stimulants can enhance the innate defense mechanisms of fish against pathogens during periods of stress, such as intensive farming practices, grading, sea transfer, vaccination and reproduction[9]. Various studies have monitored the immunological parameters after intraperitoneal injection or oral administration of plant extracts

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in distinct fish species and they found that treated fish showed increased lysozyme activity, phagocytic activity, complement activity, increased respiratory burst activity and increased plasma proteins *i.e.* globulin and albumin[10-12]. Ginger as a phytobiotic is one of the most effective natural immune-stimulants[9]. Ginger fed fish exhibited significantly increased total immune globin levels, suggesting better immune competence[13]. Roselle supplemented diets exhibited antimicrobial activity and enhanced growth of *Clarias gariepinus* (Burchell, 1822) (*C. gariepinus*) after 12 weeks feeding trial[14].

African catfish *C. gariepinus* of the family Clariidae is common in Nigerian fresh waters and also an important aquaculture species[15]. African catfish remains the preferred species for culture especially in Nigeria due to its high cultivability, hardy nature, high survivorship, reproductive ability in captivity, fast growth rate and ability to accept formulated diets[15,16]. African catfish inhabits almost any freshwater habitat including floodplains, large sluggish rivers, lakes and dams[17]. The physiology of African catfish has been widely reported[15,17,18].

There is scarce information about the potency of herbal plants in aquaculture and effect of the extracts of these plants on the biochemistry and metabolism of African catfish *C. gariepinus*. Therefore the present study evaluated the effect of ginger and roselle on serum biochemistry of *C. gariepinus*.

2. Materials and methods

2.1. Study fish

A total of 150 juveniles of *C. gariepinus* [initial weight (35.41 ± 1.53) g] were used for the experiment. The experiment was carried out in the laboratory of Department of Fisheries and Aquaculture, Federal University, Ndufu-Alike Ikwo, Nigeria. The fish were allowed to acclimate in a tarpaulin tank (4 m × 2 m × 1 m) for 2 weeks and were fed with commercial fish feed (Coppens) throughout the 2 weeks acclimation period.

2.2. Experimental design

Fish were randomly distributed to 15 aquarium plastic tanks (1 m × 1 m × 1 m) (10 fish per tank). Experimental tanks were filled with water up to 30 L. Test diets were assigned to fish groups. The fish were fed with the diet (5% of body weight) per day (8:00 a.m. and 5:00 p.m.) for 8 weeks. Quantity of feed was adjusted forth nightly after batch-weighing of experimental fish. Experimental tank water was partly removed by siphoning and replaced with fresh water every three days to avoid fouling resulting from faeces and uneaten food. Some physico-chemical parameters of experimental tank water such as temperature, dissolved oxygen and pH were monitored and measured daily using water testing kits (PRO-LABTM Flourida).

Temperature, pH and dissolved oxygen were maintained at (27.12 ± 0.04) °C, 6.98 ± 0.01 and (6.05 ± 0.21) mg/L, respectively.

2.3. Preparation and processing of herbal plant materials

Dried flower (calyx) of roselle and rhizomes of ginger were obtained from Abakpa Market located at Abakalik Town, Ebonyi State, Nigeria. Dried materials were milled into powder using electric blender (Philips, model HRI701/BC, made in China) and sieved. The resulting materials were stored separately in an air tight container until use.

2.4. Preparation of experimental diet

Five isonitrogenous diets were formulated to yield 37% crude protein (Table 1). Roselle and ginger were added into the diets as additives at different inclusion levels and coded as R1 (2%) and R2 (4%) for roselle and G1 (2%) and G2 (4%) for ginger. The control diet had 0% inclusion of both roselle and ginger. Other feed ingredients used in the diet formulation included soybean meal (SBM), wheat offal, fish meal (FM), yellow maize (YM), vitamin/mineral premix, cassava starch, palm oil, bone meal and salt (Table 1). Pearson square method was used in feed formulation. Samples of the experimental diets were sent to laboratory of International Institute for Tropical Agriculture (IITA), Ibadan, for proximate analysis. Samples were analysed following the procedure of AOAC.

Table 1

Percentage composition of experimental diet (%).

Ingredients	Control	R1	R2	G1	G2
Fish meal	26.09	26.09	26.09	26.09	26.09
Soybean meal	21.74	21.74	21.74	21.74	21.74
Yellow maize	21.74	19.74	17.74	19.74	17.74
Wheat offal	21.74	21.74	21.74	21.74	21.74
Palm oil	1.96	1.96	1.96	1.96	1.96
Vitamin/mineral premix	1.52	1.52	1.52	1.52	1.52
Cassava starch	1.74	1.74	1.74	1.74	1.74
Salt	1.52	1.52	1.52	1.52	1.52
Bone meal	1.96	1.96	1.96	1.96	1.96
Roselle	0.00	2.00	4.00	0.00	0.00
Ginger	0.00	0.00	0.00	2.00	4.00
Total	100	100	100	100	100
Calculated crude protein (%)	37.00	37.00	37.00	37.00	37.00

2.5. Collection of blood samples

Blood collection was done following the reported procedure[19]. Three fish per treatment tank were sampled for blood collection. Fish were held with hand towel and placed against the dorsal part exposing the ventral part upwards. Sterilized 3G needle and syringe of 5 mL were used to collect blood from the fish. A puncture was made at 3–4 cm from the genital opening and wiped with dry tissue paper to avoid contamination with mucus. The needle was inserted perpendicularly to the vertebral column of the fish and pushed tenderly down until blood started entering as the needle punctured a

caudal blood vessel. Blood was taken under gentle aspiration until about 3 mL had been obtained. Blood samples were immediately transferred into plain sterilized plastic tubes. Blood samples were immediately transported to the haematology laboratory unit of Federal Teaching Hospital Abakaliki (FETHA 2) for biochemical analysis.

2.6. Centrifugation of blood sample

Blood in the sterilized plastic tubes was collected and transferred into clean dry centrifuge tubes and centrifuged with Hawsley minor bench centrifuge (P spectra, Centromix No. 231254 CD7000549, Spain) at 3000 r/min for 15 min, followed by serum separation. The serum was collected by using Pasteur pipette and transferred into a plain plastic test-tube and stored at 20 °C until analysis.

2.7. Biochemical analysis

Biochemical parameters such as serum enzymes [alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino-transferase (AST)], serum protein, urea and creatinine were determined by Jaffe spectrophotometric method [20]. Analysis was done with the use of semi-automatic biochemistry analyzer (Erba Chem 7, India). Procedure for the analysis and reagents used was done according to the manufacturer's instruction.

2.8. Statistical analysis

The obtained data were subjected to statistical analysis using One-way ANOVA to test for level of significance at 5%. All analyses were performed using Statistical Package for Social Sciences (SPSS) version 23 and Excel version 16. Duncan multiple range test (DMRT) was used to separate the means.

3. Result

3.1. Proximate composition of experimental diets

Results on proximate compositions of experimental diets are presented in Figure 1. Significant differences ($P < 0.05$) were seen in proximate compositions of the experimental diets compared to the control. Higher values for crude protein, ash, dry matter, crude fat and fibre were seen in ginger and roselle based diets compared to the control diet.

3.2. Serum enzyme activities

Activities of serum enzymes (ALP, ALT and AST) in *C. gariepinus* fed with roselle and ginger based diets are presented in Figure 2. Variations in the activities of enzymes were observed between

treated group and the control. Significant increases ($P < 0.05$) in serum enzyme activities were observed in treated group compared to the control.

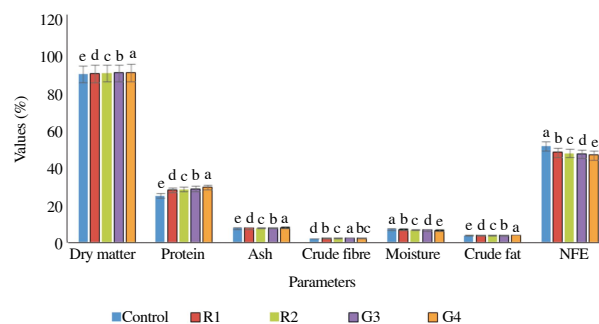


Figure 1. Proximate composition of experimental diets.

NFE: Nitrogen free extract = [100 – (crude protein + ash + crude fibre + moisture + fat)]. The bars with different letters indicate significant difference ($P < 0.05$) while bars with same letters indicate no significant difference ($P > 0.05$).

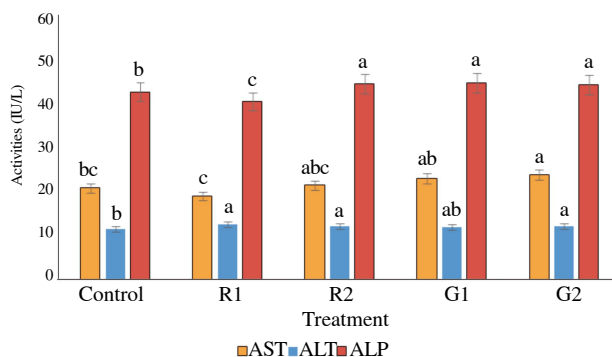


Figure 2. Activities of serum enzymes of *C. gariepinus* fed with roselle and ginger based diets.

The bars with different letters indicate significant difference ($P < 0.05$) while bars with same letters indicate no significant difference ($P > 0.05$).

3.3. Biochemistry

3.3.1. Total protein

Biochemical responses of *C. gariepinus* fed with roselle and ginger based diets are presented in Figure 3. Significant increases ($P < 0.05$) in total protein were noticed in treated groups compared to the control. Although there was no significant difference ($P > 0.05$) in treated groups in terms of total protein, higher values were noticed in fish fed with roselle based diets compared to the fish fed with ginger based diets.

3.3.2. Metabolites

In terms of blood urea content, insignificant changes ($P > 0.05$) were seen in treated groups compared to the control expect for fish fed with roselle based diet at 2 g. Result on blood creatinine level revealed that the highest values were observed in roselle fed group

when compared to ginger fed group and the control.

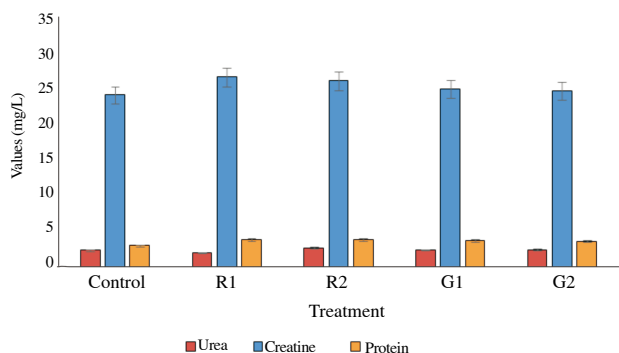


Figure 3. Result on biochemical response of *C. gariepinus* fed with roselle and ginger based diets. The bars with different letters indicate significant difference ($P < 0.05$) while bars with same letters indicate no significant difference ($P > 0.05$).

4. Discussion

4.1. Proximate composition of diets

Proximate assay indicated significant differences ($P < 0.05$) in proximate compositions of experimental diets (Figure 1). The values of dry matter, protein, ash and crude fat were observed to be highest in 4% ginger based diet followed by 2% ginger based diet while lowest values for the same parameters were seen in the control diet. The values of moisture content and crude fibre were observed to be highest in the control diet. The present study revealed that ginger based diets contained higher amount of protein and mineral elements than roselle based diets and control. The present study corroborates the findings of Kumar *et al.*[21] who reported 41.14%–42.32% crude protein for ginger based diets. Ginger contains mineral elements such as zinc, phosphorus, calcium, magnesium, iron and vitamins[3].

4.2. Serum enzymes

Result of the present study revealed that serum enzymes (ALP and ALT) activities increased in the fish group fed with ginger and roselle based diets compared to the control (Figure 2). Insignificant changes ($P > 0.05$) in the activity of AST were seen in treated groups compared to the control. Insignificant changes in AST and ALT activities were observed in *C. gariepinus* fed with walnut leaf and onion bulb supplemented diets[19]. Significant increase was seen in ALP activity when Indian catfish *Mystus montanus* (Jerdon, 1849) were fed with *Ocimum tenuiflorum*, *Zingiber officinale* and *Allium cepa* supplemented diets[21]. Insignificant changes ($P > 0.05$) were observed in serum enzymes activities when *C. gariepinus* juveniles were exposed to varying concentrations of ginger for 12 weeks[3]. Increase in ALP and ALT activities observed in the present study indicate defensive mechanism in order to protect the liver against

harmful agents and damage to the liver cells.

4.3. Biochemical responses

Evaluation of biochemical parameters (protein, urea and creatinine) in *C. gariepinus* fed with ginger and roselle supplemented diets revealed significant changes (Figure 3). Significant increase ($P < 0.05$) in serum protein was noticed in treated groups compared to the control. This could be a result of immune response to plant chemical constituents. Significant increase ($P < 0.05$) was noticed in serum protein of *C. gariepinus* fed with walnut leaf and onion bulb supplemented diets[19]. Increase in serum total protein was also observed in rohu *Labeo rohita* (Hamilton, 1822) after long term feeding with *E. viridis*[22]. Increases in serum protein, albumin and globulin levels might be a consequence of immune response to certain constituents of the extracts[23].

Urea is a metabolic product of protein. The test for urea aims at measuring the quantity of nitrogen in the blood system that comes from the waste product urea. The essence of this test is to ascertain the functionality of the kidneys. In situations that the kidneys are unable to get rid of urea from the blood normally, there is consequential rise of urea level in the blood system[18]. Creatinine is a substance that is produced by the body during metabolism. Through the kidneys filtration process, the body eliminates creatinine. Measurement of creatinine is also a precise assessment of how well the kidney filtration processes are functioning[18]. Alteration in the function of the kidneys with respect to filtration process may result in changes in creatinine levels in the blood[18]. Kidney function decreases as creatinine level in the blood system increases.

Urea nitrogen and serum creatinine are better markers of kidney function and they are used to assess the metabolic profile in organisms[18,24]. It was reported that abnormal levels of these metabolites may indicate a kidney or liver-related disease condition[18]. Authors further reported that elevation in levels of blood urea nitrogen and serum creatinine does not necessarily indicate structural renal disease.

The findings of the present study revealed significant changes ($P < 0.05$) in terms of urea and creatinine values of fish fed with roselle based diets when compared to the control. However, insignificant changes ($P > 0.05$) in terms of urea and creatinine were observed in fish fed with ginger based diets when compared to the control. The findings of this study indicate that roselle and ginger based diets had no adverse effect on the health of the fish and may further stimulate immune response in fish. It was reported decreased level of serum creatinine when *C. gariepinus* infested with gill monogenia was exposed to ginger bath treatment[25].

The findings of the present study revealed that roselle and ginger used as additives in the diet of *C. gariepinus* had no adverse effect on serum biochemistry and health of the fish.

Conflict of interest statement

We declare that we have no conflict of interest

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