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ORIGINAL RESEARCH ARTICLE

Effects of Roselle (*Hibiscus sabdariffa*) Calyx as Dietary Additive on the Growth Performance, Nutrient Utilization, and Haematological Parameters of *Clarias gariepinus* Fingerlings

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ABSTRACT

This study was conducted to investigate the effects of roselle as dietary supplements on the growth performance, nutrients utilization and hematological parameters of *Clarias gariepinus* fingerlings using standard methods and procedures for a period of three months from October to November 2021 in fish research farm of the Department of Fisheries and Aquaculture, Faculty of Renewable natural resources, Federal University Dutsin-ma (FUDMA), Katsina State located along the new market road adjacent Shema filling station at latitude 12^0.46" and longitude 7^0..49" Dutsin-ma Katsina State. Two hundred *Clarias gariepinus* fingerlings (4.25±0.13g) were allotted to four diets as treatments in a complete randomized design with roselle as additives at different inclusion levels of 0.0%, 0.4%, 0.6% and 0.8%respectively. Fish fed 0.8% roselle have significantly ($p>0.05$) higher growth performance and nutrient utilization followed by 0.6%roselle fed fish group, while 0.4%roselle fed fish had the lowest growth performance among treated groups and control. There were no significant changes ($p>0.05$) in the haematology of *C. gariepinus* fed different levels of roselle, but highest values for red blood cells (3.10±0.26), haemoglobin (9.63±0.85), packed cell volume (29.0±2.65) and mean corpuscular hemoglobin (31.1±1.05) were observed in 0.8% roselle group. Highest mean corpuscular volume (94.4±7.91) and lymphocyte (32.0±4.36) were seen in 0.6% roselle treated group. Highest mean corpuscular hemoglobin concentration (33.27±0.05) in the control. For differential white blood cell counts, higher neutrophil (71.33±7.03) and basophil (2.3±1.53) were seen in 0.8% g roselle treatment group. Highest mean corpuscular volume (94.4±7.91) and lymphocyte (32.0±4.36) were seen in 0.6% roselle treated group, while the control have highest value for monocyte (4.3±2.52) and eosinophil (2.0±1.00). This study showed that fish fed 0.8% roselle diet had better growth performance, nutrient utilization and haematological parameters and it is therefore recommended for use in fish diet.

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Additive, *Clarias gariepinus*, Growth, Haematology and Roselle.

INTRODUCTION

A promising supply of affordable and beneficial protein for human consumption is provided by aquaculture (Liao *et al.*, 2022). Due to high-density fish farming with continual contact with disease causing organisms living in water (Rocio *et al.*, 2021) the aquaculture sector's rapid growth to meet the demand for fish may cause an outbreak of numerous sorts of infectious disease (Deyan *et al.*, 2017). As a result of unregulated movement of live aquatic organisms (Jadhav *et al.*, 2006) infectious disease outbreaks have emerged as one of the key obstacles to the development of aquaculture and pose a serious threat to the industry's viability (Barman *et al.*, 2013). To avoid financial losses, a variety of drugs are utilized for the treatment and prevention of several infectious diseases in aquaculture (Deyan *et al.*, 2017). Researches for more natural substitutes, such as therapeutic plants, have been prompted by the overuse of antibiotics in fish farming and the negative effects on global health (Rocio *et al.*, 2021). Moreover, the use of antibiotics for the treatment of fish is not viable due to the rise in antibiotic-resistant bacteria, harm to the native fish microflora (Misra *et al.*, 2006) and buildup of antibiotic residues in fish tissue and the environment that pose risks to human and animal health (Barman *et al.*, 2013; Suat 2015). Finding alternatives to synthetic medications is vital for ensuring sustainable aquaculture.

Medicinal plants are appropriate candidates for antibiotic alternatives (Nik *et al.*, 2022) because they contain a variety of active ingredients like polysaccharides, alkaloids, organic acids, flavonoids, and phenols that are antibacterial and antiviral in addition to enhancing the bo-

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dy's immune system and enhancing its capacity to resist diseases (Reverter *et al.*, 2017; Nik *et al.*, 2022). Medicinal plants offer little toxicity to the aquatic environment, fewer side effects, and limited development of medication resistance as compared to synthetic pharmaceuticals and antibiotics. Majority of medicinal herbs may successfully enhance the growth performance of aquatic animals, as a result, they are valued and employed more frequently in aquaculture (Liao *et al.*, 2022).

One of the most important methods for determining the health state of various animals is hematology because, it offers a reliable assessment of organisms via non-lethal means (Satheeshkumar *et al.*, 2012). Therefore, it is recommended that the adoption of hematological analysis as a reference for assessing fish health status (Tavares-Dias *et al.*, 2006). In veterinary medicine, scientific research, and aquaculture hematological analyses are frequently used to assess the health and wellbeing of fish. According to Malgorzata *et al.* (2022), hematological parameters have shown to be extremely susceptible to a variety of environmental conditions such as diet, water quality, stress and infections.

The tropical annual dicotyledonous plant known as Roselle (*Hibiscus sabdariffa*) is an erect, herbaceous member of the Malvaceae family. (Eslaminejad *et al.*, 2011). The flavonoids, gossypetine, and sabdaretine as well as calcium, magnesium and iron are some of the flower's chemical components of the plants (Pietta, 2000; Mgaya *et al.*, 2014). Juices from roselle fruit are high in anthocyanin may protect consumers against a number of illnesses caused by the responses of free radicals (Mgaya *et al.*, 2014). A Roselle plant was found to be diuretic, diaphoretic, uricosuric, antibacterial, antifungal, mild laxative, sedative, antihypertensive, antitussive, and as agent for treating hypercholesterolemia, kidney stones and for reducing blood viscosity (Alarcon *et al.*, 2012). For many years, Roselle has been utilized as an herbal remedy in phytotherapy (Mehdi *et al.*, 2013). Roselle (*Hibiscus sabdariffa*) calyx was found to have an antibacterial activity antagonistic to pathogenic *A. hydrophila* infection in Nile tilapia (El-Mesallamy *et al.*, 2016). More so, roselle calyx demonstrated growth promoting ability in the diet of fish according to Adewole, (2014) and Ogueji *et al.* (2017). Since research on antibiotic alternatives is crucial to ensure the sustainability of aquaculture, this study assessed the effects of roselle (*Hibiscus sabdariffa*) as a dietary additive on *Clarias gariepinus* fingerling growth performance, nutrient utilization and haematological parameters to improve aquaculture development.

MATERIALS AND METHODS

Ethical approval

The procedures and ethical issues for this study were reviewed and approved in the 2019/2020 Academic Session by the Departmental Academic Board of the department of fisheries and aquaculture, Federal University Dutsin-ma.

Experimental Site

The study was carried out at the fish research farm of the Department of Fisheries and Aquaculture, Faculty of Renewable natural resources, Federal University Dutsin-ma (FUDMA) Katsina State located along the new market road adjacent Shema filling station at latitude 12^0.46" and longitude 7^0.49" Dutsin-ma Katsina State.

Collection, Processing and Calculated amount for Roselle Calyx

Dried Roselle *(Hibiscus sabdariffa)* calyx were purchased at Dutsin-ma local Market, grinded and stored in an air tight container prior to feed formulation (Adewole, 2014). The values for roselle inclusion was 4g, 6g and 8g/kg of the dry powdered calyx of *H. sabdariffa* as described by Ogueji *et al,* (2017)

Formulation of Experimental Diets and Ingredients used

Table 1 shows the ingredients used for the formulation of the experimental diets and they include Fishmeal, Soybean meal, Yellow maize, palm oil, Vitamin/mineral, premix, Cassava starch (Binder), Common salt, Bone meal and roselle calyx. All the ingredients were measured using sensitive scale, mixed, pelleted, dried, labeled and stored at room temperature until its ready for use (Sogbesan and Ugwumba 2007). Using the approved procedure, proximate analysis of the diet and fish carcasses before and after the feeding trial was conducted (AOAC, 2000).

Table 1: Ingredients with varied inclusion of Roselle calyces as additive used for the formulation of the experimental diets.

INGREDIENTS	TREATMENTS (%)			
	RM0	RM1	RM2	RM3
Fishmeal	46.24	43.36	42.5	41.21
Soybean meal	23.12	22.82	22.50	22.16
Yellow maize	20.14	19.32	18.50	18.12
Premix	1.50	1.50	1.50	1.50
Starch (binder)	2.0	2.0	2.0	2.0
Common salt	0.50	0.50	0.50	0.50
Bone meal	1.00	1.00	1.00	1.00
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Palm oil	5	5	5	5
Hibiscus sabdariffa (Roselle)	0	4	6	8
Total	100	100		100

Experimental Design and Management of Experimental fish

A Completely Randomized Design was employed, the fish were reared in a semi intensive system. The fishes were weighed in each tanks with sensitive weighing scale, in order to determine the amount of feed to be given at 5% body weight. All recommended health practices were strictly followed and water was changed after every three days.

Source of Experimental fish

A total of two hundred (200) *Clarias gariepinus* fingerlings with initial mean weight of 4.25±0.13g were obtained from Pagam Fish Farm, Kano.

Feeding of Experimental fish

The fish were given commercial feeds for 21 days in holding tanks, fed twice daily at 5% body weight for 12 weeks and randomly stocked in triplicates for each treatment as described by Sogbesan and Ugwumba (2007). The fish were starved for 24 hours before the start of the feeding trial. Before the experiment began, the average weight and length of the fish were measured and recorded.

Water source and management

The water utilized for the experiment came from the farm's well after water quality test to establish whether the water was suitable for fish growth and use. Water quality parameters were monitored and ensured that they are within recommended range throughout the experimental period.

Sampling of Experimental Fish

After feeding the fish for twelve weeks, growth performance, nutrient utilization and hematological parameters were assessed. To measure the growth rates in terms of final weights and lengths obtained for the computation of growth and nutrient utilization parameters, three (3) fingerlings from each tank were randomly selected bi-weekly.

Analysis of Growth Performance and Feed Utilization

The data obtained on the growth performance and nutrients were analyzed as follows:

i. **Specific Growth Rate (SGR)**

$$SGR = \frac{(\ln \text{final weight} - \ln \text{initial weight}) \times 100}{\text{Time (days)}}$$

Where,

W2 = mean final body weight of fish W1 = mean initial body weight of fish

T1 = final time (in days) T0 = Initial time (in days)

ii. **Feed Conversion Ratio (FCR)**

$$= \frac{\text{Feed fed (dry weight basis)}}{\text{Live weight gain}}$$

iii. **Percentage weight gain (PWG)** $= \frac{wf - wi}{wi} \times 100$

Where,

Wf = Final mean body weight of fish (g) Wi = Initial mean body weight of fish (g)

iv. **Feed intake** = Total unit of feed consumed / Total No. of days

v. **Protein Efficiency Ratio (PER)** (Falayi, 2009)

$$PER = \frac{\textit{Weight gained}}{\textit{Crude protein consumed}}$$

Where crude protein consumed = *% protein in feed x total diet consumed 100*

vi. **Net Protein Utilization (N.P.U)** (Falayi, 2009)

$$= \text{Fish } \frac{\textit{protein gained}}{\textit{Protein consumed}} \times 100$$

Where fish protein gained = final body protein – initial total body protein.

Haematology and differential white cell count

At the end of the trial, blood was collected from three fish each tank. Blood was drawn from the caudal vein and placed into tubes containing ethylene diamine tetracetic acid (EDTA). The collected blood samples were immediately taken to haematology laboratory to determine the packed cell value (PCV), while red blood cell (RBC), white blood cell (WBC) counts, haemoglobin (Hb) concentration and red cell indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). The differential white blood cell count was carried out to determine the Neutrophil (N), Lymphocyte (L), Monocyte (M), Basophils (B) and Eosinophil (E) as described by Barbara *et al*, (2011).

Data analyses

Analysis of variance (ANOVA) was used to analyze the data. Where there were significant differences between the means, the Least Significant Difference (LSD) was used to

separate the means. SAS (2002) version 9.0 was used for the statistical analyses at a confidence level of $p \geq 0.05$

RESULTS

During the period of the experiment, temperature ranges between 28.8^{0}C – 29.6^{0}C, pH ranges from 6.2 – 7 and dissolved oxygen was between 4– 5.0mg/l. The proximate composition of the experimental diets containing graded levels of roselle is presented in table 2. Four diets (40% crude protein) were formulated and titled as RM0 (Control), RM1, RM2, and RM3 respectively.
Proximate fractions were similar among treatments.

Table 2: Proximate compositions of Experimental Diets with varied inclusion of Roselle calyces as additive.

	RM0	RM1	RM2	RM3
CP	40.24	40.20	40.19	40.17
CF	3.45	3.53	3.56	3.59
Ash	5.76	5.79	5.81	5.83
% Moisture	8.21	8.24	8.23	8.25
Total crude protein	40	40	40	40

RMO- Control, RM1-Diet 1. RM2-Diet 2, RM3- Diet 3

The bi-weekly weight increments of *C. gariepinus* fingerlings fed experimental diets treated with graded levels of roselle are presented in Fig. 1. The percent bi-weekly weight increment was highest in RM3. In all the treatments evaluated weight increased steadily up to week 2. From week 4 differences in weight gain were observed with fish on treatment RM3 having higher weights compared to others.

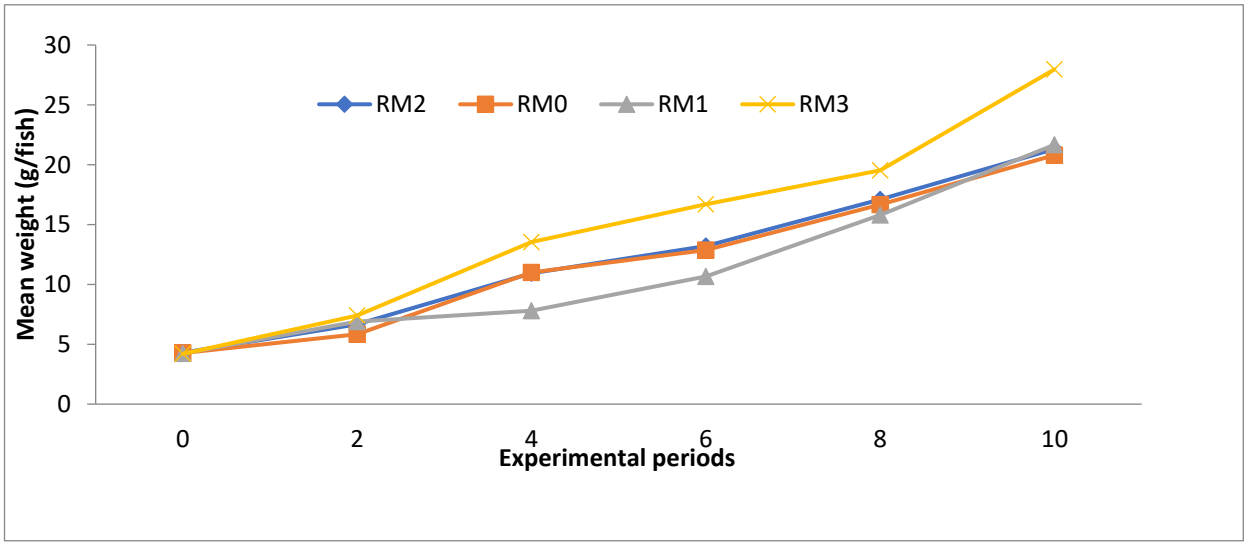

Figure 1: Bi-weekly weights of *Clarias gariepinus* fed graded levels of Roselle diets

Growth performance and nutrient utilization by *Clarias gariepinus* fingerlings fed experimental diets treated with graded levels of roselle.

The effect of levels of inclusion of roselle calyx on growth performance and nutrient utilization of *C. gariepinus* fingerlings is outlined in table 3. At the end of the study, fish fed 0.8% roselle diet had significantly higher ($p<0.05$) total final weight (97±5.7) than all the treatments and the control, followed closely by 0.6% roselle fish fed diet (21.67±11.91). The total weight gained, mean weight gain and specific growth rate followed the same similar trend as in the total final weight (Table 3). Final condition factor was also significantly ($p<0.05$) higher in fish fed 0.8% diet, followed closely by 0.6% diet, while the lowest value was

from fish fed control diet respectively. The feaed utilization indices also followed similar trend of the growth index of weight gain, with the highest feed intake and feed conversion efficiency observed in 0.8% roselle fish fed group. Highest feed conversion ratio was observed in fish fed control diet (2.13±0.34) across the treatments. Significant difference were not observed in the length of the fish fed with roselle calyx powder as dietary additive and the control, throughout the experimental period, initial length varied from 8.13cm to 9.0cm.

Table 3: Effect of levels of inclusion of roselle calyx on growth performance and nutrient utilization of Clarias gariepinus fingerlings.

Parameters	Treatments			
	RM0	RM1	RM2	RM3
Initial weight	4.30±0.10a	4.27±0.15 a	4.23±0.15 a	4.2±0.17 a
Final weight	21.31±0.45 a	20.8±2.66 a	21.67±11.91 a	27.97±5.71b
Weight gain	17.01±2.42 b	16.53±2.98 b	17.43±10.16 b	23.77±2.76 a
SGR	1.46±0.02 b	1.45±0.08 b	1.47±0.32 b	1.64±0.1a
FCR	2.13±0.34 b	2.09±0.51 b	2.06±1.19b	0.93±0.22 a
FCE	47.35±3.81c	49.56±10.85 c	70.58±58.08b	112.21±29.48a
Survival rate (%)	55.56±13.80 a	55.56±10.18 a	46.67±24.03b	24.44±3.84a
Initial Condition factor	0.49±0.0089 a	0.47±0.018a	0.52± 0.04 a	0.52±0.31a
Final Condition factor	1.38±0.66 b	1.37±0.20 b	1.43±0.76 b	1.85±0.28a
Mean weight /day	0.21±0.06 b	0.19±0.03 b	0.21±0.14 b	0.28±0.06a
Length increment	6.8±0.36 a	6.13±0.47 a	6.83±0.52 a	6.93±0.58 a
Feed intake	374.8±5.67b	343.7±4.56b	390.8±6.78b	491.5±7.56 a
Protein intake	44.20±3.02a	38.40±3.08a	24.10±2.45b	22.70±2.78b
Protein efficiency ratio	38.48±5.47c	43.08±4.67c	72.43±6.56b	104.5±5.98 a
Net protein utilization	15.75	15.79	15.87	16.57

Means along same row with different super script differ significantly ($p<0.05$).
Specific growth rate (SGR), Feed conversion ratio (FCR), Feed conversion efficiency (FCE %).

Hematological parameters and differential white blood cell count of *Clarias gariepinus* fingerlings fed experimental diets treated with graded levels of roselle.

Hematological parameters and differential white blood cell counts were analyzed (Table 4) which showed the response due to different inclusion levels of roselle to diets of *C. gariepinus* fingerlings. There were no significant changes ($P>0.05$) in hematological parameters and differential white blood cell counts of the study fish. However, highest values for PCV, HB, RBC, MCH, N and B were observed in fish fed 0.8% roselle supplemented diet as compared to other treatments and the control. MCV was higher in fish fed 0.6% roselle, while MCHC, Eosinophils and Monocytes were higher in fish fed control diet.

Table 4: Effect of different level of inclusion of roselle on mean haematological parameters of *Clarias gariepinus* fingerlings.

Parameters	Treatments (Mean ±SD)			
	RM0	RM1	RM2	RM3
PCV	25.3 ± 4.50	22.3 ± 1.52[a]	28.3 ± 7.24[a]	29.0 ± 2.65[a]
HB	8.4 ± 1.50	7.43 ± 1.52[a]	9.4 ± 2.43[a]	9.63 ± 0.85[a]
RBC	3.0 ± 0.20	2.5 ± 0.10[a]	3.0 ± 0.55[a]	3.10 ± 0.26[a]
MCV	84.3 ± 12.58	89.3 ± 4.63[a]	92.4 ± 7.91[a]	66.53 ± 49.8[a]
MCH	28.06 ± 4.21	29.7 ± 1.47[a]	30.37 ± 2.55[a]	31.10 ± 1.05[a]
MCHC	33.27 ± 0.05	33.23 ± 0.11[a]	33.13 ± 0.15[a]	33.23 ± 0.05[a]
Neutrophil	63.67 ± 7.37	70.0 ± 1.00[a]	64.67 ± 4.93[a]	71.33 ± 7.03[a]
lymphocyte	27.67 ± 5,13	25.0 ± 3.00[a]	32.0 ± 4.36[a]	24.67 ± 6.43[a]
Monocyte	4.3 ± 2.52	3.0 ± 2.66[a]	1.67 ± 0.57[a]	1.0 ± 0[a]
Basophils	1.67 ± 1.53	1.0 ± 1.00[a]	1.67 ± 0.57[a]	2.3 ± 1.53[a]
Eosinophil	2.0 ± 1.00	1.0 ± 1.00[a]	0 ± 0[a]	0.67 ± 0.57[a]

PCV: Packed cell value; Hb: haemoglobin; RBC: red blood cell, WBC: while blood cell, MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular haemoglobin concentration.
RM0 – control, RM1 –0.4% roselle, RM2- 0.6% roselle, RM3-0.8% roselle.

DISCUSSION

The physiochemical parameters including temperature, pH and dissolved oxygen were consistent with the findings of Balogun *et al.* (2004) who reported little fluctuations of the parameters throughout the period of the study. From this study, highest live-weight changes were obtained from the fish on treatment RM3. The weight gained might be due to the addition of roselle in the diets of the fish. Earlier studies by Ogueji *et al.* (2017) and Kumar *et al.* (2014) reported that plants have growth promoters for use in fish nutrition. Findings from this study agrees with Ogueji *et al.* (2017) who obtained higher growth performance from *C. gariepinus* juvenile fed roselle and ginger supplemented diets compared to the control. Furthermore, findings of this study agrees with earlier report by Adewole, (2014) who observed increased in growth parameters in *C. gariepinus* fed diet incorporated with roselle compared with the control. The progressive response in the growth of treated fish especially at 0.8% was associated with the presence of roselle which Pietta (2000), Mahadevan and Pradeep (2009)) reported that the plant contain minerals, vitamins, flavonoids, gossypetine, sabdaretine and virtuous number of phytochemical components that boosts the growth and health status of animals. Feed utilization values also followed similar pattern with the growth parameters with the highest feed intake from RM3 diet which was in agreement with findings of Adewole (2014) who reported that roselle fed to *Clarias gariepinus* gave the highest feed utilization indices.

The physiological and nutritional state of animals can be accurately predicted by blood analysis. Haematological parameters have been used to clarify the effects of dietary components or supplements given to live organisms (Majid, 2010). They can also be used to describe how chemical components such as plant extracts behave in relation to blood (Bashir *et al.*, 2015).

Consuming roselle fruit with a high anthocyanin content helps protect against a number of diseases due to the presence of free radicals (Mgaya *et al.*, 2014). In this study, fish fed 8% roselle-supplemented diets have higher PCV, HB, RBC, MCH, N, and B levels compared to other treatments and the control. However, these differences were not statistically significant. The findings from this study were in agreement with the report of Olatunji (2005), who administered extract of *H. sabdariffa* calyx and found no significant differences, though with higher haematological values, red blood cell and platelet count. This findings also corroborated with the work of Yahaya *et al.* (2012) who found medicinal plants (roselle, moringa, ginger and fluted pumpkin) incorporated in the diets of albino rat to boost their haematological parameters..

CONCLUSSION

The study revealed that roselle supported the growth and nutrient utilization of *C. gariepinus* fingerlings especially 0.8% roselle supplemented diets. However, data on hematology and carcass composition will not be influenced significantly by inclusion of 0.8% roselle in the diet of *C. gariepinus*.

Recommendations:

I. Findings from this study recommend the inclusion of 0.8% roselle in the diet of *Clarias gariepinus* fingerlings because it have better growth and haematological profile.
II. Furthermore, more research work should be carried out on roselle to determine its suitability in other species of fish to enable commercial use.
III. Natural plants materials such as roselle should be promoted for used as fish growth promoters /additives.

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