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Research Article

Fenugreek Supplementation in Hybrid African Catfish Diets: Effect on Growth Performance, Physio-Metabolic Response, and Antioxidant Enzyme Capacity

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The impact of dietary fenugreek (FN) supplementation on growth performance, physio-metabolic enzyme response, and antioxidant enzyme activities in hybrid African catfish (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂) was investigated in this study. Five isonitrogenous diets (38% crude protein) were developed and fed to the fish for 60 days. The FN was incorporated into the diets at 0%, 0.5%, 1.0%, 1.5%, and 2%. Two hundred twenty-five fingerlings of hybrid African catfish (average weight 4.01 g ± 0.02) were used for the study and randomly distributed to five dietary groups in triplicate. FN supplementation improved growth responses and survival rates; the dietary groups considerably ($p < 0.05$) outperformed the control group. FN-based diets considerably increased the carcass protein content ($p = 0.020$) and improved protein retention ($p = 0.015$). FN supplementation markedly ($p < 0.001$) and dose-dependently reduced the carcass's lipid content and substantially ($p < 0.001$) increased the levels of metabolic enzymes. The fish antioxidant capacity was significantly enhanced ($p < 0.001$), and hepatic stress enzymes were greatly ($p < 0.001$) lowered in FN-based diets. In summary, the second-order polynomial regression analysis indicates that the optimal dose of FN supplementation to improve hybrid catfish growth without negatively affecting the fish's physio-metabolic response or overall wellbeing is 1% dietary inclusion level.

Keywords: carcass protein; catfish; malondialdehyde; phytogenic; stress enzyme

1. Introduction

Aquaculture production has witnessed considerable growth in recent times to meet the growing consumer demand for food fish protein. In 2022, aquaculture productivity exceeded capture fisheries for the first time in history, accounting for 51% of all aquatic animal production and 57% of fish intended for human consumption [1]. This growth and expansion were necessitated in part by the intensive fish production techniques employed by the farmer, which often increase stress as well as the danger of disease and loss to the farmer [2]. Chemicals, growth hormones, and medications are frequently utilized as remedies to address some of the problems arising from intensive aquaculture. However, this approach poses a threat to aquatic ecosystem health. More so, consumers are prioritizing quality and safety as well as the absence of pollutants, antibiotics, and cancer-causing agents in farmed food fish [3]. According to Tran et al. [4], consumers in Nigeria are willing to pay a price increase that ranges from 3.1% to 18.8% for catfish that has been reared in a secure, regulated farm setting. Dietary adjustments, such as

adding medicinal herbs to fish diets to boost health and growth, have therefore been suggested as a potential alternative [5, 6].

The use of herbal plants or their extracts improves the health and performance of fish, according to numerous research [7, 8]. Numerous studies have investigated the functional role of different plant materials like kenaf, *Hibiscus sabdariffa* [9] coffee bean, *Coffea arabica* [10] roselle, *H. sabdariffa* [11, 12], and turmeric, *Curcuma longa* [13] on fish performance, immunity, and disease resistance. Recently, fish have been treated with medicinal plant products (powder plants, essential oils, extracts, and isolated compounds) to counteract the negative impacts of stressors like toxicants and unpleasant environmental variables [14, 15]. Phytochemicals are biologically active substances derived from plants that have immune-boosting, biocidal, stress-relieving, and growth-promoting properties. The most common plant-derived substances are steroids, alkaloids, terpenoids, pigments, phenolics, tannins, flavonoids, and essential oils [8, 16–18]. To promote growth, fish were fed dietary supplements made from medicinal plants that contained a variety of phytochemicals [19, 20].

A potent herb with a long history of usage in traditional medicine is fenugreek (FN) [21]. FN seeds (*Trigonella foenum-graceum*, Leguminosae/Fabaceae) contain lipids, biogens, and protein with a good amino acid composition. Additionally, FN possesses various bioactive components such as saponins, flavonoids, choline, carotene, essential oils with trigonelline, etc., which have been reported to lower blood glucose and cholesterol levels and enhance antioxidant functions in fish [22–25]. The digestive system benefits from FN's laxative, intestinal lubricant, and tonic properties [26]. Recently, Diab et al. [27] reported that dietary FN seed extract fed to Nile tilapia significantly improved the growth performance, feed efficiency, and superoxide dismutase (SOD) enzyme response. Furthermore, it was noted that the supplementation of FN extract enhanced the lysozyme and phagocytic activities and upregulated immune-related genes such as IL-1β, TGF-β, and TNF-α relative to the control group. Other studies have also reported the growth-promoting, anti-inflammatory, and immune enhancement functions of FN in different fish species [28–30]. Nevertheless, studies on the dietary use of FN seeds as an additive in African catfish, the most commonly consumed fish in Nigeria, are scanty, so there is a need to delineate its functional role in African catfish aquaculture. In view of this, the current study examined the effect of FN powder on the growth, physio-metabolic, and antioxidant enzyme responses of hybrid African catfish (*Clarias gariepinus* ♀ × *Heterobranchus bidorsalis* ♂).

2. Materials and Methods

2.1. Experimental Diet. The powdered FN seeds were bought from commercial sources, processed, and individually screened to fine particle size. The proximate composition of the triplicate samples of each feedstuff was then conducted [31]. Based on the results of the ingredients proximate, a diet formulation program created by the Network of Aquaculture Centers in Asia-Pacific was used to develop the diets [32]. FN was combined with dietary feedstuffs and added at concentrations of 0.5%, 1.0%, 1.5%, and 2.0%. FN-free diets were used as the control. The experimental diets were labeled control (FN-free diet), 0.5% FN (Control + 0.5% FN), 1.0% FN (Control + 1% FN), 1.5% FN (Control + 1.5% FN), and 2.0% FN (Control + 2% FN) (Table 1). The mixture was moistened with hot water, after which it was run through a mesh (2 mm Ø die) to form pellets, which were subsequently air-dried at 30–32°C. A Ziploc bag was used to store the dried feeds in a refrigerator (4°C) after they had been dried to a final moisture content of less than 10%. To ensure that the diets' desired protein and fat levels were met, representative diet specimens were taken for proximate analysis.

Table 1: Gross composition (g/100 g) of fenugreek supplemented diet fed to hybrid African catfish (*C. gariepinus* ♀ × *H. bidorsalis* ♂).

Ingredient composition (%)	Control	Fenugreek supplemented diets			
		0.5%	1.0%	1.5%	2.0%
Fishmeal@72% cp	27.80	27.80	27.80	27.80	27.80
SBM@44% cp	44.50	44.50	44.50	44.50	44.50
Yellow maize@10% cp	4.00	4.00	4.00	4.00	4.00
Fenugreek	0.00	0.50	1.00	1.50	2.00
‖Fish premix	5.00	5.00	5.00	5.00	5.00
Fish oil	2.50	2.50	2.50	2.50	2.50
Soy oil	2.50	2.50	2.50	2.50	2.50
Starch	13.70	13.20	12.70	12.20	11.70
Proximate composition (%)					
Moisture	8.14	8.12	8.10	8.08	8.06
Crude protein	37.82	38.00	38.19	38.38	38.58
Crude lipid	12.31	12.40	12.50	12.59	12.69
Ash	7.39	7.42	7.44	7.47	7.50
Crude fiber	5.91	5.92	5.93	5.94	5.95
NFE	28.44	28.14	27.84	27.54	27.24
Amino acid composition (%)					
Arginine	3.00	3.01	3.03	3.04	3.06
Histidine	0.94	0.94	0.94	0.95	0.95
Isoleucine	1.84	1.85	1.85	1.86	1.87
Leucine	3.00	3.01	3.03	3.04	3.06
Lysine	2.82	2.83	2.85	2.86	2.88
Methionine	0.86	0.87	0.87	0.88	0.88
M + C	1.38	1.39	1.39	1.40	1.41
Phenylalanine	1.76	1.77	1.78	1.79	1.80
P + T	3.05	3.07	3.08	3.10	3.11
Threonine	1.78	1.79	1.80	1.81	1.82
Tryptophan	0.47	0.47	0.47	0.47	0.48
Valine	2.00	2.01	2.02	2.03	2.04

Abbreviations: M + C, methionine + cysteine; NFE, nitrogen-free extract; P + T, phenylalanine + tyrosine.

‖Each kg of the Agri-mix fish premix contains vitamin A 1,500,000 i.u, vitamin B1 800 mg, vitamin B2 1600 mg, vitamin B6 1500 mg, vitamin B12 4 mg, vitamin D3 3,000,000 i.u, vitamin E 200,000 mg, vitamin C 240 g, folic acid 300 mg, niacin 5970 mg, biotin 40 mg, pantothenic acid 4000 mg, copper 800 mg, iodine 191 mg, iron 1200 mg, manganese 20,323 mg, selenium 60 mg, zinc 15,996 mg, choline 87,000 mg. Manufactured by Agri-Dom 20/22 Kolawole Shonibare Street, Ajao Estate, Lagos.

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2.2. Chemical Analysis. The feed samples were heated to 105°C for 24 h in an oven to assess their moisture content. The amount of ash was calculated by burning the samples at 600°C for 4 h (Omegalux LMF-3550) in a muffle furnace. A Kjeldahl protein autoanalyzer (Foss Tecator Kjeltec 8400) was used to determine the nitrogen content after acid digestion. A factor of 6.25 was used to convert the nitrogen into crude protein. In order to quantify crude lipid, Soxhlet extraction equipment (Foss Tecator Soxtec 8000) was employed. The raw fiber was examined with a hot extraction fiber analyzer (Foss Tecator Fibertec 2010). All the analyses were done in triplicates.

2.3. Rearing Condition. The feeding trial was conducted at the wet laboratory of the Aquaculture and Fisheries Department, Faculty of Agriculture University of Ilorin. University of Ilorin Aquaculture and Fisheries hatchery provided the fingerlings of hybrid African catfish (*C. gariepinus* ♀× *H. bidorsalis* ♂), which were then acclimated to laboratory conditions in a 1000-L circular plastic tank for 15 days being given a commercial feed (1.8 mm Skretting catfish starter feed). Following acclimation, a triplicate batch of fish (4.01 ± 0.02 g per fish) was bulk weighed, with 15 fish per tank being randomly assigned to each of the five dietary treatments. Before the feeding experiment, all of the fish were fasted for 24 h. To prepare their gastrointestinal tracts for the experimental diet, increase their appetite for the new diet, and reduce stress while weighing and stocking. The 15 rectangular plastic tanks were filled with water up to 70 L capacity and aerated. The fish were fed manually twice daily, at 9:00 a.m. and 5:00 p.m. To adjust feeding and monitor fish performance, bulk weighing was performed every 2 weeks. During the culture time, the normal water quality values were maintained: 6.75 ± 0.35 mg/L dissolved oxygen; pH 6.57 ± 0.29; temperature 27.26 ± 0.48°C

2.4. Assessment of Growth. The assessment of growth responses was calculated using the formula below:

$$\text{Mean weight gain (MWG, g)} = \text{Final mean weight (g)} - \text{initial mean weight (g)},$$

$$\text{Percentage weight gain (PWG, \%)} = \frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{Initial weight (g)}} \times 100,$$

$$\text{Specific growth rate (SGR, \% d}^{-1}\text{)} = \frac{[\ln \text{final weight} - \ln \text{initial weight}]}{\text{Time of culture}} \times 100,$$

$$\text{Feed conversion ratio} = \frac{\text{Total feed fed (g)}}{\text{Weight gain (g)}},$$

$$\text{Protein efficiency ratio} = \frac{\text{Weight gain (g)}}{\text{Total protein fed (g)}},$$

$$\text{Total protein fed} = \text{Total feed fed} \times \text{crude protein of the diet},$$

$$\text{Survival (\%)} = \frac{(\text{Total initial number of fish} - \text{mortality})}{\text{The total initial number of fish}} \times 100.$$

2.5. Body Composition and Nutrient Retention. Following AOAC guidelines [33], the whole body proximate composition of the initial fish ($n = 6$) and final fish ($n = 3$ fish/replicate tank) samples were analyzed. The formula below were applied to the data to measure nutrient retention.

$$\text{Protein retention (\%)} = \frac{\text{Final carcass protein} - \text{initial carcass protein}}{\text{Food fed} \times \text{crude protein content of the feed}} \times 100,$$

$$\text{Lipid retention (\%)} = \frac{\text{Final carcass lipid} - \text{initial carcass lipid}}{\text{Food fed} \times \text{crude lipid content of the feed}} \times 100,$$

$$\text{Energy retention (\%)} = \frac{\text{Final carcass energy} - \text{initial carcass energy}}{\text{Food fed} \times \text{energy content of the feed}} \times 100.$$

2.6. Preparing Tissue for a Biochemical Test. The livers of experimental fish ($n = 3$ fish/replicate), which were chosen at random, were examined for the presence of antioxidants, stress enzymes, total protein, and metabolic enzyme activities. To maintain the cold chain, liver tissue was homogenized in a sucrose solution (250 mM) while submerged in ice [34–36]. Centrifuging was done on the homogenized tissue for 5 min at 3000 rpm. Following the standard manufacturer's recommendations, the produced supernatant was employed for a biochemical analysis with a commercial kit (Randox, United Kingdom). The hepatic electrolytes (Na^+, Ca^{2+}, K^+, and Cl^{-1}) were determined using an atomic absorption spectrophotometer following the manufacturer's procedure (Randox, United Kingdom).

2.7. Analytical Statistics. Following the Levene test for homogeneity of variance being passed by the data, a one-way analysis of variance was utilized in the statistical analysis, and Tukey's *b* was employed to distinguish between the treatment means. All of the analyses were conducted using SPSS version 17.0. The experiment's data were presented as the mean ± standard error of means. To determine the optimal level of FN that supported maximum weight, second-order polynomial regression was used. Microsoft Excel was used to construct the chart showing changes in weekly mean weight.

3. Results

3.1. Survival and Growth Performance. Hybrid African catfish fed the different experimental diets were measured for growth performance, as shown in Table 2. The fish fed 1% FN diet had the highest value of growth performance (mean weight gain [MWG], 14.94 ± 0.05; percentage weight gain [PWG], 376.3 ± 4.36, and specific growth rate [SGR], 2.79 ± 0.02) parameters ($p < 0.05$) among the dietary groups. The

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Table 2: Growth performance of hybrid African catfish (*C. gariepinus* ♀ × *H. bidorsalis* ♂) fed fenugreek-based dietary treatments.

Parameter	Control	Fenugreek (FN) supplemented diets				*p* Value
		0.5%	1.0%	1.5%	2.0%	
Initial mean weight (g)	3.98 ± 0.02^{a}	4.04 ± 0.03^{a}	3.97 ± 0.04^{a}	3.97 ± 0.05^{a}	4.09 ± 0.01^{a}	0.290
Final mean weight (g)	15.69 ± 0.03^{c}	18.05 ± 0.21^{b}	18.92 ± 0.05^{a}	17.65 ± 0.25^{b}	16.11 ± 0.20^{c}	<0.001
Mean weight gain (g)	11.71 ± 0.02^{c}	14.01 ± 0.22^{b}	14.94 ± 0.05^{a}	13.68 ± 0.21^{b}	12.02 ± 0.19^{c}	<0.001
Percentage weight gain (%)	294.55 ± 1.77^{c}	346.60 ± 7.58^{b}	376.3 ± 4.36^{a}	344.64 ± 2.68^{b}	294.04 ± 4.37^{c}	<0.001
Specific growth rate (% d^{-1})	2.45 ± 0.01^{c}	2.67 ± 0.03^{b}	2.79 ± 0.02^{a}	2.66 ± 0.01^{b}	2.45 ± 0.02^{c}	0.002
Feed intake (g/fish)	25.30 ± 0.46^{a}	22.07 ± 0.17^{b}	19.11 ± 0.25^{c}	21.91 ± 0.09^{b}	21.77 ± 0.27^{b}	<0.001
Feed conversion ratio	2.16 ± 0.04^{a}	$1.58 \pm 0.0.03^{c}$	1.28 ± 0.02^{d}	1.60 ± 0.03^{c}	1.81 ± 0.05^{b}	<0.001
Protein-fed (g)	9.61 ± 0.18^{a}	8.39 ± 0.07^{b}	7.26 ± 0.09^{c}	8.33 ± 0.03^{b}	8.27 ± 0.10^{b}	<0.001
Protein efficiency ratio	1.21 ± 0.02^{d}	1.67 ± 0.03^{b}	2.06 ± 0.03^{a}	1.64 ± 0.03^{b}	1.45 ± 0.04^{c}	<0.001

Note: Row values (means with standard errors, $n = 3$) with different superscripts differ from one another considerably ($p < 0.05$).

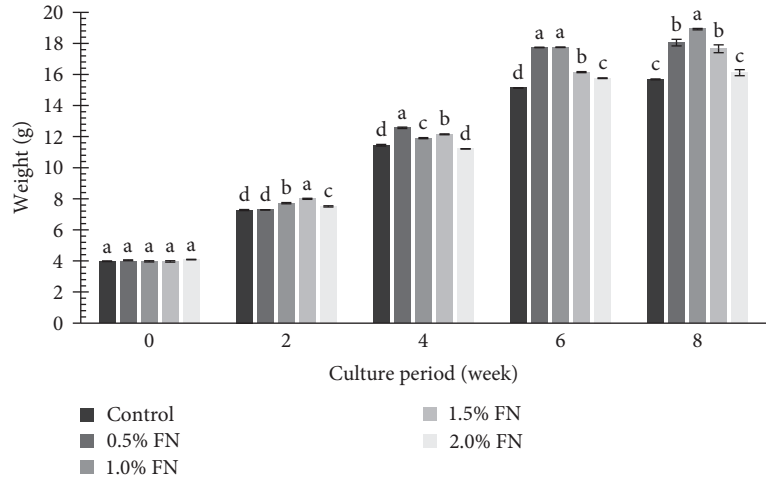


Figure 1: Weekly variations in weight (mean ± standard error of mean; $n = 3$ groups of fish) of hybrid African catfish (*C. gariepinus* ♀ × *H. bidorsalis* ♂) fed fenugreek supplemented diets for 8 weeks. Bar values (means with standard errors, $n = 3$) with distinct letters are substantially different (Tukey's-*b* test; $p < 0.05$) from each other.

biweekly weight variations during the culture period are shown in Figure 1. Additionally, the optimal level of FN with respect to final weight is 1.0% based on the second-order polynomial regression (Figure 2). The percentage survival (Figure 3) of the FN dietary groups was significantly higher ($p < 0.001$) than the control group. There was no discernible difference in the survival percentage among the fish-fed FN-based diets.

3.2. Whole Body Composition and Nutrient Retention. The body composition of hybrid African catfish and their nutrient retention were examined, and the results (Table 3) revealed substantial differences between the various dietary groups. A significantly ($p = 0.02$) higher whole-body crude protein level was recorded in fish fed FN 1.0% (17.63 ± 0.36) compared to other dietary groups, except 0.5% (16.83 ± 0.46) and 1.5% (16.52 ± 0.30) FN-fed fish. In comparison to the FN dietary groups, the control (9.98 ± 0.05) group had the highest whole-body lipid, which was statistically ($p < 0.001$) significant. The whole-body lipid of the FN-fed groups showed a considerable reduction in dose-dependent patterns.

The protein retention result showed that the fish fed FN 1.0% (36.50 ± 2.65) registered the highest value, which is similar to FN 0.5% (30.28 ± 3.48) but differs significantly from other dietary groups ($p = 0.015$). Between the control (69.31 ± 1.01) group and the FN-fed group, the lowest ($p < 0.001$) lipid retention value was recorded in 2.0% (46.41 ± 0.54) FN-fed fish compared to other dietary groups. However, no difference was noticed between the control (69.31 ± 1.01), 0.5% (67.16 ± 0.77), and 1.0% (66.66 ± 0.64) FN groups ($p > 0.5$). The control (36.12 ± 1.02) group's energy retention and that of the 0.5% (38.16 ± 0.53) and 1.0% (40.83 ± 0.97) FN dietary groups did not differ significantly ($p > 0.05$).

3.3. Hepatic Metabolic Enzymes. Substantial variations were observed in the hepatic metabolic enzyme activities of African catfish hybrids, as shown in Table 4. The 1.0% FN dietary group's AST (65.80 ± 0.08), ALT (79.54 ± 0.08), and ALP (18.43 ± 0.03) levels were the highest and substantially ($p < 0.001$) different from those of the other dietary groups. The lowest concentrations of these enzymes are found in the control group. The liver total protein did not vary significantly among the dietary groups ($p = 0.061$).

The control group had the highest level of reactive oxygen species (ROS) (6.86 ± 0.04), malondialdehyde (MDA)

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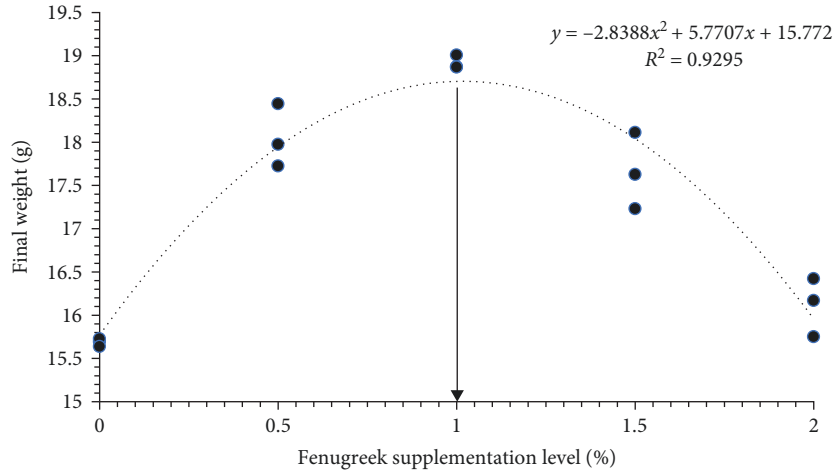


FIGURE 2: Second-order polynomial of final weight and fenugreek supplementation level in the diet of hybrid African catfish (*C. gariepinus* ♀ × *H. bidorsalis* ♂).

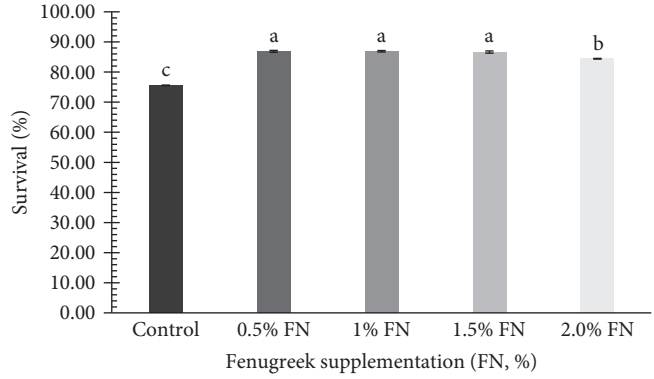


FIGURE 3: Percentage survival of hybrid African catfish (*C. gariepinus* ♀ × *H. bidorsalis* ♂) fed fenugreek-supplemented diets. Bar values (means with standard errors, $n = 3$) with distinct letters are substantially different (Tukey's-*b* test; $p < 0.05$) from each other.

TABLE 3: Whole body nutrient composition (g 100 g^{-1}) and nutrient retention (%), energy content (kcal/100 g) of hybrid African catfish (*C. gariepinus* ♀ × *H. bidorsalis* ♂) fed diets containing graded levels of Fenugreek powder.

Parameters	Initial	Control	Fenugreek (FN) supplemented diets				*p* Value
			0.5%	1.0%	1.5%	2.0%	
Moisture	76.47 ± 0.08	68.25 ± 0.04^{b}	67.24 ± 0.05^{c}	67.01 ± 0.03^{c}	68.36 ± 0.32^{b}	71.63 ± 0.06^{a}	<0.001
Crude protein	12.92 ± 0.03	15.78 ± 0.34^{b}	16.83 ± 0.46^{ab}	17.63 ± 0.36^{a}	16.52 ± 0.30^{ab}	15.91 ± 0.18^{b}	0.020
Crude lipid	5.89 ± 0.01	9.98 ± 0.05^{a}	9.84 ± 0.03^{b}	9.82 ± 0.02^{b}	9.47 ± 0.03^{c}	8.62 ± 0.03^{d}	<0.001
Ash	4.72 ± 0.04	5.80 ± 0.03^{a}	6.11 ± 0.03^{a}	5.57 ± 0.03^{a}	5.63 ± 0.31^{a}	5.01 ± 0.06^{b}	0.004
Energy	463.96 ± 3.22	631.47 ± 0.76^{bc}	640.96 ± 2.32^{ab}	653.34 ± 2.71^{a}	620.55 ± 4.15^{c}	559.28 ± 6.97^{d}	<0.001
Nutrient retention (%)							—
Protein retention		22.19 ± 2.90^{b}	30.28 ± 3.48^{ab}	36.50 ± 2.65^{a}	25.46 ± 2.07^{b}	23.14 ± 1.19^{b}	0.015
Lipid retention		69.31 ± 1.01^{a}	67.16 ± 0.77^{a}	66.66 ± 0.64^{a}	60.15 ± 0.24^{b}	46.41 ± 0.54^{c}	<0.001
Energy retention		36.12 ± 1.02^{ab}	38.16 ± 0.53^{ab}	40.83 ± 0.97^{a}	33.78 ± 1.75^{b}	20.57 ± 2.20^{c}	<0.001

Note: Row values (means with standard errors, $n = 3$) with different superscripts differ from one another considerably ($p < 0.05$).

(3.09 ± 0.04), and lactate dehydrogenase (LDH) (319 ± 53.68) enzyme activity, which was statistically different ($p < 0.001$) from those of the FN-fed groups; however, no differences were observed among those FN-based diets, irrespective of the supplementation level. This group fed 2.0% FN (3.01 ± 0.08) diet shared statistical similarity ($p > 0.05$) in MDA with the control group. In contrast, the 1.0% dietary group had the significantly highest levels of both first-line defense antioxidants (SOD, 457.64 ± 2.39; catalase [CAT], 887.97 ± 2.15; and glutathione peroxidase [GPx], 204.69 ± 1.36) and second-line defense

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Table 4: Hepatic enzyme activities of hybrid African catfish (*C. gariepinus* ♀ × *H. bidorsalis* ♂) fed fenugreek-supplemented diets.

Metabolic enzymes	Control	Fenugreek (FN) supplemented diets				*p* Value
		0.5%	1.0%	1.5%	2.0%	
Total protein (g/dL)	4.08 ± 0.11^{a}	$4.15 \pm 0.0.06^{a}$	4.34 ± 0.04^{a}	4.13 ± 0.05^{a}	4.12 ± 0.09^{a}	0.061
AST (U/L)	41.84 ± 0.02^{e}	64.96 ± 0.07^{b}	65.80 ± 0.08^{a}	55.83 ± 0.04^{c}	54.74 ± 0.08^{d}	<0.001
ALT (U/L)	71.10 ± 0.09^{e}	76.77 ± 0.06^{b}	79.54 ± 0.08^{a}	74.46 ± 0.03^{c}	71.73 ± 0.07^{d}	<0.001
ALP (U/L)	10.58 ± 0.02^{e}	17.95 ± 0.02^{b}	18.43 ± 0.03^{a}	16.55 ± 0.06^{c}	16.10 ± 0.05^{d}	<0.001
Oxidative stress indices						—
ROS (mg/L)	6.86 ± 0.04^{a}	6.26 ± 0.05^{c}	6.21 ± 0.08^{c}	6.27 ± 0.05^{c}	6.56 ± 0.10^{b}	<0.001
MDA (U/mg protein)	3.09 ± 0.04^{a}	2.48 ± 0.01^{c}	1.89 ± 0.04^{d}	2.89 ± 0.03^{b}	3.01 ± 0.08^{a}	<0.001
LDH (U/L)	319 ± 53.68^{a}	72.66 ± 3.26^{c}	42.11 ± 1.00^{d}	208.30 ± 7.49^{b}	218.02 ± 1.36^{b}	<0.001
Antioxidants enzymes						—
GSH (U/mg protein)	20.45 ± 0.27^{b}	21.80 ± 0.39^{b}	25.40 ± 0.70^{a}	21.38 ± 0.75^{b}	20.57 ± 0.35^{b}	<0.001
GPx (U/mg protein)	142.65 ± 2.44^{d}	192.15 ± 5.80^{b}	204.69 ± 1.36^{a}	169.59 ± 2.45^{c}	161.23 ± 2.88^{c}	<0.001
GST (nmole/CDMB)	2.14 ± 0.05^{d}	2.83 ± 0.02^{a}	2.98 ± 0.04^{a}	2.57 ± 0.03^{b}	2.36 ± 0.05^{c}	<0.001
SOD (U/mg protein)	208.18 ± 4.45^{d}	311.57 ± 1.26^{b}	457.64 ± 2.39^{a}	303.86 ± 4.47^{b}	274.60 ± 2.85^{c}	< 0.001
CAT (U/mg protein)	351.78 ± 10.64^{d}	872.65 ± 1.92^{a}	887.97 ± 2.15^{a}	750.46 ± 8.61^{b}	446.24 ± 8.67^{c}	< 0.001

Note: Row values (means with standard errors, $n = 3$) with different superscripts differ from one another considerably ($p < 0.05$).
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; GPX, glutathione peroxidase; GSH, glutathione; GST, glutathione S-transferase; LDH, lactate dehydrogenase; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase.

antioxidants (glutathione [GSH], 25.40 ± 0.70 and glutathione S-transferase [GST], 2.98 ± 0.04), even though there was no statistically remarkable ($p > 0.05$) difference between CAT and GST of the 0.5% and 1.0% FN dietary groups.

3.4. Liver Electrolytes. The liver electrolytes of the differently-fed fish are depicted in Figure 4. The concentrations of the electrolytes Na^+, Cl^-, $Ca^{2+}$and K^+ were statistically remarkably ($p < 0.001$) elevated in the 1.0% FN dietary group. Hepatic Cl^- displayed a similar trend of results as hepatic Na^+. The 2.0% FN dietary group and the control group showed statistical similarities in hepatic Na^+ and hepatic Cl^-. The hepatic Ca^{2+} and K^+ of these dietary groups were also statistically similar.

4. Discussion

Herbal supplementation in fish diets has been promoted as a means of enhancing growth performance, regulating physiological conditions, boosting immunity, and enhancing antioxidative status in fish [8, 37, 38]. In the current study, the dietary supplementation of FN improves the growth performance, nutrient utilization efficiency, and survival rate of hybrid African catfish. The observed growth-promoting effect of FN could be related to the bioactive components, such as flavonoids, steroidal saponins, alkaloids, and polyphenols, which have the potency to influence intestinal enzyme activity and improve feed digestion for optimum nutrient absorption [39, 40]. The improved growth recorded in the FN-fed fish, especially the 1% group, clearly aligns with the nutrient utilization (FCR and PER) indices, suggesting that the FN did elicit the digestive enzyme function necessary for effective nutrient breakdown and uptake via the intestinal wall. Furthermore, the fish fed the FN-based diet consumed less feed, as indicated by the low feed intake per unit gain in weight (low FCR), which implies that the FN-fish convert feed more efficiently than those fed the control diet. As a result, the use of FN as a feed supplement could help reduce production costs due to less feed input, which is desirable for fish farming operations. Our observation is in congruent with Diab et al. [27], who reported that the methanolic extract of FN enhanced the growth performance and feed conversion efficiency in Nile tilapia. Similarly, Mehboob et al. [41] also reported that 1% FN supplementation supported optimal growth performance and digestibility coefficient in striped catfish. The best growth performance recorded in the fish-fed 1.0% FN diet is comparable to that of Mostafa et al. [42] for Nile tilapia and Roohi et al. [43] for common carp. Additionally, the level of FN that supported good growth was optimized using second-order polynomial regression, and the optimal level was observed at the 1% supplementation level for hybrid African catfish. The coefficient of determination (R^2) of 0.9295 better explains the relationship; 92.95% of the variations in the final weight of hybrid African catfish were explained by FN supplementation level [44].

Body nutrient composition and retention data showed similar tendencies to those seen in the growth performance parameters. The highest total body protein and energy content was found in the 1.0% dietary group, which is consistent with Abdelhamid and Soliman [45] submission that FN greatly enhances the nutritional composition of fish. The hypolipidemic effect of FN, reported by Abd El-Salam et al. [46], was supported by the considerable reduction in total body lipids observed in this investigation, which showed a dose-dependent trend. In contrast to these findings, it was stated in Abdelhamid and Soliman [45] that a nonsignificant decrease in carcass lipid was seen in fish given FN-based diets. The nutrient retention data exhibited similar trends to those found for the fish's nutrient content.

Although there were statistical similarities among the different dietary groups, the FN dietary groups revealed elevated amounts of hepatic total protein than the control group. Numerically, the 1% dietary group had the highest concentration of

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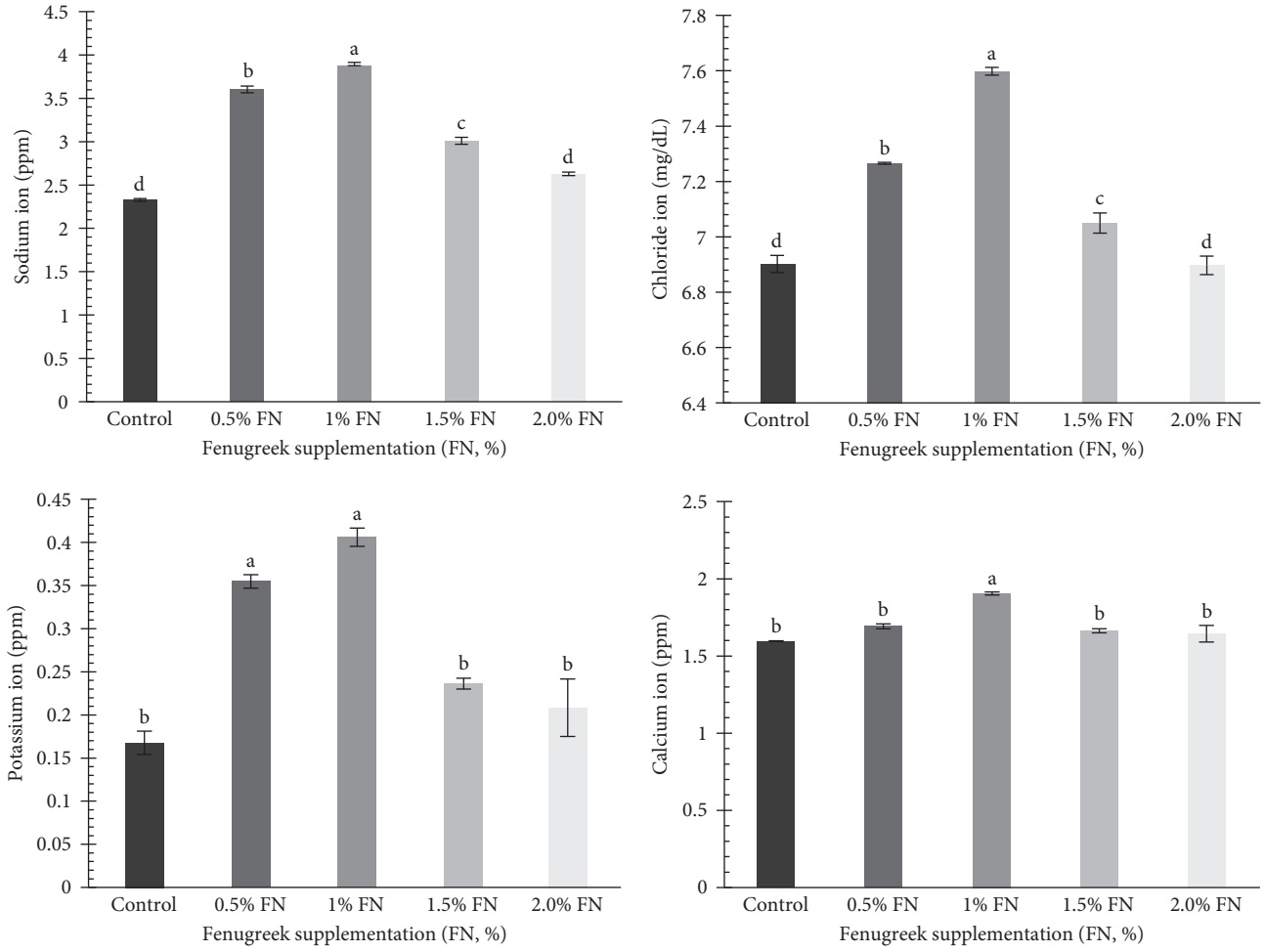


FIGURE 4: Liver electrolytes of hybrid African catfish (*C. gariepinus* ♀ x *H. bidorsalis* ♂) fed fenugreek-supplemented diets. Bar values (means with standard errors, $n = 3$) with distinct letters are substantially different (Tukey's-*b* test; $p < 0.05$) from each other.

total protein. The lack of a statistically remarkable difference between the amounts of hepatic protein generated by the liver in this study across different dietary groups suggests that the fish in each group used the various amino acids in their feed similarly, demonstrating that protein intake was enough to ensure that the required amount of amino acids was supplied in each diet. Eisenstein and Harper [47] reported that when protein intake is lower than what the body needs, the availability of amino acids may limit the pace of hepatic protein synthesis. The synthesis of nearly all plasma proteins, such as albumin, globulin, etc., relies greatly on the liver. Hepatic total protein, which includes albumin and globulin, is known to be one of the essential components of the nonspecific innate immune system [48]. A significant variation was observed in the metabolic enzymes (AST, ALT, and ALP) in the liver of hybrid African catfish in this study. Metabolic enzyme levels were substantially higher in the FN-fed groups than in the control group, demonstrating the enhancement of physio-metabolic responses by FN supplementation. The AST, ALT, and ALP values in the 1% dietary group were the highest and remarkably higher than those in the other dietary groups. The lowest concentrations of these enzymes were found in the control group. For the liver to function at its best in terms of growth and energy use, hepatic AST, ALT, and ALP act as metabolic intermediaries between protein and carbohydrate catabolism [49, 50]. When a protein's non-nitrogenous portion is no longer required by the bodily system, ALT enzymes convert it to fat or glucose before eventually turning it into glycogen [51]. When the body system needs some nonessential amino acids, AST enzymes aid in transamination by transferring amino groups to keto acid groups, while ALP dephosphorylates substances as part of its physiological function [34,34]. According to Cheng et al. [52], the production of keto acids decreases in response to dietary deficiencies in some essential amino acids, which lowers AST enzyme activity. Furthermore, Fawole et al. [50] reported that the higher ALP activity signifies better phosphorus metabolism and nutrient absorption in the intestine, resulting in improved phosphorus utilization and growth. Therefore, the much higher levels of AST, ALT, and ALP enzymes observed in the FN-based groups imply that the essential amino acids were present in those diets in appropriate amounts and optimal transport of nutrients.

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The FN dietary groups had considerably lowered activity of the stress enzymes (ROS, MDA, and LDH), with the lowest level being observed in the 1% dietary group. These stress enzyme activities were highest in the control group. Protein, lipid, and DNA damage may result from increased activity of these stress enzymes, resulting in numerous diseases [53, 54]. Another widely used biological indicator of oxidative stress is lipid peroxidation, which typically correlates favorably with MDA levels [55]. A physiological imbalance favoring oxidants (free radicals or reactive species) over antioxidants causes the cellular phenomena or state known as oxidative stress. The lowered stress enzyme activities recorded among the FN dietary groups might not be unconnected to significantly greater levels of both first-line defense antioxidants (SOD, CAT, and GPx) and second-line defense antioxidants (GSH and GST). The fact that there was no statistically noteworthy difference in the ROS between the dietary groups of 0.5% up to 1.5% suggests that these FN dietary groups showed no signs of stress. Antioxidants play a crucial role in the body's overall defensive strategy, notably in relation to the superoxide anion radical that is constantly produced during routine bodily metabolism. The antioxidant enzyme system of fish has been demonstrated to be improved by a number of herbs, presumably due to the bioactive chemicals they contain [56]. First-line antioxidants work to reduce or stop the production of free radicals or reactive species in cells. They break down hydrogen peroxide, hydroperoxide, and superoxide radicals into benign compounds, respectively. Second-line antioxidants are in charge of contributing an electron to neutralize or destroy free radicals, and in the process, they transform into new, less dangerous free radicals [53]. Similarly, Guardiola et al. [28] reported that FN inclusion in the diet of gilthead seabream (*Sparus aurata*) enhanced hydroxyl radical scavenging capacity and imparted extremely high antioxidant activity on the fish.

The maintenance of fish cells', tissues', or organs' normal physiological function depends on the management of liver electrolytes and any other fish tissues. In order to maintain internal homoeostasis, hepatic electrolytes are essential [57]. According to Karthikeyan et al. [58], they are critical elements of cellular metabolism. The concentrations of the electrolytes Na^+, Cl^-, Ca^{2+}, and K^+ were statistically substantially ($p < 0.05$) elevated in the 1% dietary group. According to Gabriel, Obomanu, and Edori [59], a considerable decrease in Na^+ concentration in the liver could impair its iso-osmolarity, which would lead to stress. 0.5%–1.5% dietary group had a significantly higher hepatic Na^+ concentration, further establishing no stress condition among the FN dietary groups. The trend in liver electrolyte data revealed that the FN-fed fish had an enhanced or comparable level in electrolytes with control, further demonstrating no stress state in the liver of the dietary group. The trend of results in electrolytes may be due to the absence of a physiological stress condition in the FN dietary group, which was associated with a much higher level of antioxidant capacity in the liver of the fish group.

5. Conclusion

Conclusively, in terms of growth responses and survival rates, the FN dietary groups outperformed the control group. Diets based on FN increased protein retention and improved carcass nutritional composition. Supplemental FN significantly and dose-dependently decreased the fat content of the carcass, as well as increased metabolic enzymes. FN-based meals dramatically decreased hepatic stress enzymes while enhancing the fish's antioxidant defenses; hence, based on the results obtained in the current findings and the polynomial regression analysis, the addition of FN at 1.0% inclusion would augment the growth performance and well-being of hybrid African catfish without any deleterious impact on the fish's physiological status.

Data Availability Statement

Upon reasonable request, the data which supports the study's outcomes, will be made available by the corresponding author.

Ethics Statement

Standard operating procedures on fish transportation, welfare, and housing in accordance with University of Ilorin policies and procedures for the handling and use of laboratory animals before, during, and after the experiment were followed in accordance with the experimental protocol and procedures approved by the Departmental Ethical Review Committee (DERC), Department of Aquaculture and Fisheries, Faculty of Agriculture, University of Ilorin, Nigeria; Protocol Identification number DERC/AQF/0014, DERC Approval Number: DERC/AQF/2020/1214. The reporting adhered to ARRIVE's rules (Animal Research: Reporting of In-vivo Experiments). The version that was submitted has the approval of all the people who are listed as writers and are eligible to be authors. The work is unique, and no other journals are considering it.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Wasiu Adeyemi Jimoh was responsible for conceptualization, supervision, and writing—final review and editing. Femi John Fawole was responsible for data curation, formal analysis investigation, and writing—review and editing. Ayodeji Ahmed Ayeloja was responsible for data curation, formal analysis investigation, and writing—review and editing. Olayinka Abosede Ojo was responsible for data curation, formal analysis investigation, and writing—review and editing. Boluwatife Mary Ijalaye was responsible for project administration and Writing—original draft. Aishat Yetunde Adesaogun was responsible for project administration and writing—original draft. Emmanuel Oladele Olaitan was

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responsible for project administration and writing—original draft.

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