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Research Article

Effect of Dietary Polyherbal Mixture on Growth Performance, Haemato-Immunological Indices, Antioxidant Responses, and Intestinal Morphometry of African Catfish, *Clarias gariepinus*

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A 56-day feeding trial was conducted to evaluate the dietary effect of Nigeria polyherbal mixture (PHB) on growth, haemato-immune parameters, antioxidant enzyme activities, and intestinal morphometry of African catfish, *Clarias gariepinus*. Four diets were formulated with PHB powder at inclusion of 0 g/kg (control), 0.5 g/kg (PHB 0.5), 1.0 g/kg (PHB 1.0), and 2.0 g/kg (PHB 2.0) and fed to African catfish (6.32 ± 0.02 g/fish, 15 fish/50 L tank) in triplicates. Fish fed PHB 2.0 recorded higher final weight, weight gain, and specific growth rate ($p < 0.05$) compared to the control. There were no statistical differences ($p > 0.05$) in feed conversion ratio and protein efficiency ratio among the dietary groups. The haematological profile indicates that the fish fed PHB-1.0 had higher Hb, RBCs count, and Hct, and blood performance ($p < 0.05$) compared to the control. ALT levels were found higher in the control and the lowest values recorded in PHB groups ($p < 0.05$); however, AST did not differ significantly among the groups ($p > 0.05$). The highest heterophil counts and respiratory burst activity were recorded in PHB-1.0 and PHB-0.5 and PHB-2.0, respectively, while a numerically improved total immunoglobulin value was recorded in PHB-0.5 compared to the control ($p > 0.05$). Antioxidant enzymes such as superoxide dismutase and catalase had a significant improvement in fish fed PHB-based diet compared to the control ($p < 0.05$), while glutathione peroxidase showed no statistical differences between the groups ($p > 0.05$). Intestinal morphometric measurements showed that the fish fed PHB-1.0 had the highest villus height, area of absorption, and villus height/cryptal depth ratio compared to the control. Muscle thickness increased ($p < 0.05$) with increasing PHB level with the highest value recorded PHB-2.0. In conclusion, dietary PHB could improve growth, blood profile, immunity, antioxidant enzyme capacity, and intestinal morphometry of African catfish without any significant alteration in the liver function enzymes.

1. Introduction

Aquaculture is the world's fastest-growing food-producing sector, and its sustainability must be enhanced in order for it to continue to grow. The most recent global statistics on aquaculture fish output reached a new high of 82.1 million tonnes (MT) (114.5 MT including aquatic plants and mollusks) and accounted for about 52% of the global food fish for human consumption [1]. However, to meet the projected human population demands by 2050, aquaculture contribution to food fish must be increased beyond its current level [1]. According to Naylor et al. [2], global fish consumption will need to increase by nearly 80% in the coming decades to meet the growing population. Due to spatial constraint, this projected increase can only be achieved through a high-density intensive farming method, and this is usually accompanied by numerous technical problems such as oxygen depletion, husbandry-associated stress, immune system depression, diseases, growth reduction, and mortality [3, 4]. With little barriers to prevent diseased fish from infecting others,

Table 1: Biological functions of Nigerian herbs used for polyherbal preparation.

Herbals	Family	Biological function	Reference
Psidium guajava	Myrtaceae	Promote growth, antioxidant, antimicrobial activity.	Fawole et al. [9, 55], Giri et al. [56] Olusola and Olorunfemi [22]
Parquetina nigrescens	Asclepiadaceae	Strengthen immunity, stimulate blood production, anti-inflammatory, antioxidant	Airaodion et al. [57] Ighodaro et al. [19] Ayoola et al. [24]
Anacardium occidental	Anacardiaceae	Antioxidant, anti-inflammatory, antibacterial, antiparasitic	Encarnacao et al. [23] Abreu et al. [58] Andrade et al. [59]
Cymbopogon citratus	Poaceae	Improve growth, strengthen immunity, anti-inflammatory, antioxidant	Adebayo et al. [21] Awe et al. [60]
Mangifera indica	Anacardiaceae	Anti-inflammatory, antimicrobial, antioxidant	Kumar et al. [20]; Fawole et al. [9]
Ocimum gratissimum	Lamiaceae	Promote digestion and growth, antioxidant, improve innate immunity and survival	Abdel-Tawwab et al. [14]
Carica papaya	Caricaceae	Promote digestion, antioxidant, anti-inflammatory, improve growth performance	Fawole et al. [4]; Hamid et al. [61]

antibiotics have become an inevitable disease management tool for fish farmers [5]. However, this measure has received a number of criticisms due to numerous side effects [6].

Natural compounds of plant origin are becoming increasingly popular in aquaculture as an alternative to antibiotics, both in terms of efficacy and cost, and are superior to other additives because they contain high levels of organic constituents that are safe for fish and humans, as well as the environment [7, 8]. Herbs and their derivatives have been reported in fish as growth enhancers and activators of the innate immune system and have antioxidant and antibacterial properties due to the presence of several bioactive principles [3, 9–12]. For instance, in rohu (*Labeo rohita*), Fawole et al. [9, 13] showed that the use of guava and mango leaf extract effectively improves the growth, white blood cells, respiratory burst and lysozyme activities, and resistance against *Aeromonas hydrophila*. Similarly, the use of *Ocimum gratissimum* significantly improved the growth, innate immunity, and survival of African catfish after challenge with *Listeria monocytogenes* [14].

In folklore medicine in Nigeria, different types of herbs are usually mixed together to improve efficacy and potency, and this method is widely applied in alternative medicine practice. Polyherbal formulations, as widely used in Nigerian folklore medicine, are made up of different plant constituents and unpurified extracts that have medicinal properties and are used for the treatment of various ailments, as well as to maintain well-being [15, 16]. This is premised on the notion that a mixture of different herbs, with varied bioactive constituents, could work synergistically and have a greater beneficial effect than a single preparation. Combinations of turmeric and black pepper powder were reported to improve growth and ameliorate the hepatotoxic, reprotoxic, and nephrotoxic effects of cadmium in *Clarias gariepinus* [16]. In aquaculture, several attempts have been made to examine the efficacy of different polyherbal mixtures [4, 11, 12, 17], and the results obtained have been of great interest for antibiotic-free aquaculture. However, despite the great potential of polyherbal mixture for the treatment of ailments in Nigeria, little has been done in aquaculture. For instance, study has shown that lemon grass (*Cymbopogon citratus*) has potent antioxidants, anti-bacterial, anti-inflammatory, and antifungal effects [18]. Similarly, *Parquetina nigrescens* has been reported to stimulate and enhance blood production [19]. *Mangifera indica* leaf is another herb commonly used in Nigerian traditional medicine due to its antidiabetic, antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, and immunostimulatory effects [9, 20]. Despite the documented biological functions of these herbs (Table 1), little emphasis has been placed on their potential synergistic effect on fish, especially African catfish. Huang et al. [11] showed that twelve dietary Chinese herbal mixtures improve growth, stimulate digestive enzyme activity, and enhance the antioxidant and immune response of European eels (*Anguilla anguilla*). Similarly, dietary mixture of *Coriandrum sativum*, *Malva sylvestris*, and *Quercus brantii* elevated growth, non-specific immunity, and improved resistance of common carp against *Aeromonas hydrophila* [12]. On the contrary, the combination of onion peel and pawpaw seed powder did not elicit a growth response nor improve the blood profile in African catfish [4]. Nevertheless, based on individual biological functions, we hypothesized that seven different herbs mixed together in the current study may work synergistically to improve efficacy, promote growth, and enhance fish health status. Therefore, this study examined the dietary effects of a polyherbal mixture on growth performance, haemato-immune parameters, and intestinal morphometry of African catfish, *Clarias gariepinus*, the most important aquaculture fish species in sub-Saharan Africa.

2. Materials and Methods

2.1. Collection, Identification, and Preparation of Plant Material. The leaves of 7 different plants were collected according to their biological functions (Table 1) from the

local medicinal herbal dealers in Ilorin, Nigeria. The collected herbs were taken to the herbarium of the Department of Plant Biology of the University of Ilorin, for proper identification and authentication. The herbs were later air dried under shade, after which they were powdered using a kitchen blender. The finely powdered lemongrass leaf (*Cymbopogon citratus*), guava leaf (*Psidium guajava*), mango leaf (*Mangifera indica*), pawpaw leaf (*Carica papaya*), cashew leaf (*Anacardium occidental*), bullock leaf (*Parquetina nigrescens*), and scent leaf (*Ocimum gratissimum*) were thoroughly mixed together in a ratio of 1 : 1 : 0.5 : 0.25 : 0.5 : 0.5 : 1 on a dry *w*/*w* basis, respectively, decided based on previous findings [4, 9, 14, 21–24]. The mixed polyherbal sample, hereafter referred to as PHB, was packed in a Ziploc bag and stored at 4°C until use.

2.2. Experimental Diets and Proximate Analysis. Four isonitrogenous (388.45 g/kg crude protein) and isolipidic (88.22 g/kg crude lipid) experimental diets were prepared to include control (basal diet (BD) without polyherbal mixture (PHB), PHB 0.5 g/Kg (BD + PHB 0.5), PHB 1.0 g/Kg (BD + PHB 1.0), and PHB 2.0 g/Kg (BD + PHB 2.0). Other ingredients were purchased from a local feed mill in Ilorin, grinded into fine powder and homogeneously mixed. Hot water was added to the homogenous feed mixture to form dough. The trial diets were pelleted at the Department of Aquaculture and Fisheries, University of Ilorin, Nigeria, using a pelletizer fitted with a 2 mm diameter. Feeds were dried to final moisture of less than 10% and then stored in a refrigerator (4°C) in a Ziploc bag. Diet samples were collected for proximate analysis before the start of the feeding trial to ensure that the expected values were achieved. The dietary ingredient and chemical composition of the experimental diets is given in Table 2.

Diet samples were dried in a convection oven at 103°C for 12 h to determine the moisture level according to AOAC [25]. The moisture free samples were finely ground by mortar and pestle, and crude protein (total nitrogen ×6.25) was analyzed using a Kjeldahl's method. The ether extract was analyzed following AOAC [25] method. Ash was analyzed by incineration at 550°C in a muffle furnace for 5 h.

2.3. Experimental Animal and Feeding Protocols. Two hundred and twenty-five (225) healthy African catfish (*C. gariepinus*) fingerlings were obtained from a reputable fish hatchery in Ilorin, Kwara State, Nigeria. Fish were transported to the wet laboratory of the Department of Aquaculture and Fisheries, University of Ilorin, in a plastic tank and were acclimatized in the rearing tank for 17 days during which they were fed control diet (2 mm). At the beginning of the feeding, fifteen fish (6.32 ± 0.02 g/fish) were randomly stocked in each of fifteen 50 L rectangular tanks in triplicates. Fish were hand fed to apparent satiation two times daily at 8:00 h and 17:00 h during the 56-day trial. A completely randomized design was used to assign diets to account for any effects on tank position. Photoperiod was maintained at 12 h light : 12 h dark cycle. During the trial, the water temperature range between 26.93 and 27.11°C, oxygen 5.5–6.13 mg/L, and pH 7.58–7.63. The water temperature was measured with a clinical thermometer; the pH was measured with a portable pH meter (Model pHep, Hanna Instruments, Romania). The fish were weighed and counted every two weeks to monitor growth and adjust feeding accordingly. Feeding was stopped for 24 h before each measurement to avoid including the ingested feed in the weight measurement.

2.4. Growth and Nutrient Utilization Parameters. After 56 days, the fish were fasted for 24 h, and the individual weight of the fish was measured to determine the growth and nutrient utilization parameters following the equation described below.

$$
\begin{aligned}
\text{Weight gain (WG, g)} &= \text{final body weight (g)} - \text{initial body weight (g)},\\
\text{Specific growth rate (SGR, \%/day)} &= \left[\frac{(\ln \text{finalbody weight (g)} - \ln \text{initialbody weight (g)})}{\text{duration of feeding}}\right] \times 100,\\
\text{Feed intake (g/fish)} &= \frac{\text{total dry feed given (g)}}{\text{number of surviving fish}},\\
\text{Feed conversion ratio (FCR)} &= \left[\frac{\text{dry feed consumed (g)}}{(\text{final biomass (g)} - \text{initial biomass (g)} + \text{dead fish weight (g)})}\right],\\
\text{Protein efficiency ratio (PER)} &= \left[\frac{\text{net weight gain (g)}}{\text{protein fed (g)}}\right],\\
\text{Hepatosomatic index (HSI, \%)} &= 100 \times \frac{\text{wet weight of liver (g)}}{\text{whole body weight of fish (g)}},\\
\text{Viscerosomatic index (VSI, \%)} &= 100 \times \frac{\text{wet weight of visceral (g)}}{\text{whole body weight of fish (g)}},\\
\text{Condition factor } \left(\text{CF, g/cm}^3\right) &= \frac{\text{Body weight of fish (g)}}{(\text{Body length of fish (cm)})^3} \times 100.
\end{aligned}
\tag{1}
$$

Table 2: Ingredient composition of the experimental diets containing graded levels of polyherbal mixture fed to *C. gariepinus* juveniles.

Ingredients (g/kg)	Control	PHB-0.5	PHB-1.0	PHB-2.0
Fishmeal	100	100	100	100
Soyabean meal	370	370	370	370
Poultry byproduct meal	250	250	250	250
Maize	80	79.5	79	78
Wheat offal	106.9	106.9	106.9	106.9
Vegetable oil	50	50	50	50
Vitamin-mineral premix	20	20	20	20
Dicalcium phosphate	10	10	10	10
Vitamin-C	0.1	0.1	0.1	0.1
Binder	10	10	10	10
Lysine	1	1	1	1
Methionine	2	2	2	2
Polyherbal mixture	0	0.5	1	2
Total	1000	1000	1000	1000
Proximate composition (% as-is basis)				
Moisture	7.10	7.00	6.40	6.90
Crude protein	39.26	39.60	38.45	38.07
Crude lipid	9.20	9.74	8.07	8.28
Ash	10.20	10.69	12.06	11.34
*Nitrogen-free extract	32.75	32.02	34.24	34.06
**Gross energy (kJ/g)	18.73	18.26	18.75	18.71

Vitamin-mineral mix (mg/kg): vitamin A, 20,000 IU/kg feed; vitamin D3, 4000 IU/kg feed; vitamin E, 200 mg/kg feed; vitamin K3, 8 mg/kg feed; vitamin B1, 20.5 mg/kg feed; vitamin B2, 15 mg/kg feed; vitamin B6, 19.5 mg/kg feed; vitamin B12, 0.015 mg/kg feed; vitamin B3, 90 mg/kg feed; vitamin C, 300 mg/kg feed; folic acid, 4 mg/kg feed; calcium d-pantothenate, 40 mg/kg feed; biotin, 0.5 mg/kg feed; inositol, and 50 mg/kg feed. Fe, 40 mg/kg; Cu, 4 mg/kg; Mn, 30 mg/kg; Zn, 40 mg/kg; I, 2 mg/kg; Se, 0.2 mg/kg; and Co, 0.75 mg/kg. lysine, 50 mg/kg feed; methionine, 50 mg/kg feed; antioxidant, 125 mg/kg feed. *Nitrogen – free extracts (NFE) = 100 – (crude lipid + crude ash + crude protein). **Calculated gross energy was calculated as 23.9, 39.8, and 17.6 kJ/g for protein, lipid, and carbohydrates, respectively.

2.5. Sampling for Haemato-Biochemical Assay. Two fish per tank ($n = 6$) were randomly selected for blood collection through the caudal vein using a 2 mL hypodermic syringe previously rinsed with EDTA. The collected blood was transferred to an EDTA-coated tube for the haematological assay. For serum collection, three fish per tank ($n = 9$) were sampled, and blood was immediately transferred to a plain tube and kept in the refrigerator (4°C) for 24 h. Serum was separated by centrifugation in a cooling centrifuge at 3000 ×g for 10 min; the sera samples were collected and kept at -20°C until further analysis.

Haematological indices such red blood cells (RBC), haemoglobin level, haematocrit value, and white blood cells (WBC) were measured following standard techniques [26]. The smears obtained from EDTA treated samples were air-dried, fixed in 96% ethanol for 30 min, stained with Giemsa, and examined by light microscopy to determine differential leukocyte counts (heterophils and lymphocytes) [27]. Biochemical parameters and antioxidant enzymes such as total protein, glucose, and aminotransferases (ALT & AST) were analyzed using a Fortress kit (Fortress Diagnostics Limited, United Kingdom) while superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were determined using a reagent kit (Elabscience®, USA). The blood performance was determined following the formulae of Esmaeili [28]:

$$\text{Blood performance (BP)} = \text{Ln Hb} + \text{Ln Hct} + \text{Ln RBC} + \text{Ln WBC} + \text{Ln TP}, \quad (2)$$

where Hb is the haemoglobin; Hct is the haematocrit; RBC is the red blood cells; WBC is the white blood cells; and TP is the total protein.

2.6. Innate Immune Parameters. The total immunoglobulin (Ig) concentration was determined following the method described by Anderson and Siwicki [29]. The respiratory burst activity of the phagocytes was measured using the nitroblue tetrazolium assay (NBT, Himedia India) according to the method described by Secombes [30].

2.7. Intestinal Histomorphometry. At the end of the experimental trial, two fish per tank ($n = 6$) were randomly selected for histological examination of the distal intestine according to the method described by Kumar et al. [31]. The distal intestine was fixed in 10% neutral-buffered formaldehyde (pH, 7.4) for 24 hours, after which the samples were dehydrated and embedded in melted paraffin wax as per the conventional histological procedure. The tissue was sectioned (5 μm) and stained with hematoxylin-eosin (H&E). The sections were observed and photographed using an Olympus CX21 light microscope equipped with Amscope MU900 digital camera, and morphological measurements were carried out using Topview software.

2.8. Statistical Analysis. Data were checked for normality and homogeneity of variance using the Kolmogornov–Smirnov and Levene's test, respectively. When normal assumptions were met, data was statistically analyzed by one-way analysis of variance (ANOVA) using the general linear model, and polynomial contrast was used to determine the overall, linear, and quadratic trends of the parameters in relation to the dietary levels of the polyherbal mixture. Duncan's multiple range test was used to separate means when significant differences were found. Mean differences were considered statistically significant with a 95% confidence level. All data were presented as means ± pooled standard error of the mean (SEM). SPSS 22.0 for Windows was used for all analyses (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Growth Performance, Nutrient Efficiency, and Somatic Indices. The growth performance, feed utilization, and somatic indices of African catfish (*C. gariepinus*) fed different experimental diets are shown in Table 3. The final weight (FW) and weight gain (WG) among the dietary groups showed overall significant effects ($p < 0.05$) and followed a linear trend ($p < 0.01$). The specific growth rate (SGR) follows a similar trend and shows linear and quadratic trends

Table 3: Growth performance, nutrient utilization, and somatic indices of *C. gariepinus* fed with polyherbal mixture at different inclusion levels for 56 days.

Parameters	Dietary groups							
	Control	PHB 0.5	PHB 1.0	PHB 2.0	SEM	Overall p-value	Linear	Quadratic
IW (g/fish)	6.31	6.36	6.31	6.31	0.04	0.859	0.829	0.631
FW (g/fish)	15.77^{bc}	15.13^{c}	17.14^{ab}	18.35^{a}	0.58	0.017*	0.005*	0.147
WG (g)	9.46^{bc}	8.78^{c}	10.83^{ab}	12.04^{a}	0.58	0.018*	0.006*	0.144
SGR (%/day)	1.63^{bc}	1.53^{c}	1.72^{ab}	1.81^{a}	0.04	0.005*	0.003*	0.039*
FCR	2.50	3.15	2.25	2.44	0.41	0.476	0.569	0.584
PER	1.13	0.92	1.24	1.20	0.17	0.563	0.496	0.627
FI (g/fish)	23.36	27.60	24.49	29.37	3.81	0.715	0.450	0.983
HSI (%)	1.09	0.89	0.81	0.82	0.12	0.327	0.114	0.378
VSI (%)	4.40	4.25	3.97	3.89	0.39	0.775	0.330	0.933
CF (g/cm^3)	0.78^{a}	0.67^{b}	0.76^{a}	0.70^{ab}	0.02	0.041*	0.248	0.295

Mean values ($n = 3$) with different superscript are significantly different ($p < 0.05$) from each other. SEM: pooled standard errors of the means; PHB: polyherbal mixture; PHB – 0.5 = 0.5 g/kg; PHB – 1.0 = 1.0 g/kg; PHB – 2.0 = 2.0 g/kg. IW: initial weight; WG: weight gain; SGR: specific growth rate; FCR: feed conversion ratio; FI: feed intake; PER: protein efficiency ratio; HSI: hepato-somatic index; VSI: viscerosomatic index; CF: condition factor.

($p < 0.05$). The group fed PHB-2 had the highest FW, WG, and SGR, which is similar to PHB-1 but differs significantly from PHB-0.5 and the control. No significant difference ($p > 0.05$) was observed in the FCR, PER, FI, HSI, and VSI values between the dietary groups, and no observable trends were recorded. The CF was significantly higher in the control and PHB-1 similar to PHB-2 but differ from the PHB-0.5 ($p < 0.05$).

3.2. Haematological Parameters. The haematological parameters of African catfish in different dietary groups are presented in Table 4. Compared to the control group, the fish fed PHB-1.0 had the highest Hb, RBCs count, Hct, and blood performance ($p < 0.05$) and showed a quadratic trend, but no linear trend was observed. There was no significant variation ($p > 0.05$) in the WBC, MCV, and lymphocyte counts between the various dietary groups, and no defined trends were noticed. MCH and MCHC values among the groups showed a linear trend ($p < 0.05$) but there were no overall significant effects ($p > 0.05$). Compared to the control, the fish fed PHB-1 and PHB-0.5 had the highest heterophil count ($p < 0.05$) and followed a quadratic trend ($p = 0.002$; Table 4). Using the 2^{nd}-order polynomial regression, the relationship between dietary PHB levels and blood indices (Hb, RBC, and Hct) was best fitted as follows (Figure 1): $y = -2.1455x^2 + 3.8047x + 4.7693$, $R^2 = 0.9016$; $y = -1.0491x^2 + 1.9005x + 2.3115$, $R^2 = 0.8771$; $y = -6.3645x^2 + 11.452x + 14.418$; $R^2 = 0.9026$; and the blood performance (BP) $y = -1.4973x^2 + 2.7346x + 8.2474$, $R^2 = 0.961$. These relationships showed that the optimal inclusion levels of PHB for African catfish are 0.90, 0.91, 0.90, and 0.92 g/kg for Hb, RBC, Hct, and BP, respectively. The optimal inclusion level that shows higher heterophil counts was found to be 0.99 g PHB/kg diet (Figure 2).

3.3. Biochemical and Innate Immunity Parameters. The overall, linear, and quadratic trends of serum total protein were not significantly affected as a result of feeding PHB-based diets compared with the control ($p > 0.05$; Table 5). The liver function enzymes such as ALT and AST were determined in the serum, and the result is shown in Table 5. The fish fed control had the highest serum ALT compared to those fed PHB-based diets and follows linear and quadratic trends ($p < 0.05$). AST level did not differ significantly ($p > 0.05$) among the groups but follows a linear trend. The dietary impact of PHB on total immunoglobulin does not follow a dose-dependent pattern, but a numerically higher value was observed in the PHB 0.5 group, which was not different from the control or PHB 1.0, but significantly different from PHB 2.0 ($p < 0.05$; Table 5).The respiratory burst activity showed linear and quadratic trends, with a significantly ($p < 0.05$) highest value recorded in fish fed PHB-2 compared to other dietary groups. No significant difference ($p > 0.05$) was noticed for serum glucose concentration among the groups.

3.4. Antioxidant Enzyme Activity. SOD activity was found to show an overall significant effect and followed a quadratic trend (Table 5). Compared to the control, the group fed PHB-0.5 had the highest SOD activity. Catalase activity registered a significantly higher value in fish fed PHB-0.5 similar to PHB-1 and PHB-2 but differs significantly from the control ($p < 0.05$). GPx activity did not show statistical differences among the dietary groups ($p > 0.05$).

3.5. Intestinal Morphometry. The overall, linear, and quadratic trends of the intestinal morphology of African catfish fed different diets are shown in Table 6. The height of the villus in fish fed the PHB-1 diet was significantly higher than those of the control group ($p < 0.05$) while PHB-0.5 records the highest villus width compared to the lowest value seen in PHB-1.0 and PHB-2.0. However, a higher area of absorption was found in fish fed PHB-1.0 followed by PHB-0.5 and the lowest in the control and PHB-2.0. The cryptal depth in fish fed PHB-0.5 was found to be higher than in other dietary

Table 4: Haematological parameters of African catfish (*C. gariepinus*) fed polyherbal mixture at different inclusion levels for 56 days.

Parameters	Dietary groups							
	Control	PHB 0.5	PHB 1.0	PHB 2.0	SEM	Overall p value	Linear	Quadratic
HB (g/dL)	4.97^{bc}	5.60^{ab}	6.83^{a}	3.73^{c}	0.47	0.010*	0.273	0.004*
RBC ($\times 10^{12}$/L)	2.42^{bc}	2.71^{ab}	3.38^{a}	1.88^{c}	0.22	0.009*	0.369	0.004*
Hct (%)	15.00^{bc}	17.00^{ab}	20.67^{a}	11.67^{c}	1.34	0.009*	0.323	0.003*
WBC ($\times 10^{9}$/L)	6.92	6.50	7.89	6.35	0.91	0.644	0.939	0.554
MCV (fl)	62.10	62.77	61.07	62.17	0.44	0.125	0.466	0.634
MCH (pg)	20.53	20.60	20.20	19.87	0.22	0.146	0.040*	0.388
MCHC (g/dL)	33.03	32.87	33.03	31.97	0.28	0.077	0.042*	0.147
Lymphocyte (%)	65.33	60.67	60.00	65.00	2.41	0.323	0.881	0.080
Heterophil (%)	28.00^{b}	38.67^{a}	37.67^{a}	30.33^{b}	2.00	0.012*	0.521	0.002*
Blood performance	8.33^{bc}	9.02^{ab}	9.65^{a}	7.70^{c}	0.38	0.031*	0.484	0.008*

Mean values ($n = 3$) with different superscript are significantly different ($p < 0.05$) from each other. SEM: pooled standard errors of the means; PHB: polyherbal mixture; PHB – 0.5 = 0.5 g/kg; PHB – 1.0 = 1.0 g/kg; PHB – 2.0 = 2.0 g/kg. Hb: haemoglobin; RBC: red blood cells; Hct: haematocrit; WBC: white blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration.

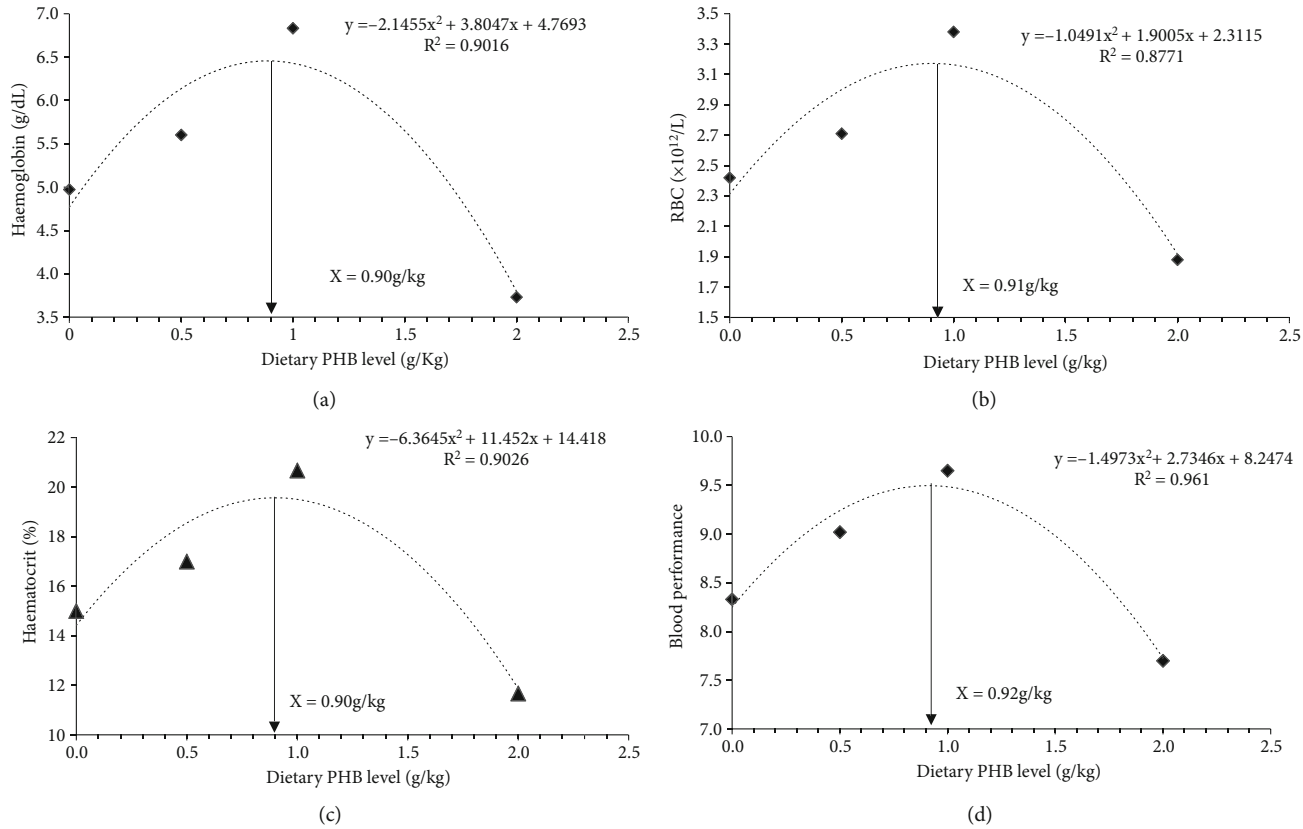

Figure 1: Relationships between the dietary PHB levels and blood parameters of African catfish.

groups, while PHB-1 recorded the highest villus height/cryptal depth ratio ($p < 0.05$). Muscle thickness increased with increasing level of PHB with the highest recorded value in fish fed PHB-2 compared to the control that recorded the lowest value ($p < 0.05$).

4. Discussion

Several herbal plants have been examined and used in the aquatic animal diet to improve fish performance and health status [32]. In the current study, growth performance of

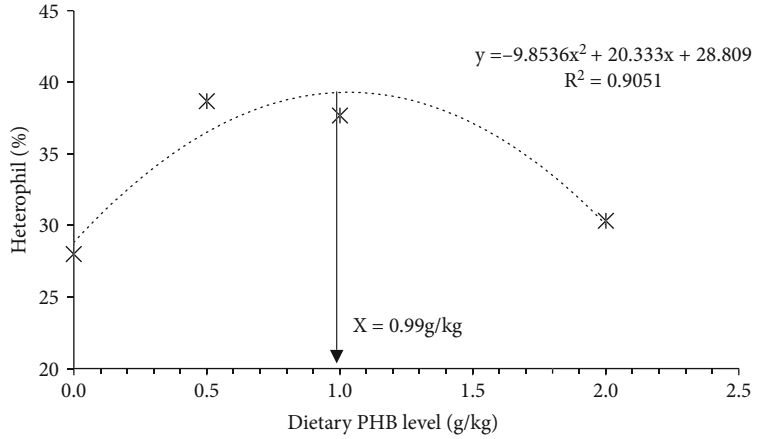

Figure 2: Relationship between PHB levels and heterophil count of African catfish fed for 56 days.

Table 5: Nonspecific immunity, glucose concentration, and antioxidant enzyme activities of African catfish (*C. gariepinus*) fed polyherbal mixture at different inclusion levels for 56 days.

Parameters	Dietary groups							
	Control	PHB 0.5	PHB 1.0	PHB 2.0	SEM	Overall p value	Linear	Quadratic
Total protein (g/dL)	3.77	5.15	4.18	4.34	0.37	0.142	0.677	0.141
ALT (U/L)	73.42^{a}	23.27^{c}	36.96^{b}	23.42^{c}	2.07	<0.001*	<0.001*	<0.001*
AST (U/L)	53.74	40.66	46.22	27.15	5.53	0.057	0.024*	0.608
Total immunoglobulin (g/dL)	1.24^{a}	1.56^{a}	0.66^{ab}	0.14^{b}	0.28	0.031*	0.010*	0.175
Respiratory burst activity	0.33^{b}	0.33^{b}	0.34^{b}	0.35^{a}	0.03	0.004*	0.004*	0.011*
Glucose (mmol/L)	2.39	2.66	3.56	3.56	0.53	0.357	0.107	0.822
SOD (U/mL)	1.11^{bc}	1.58^{a}	1.37^{ab}	0.93^{c}	0.13	0.033*	0.223	0.008*
Catalase (Umol/mL/min)	9.99^{b}	14.45^{a}	11.60^{ab}	12.51^{ab}	0.92	0.048*	0.282	0.089
GPx (U/L)	67.52	73.15	58.52	45.01	9.78	0.270	0.097	0.357

Mean values ($n = 3$) with different superscript are significantly different ($p < 0.05$) from each other. SEM: pooled standard errors of the means; PHB: polyherbal mixture; PHB – 0.5 = 0.5 g/kg; PHB – 1.0 = 1.0 g/kg; PHB – 2.0 = 2.0 g/kg. ALT: alanine aminotransferase; AST: aspartate aminotransferase; SOD: superoxide dismutase; GPx: glutathione peroxidase.

Table 6: Effect of dietary polyherbal mixture on the intestinal morphometry parameters of African catfish (*C. gariepinus*) after 56 days feeding trial.

Parameters	Dietary groups							
	Control	PHB-0.5	PHB-1.0	PHB 2.0	SEM	Overall p value	Linear	Quadratic
Villus height (μm)	684.13^{d}	1028.20^{b}	1429.13^{a}	816.00^{c}	8.88	<0.001*	<0.001*	<0.001*
Villus width (μm)	202.30^{b}	245.69^{a}	188.57^{c}	185.62^{c}	2.90	<0.001*	<0.001*	<0.001*
Area of absorption (μm^2)	138401.27^{c}	252731.30^{b}	269497.81^{a}	151461.56^{c}	4805.78	<0.001*	0.031*	<0.001*
Crypt depth (μm)	246.46^{d}	346.39^{a}	288.81^{c}	308.46^{b}	3.86	<0.001*	<0.001*	<0.001*
Villus height/crypt depth ratio	2.78^{bc}	2.97^{b}	4.95^{a}	2.65^{c}	0.60	<0.001*	<0.001*	<0.001*
Muscle thickness (μm)	204.99^{d}	247.75^{c}	302.65^{b}	320.35^{a}	3.34	<0.001*	<0.001*	0.006*

Mean values ($n = 3$) with different superscript are significantly different ($p < 0.05$) from each other. SEM: pooled standard errors of the means; PHB: polyherbal mixture; PHB – 0.5 = 0.5 g/kg; PHB – 1.0 = 1.0 g/kg; PHB – 2.0 = 2.0 g/kg.

African catfish in terms of FW, WG, and SGR were found to improve at higher inclusion of PHB (1-2 g/kg diet). The increase in weight gain in fish fed PHB-2 and PHB-1 represents 21.43% and 12.65% compared to fish fed control diets. Similar results were reported in Japanese seabass [17] and European eels [11] fed diets supplemented with Chinese herbal medicine mixture. Raissy et al. [12] reported that 5% of combined herb extracts significantly improved growth performance and feed conversion efficiency in *Cyprinus carpio*. In Japanese flounder, the supplementation of combined herbs (*Massa medicata fermentata*, *Artemisia capillaries*, *Cnidium officinale*, and *Crataegi fructus*) significantly enhanced the growth performance and survival compared to the control [33]. However, in our previous study, the combination of onion peel and pawpaw seed powder did not have a growth promoting effect on African catfish [4]. Also, dietary gotu kola, *Centella asiatica*, powder did not improve the growth performance and FCR in Nile tilapia [34]. Fawole et al. [4] reported that phytogenic compounds could antagonize each other when mixed together, thereby resulting in growth depression or indifference. Thus, the better performance seen in this study indicates that the biologically active compounds present in the mixed herbs may not antagonize each other but rather work in synergy to improve the growth of African catfish. Furthermore, the insignificant differences observed in the FCR, PER, and FI indicate that the herbal mixture would not impair feed palatability or acceptance by the fish. The hepatosomatic index (HSI) is often used as a marker of dietary impacts on liver functionality and liver health [35]. In this study, the HSI and VSI values among the dietary groups did not show variation, which implies that the inclusion of PHB would not cause excessive fat accumulation in the liver and intraperitoneal cavity of African catfish. Congruent with this study, Huang et al. [11] found no significant differences in European eel fed mixed Chinese herbal medicine compared to the control.

Blood has been regarded as a pathophysiological reflector of the entire body, and it has been utilized in various studies to assess the health condition and nutritional status of fish in response to feed supplements [4, 12, 36, 37]. The higher Hb, RBCs count, Hct, and blood performance in fish fed PHB-1.0 g/kg suggests improved physiological condition. *Parquetina nigrescens* leaf has been reported to stimulate red blood cells production in anemic rat [19]. Hence, the higher response observed in fish fed PHB, especially PHB-1.0 and PHB-0.5, signifies the potency of the mixed herbs to work together to promote fish blood production and improve oxygen carrying capacity of the blood. The lower Hb, RBCs count, and Hct values in PHB-2.0 compared to the controls may be due to the presence of higher quantities of polyphenolic compounds such as tannins, alkaloids, terpenoids, saponins, etc., which have previously been linked to a considerable decrease in RBC count [38]. Additionally, the reduction could be due to the presence of *Anacardium occidentale* in the PHB mixture, which has been reported to cause a significant decrease in Hb and Hct in rats [39]. Nevertheless, lower inclusion of PHB (0.5 and 1.0 g/kg) improved red blood cell production compared with the control, and based on the polynomial regression analysis, the optimal inclusion level that supported better blood production/performance ranged from 0.90 to 0.92 g/kg diet. Interestingly, the fish with a higher blood profile (PHB 1.0) were found to have higher blood performance than the control, which indicates better fish health. Similar results were reported for combined medicinal herbs [12] and figwort, *Scrophularia striata,* fed to common carp [40]. Furthermore, the considerable rise in the heterophil counts observed in PHB-0.5 and PHB-1.0 could be indicative of innate immune system stimulation and the PHB's potential to activate heterophils in African catfish.

Damage to the liver often results in the release of protein metabolism enzymes like ALT and AST into the blood, which are typically used to evaluate liver health conditions [41]. In the current study, the ALT level was found to be lower in fish fed PHB-based diet compared to the control group. Similar results were reported in *L. rohita* [37] and *Carassius auratus* [32] fed with *Hybanthus enneaspermus* extract and *Flos populi* extract, respectively. Although no significant variation was noticed in the AST level among the groups, but fish fed PHB diets showed a numerically lower value, especially for PHB-2.0. Furthermore, because ALT is found mostly in the liver and has a longer half-life, it is more specific for liver damage/disease than AST, which is detected in a variety of organs in the body. As a result, ALT is a better predictor of liver injury than AST [42, 43]. Based on this assertion, it could be inferred that the polyherbal supplement fed to the African catfish may not have hepatotoxic effect. Herbal extracts have been shown to have immunostimulatory properties in numerous investigations [9, 11, 12, 17, 44]. Innate immunity is the primary defense mechanism in fish, and respiratory burst, lysozyme, immunoglobulins, and complement system are among the most frequently investigated innate immune response parameters in fish [45, 46]. In the current study, dietary PHB stimulated total immunoglobulin (Ig) and respiratory burst activity (RBA), with the highest response seen in PHB-0.5 and PHB-2.0 g/kg, respectively. Although the Ig value did not follow a dose-dependent pattern, the fish in PHB 0.5 had a numerically higher value compared to the control and other groups, and a similar observation was noticed in phagocytosis activity in red hybrid tilapia fed with *Isochrysis galbana* [47]. Hence, since both the RBA and Ig are indicators of the immune system in fish, their higher response in groups fed PHB-based diets is indicative of immune system activation and the potency of the supplement in strengthening immune responses in African catfish. Consistent with this study, dietary administration of plant extracts increased plasma and mucus total Ig in striped catfish [48]. Moreover, dietary supplementation with mango and guava leaf extracts significantly improves the RBA, globulin, and lysozyme activity in *L. rohita* [9]. The RBA and IgM were also found to be enhanced in rainbow trout fed lemon verbena (*Aloysia triphylla*) extract compared with the control [49].

The antioxidant defense system protects cells from oxidative damage and is closely related to the immune system and health status of fish [50, 51]. In this study, fish fed a diet based on PHB showed a higher induction of SOD and CAT enzymes, especially in PHB-0.5 and PHB-1.0, compared to the control, and this indicates that PHB has the ability to

enhance the antioxidant defense system in African catfish. Similarly, supplementation with the Chinese herbal medicine mixture significantly improved the activities of SOD and CAT in European eel [11] and Japanese seabass [17]. Other studies have also reported the enhancing effects of different herbs, singly or in combination, on the fish antioxidant enzyme system [4, 12, 44, 51]. Therefore, the higher activities of SOD and CAT correspond to the higher heterophil counts recorded in PHB-0.5 and PHB-1.0, which means that PHB has the potential to modulate nonspecific immunity and antioxidant enzyme responses in African catfish, and this could be related to the biological active compounds present in the mixed herb.

In fish, the brush border membrane and enterocyte of the small intestine are primarily important for nutrient digestion and absorption, and this can be assessed by gut histomorphometry [52, 53]. Duan et al. [54] reported that the length and width of the intestinal villi influence the contact area between mucosal epithelial cells and the chyme. The higher villi height and greater surface area recorded in fish fed PHB, especially PHB-1.0, is indicative of increased nutrient absorption and improved brush border integrity. Furthermore, the villus height/cryptal depth ratio, which indicates the small intestine's absorption ability and better growth rate, was found higher in PHB-1.0, and this suggests that PHB at 1.0 g/kg diets would enhance the African catfish small intestinal structure and eventually promote growth and feed utilization by the fish. Our result agrees with that of Adeshina et al. [44] who reported that *Mitracarpus scaber* leaf extract supplementation increased villus height and improved the intestinal area of absorption with a resultant increase in growth of Nile tilapia. Adejonwo et al. [53] also reported that mushroom supplementation increased villus height and the absorptive capacity of the gastrointestinal tract in African catfish. Furthermore, the higher depth of the crypt and the increased muscle thickness recorded in this study further reinforce our assertion that the addition of PHB would improve intestinal structural integrity and enhance the growth of African catfish.

5. Conclusions

Our study has shown that the addition of a Nigerian polyherbal mixture (PHB) has the potential to improve the growth performance and haematology profile of African catfish. Furthermore, dietary PHB showed an immunostimulatory effect by enhancing heterophil counts, respiratory burst activity, antioxidant enzyme responses, and a numerical increase in the total immunoglobulin at a lower inclusion level without causing any alteration to the intestinal integrity of the fish. Based on the current findings, PHB can be added to the diet of African catfish at a rate of 0.9-1.0 g/kg.

Data Availability

The data published in this research article is available upon reasonable request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

F.J.F designed the research project and diet formulation, performed the statistical analysis, and wrote the manuscript. R.O. and O.J. contributed to the design and planning of the experiment and conducted the feeding trial, fish sampling, and laboratory analyses. A.O.A contributed to the planning of the experiment. I. A performed the regression analysis. B.O.E contributed to planning of the experiment and interpretation of histological results. All authors read the draft and corrected and approved the final version of the manuscript for submission.

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