HEALTH STATUS AND PHYSIOLOGICAL RESPONSES OF *Clarias gariepinus* FED GRADED LEVELS OF HERBAL COMBINATION SUPPLEMENTED DIETS

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****Olusola, Sunday Emmanuel, Fatokun, Timothy Abiodun and Olowojuni, Olufemi**
Department of Fisheries and Aquaculture Technology, School of Agriculture, Food and Natural Resources, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria
**Corresponding author: se.olusola@oaustech.edu.ng; belloolus@yahoo.com, +2348034110139

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Abstract: To improve fish production and curb the losses from diseases occurring in fish farming, fish farmers resort to the use of phytogenic additives as growth promoters and health management to inhibit the growth of disease-causing organisms, to enhance food security. This study was undertaken to investigate the sustainable effect of the herbal mixture (date palm, tiger nut, thorn apple seed, maca root and snot apple) supplemented diets on health status and physiological responses of *Clarias gariepinus* at different levels of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% for 56 days. The experiment was based on a completely randomized design with each treatment having 20 *C. gariepinus* (mean weight 450± 0.04g), replicated twice and fed at 3% body biomass. The phytochemical screening of herbal plants, internal organs and morphometric traits of *C. gariepinus* were measured using standard methods. The result of the phytochemical screening of the herbal plants revealed the presence of saponins, tannins, alkaloids, steroids, phenol and flavonoids. The results of the internal organ such as gills, liver, kidney, ovary, spleen and heart show significant variation ($P<0.05$) among the dietary groups and the treated groups performed better than the control. The result of morphometric traits of male and female *C. gariepinus* indicated that significantly higher values ($P<0.05$) were obtained in the treated groups compared to the control. It can be concluded that herbal mixture-supplemented diets impact internal organs and morphometric traits of *C. gariepinus* that can influence the productivity of organic fish farming for sustainable agriculture

Keywords: Internal organs; Morphometric traits; *Clarias gariepinus;* Herbal mixture; Phytochemical screening

Introduction

Aquaculture has contributed immensely to the availability of food globally in Nigeria (Food and Agriculture Organization, FAO, 2012). Aquaculture has the potential for growth to meet the natural fish demand, thereby reducing importation, providing employment, alleviating poverty, and helping to meet the millennium development goals (Williams *et al.*, 2007). The annual fish demand in Nigeria as of 2012 is 2.66 million metric tonnes with supply being only 1.32 million metric tonnes, local production is 0.62 million metric tonnes while 0.7 million metric tonnes is from importation, the total fish supply account for only 200,000 metric tonnes from aquaculture. The current state of aquaculture production in Nigeria is far less than its potential production of 2.5 -4.0 million metric tonnes (FAO, 2006). Nigeria has a land mass of 923,766 km2 with about 175 million hectares of suitable sites for aquaculture development with a coastal state bordered in the south by the Atlantic Ocean. The aquaculture sub-sector is considered a very viable alternative to meeting the nation's need for self-sufficiency in fish production. The Food and Agriculture Organization (FAO,1990) recommended that an individual takes 35 g per caput of animal protein per day for sustainable growth and development. In Nigeria, the annual protein consumption is less than 8 g per person per day which is less than FAO minimum requirements. However, the major animal protein sources in Nigeria include fish, cattle, goats, sheep and poultry. Fish and fish products account for more than 60% of total protein intake (Adekoya *et al*, 2004).

Medicinal plants (phytobiotics) play a significant role as appetite enhancers, growth promoters, pro-fertility agents, antimicrobials, antifungal, antiviral and immunostimulatory effects for aquatic animals (Anthony, 2023; Uguh, 2019; Harikrishnan *et al.*, 2011). Therefore, the concept of functional feed is novel to the aquaculture industry as it goes beyond satisfying basic nutritional requirements of the culture organisms but also, health management (Bello *et al*., 2012). Plants such as tiger nut, maca root, snot apple, thorn apple, and date palm have scanty documentation in utilization on indigenous fish species such as *Clarias gariepinus* which command high economic returns in Nigeria due to high survival rate, fast growth, feeding habits, high feed to flesh conversion, and tolerance to high environmental condition. Hence, this study was carried out to assess the sustainable effect of the herbal mixture (date palm, tiger nut, thorn apple seed, maca root and snot apple) supplemented diets on internal organs and morphometric traits of *Clarias gariepinus.*

Materials and Methods

Experiment Location and Plant Identification

This study was carried out at the Teaching and Research Farm of the Department of Fisheries and Aquaculture Technology, Olusegun Agagu University of Science and Technology, Okitipupa. An herbal mixture such as tiger nut, thorn apple, and date palm was obtained in Oja–Oba, Ibadan while maca root and snot apple were obtained from Jigawa State. The plants were identified at the Department of Biological Sciences (Botany Programme) of the same institution.

Preparation of plant materials

The Polyherbal mixture {maca root, *Lepidium meyenii*; Snot apple, *Azanza garckeana;* tiger nut, *Cyperus esculentus;* date palm, *Phoenix dactylifera;* thorn apple, *Datura stramonium*} were air-dried for four weeks after which it was grinded into fine powder, polyherbal mixture were thoroughly mixed together in a ratio of 0.5: 1:

1:0.5:0.5 on a dry *w*/*w* basis, respectively, decided based on previous findings and was stored at room temperature until required.

Feed Formulation

Fishmeal, soybean, groundnut cake, maize, wheat bran, vitamin-mineral premix, starch, vegetable oil, Dicalcium phosphate, lysine and methionine were purchased from Adom feed mill, Ibadan, Oyo State and weighed using sensitive weighing balance. Herbal mixture (HM) (date palm, tiger nut, thorn apple seed, maca root and snot apple) were incorporated at different inclusion levels of 0.5, 1.0, 1.5, 2.0, 2.5 and 3%. Seven experimental diets were formulated using Pearson's square method and Hobart A - 200T pelleting machine which include; Control (without herbal mixture), HM_2 (0.5%), HM_3 (1.0%), HM_4 (1.5%), HM_5 (2.0%), HM_6 (2.5%) and HM_7 (3.0%) and analyzed to produce 40% crude protein, 7% moisture, 10% crude fibre, 16% ash content, 5% ether extract and 22% nitrogen free extract.

Source of Fish and Experimental Procedure

The experimental fish were purchased from a farm at Okitipupa and transported in an oxygenated bag to the Teaching and Research Farm of the Department of Fisheries and Aquaculture, Olusegun Agagu University of Science and Technology, Okitipupa. The fish were acclimatized for two weeks before the commencement of the study and were fed a commercial diet (2 mm Coppens). Feeding was stopped 24 hours before the commencement of the feeding trials. One hundred and forty *C. gariepinus* (450.00±0.04g) were randomly assigned to seven dietary treatments in a completely randomized design. Each treatment was replicated twice with us10 fish per replicate. The water level was maintained at a volume of 40 litres throughout the experimental period. Water in each tank was replaced every three (3) days throughout the experiment to maintain relatively uniform physiochemical parameters and also to prevent fouling of the result from the faecal and feed residues. The experiment lasted for 8 weeks during which the fish was fed at 5% body weight daily. The diet per day was divided into two; 1.5% given in the morning by 8:00 am and 1.5% in the evening by 5:00 pm.

Viscero – Intestino Somatic Index

Organ index such as gill, liver, intestine, ovary, testis, heart and spleen of the fish were collected and weighed to calculate the viscero-somatic index (VSI) and intestine-somatic index (ISI) at the end of the feeding trial. They were calculated as described by Fox *et al*., (1997);

VSI (%) = 100 x (viscera weight [g]/whole fish weight [g])

ISI (%) = 100 x (intestinal weight [g]/whole fish weight [g])

Morphometric characteristic of Clarias gariepinus

Total Length (TL), Standard Length (SL), Head Length (HL), Dorsal Width (DW) and Height (H) were measured as shown in plate 1.

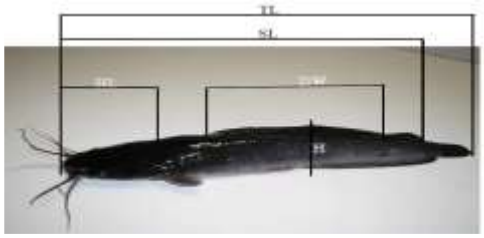

Plate 1: Morphometric characteristic of *C. gariepinus juveniles*

Determination of Phytochemical Screening of Herbal Plants

Phytochemical constituents such as saponins, phenols, tannins, flavonoids, glucosinolates, triterpenes and steroids and proteins were done as described by Olusola *et al*., (2017)

Statistical Analysis

Data resulting from the experiment were subjected to a one-way analysis of variance (ANOVA) using Python (version 3.0). Tukey's HSD (honest significant difference) was used to compare differences among individual mean at P= 0.05.

Results

Determination of phytochemicals in date palm, tiger nut, thorn apple seed, maca root and snot apple

The phytochemical screening of the herbal mixture (date palm, tiger nut, thorn apple seed, maca root and snot apple) revealed the presence of saponins, flavonoids, alkaloids, protein, tannins, steroids, protein and glycosides. Phenol was not detected in date palm and thorn apple, alkaloids were not detected in date palm and tiger nut and flavonoids were not detected in maca root. The value of these metabolites was low (+), and moderate (++) as shown in Table 1.

Table 1: Phytochemical screening of date palm, tiger nut, thorn apple seed, maca root and snot apple

Phytochemicals	**Date palm**	**Tiger nuts**	**Thorn apple seed**	**Maca root**	**Snot apple**
Saponins	++	++	++	+	+
Flavonoids	++	++	++	-	+
Phenol	-	++	-	+	++
Alkaloids	-	-	++	++	++
Tannins	+	+	+	+	+
Glucosinolates	+	+	++	++	+
Steroids	+	++	++	+	++
Protein	++	+	+	+	+

Keys: low (+), moderate (++)

Table 2: Performance evaluation of *C. gariepinus* fed herbal mixture supplemented diets for 8 weeks

		Survival rate	Initial condition factor	Final condition factor	Difference
MALE	Control	60.00±1.89^{a}	2.18±0.01^{a}	1.35±0.02^{a}	0.83±0.04^{e}
	HM_2	60.00±1.56^{a}	2.18±0.00^{a}	1.61±0.03^{cd}	0.57±0.01^{bc}
	HM_3	80.00±2.43^{b}	2.18±0.02^{a}	1.91±0.05^{e}	0.27±0.03^{a}
	HM_4	80.00±2.01^{b}	2.18±0.00^{a}	1.66±0.01^{d}	0.52±0.02^{b}
	HM_5	100.00±0.00^{c}	2.18±0,01^{a}	1.35±0.00^{a}	0.83±0.01^{e}
	HM_6	100.00±0.00^{c}	2.18±0.00^{a}	1.54±0.05^{b}	0.64±0.03^{d}
	HM_7	80.00±2.24^{b}	2.18±0.00^{a}	1.55±0.04^{bc}	0.63±0.04^{cd}
FEMALE	Control	100.00±0.00^{a}	1.98±0.01^{a}	1.79±0.07^{c}	0.19±0.01^{a}
	HM_2	100.00±0.00^{a}	1.98±0.01^{a}	1.72±0.04^{b}	0.26±0.02^{b}
	HM_3	100.00±0.00^{a}	1.98±0.00^{a}	1.57±0.03^{a}	0.41±0.04^{c}
	HM_4	100.00±0.00^{a}	1.98±0.01^{a}	1.84±0.06^{c}	0.14±0.00^{a}
	HM_5	100.00±0.00^{a}	1.98±0.00^{a}	1.59±0.05^{a}	0.39±0.02^{c}
	HM_6	100.00±0.00^{a}	1.98±0.01^{a}	1.81±0.08^{c}	0.17±0.01^{a}
	HM_7	100.00±0.00^{a}	1.98±0.00^{a}	1.84±0.05^{c}	0.14±0.02^{a}

Means (n= 2) in the same column with similar superscripts are not significantly different (p>0.05)**,** HM = Herbal mixture

Performance evaluation of C. gariepinus fed herbal mixture supplemented diets for 8 weeks

The results of this study revealed that the value of survival rate of male *C. gariepinus* was highest in HM 5 and HM 6 (100%) and lowest in control and HM 2 (60%), there was a significant difference (P<0.05) among the dietary groups while 100% survival rate was recorded in female and there was no significant difference (P>0.05) among the dietary groups. The value of the final condition factor in the study was highest in control and HM 5 (0.83) and the lowest in HM 3 for male *C. gariepinus* while the highest (0.41) was recorded in HM 3 and lowest (0.14) for HM 4 and HM 7 for the female *C. gariepinus*, there was a significant difference (P<0.05) among the dietary groups (see table 2).

Viscero - Intestino Somatic Index of Male C. gariepinus fed Herbal Mixture Supplemented Diets for 8 Weeks

The results of the organo somatic index revealed an increase in the value of these organs at the end of the study compared to the value obtained at the commencement of the study. There was a significant difference (P<0.05) among the dietary groups except for testis (Table 3).

Viscero - intestino somatic index of female C. gariepinus fed herbal mixture supplemented diets for 8 weeks

The value of the liver, kidney, ovary, spleen, heart, and gills recorded in this study shows a better value at the end of the experiment compared to the value obtained before the commencement of the study and there was a significant difference (P<0.05) in the parameters among the dietary groups (Table 4).

Table 3: Organ weight of male *C. gariepinus* fed herbal mixture supplemented diets for 8 weeks

Parameters		HM_1	HM_2	HM_3	HM_4	HM_5	HM_6	HM_7
Gill	Initial	21.10±0.00	21.10±0.00	21.10±0.00	21.10±0.00	21.10±0.00	21.10±0.00	21.10±0.00
	Final	34.55±0.03^{b}	22.03±0.03 ^{a}	26.51±2.83^{ab}	22.90±0.03^{ab}	25.69±0.03^{ab}	24.90±0.03^{ab}	25.40±0.03^{ab}
Liver								
	Initial	7.10±0.00	7.10±0.00	7.10±0.00	7.10±0.00	7.10±0.00	7.10±0.00	7.10±0.00
	Final	11.97±0.01^{b}	7.10±2.83 ^{a}	10.14±0.03^{ab}	8.11±0.03^{ab}	11.44±0.03^{b}	12.00±0.03^{b}	9.50±0.28^{ab}
Intestine								
	Initial	14.20±0.00	14.20±0.00	14.20±0.00	14.20±0.00	14.20±0.00	14.20±0.00	14.20±0.00
	Final	20.58±0.03^{e}	20.60±0.03^{e}	14.84±0.04^{b}	17.82±0.03^{d}	10.48±0.03 ^{a}	16.20±0.03^{c}	16.22±0.03^{c}
Testis								
	Initial	4.60±0.00	4.60±0.00	4.60±0.00	4.60±0.00	4.60±0.00	4.60±0.00	4.60±4.35
	Final	5.94±2.64 ^{a}	5.27±1.00 ^{a}	4.70±2.31 ^{a}	5.75±1.45 ^{a}	5.91±2.33 ^{a}	4.64±1.10 ^{a}	4.35±0.69 ^{a}
Spleen								
	Initial	1.60±0.57	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
	Final	1.72±0.03^{d}	0.54±0.03 ^{a}	0.91±0.03^{b}	0.65±0.03 ^{a}	0.84±0.03^{b}	0.65±0.03 ^{a}	1.06±0.03^{c}
Heart								
	Initial	0.60±0.00	0.60±0.00	0.60±0.00	0.60±0.00	0.60±0.96	0.60±0.00	0.60±0.00
	Final	1.13±0.03^{d}	0.97±0.03^{c}	0.91±0.01^{bc}	0.87±0.03^{bc}	0.96±0.03^{c}	0.80±0.03^{b}	0.57±0.04 ^{a}

Mean (n =2) with the same superscript along the rows are not significantly different at p>0.05, HM = Herbal mixture

Table 4: Organ weight of female *C. gariepinus* fed herbal mixture supplemented diets for 8 weeks

Parameters		HM_1	HM_2	HM_3	HM_4	HM_5	HM_6	HM_7
Gill	Initial	21.90±0.0	21.90	21.90±0.00	21.90±0.00	21.90±0.00	21.90±0.00	21.90±0.00
	Final	0	±0.00	34.89±0.03^{b}	34.56±0.03^{b}	29.20±0.03^{a}	28.04±0.03^{a}	29.45±0.03^{a}
Liver		35.21±0.2	36.60					
	Initial	8^{b}	±2.83	12.30±0.00	12.30±0.00	12.30±0.00	12.30±0.00	12.30±0.00
	Final		^{b}	7.59±0.03^{f}	6.44±0.03^{c}	6.54±0.03^{d}	9.54±0.03^{g}	3.89±0.03^{a}
Intestine		12.30±0.0						
	Initial	0	12.30	6.90±0.00	6.90±0.00	6.90±0.00	6.90±0.00	6.90±0.00
	Final	5.71±0.03	±0.00	16.15±0.03^{f}	13.83±0.03^{c}	13.21±0.03^{b}	17.20±0.03^{g}	14.25±0.03^{d}
Kidney		^{b}	6.96±					
	Initial		0.03^{e}	1.10±0.00	1.10±0.00	1.10±0.00	1.10±0.00	1.10±0.00
	Final	6.90±0.00		3.75±0.03^{d}	1.90±0.03^{a}	3.31±0.03^{c}	3.90±0.03^{e}	4.86±0.03^{g}
Ovary		11.90±0.0	6.90±					
	Initial	3^{a}	0.00	13.40±0.00	13.40±0.00	13.40±0.00	13.40±0.00	13.40±0.00
	Final		16.08	73.28±2.83^{a}	118.81±2.83^{d}	145.11±2.83^{e}	99.68±2.83^{b}	110.12±2.83^{c}
Spleen		1.10±0.00	±0.03					
	Initial	2.85±0.03	^{e}	1.10±0.00	1.10±0.00	1.10±0.00	1.10±0.00	1.10±0.00
	Final	^{b}		1.23±0.03^{c}	1.88±0.00^{f}	1.14±0.03^{b}	1.01±0.03^{a}	1.10±0.03^{b}
Heart			1.10±					
	Initial	13.40±0.0	0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00
	Final	0	4.98±	1.09±0.14^{d}	1.01±0.03^{c}	0.76±0.03^{a}	0.89±0.03^{b}	0.81±0.03^{a}
		123.85±2.	0.03^{g}					
		83^{d}						
			13.40					
		1.10±0.00	±0.00					
		1.49±0.03	118.7					
		^{d}	4±2.8					
			3^{d}					
		0.70±0.00						
		1.08±0.03	1.10±					
		^{d}	0.00					
			1.64±					
			0.03^{e}					
			0.70±					
			0.00					
			0.97±					
			0.03^{c}					

Mean (n = 2) with the same superscript along the rows are not significantly different at p>0.05, HM = Herbal mixture

Morphometric traits of male C. gariepinus fed herbal mixture supplemented diets for 8 weeks

The result of the morphometric traits of male *C. gariepinus* shows that there was an increase in the value of head length, standard length, total length, dorsal length, height and length of the spine as the week of feeding trials increased and there was a significant difference ($p < 0.05$) among the dietary groups (Table 5).

Table 5: Measurement of morphometric traits (cm) of male *C. gariepinus* fed herbal mixture supplemented diets for 8 weeks

Parameters	Week	HM_1	HM_2	HM_3	HM_4	HM_5	HM_6	HM_7
Head length	2	10.85±0.49^{a}	11.75±1.07^{a}	10.75±0.35^{a}	11.75±0.35^{a}	11.50±0.71^{a}	11.35±0.50^{a}	11.00±0.00^{a}
	4	11.65±0.21^{ab}	12.05±0.07^{b}	11.25±0.35^{a}	13.00±0.00^{c}	13.25±0.35^{c}	13.50±0.00^{c}	13.00±0.28^{c}
	6	12.25±0.35^{a}	13.25±0.35^{bc}	13.00±0.00^{b}	14.10±0.14^{de}	14.00±0.00^{de}	14.40±0.07^{e}	13.70±0.14^{cd}
	8	14.25±0.35^{a}	14.40±0.14^{a}	14.00±1.41^{a}	14.50±0.71^{a}	14.40±0.14^{a}	14.45±0.07^{a}	14.15±0.21^{a}
Height	2	6.15±0.21^{ab}	6.00±0.28^{a}	7.40±0.14^{c}	6.05±0.63^{a}	6.90±0.14^{b}	6.35±0.21^{ab}	6.25±0.35^{ab}
	4	6.95±0.07^{a}	7.25±0.35^{a}	7.35±0.21^{a}	7.40±0.57^{a}	7.75±0.35^{a}	7.50±0.00^{a}	7.25±0.35^{a}
	6	8.10±0.14^{a}	8.25±0.21^{a}	8.50±0.00^{ab}	9.00±0.00^{c}	8.75±0.35^{bc}	9.00±0.00^{c}	8.50±0.00^{ab}
	8	8.25±0.35^{c}	8.75±0.35^{c}	8.75±0.35^{c}	9.00±0.00^{c}	8.75±0.35^{c}	9.15±0.21^{c}	8.50±0.00^{c}
Standard length	2	38.25±2.48^{a}	40.00±0.00^{a}	39.50±2.12^{a}	39.75±1.77^{a}	41.25±1.07^{a}	41.75±1.77^{a}	39.50±0.71^{a}
	4	40.00±1.41^{a}	42.25±3.89^{a}	40.75±0.35^{a}	42.35±0.92^{a}	39.45±0.64^{a}	39.00±0.35^{a}	39.35±0.49^{a}
	6	42.00±4.24^{a}	40.00±1.41^{a}	39.50±3.54^{a}	42.90±1.56^{a}	43.50±4.95^{a}	39.75±0.35^{a}	41.50±0.71^{a}
	8	41.50±3.54^{a}	40.00±0.00^{a}	39.50±3.54^{a}	42.50±0.71^{a}	42.00±0.00^{a}	41.25±0.35^{a}	41.75±0.35^{a}
	2	43.00±0.00^{a}	45.50±0.71^{ab}	46.40±2.26^{abc}	47.50±2.12^{b}	49.15±0.92^{c}	46.50±0.71^{abc}	45.00±1.41^{ab}
Total length	4	46.75±1.77^{ab}	42.90±4.10^{a}	47.00±0.00^{ab}	48.85±1.63^{b}	47.00±0.99^{ab}	46.00±2.12^{ab}	46.00±0.00^{ab}
	6	49.25±5.30^{ab}	46.75±1.06^{ab}	46.25±0.35^{a}	49.00±1.41^{b}	47.60±0.57^{ab}	46.25±1.06^{a}	47.75±0.35^{ab}
	8	23.00±1.41^{a}	47.00±0.00^{a}	46.00±4.24^{a}	49.75±1.06^{a}	48.75±0.35^{a}	48.25±0.35^{a}	47.75±0.35^{a}
	2	23.00±1.41^{a}	25.25±0.35^{ab}	23.50±0.71^{a}	26.50±0.71^{ab}	28.50±2.12^{b}	25.50±2.12^{ab}	27.00±2.83^{ab}
Dorsal length	4	25.00±1.41^{ab}	25.50±0.71^{ab}	25.40±0.85^{ab}	28.50±0.71^{b}	26.50±0.71^{ab}	26.00±1.41^{ab}	25.85±0.21^{ab}
	6	27.00±1.41^{abc}	26.50±0.71^{ab}	25.40±0.57^{a}	29.00±1.41^{c}	28.50±0.71^{bc}	26.40±0.57^{ab}	27.25±0.35^{abc}
	8	27.50±2.12^{a}	27.00±0.00^{a}	27.00±1.41^{a}	28.50±0.71^{a}	28.50±0.00^{a}	27.25±0.35^{a}	27.75±0.35^{a}
Length of spine	2	4.30±0.14^{a}	5.20±0.00^{a}	4.70±0.14^{a}	6.50±0.71^{b}	5.35±0.21^{a}	4.60±0.85^{a}	5.35±0.21^{a}
	4	5.30±0.42^{ab}	5.10±0.57^{a}	5.85±0.07^{ab}	6.15±0.07^{b}	5.95±0.07^{ab}	5.60±0.14^{ab}	5.75±0.50^{ab}
	6	5.80±0.28^{ab}	5.40±0.00^{ab}	5.80±0.28^{ab}	6.10±0.14^{b}	6.10±0.14^{b}	5.30±0.42^{a}	6.10±0.42^{b}
	8	6.10±0.99^{a}	5.50±0.14^{a}	6.00±0.00^{a}	6.20±0.00^{a}	6.25±0.07^{a}	5.95±0.07^{a}	6.30±0.14^{a}

Mean (n = 2) with the same superscript along the column are not significantly different at p > 0.05, HM = Herbal mixture

Morphometric traits of female C. gariepinus fed herbal mixture supplemented diets for 8 weeks

The results of the head length, height, length of the spine, standard length and total length recorded in this study show that there was an increase in the value obtained at the end of the experiment compared to the value obtained at the commencement of the study. There was a significant difference (P<0.05) among the dietary groups (Table 6).

Table 6: Measurement of morphometric traits (cm) of female *C. gariepinus* fed herbal mixture supplemented diets for 8 weeks

Parameters	Week	HM_1	HM_2	HM_3	HM_4	HM_5	HM_6	HM_7
Head length	2	10.85±0.50^{a}	11.75±1.06^{a}	10.75±0.35^{a}	11.75±0.35^{a}	11.50±0.71^{a}	11.35±0.50^{a}	11.00±0.00^{a}
	4	12.25±0.35^{ab}	11.50±0.71^{a}	12.25±0.35^{ab}	12.15±0.50^{ab}	12.75±0.35^{ab}	13.00±0.00^{b}	13.25±1.07^{b}
	6	13.25±0.35^{ab}	12.90±0.57^{a}	13.80±0.00^{ab}	13.50±0.00^{ab}	13.75±0.35^{ab}	13.65±0.21^{ab}	14.00±0.71^{b}
	8	13.50±0.00^{a}	14.35±0.50^{bc}	15.15±0.50^{c}	14.25±0.35^{ab}	14.40±0.14^{bc}	14.25±0.35^{ab}	14.00±0.00^{ab}
Height	2	6.15±0.21^{ab}	6.00±0.28^{a}	7.40±0.14^{c}	6.05±0.64^{a}	6.90±0.14^{bc}	6.35±0.21^{ab}	6.25±0.35^{ab}
	4	7.25±0.35^{a}	7.15±0.50^{a}	7.90±0.14^{ab}	7.80±0.28^{a}	7.90±0.14^{ab}	7.75±0.35^{a}	8.60±0.14^{b}
	6	8.25±0.35^{a}	8.75±0.35^{ab}	9.00±0.00^{b}	8.75±0.35^{ab}	9.00±0.00^{b}	8.50±0.00^{ab}	8.60±0.14^{ab}
	8	8.50±0.71^{a}	9.25±0.35^{a}	9.25±0.35^{a}	9.25±0.35^{a}	9.40±0.14^{a}	9.35±0.07^{a}	9.20±0.42^{a}
Standard length	2	38.25±2.48^{a}	40.00±0.00^{a}	39.50±2.12^{a}	39.75±1.77^{a}	41.25±1.07^{a}	41.75±1.77^{a}	39.50±0.71^{a}
	4	37.75±1.77^{a}	42.00±4.24^{a}	38.00±1.41^{a}	39.30±1.83^{a}	38.00±1.41^{a}	38.40±0.85^{a}	38.50±2.12^{a}
	6	42.50±4.95^{a}	41.25±0.35^{a}	41.50±0.70^{a}	41.50±0.71^{a}	38.75±0.35^{a}	40.50±0.71^{a}	39.25±0.35^{a}
	8	38.50±0.71^{a}	40.00±1.41^{ab}	42.00±1.41^{b}	40.00±1.41^{ab}	40.00±0.00^{ab}	39.00±0.00^{a}	39.00±0.00^{a}
Total length	2	43.00±0.00^{a}	45.50±0.71^{ab}	46.40±2.26^{abc}	47.50±2.12^{b}	49.15±0.91^{c}	46.50±0.71^{abc}	45.00±1.41^{ab}
	4	44.40±2.26^{a}	45.15±3.32^{a}	44.50±2.12^{a}	45.05±1.06^{a}	44.45±1.77^{a}	44.65±1.48^{a}	45.05±2.05^{a}
	6	45.50±0.71^{a}	47.60±0.85^{a}	47.25±0.35^{a}	47.00±2.82^{a}	45.00±1.41^{a}	46.15±0.21^{a}	45.50±0.71^{a}
	8	44.75±1.06^{a}	47.50±2.12^{ab}	49.00±0.71^{b}	46.50±2.12^{ab}	46.75±0.35^{ab}	46.25±0.35^{ab}	46.00±0.00^{ab}
Dorsal length	2	23.00±1.41^{a}	25.25±0.35^{ab}	23.50±0.71^{ab}	26.50±0.71^{ab}	28.50±2.12^{b}	25.50±2.12^{ab}	27.90±0.14^{ab}
	4	25.00±1.41^{a}	25.25±0.71^{a}	25.50±0.71^{a}	25.10±1.27^{a}	25.90±0.14^{a}	26.40±0.57^{a}	25.90±0.14^{a}
	6	25.50±2.12^{a}	27.00±1.41^{a}	27.25±0.35^{a}	27.00±1.41^{a}	25.50±0.71^{a}	25.75±1.06^{a}	25.90±0.14^{a}
	8	25.50±2.12^{a}	27.50±0.71^{ab}	28.00±0.00^{b}	27.25±0.35^{ab}	26.50±0.71^{ab}	26.75±1.06^{ab}	25.90±0.14^{ab}
Length of spine	2	4.30±0.14^{a}	5.20±0.00^{a}	4.70±0.14^{a}	6.50±0.71^{a}	5.35±0.21^{a}	4.60±0.85^{a}	5.35±0.21^{a}
	4	6.10±0.14^{b}	6.15±0.50^{b}	5.80±0.14^{b}	5.55±0.07^{a}	5.80±0.00^{ab}	5.45±0.07^{a}	5.80±0.00^{ab}
	6	5.80±0.00^{a}	5.70±0.14^{a}	5.80±0.28^{a}	5.90±0.14^{a}	5.80±0.28^{a}	5.85±0.07^{a}	5.70±0.14^{a}
	8	5.30±0.14^{a}	5.90±0.14^{b}	6.02±0.21^{b}	5.90±0.14^{b}	6.05±0.07^{b}	5.70±0.28^{ab}	6.05±0.21^{b}

Mean (n = 2) with the same superscript along the column are not significantly different at p>0.05, HM = Herbal mixtur

Discussion

The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, phenolics, proteins and flavonoids. This result was similar to the report of Bando *et al*., (2020); Elshiekh and Ali (2020); Lee and Chang, (2019); Al-Samarai *et al*., (2016); Banso and Adeyemo (2006); Nwaoguikpe, (2010), who reported the presence of Saponins, flavonoids, glycosides, alkaloids and tannins in tiger nut (*Cyperus esculentus*). The phenolic compound constituents like tannins and flavonoids act highly as antioxidants (protection and regeneration of other dietary antioxidants) and growth-promoting chemicals in animals (Bello, 2014), the increase in the weight gain of the fish-fed treated groups can be attributed to this factor.

The result of this study shows significantly higher (p <0.05) values of survival rate in the treated groups of the male *C. gariepinus* fed graded levels of the herbal mixture while 100% survival was recorded both in the treated groups and control of the female *C. gariepinus* and there was no significant difference (p>0.05) among the dietary groups. The general well–being of the fish fed different graded levels of the herbal mixture–based diets as expressed by the condition factor (k) was best in the treated groups when compared to the control. There were significant differences (P< 0.05) among the dietary groups. All the fish were in a better state of well-being at the end of the experiment than they were at the beginning (see Table 3). The presence of essential nutrients in the herbal plants as well as having antioxidant properties in the plants of the treated groups compared to the control could have resulted in better survival and well-being of the fish

The morphometric index shows a significantly higher value in head length, standard length, height, dorsal length and length of spine in *C. gariepinus* fed herbal mixture supplemented diets in comparison with the control at 2, 4, 6 and 8 weeks. The value obtained in these parameters increases as the period of feeding trials increases and this was similar to the report of Olusola *et al*., (2022). No significant differences (p>0.05) were recorded in standard length of the female *C. gariepinus* at 2, 4, and 6 weeks, 2 and 8 weeks for total length, 4 and 6 weeks for dorsal length, 6 weeks for length of spine, 8 weeks for height among the dietary groups. The value obtained in the male revealed that no significant differences (p>0.05) were observed at 2 and 8 weeks for head length, 4 weeks for height, 2, 4. 6 and 8 weeks for standard length, 8 weeks for total length, dorsal length and length of spine among the dietary groups. It is interesting to know that the value of the treated groups performed better than the control and the treatment supplemented with 2.0% of the herbal mixture had the best performance in morphometric indices in males while there was variation observed in the female although the treated groups had better values than the control. The reason for the better performance might be as a result of phytoconstituents present in the herbal mixture (flavonoids, saponins, and alkaloids). This study accords with the report of Olusola *et al*., (2022) who observed an increase in the head length, total length, standard length, dorsal length and height of *C. gariepinus* fed cinnamon bark at different graded levels when compared to the control. Also, this study agrees with the work of Olusola and Akinbiodun, (2021) who reported an increase in the morphometric indices of *C. gariepinus* juveniles fed nutmeg seed when compared to the control.

Fox *et al.*, (1997) reported that the internal organ indices as indicators of health which could be used to predict the health status of fish. The value of the organ index of male and female *C. gariepinus* fed herbal mixture revealed that the treated groups had better performance than the control and they were in a better state of well-being than the control. There were increases in the value of gill, liver, spleen, kidney, intestine, ovary, testis, and heart of male

and female *C. gariepinus* and there was a significant difference ($p < 0.05$) among the dietary groups. The organ index of the female *C. gariepinus* fed herbal mixture supplemented diets was significantly higher ($p<0.05$) than the control, slight variation was recorded in these parameters among the dietary groups. This present study was in agreement with the observation of Olusola *et al*., (2022) who reported an increase in the internal organ of *C. gariepinus* juveniles fed cinnamon bark compared to the control and the values obtained before the commencement of the study. Also, the report of Nugroho *et al*., (2019) who indicated an increase in the values of the internal organ of striped catfish fed *Myrmeco diatuberosa*. Ayebidun and Olusola, (2023) reported that the organ index of *C. gariepinus* juveniles after the experiment showed significant differences ($P<0.05$) in the liver, kidney, gill, muscle, intestine and spleen which was similar to this present finding.

The liver of the male and female *C. gariepinus* shows that the herbal mixture enhances the hepaprotective effect compared to the control. Also, the spleen revealed a lower value in the treated groups compared to the control which means that the treated groups had better immune response than the control. There significant difference ($p<0.05$) among the parameters tested except for testis shows no significant difference ($p>0.05$) among the dietary groups. Bello, (2014) reported that the liver is associated with the detoxification of drugs and toxins, the biotransformation process due to its function such as metabolites fat, cholesterol and reduces and conjugates adrenocortical and gonadal steroids hormones. The liver receives about 30% of the total cardiac output so this is susceptible to chemicals present in systemic circulation (Bridget, 1990). There were no traces of oedema among the dietary groups, therefore the inclusion of herbal mixture in the diet of *C. gariepinus* could be said to be safe and non-toxic to the cultured organisms because of the metabolites present in this herbal combination (saponins, phenol and flavonoids).

Conclusion

Healthy fish and a higher survival rate a novel and paramount asset to sustain livelihoods/ food security in the production of fish in Nigeria and the world at large. However, this study revealed that the inclusion of herbal mixture in the diet of *C. gariepinus* could enhance global food safety and sustainability, survival and health of *C. gariepinus* in sustainable aquaculture.

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