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Growth and Physiological responses of Juvenile African Catfish (*Clarias gariepinus*) Fed with Dietary Supplementation of Garlic-Ginger Mixture

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ABSTRACT: A-*84-days study was conducted to evaluate the growth and physiological responses of juvenile Clarias gariepinus fed diets supplemented with garlic-ginger mixture. The garlic-ginger mixture was included in a base diet at 0%, 1%, 2%, and 3%; corresponding to treatments D0 or control, D1, D2 and D3. A total of 120 juveniles (46.55±3.66g) were randomly distributed in triplicate to 12 plastic tanks and fed three times daily with biweekly intermediate sampling. The results indicate that D2 fed fish had the most impressive weight gain (WG) of 218.74±6.42g and feed conversion ratio (FCR) of 1.55±0.05, compared to the control (WG=99.53±0.03g, FCR=2.45±0.18). Furthermore, D2 treatment induced significant improvements in whole body bromatological composition, nutrient retention, organosomatic indexes, and serum biochemical profile in fish compared to control. The growth improvement observed could be due to the combined action of the bioactive components of ginger and garlic to enhance the functioning of the organs of the digestive system as well as the homeostasis balance of the body. Therefore, garlic and ginger can be used as a feed additive to enhance the production of African catfish.*

KEYWORDS: *Clarias gariepinus,* Garlic, Ginger, Growth, Physiological responses

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INTRODUCTION

Fish is the most widely consumed source of animal protein in the world, certainly because it is highly nutritious and rich in essential nutrients. Improving fish production to meet the growing demand of populations as well as human needs requires the provision of a quality feed containing not only a high protein content with essential nutrients, but also feed additives to maintain the dietary characteristics of the feed ingredients, improve the quality of the feed and thus promote rapid growth of the fish to reach maximum weight within a reasonable time (Gwangyeol Yoo *et al*., 2021). One approach to improving feed quality is to include natural substances such as plant-derived products to improve feed conversion efficiency and raise the general conditions for fish growth and maintenance. Plant-derived products such as spices are natural sources of bioactive compounds such as alkaloids, tannins, essential oils, flavonoids, phenols, coumarin, organic acids and saponins that have been reported to promote various physiological activities such as anti-stress, appetite stimulation, growth promotion and improved flesh quality in farmed fish (Sivaram *et al*., 2004; Bhosale *et al*., 2010).

Garlic (*Allium sativum*) is a perennial bulb-forming plant that belongs to the genus Allium in the family Liliaceae. It has been used for decades as an aromatic agent and functional food to improve physical and mental health. Research carried out on garlic extracts (aqueous, ethanol) and dried powder has shown that it contains a variety of organo-sulfur compounds like allicin, ajoene, S-allylcysteine, diallyl disulfide, Smethylcysteine sulfoxide and S-allylcysteine (Chi *et al*., 1982). In addition, biochemical analyses also revealed the presence of minerals such as phosphorus and calcium as well as vitamins A, C and B, linolenic acid, carbohydrates and other valuable compounds such as iodine and silicates whose beneficial effects on the circulation and skeleton are known. Earlier studies have reported that garlic, as a feed additive in fish feed, stimulates growth, improves antioxidant status, and enhances immunological, hematological and serum biochemical parameters (Yilmaz and Ergün, 2012).

Ginger (*Zingiber officinale*) belongs to Zingiberaceae family. The used part of the plant is the rhizome. Ginger is used worldwide as a spice in the culinary industry. Pharmacological and nutritional studies have revealed the presence of alkaloids, flavonoids, polyphenols, saponins, steroids and tannins, as well as fiber, carbohydrates, vitamins, carotenoids and minerals. It is also rich in gingerols, shogaols and zingerone, known to be natural antioxidant compounds (Yashin *et al*., 2017). The inclusion of ginger in the diet of farmed fish has been shown to promote growth by stimulating digestion, improving protein and lipid metabolism, and through its antioxidant, anti-hyperglycaemic, antiviral, antimicrobial and parasitic properties (Mohammadi *et al*., 2020).

The combination of garlic and ginger is widely used in households and restaurants to spice up meals. Small amounts are also used as food additives, preservatives or flavours to improve the taste of food and as an appetite stimulant by increasing the secretion of gastrointestinal juices (Lawal *et al*., 2018). The physiological and pharmacological properties of the various active compounds of ginger and garlic make them safe medicinal plants with little known adverse effects on humans, livestock and fish (Mekuriya and Mekibib, 2018). According to Gabor *et al*. (2012), feeding rainbow trout (*Oncorhychus mykiss*) with a combination of garlic and ginger improves growth parameters such as weight gain, specific growth rate and feed conversion ratio. Nyadjeu *et al*. (2021a) also reported that dietary inclusion of the garlic-ginger mixture improved growth and feed utilization parameters as well as survival and body composition of Clarias gariepinus fry. In order to enrich these few existing studies regarding the combined effects of garlic and ginger as phytogenic feed additive in the grower feeds of *Clarias gariepinus*, In order to enrich these few existing studies on the effects of using garlic-ginger mixture as dietary phytoadditives in the diet of *Clarias gariepinus*, the present study was carried out to evaluate the growth, feed nutrient utilization, serum biochemical profile and whole body proximate composition of *Clarias gariepinus* juveniles fed diets supplemented with garlic-ginger mixture.

MATERIAL AND METHODS

Fish and rearing condition

One hundred and forty-four juvenile *Clarias gariepinus* weighing 46.55±3.66g used in this experiment were purchased from a private fish farm and transported to the laboratory of the Agricultural Research Station of the Institute of Agricultural Research for Development at Batoke-Limbe in the Fako

Division of the South West Region of Cameroon. The experiment was conducted in closed recirculating culture system consisting of 12 rectangular plastic water tanks (50L) connected to biofilters and supplied with borehole water. Forty-four fish were randomly allocated to each rearing tank and acclimatized prior to the start of the feeding trial.

Preparation of the experimental diets

Diets were formulated according to the nutritional requirements of Clarias gariepinus juveniles. The ingredients such as fish meal, soybean cake, groundnut cake, wheat bran, corn flour, vitamin premix were purchased from feed retailers and Belgocam; while ginger (*Zingiber officinale*) and garlic bulbs (*Allium sativum*) used as phytoadditive were purchased from the local market, washed, the dry skin removed, cut into small pieces, air dried then ground using a household grinder and sieved to obtain a fine powder. Four isoproteinic diets were prepared with varying levels of garlic-ginger mixture at 0%, 1%, 2% and 3% corresponding to treatments D0, D1, D2 and D3 (table 1). To prepare the different diets, each group of ingredients was ground, sieved (40 mesh), gradually mixed with water and oil, and then pelleted into a 2.0-3.0 mm diameter pellet using a pellet machine (9KLP-150 pellet feed press machine, serial number JB/T5161-2013, Zhengzhou Xinzheng Yongyang Machinery Equipment, China). The feeds were then air dried and then 10g of each diet was taken for biochemical analysis while the rest was packed in airtight polyethylene bags and stored until use for the feeding trial.

Table 1: Ingredients, and proximate composition of different diets (g/100g dry mater).

Ingredients	**D0: 0% A.s-Z.o**	**D1: 1% A.s-Z.o**	**D2; 2% A.s-Z.o**	**D3: 3% A.s-Z.o**
Fish meal	35	35	35	35
Garlic meal	0	0.5	1	1.5
Ginger meal	0	0.5	1	1.5
Soybean cake	25	25	25	25
Groundnut cake	10	10	10	10
Wheat bran	14	14	14	14
Corn flour	10.5	10.5	10.5	10.5
Vitamin premix 2.5%	2.5	2.5	2.5	2.5
vegetable oil	2	2	2	2
Salt	1	1	1	1
	Proximate composition (%.DM)			
Protein	45.07±2.30	45.29±0.49	46.39±1.15	46.76±0.45
Lipid	6.50±0.71	4.50±0.71	5.00±0.00	5.00±1.45
Ash	13.50±0.71	13.00±0.00	13.50±0.71	13±0.00
Moisture	6.00±0.00	5.00±0.00	4.00±0.00	5.00±0.00
Energy (kcal/100g DM)	378,50±6.36	370.50±3.54	371.00±2.83	373.00±7.07

A.s: *Allium sativum* (garlic) and Z.o: *Zingiber officinale* (ginger)

Experimental design

At the end of the acclimatization period of two weeks during which fish were fed with a commercial feed, the 12 rearing tanks containing 32 fish each were randomly divided in four triplicates corresponding to the aforementioned treatments D0, D1, D2 and D3. Before the start of the feeding trial, 6 fish from each treatment were sacrificed and kept in a freezer at -20°c for the initial body composition. The remaining fish in each treatment were then fed with various supplementation levels of garlic-ginger mixture named 0% (control) and 1%, 2% and 3% for the experimental diets, corresponding respectively to treatments D1, D2 and D3. Fish were fed three times daily (09:00, 13:00 and 17:00) at the feeding rate of 5-3% of their body weight for 84 days. During the experimental period, water temperature (T˚C), pH, dissolved oxygen (D.O), ammonia (NH^3), nitrite (NO^{2-}) and nitrate (NO^{3-}) was measured daily before feeding (table2). Intermediate sampling was conducted every fourteen days during which all fish from each rearing tank were counted and measurements taken after fasting for 24 h.

Table 2: Water quality parameters (Mean±SD) during 84 days of the experimental period.

Parameters	Rearing time (days)						
	0	14	28	42	56	70	84
T°C (°C)	25±0	26.49±0.22	25.85±0.08	25.51±0.1	24.68±0.39	26.9±0.21	26.64±0.09
pH	7.60±0.02	7.75±0.21	7.80±0.21	7.78±0.36	7.50±0.38	7.48±0.35	7.23±0.31
D.O (mg/l)	5.84±0.06	5.84±0.06	5.86±0.05	5.90±0.05	6.09±0.08	5.60±0.05	5.74±0.05
NH_3 ($mg.l^{-1}$)	0.27±0.06	0.27±0.06	0.29±0.1	0.28±0.09	0.25±0	0.31±0.12	0.25±0.00
NO_2^- ($mg.l^{-1}$)	0.12±0.02	0.12±0.02	0.12±0.02	0.13±0,02	0.14±0.02	0.12±0.03	0.10±0.02
NO_3^- ($mg.l^{-1}$)	12.5±0.00	12.5±0.00	12.5±0.00	12.5±0.00	12.5±0.00	12.5±0.00	12.5±0.00

Fish measurement, blood analysis and proximate composition of diets and fish

The total length and weight of the fish were measured at the beginning, during intermediate sampling and at the end of the experiment. Prior to weight measurement, all fish were starved for 24 h to remove any remaining metabolites from the gut. At the end of the feeding period, 18 fish of both sexes were randomly selected from each treatment group. Six of them were sacrificed for final whole body bromatological composition. For the remaining 12 fish, blood obtained by cutting the caudal fin was collected in non-heparinized tubes and left in the open air for 5 hours. Afterwards, the serum samples were transferred to other tubes and stored at -20°C for biochemical analysis. The serum biochemical parameters included total protein (Biuret method), triglycerides (colorimetric enzymatic method GPO-PAP), total cholesterol (colorimetric enzymatic Trinder method) and HDL cholesterol (PEG 6000 precipitating method) was performed using commercial kits SGM ITALIA®. The liver, stomach, intestine, spleen, and gonads were removed immediately after blood sampling and weighed to determine the organosomatic indices.

For analysis of the biochemical composition of the diets and that of the whole body of fish, the diet and fish samples from each treatment were subjected to proximal analysis according to the method of Association of Official Analytical Chemistry (AOAC, 1990) to determine the percentage composition of the various components of both the feed and fish. Moisture was analyzed by drying the sample in an air convection oven at 105 °C overnight. Crude protein was analyzed by the Kjeldahl method after acid digestion (% crude protein = % nitrogen x 6.25), while the crude lipid was determined by extraction with petroleum ether using the Soxhlet method. The ash content in the diet was analyzed by combustion of samples in a muffle furnace at 550 °C for 12 h.

Growth and feed efficiency parameters

Growth performances, survival rate, feed utilization and nutrients retention were assessed for each treatment by determination of weight gain (WG), length gain (LG), specific growth rate (SGR), survival rate (SR), condition factor (K), feed conversion ratio (FCR), lipid efficiency ratio (LER), protein efficiency ratio (PER) and nutrients retention (NR). Calculations were carried out using the following formulae:

- WG (g) = Wf – Wi, where Wf is the final weight and Wi is the initial weight
- LG (cm) = Lf – Li, where Lf is the final length and Li is the initial length
- SGR (% /day) $= \frac{(\ln Wf - \ln Wi)}{t} \times 100$, where t is the number of days
- (SR (%) $= \frac{\text{Final number of fish}}{\text{Initial number of fish}} \text{X } 100$;
- $K = \frac{\text{Weight(g)}}{\text{Length(cm)}^3} X\ 100$
- FI (g/fish) $= \frac{\text{dry feed distributed}}{\text{number of fish}}$;
- $\text{FCR} = \frac{\text{weight of feed consumed}}{\text{Fish Weight Gain}}$;
- $\text{LER} = \frac{\text{Fish Weight Gain}}{\text{Lipid fed}}$;
- $\text{PER} = \frac{\text{Fish Weight Gain}}{\text{Protein fed}}$;

where, Protein fed (g) $= \frac{\text{Total feed consumed x Crude protein in feed}}{100}$;
- NR (% dry feed intake) $= \frac{\text{Final carcass composition} - \text{Initial carcass composition}}{\text{Amount of nutrient fed}} X\ 100.$

Organosomatic indices

- Stomachtosomatic index (StSI, %) $= \frac{\text{Stomach weight}}{\text{Body weight}} \text{X } 100$;
- Intestinosomatic index (ISI, %) $= \frac{\text{Intestine weight}}{\text{Body weight}} \text{X } 100$;
- Spleenosomatic index (SpSI, %) $= \frac{\text{Spleen weight}}{\text{Body weight}} \text{X } 100$;
- Hepatosomatic index (HSI, %) $= \frac{\text{Liver weight}}{\text{Body weight}} \text{X } 100$;
- Gonadosomatic index (GSI, %) $= \frac{\text{Gonads weight}}{\text{Body weight}} \text{X } 100$.

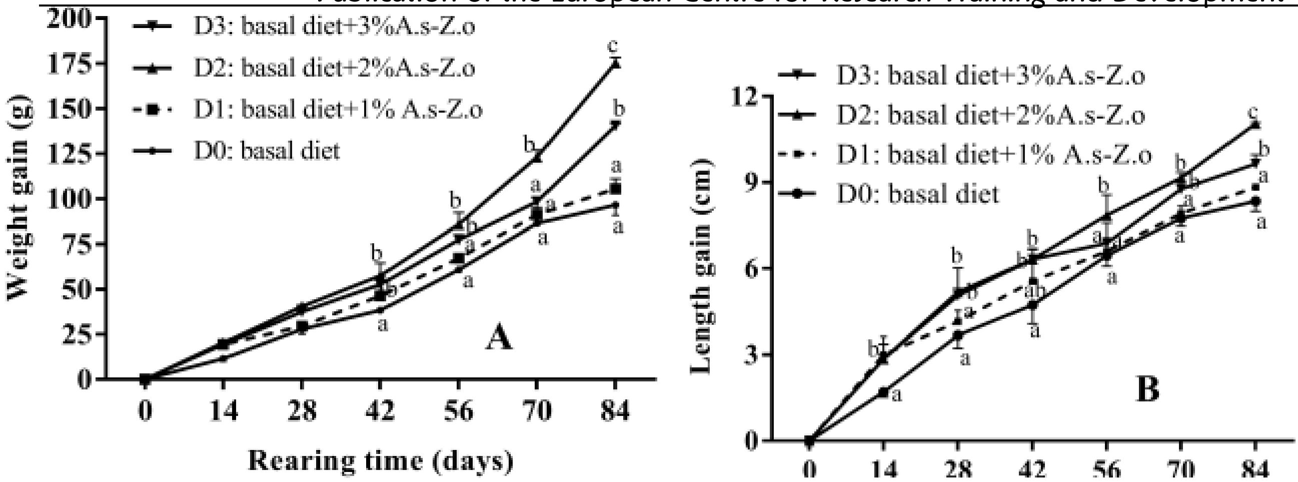
Statistical analyses

All results were expressed as mean ± SD. The data collected during every fish sampling were analysed by one-way analysis of variance (*ANOVA*-1) repeated measure followed by Tukey's multiple comparisons test with n=3 replications containing 30 fish each. Differences were regarded as significant when $p<0.05$. All statistical analyses were conducted using *GraphPad Prism* version 6.0.

RESULTS

Growth performance

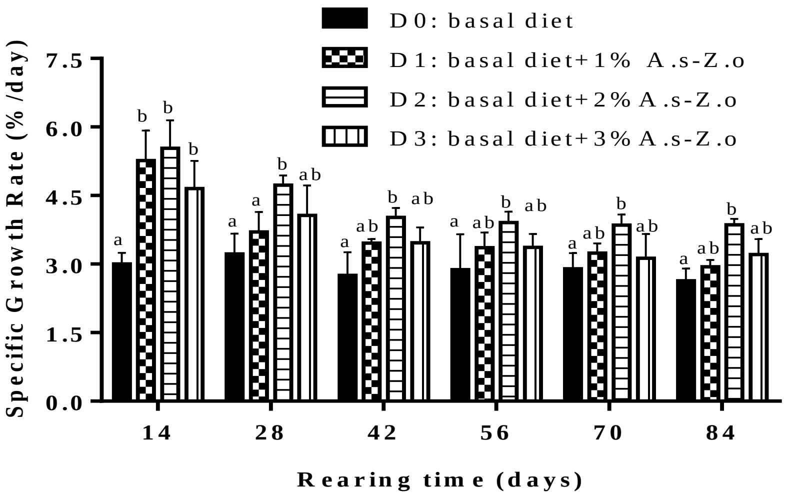
Dietary inclusion of the garlic-ginger mixture induced significant effects ($p < 0.05$) throughout the feeding period on the growth performance of juvenile *Clarias gariepinus* in terms of weight gain, length gain and specific growth rate independent of the level of inclusion. At the end of the feeding trial, the best performance was obtained in fish fed a diet containing 2% of the garlic-ginger mixture. The weight gain of 175.38±3.05g and length gain of 11.04±0.04cm were 44.96% and 24.46% higher than those of fish fed the control diet, respectively (fig 1).



A.s: *Allium sativum* (garlic) and Z.o: *Zingiber officinale* (ginger)

Figure 1: Effect of dietary supplementation of garlic and ginger mixture on weight gain (A) and length gain (B) of *Clarias gariepinus* juveniles for 84 days. Means on the same rearing time carrying different superscripts are significantly different from each other at p<0.05.

The specific growth rate of juvenile *Clarias gariepinus* fed with the experimental diet D2 was the highest of the entire experimental period (fig 2). At the end of the feeding trial, the specific growth rate of 3.86±0.13%/d was 31.86% significantly (p<0.05) higher than that of fish fed the control diet (2.63±27%/d).



A.s: *Allium sativum* (garlic) and Z.o: *Zingiber officinale* (ginger)

Figure 2: Effect of dietary inclusion of garlic-ginger mixture on specific growth rate of *Clarias gariepinus* juveniles for 85 days. Means on the same rearing time carrying different superscripts are significantly different from each other at p<0.05.

Survival rate, feed and nutrients utilization

The survival rate, feed and nutrients utilization of *C. gariepinus* juveniles obtained at the end of the rearing trial are presented in Table 3. Survival rate (SR), feed intake (FI), protein intake (PI) as well as lipid intake (LI) and condition factor (K) did not differed significantly (p>0.05) among treatment groups. However, feed conversion ratio (FCR), protein efficiency ratio (PER) and lipid efficiency ratio

(LER) of *C. gariepinus* juveniles were significantly ($p<0.05$) affected by dietary supplementation with garlic-ginger mixture. The best performances were obtained with the diet containing 2% garlic-ginger mixture. The FCR (1.55±0.05), PER (1.39±0.05) and LER (12.92±0.38) differed respectively by 37%, 34.53% and 51.31% from those of the control diet (FCR=2.46±0.18, PER=0.91±0.07 and LER=6.29±0.45).

Table 3: Survival rate, feed and nutrients utilization of juveniles *Clarias gariepinus* fed garlic-ginger mixture supplemented diets for 84 days.

Parameter	D0: 0% A.s-Z.o	D1: 1% A.s-Z.o	D2; 2% A.s-Z.o	D3: 3% A.s-Z.o	P
Ni	30	30	30	30	ns
Nf	29	27	30	29	ns
Wi (g)	49.78±9.06	43.39±5.82	43.36±3.52	49.65±6.11	ns
Wf (g)	149.31±5.05	149.01±4.54	218.74±6.42	190.22±5.60	***
Li (cm)	18.53±1.41	17.81±0.62	17.99±0.59	18.42±1.13	ns
Lf (cm)	26.87±1.1	26.65±0.61	29.03±0.29	28.07±1.12	ns
FI (g/fish)	243.9±26.32	243.7±28.87	271.6±11.54	271.3±7.56	ns
PI (g/fish)	109.9±11.86	110.4±13.07	126±5.36	126.9±3.53	ns
LI (g/fish)	15.85±1.71	10.87±1.30	13.58±0.58	13.57±0.38	ns
K	0.77±0.05	0.79±0.06	0.9±0,01	0.86±0.08	ns
SR (%)	96.67±5.77	90±17.32	100±0	96.67±5.77	ns
FCR	$2.46±0.18^{a}$	$2.33±0.27^{a}$	$1.55±0.05^{b}$	$1.94±0.13^{c}$	**
PER	$0.91±0.07^{ac}$	$0.96±0.1^{a}$	$1.39±0.05^{b}$	$1.11±0.07^{c}$	***
LER	$6.29±0.45^{a}$	$9.66±1.07^{b}$	$12.92±0.38^{c}$	$10.36±0.66^{b}$	**

A.s: *Allium sativum* (garlic) and Z.o: *Zingiber officinale* (ginger)
Values are mean ± standard deviation of three replicates of 10 fish each. Mean within a row with different superscripts are significantly different each other at p<0.05. ns, p≥0.05; *, p<0.05; **, p<0.01; ***, p<0.001.
Ni, initial number of fish; Nf, final number of fish; Wi, initial body weight of fish; Wf, final body weight of fish; Li, initial length of fish; Lf, final length of fish; FI, feed intake; PI, protein intake; LI, lipid intake; SR, survival rate; K, condition factor; FCR, feed conversion ratio; PER, protein efficiency ratio; LER, lipid efficiency ratio.

Whole body biochemical composition and nutrient retention
Whole body composition and nutrient retention of *C. gariepinus* pre-adults obtained after 84 days of feeding are shown in Table 4. Apart from the moisture content which decreased non-significantly ($p>0.05$) in C. gariepinus pre-adults in all treatment groups compared to that of the start juveniles, opposite effects were observed for body ash, protein, lipid and energy content which increased significantly ($p<0.05$) with the best values obtained with treatment D2. For the whole body nutrient retention, the results obtained indicate that apart from treatment D3 which induced the highest retention in ash compared to the rest of the treatments, one more time, the highest retention in protein, lipid and energy are obtained with treatment D2, that induced significant ($p<0.05$) increases of 45%, 66.72% and 44.56%, respectively compared to the control.

Table 4: Proximal composition (% or kcal/100g WW) of fish obtained after 84 days of feeding with garlic-ginger mixture supplemented diets.

Parameters	initial	D0: 0% A.s-Z.o	D1: 1% A.s-Z.o	D2; 2% A.s-Z.o	D3: 3% A.s-Z.o	P
Whole body Composition (% or kcal/100g WW)						
Moisture	76±0	76±0	74±0	75±0	71 ±0	ns
Ash	3.36±0.02[a]	3.12±0.02[a]	4.16±0.03[b]	3.63±0.12[a]	4.35±0.04[b]	**
Protein	13.94±0.42[a]	14.71±0.44[b]	16.82±0.34[c]	16.59±0.08[bc]	19.01±0.15[d]	**
Lipid	2.88±0[a]	2.76±0.36[ab]	2.73±0.13[ab]	3.63±0.13[b]	2.9±0.29[ab]	ns
Energy	96.96±0 [a]	97.32±1.8 [ab]	101±0.65 [b]	103.6±0.13 [b]	113.1±1.45 [c]	*
Nutrient Retention (% dry feed)						
Ash		12.22±0[a]	20.09±0[b]	24.63±1.04[c]	25.04±0[c]	**
Protein		18.42±0.55[a]	23.13±0.4[b]	33.48±0.39[c]	30.81±0.08[d]	**
Lipid		22.84±4.57[a]	34.5±2.37[a]	68.63±2.81[b]	40.28±5.44[ab]	*
Energy		14.17±0.39[a]	16.13±0.15[a]	25.56±0.04[b]	22.07±0.37[c]	**

A.s: *Allium sativum* (garlic) and Z.o: *Zingiber officinale* (ginger)
Values are mean ± standard deviation of three replicates of 35 fish each. Mean within a row with different superscripts are significantly different each from other at p<0.05. ns, p≥0.05; *, p<0.05; **, p<0.01.

Organosomatic indices and serum biochemical parameters

The organosomatic indices and serum biochemical parameters determined at the end of the experimental period (Table 5) indicated a significant difference (p<0.05) only for the female gonadosomatic index (FGI). The highest female gonadosomatic indices obtained in treatments D1 and D2 were 91.83% and 82.89% higher than the control respectively. However, the male gonadosomatic index (MGSI), intestinosomatic index (ISI) and hepatosomatic index also increased, although not significantly, in all experimental diets compared to the control. The serum biochemical composition of *C. gariepinus* pre-adults obtained after 84 days of feeding was also significantly (p<0.05) affected by the level of dietary inclusion of the garlic-ginger mixture. The concentrations of total protein (TP), total triglycerides (TG), total cholesterol (TC) and the ratio of high-density lipoprotein cholesterol to low-density lipoprotein cholesterol (HDL-C/LDL-C) in the control group were significantly lower than in the test groups. Although the highest value of TG is recorded in treatment D3, representing an increase of 39% compared to the control, the levels of TP, TC and HDL-C/LDL-C ratio in the blood serum of fish in treatment D2 are the highest with significant increases of 24.92%, 62.65% and 33.10% respectively compared to those in treatment D0.

Table 5: Organosomatic indices and serum biochemical parameters of *Clarias gariepinus* pre-adults obtained after 84 days feeding with garlic-ginger mixture supplemented diets.

Parameter	Experimental diets				*P*
	D0: 0% A.s-Z.o	D1: 1% A.s-Z.o	D2; 2% A.s-Z.o	D3: 3% A.s-Z.o	
		Organoomatic indices			
StSI (%)	0.73±0.04	0.73±0.02	0.72±0.06	0.73±0.05	ns
ISI (%)	1.49±0.03	1.52±0.15	1.52±0.15	1.61±0.2	ns
SpSI (%)	0.03±0	0.04±0.07	0.03±0	0.04±0.01	ns
HSI (%)	0.87±0.02	0.9±0.03	0.93±0.06	0.9±0,06	ns
MGSI (%)	0.33±0.06	0.38±0.04	0.35±0.04	0.35±0.05	ns
FGSI (%)	1.02±0.37^{a}	12.48±0.59^{b}	6.75±0.64^{c}	4.01±0.8^{c}	**
		Serum biochemical profile			
TP (g/dl)	13.53±0.27^{a}	17.36±0.01^{b}	18.02±0.03^{b}	17.99±0.03^{b}	**
TG (mg/dl)	225.7±0.09^{a}	303.8±0.32^{b}	263.7±0.42^{c}	369±0.31^{d}	***
TC (mg/dl)	199.5±0.87^{a}	432.7±0.19^{b}	534.1±0.02^{c}	341.6±0.28^{d}	***
HDL-C (mg/dl)	98.01±0.56^{a}	244.70±0.26^{b}	347.50±0.06^{c}	137±0.01^{d}	***
LDL-C (mg/dl)	56.37±1.44^{a}	130.3±0.39^{b}	133.8±0.04^{b}	130,7±0.26^{b}	***
HDL-C/LDL-C	1.74±0.05^{a}	1.86±0.01^{a}	2.60±0.01^{b}	1.05±0.01^{c}	**

A.s: *Allium sativum* (garlic) and Z.o: *Zingiber officinale* (ginger)
Values are mean ± standard error of the mean of three replicates of 4 fish each. Mean within a row with different superscripts are significantly different each from other at p<0.05. ns, p≥0.05; *, p<0.05; **, p<0.01; ***, p<0.001.
HSI: Hepatosomatic index, SpSI: Spleenosomatic index, StSI: Stomachtosomatic index, ISI: Intestinosomatic index, MGSI: Male gonadosomatic index, FGSI: Male gonado-somatic index, TP: total protein, TG: triglycerides, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol.

DISCUSSION

The general homeostasis as well as nutritional metabolism in fish are highly influenced by the water quality in which they live and exchange gases and non-gaseous particles. During this work, the values of the main physicochemical parameters such as temperature, pH, dissolved oxygen, ammonia, nitrite and nitrate recorded in the rearing tanks were in the appropriate range for the growth of *C. gariepinus* (Bhatnagar *et al*., 2004). These values were in the same ranges with those recorded by Nyadjeu *et al*. (2021a) while evaluating the effect of adding *Zingiber officinale* and *Allium sativum* mixture in the diet of *Clarias gariepinus* fry, with the exception of the pH whose value is higher (above 7). This indicates that the quality of the rearing water was better and therefore favourable to the harmonious development of the fish; thus justifying the high survival rate as well as growth optimization observed, certainly due to a good assimilation of the feed nutrient ingested. Thus, the few mortalities recorded would have other causes than those related to water quality or treatments. According to the FAO. (2020), world aquaculture production reached a record level of 114.5 million tonnes live weight in 2018. This production is constantly increasing, mainly due to the quality of the feed used, which in turn depends on the quality of the ingredients used. Among the essential components of aquafeeds are feed additives whose multiple properties are the subject of great attention by researchers in aquaculture nutrition. Feed additives, and more specifically phytoadditives, are rich in bioactive compounds that have a stimulating effect on the growth and health of fish (Rezaei *et al*., 2022). It is well documented that dietary supplementation with *Allium sativum* (garlic) or *Zingiber officinale* (ginger) boosts the growth performances of major freshwater species commonly farmed in sub-Saharan African countries such as Nile tilapia, Common carp and African catfish (Jafarinejad *et al*., 2018; Iheanacho *et al*., 2018; Santos et., 2020). On the other hand, very few studies have been conducted on the combined effect of the above mentioned phytoadditives on growth performances in relation to the physiological state of freshwater fish such as African catfish, *Clarias gariepinus*.

The results obtained in this study revealed the positive effects of using the garlic-ginger mixture as a feed additive on growth, feed nutrient utilization, whole-body biochemical content, organosomatic indices and serum biochemical profile. In the feeding trial, it was observed that juveniles fed the basal diet enriched with the garlic-ginger mixture, in addition to being more restless than those fed the control diet, also recorded the highest feed consumption values associated with the lowest feed conversion ratio; this indicates that in addition to being more easily digestible and convertible to flesh, the experimental diets would have been more attractive to the juveniles than the control diet. These observations suggest that the inclusion of the garlic-ginger mixture in the growth diet of *C. gariepinus* would have not only improved the taste of the feed, but would have also induced an appetite stimulation; thus corroborating previous results in which a significant decrease in feed conversion ration followed by an increase in feed consumption and vigour was noted in *C. gariepinus* post-larvae and fry fed with a diet enriched with the combination of garlic and ginger powder (Nyadjeu *et al*., 2021a). Indeed, studies have shown that products or by-products of plant origin used as additives in fish feed can improve the taste or palatability of feed and therefore stimulate appetite (Iheanacho, *et al*., 2018). In addition to the likely appetite-stimulating effect, growth parameters and feed nutrient utilization performance, such as weight gain, length gain, specific growth rate, protein and lipid efficiency as well as body protein and lipid retention, were also significantly improved in fish fed diets containing phytoadditives compared with control fish. The highest response was observed in fish fed the basal diet containing the 2% inclusion of the garlic-ginger mixture. These results are consistent with some previous studies that have demonstrated positive effects of dietary addition of garlic-ginger mixture on growth, feed utilization and nutrient retention in post-larvae, fry or juvenile *C. gariepinus*, as well as in other fish species such as *Oncorhychus mykiss* juveniles, juvenile great sturgeon (*Huso huso*) and Sobaity Sea Bream (*Sparidentex hasta*) fry (Jahanjoo *et al*., 2018; Nyadjeu *et al*., 2021b). The growth improvement observed in *C. gariepinus* juveniles fed with the experimental diet would be attributed more to the synergistic effects of the bioactive compounds contained in the two phytoadditives than to their nutritional contribution. In fact, it has been shown that the bioactive molecules contained in ginger, such as 6-gingerol, flavonoids and phenolic acids, as well as promoting the balance of intestinal bacteria, also have the power to stimulate pancreatic and biliary secretions, helping to speed up the digestion of feed (Ghasemzadeh *et al*., 2010). Ginger also contains proteolytic and lipolytic enzymes, which improve the digestion of protein and dietary fats (Venkataramalingam *et al*., 2007). Furthermore, previous work has suggested that these functions are also attributable to the bioactive components of garlic, which, in addition to the flavonoids and phenolic acids it contains, has allin and allicin as its main sulphuric components. Allicin being the most abundant, account for nearly 70% is known to improve the performance of the intestinal flora, thereby improving digestion (Khalil *et al*., 2001). As a result, energy utilisation is improved, leading to better growth. Thus, it is very likely that feeding *C. gariepinus* juveniles with the dietary inclusion of the garlic-ginger mixture would have improved feed nutrient utilization characterized by low feed conversion ratio, high protein and lipid efficiency ratios and greater nutrient retention; which finally resulted in significantly high somatic growth. The observed growth improvement could therefore be attributed more to the pharmacological properties of the two phytoadditives than their nutritional contributions. Indeed, the bioactive compounds of garlic and ginger, in addition to their possible morphometric effects on the gastrointestinal tract to improve the process of assimilation of dietary nutrients, would also improve the physiological condition of the fish, probably by enhancing their immune and antioxidant systems. (Udu-Ibiame *et al*., 2014).

According to Brum *et al*. (2018), the ability to assimilate dietary nutrients in fish depends on gut morphology, which plays an important role in the digestive and absorption functions of the digestive tract, thus implying a direct correlation with somatic growth and welfare in animal production. Gut morphology can be determined by histomorphometric analysis or macroscopically by simple determination of gut length or weight. In the present study, the change in gut weight expressed in terms of intestinosomatic index indicated an increase in treated fish compared to the control. These results indicate that, bioactive compounds such as polyphenols compounds, flavonoids and saponins contained in ginger and garlic would have modified the morphology of the gastrointestinal tract especially the gut structure by increasing the height and width of the villi, thereby increasing the intestinal absorption surface. It has been shown that any increase in the size (height and width) of the intestinal villi is due to an improvement in mucosa integrity (Chung *et al.*, 2021) indicating the possible beneficial effects of the above mentioned bioactive molecules on the gastrointestinal mucosa integrity. The intestine and hepatopancreas are the main sites of digestion and nutrient absorption (Wang, 2007). As previously observed, the intestinosomatic index and the hepatosomatic index of fish fed diets containing the garlic-ginger mixture was higher than that of fish fed the basal diet. These results, while corroborating those obtained by Iheanacho *et al*. (2018), lead to the hypothesis of a possible synergistic action of the bioactive compounds contained in garlic and ginger on the accumulation of liver energy reserves due to the increased availability of dietary nutrients and favourable physiological conditions, thus reflecting the welfare state of the treated fish.

Blood biochemical indices such as total protein, and lipid profile is physiological indicators for prognosis of any problem in fish or to explain their health status. The improvement of blood biochemical parameters in fish is mainly influenced by environmental factors and dietary supplementation (Rao *et al*., 2017). Serum total protein is an important nonspecific immune variable (Magnadóttir, 2006); its concentration in fish blood serum is less stable than in mammals and any disturbance of the physiological state of the fish due to stress or infection can result in a reduction in its concentration. Thus, maintaining a high concentration of blood total protein is likely to make the fish more tolerant of stressful conditions and more resistant to pathogens. In the present study, feeding juvenile *C. gariepinus* with diets containing graded levels of the garlic-ginger powder mixture significantly increased total protein compared to the control group. These results suggest an important role of the bioactive compounds of garlic and ginger through a synergistic action in modulating serum proteins; this partially explains that growth observed in treated fish would have also been one of the positive consequences of the aforementioned active compounds of garlic and ginger on the strengthening of the fish's non-specific immune system. Although very few studies have shown the combined effects of garlic and ginger powders on serum total protein levels, the individual effects of each of these phytoadditives on increasing total protein have, however, been confirmed by numerous studies on different fish species at different stages of development (Metwally 2009; Sevdan and Ergün, 2012). The results on the lipid profile in treated fish revealed a significant increase in serum concentrations of triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C); with the HDL-C/LDL-C ratio also significantly elevated compared to the control. Though many authors obtained conflicting results regarding the individual or combined effects of dietary garlic powder/extract and dietary ginger powder/extract on the lipid profile of different species of rearing fish (Chung *et al*., 2021; Adeniji *et al*., 2019 Jahanjoo *et al*., 2018), the results of the present work are however in agreement with those of Yılmaz and Ergün. (2012) who evaluated the effects of combined garlic and ginger oils on juvenile sea bass (*Dicentrarchus labrax*). Indeed, triglycerides are the main energy reserve in fish and are transported in the bloodstream for storage in a wide range of tissues such as liver tissue, muscle tissue and perivisceral adipose tissue (Henderson and Tocher, 1987). As an important functional substance in the body, cholesterol is an essential lipid involved in fish growth and development. It is mainly transported by lipoproteins (HDL-C and LDL-C) in the bloodstream. HDL-C and LDL-C have opposite physiological functions in cholesterol transport, with HDL-C transporting bloodstream cholesterol into the liver and LDL-C performing the reverse action. Therefore, an elevation or reduction in the HDL-C/LDL-C ratio indicates whether blood cholesterol is entering the liver to participate in metabolic activity or being transported from the liver to be deposited in tissues (Xiang *et al*., 2011). In the present study, the HDL/LDL ratio increased significantly in the treated fish suggesting that including the garlic-ginger mixture in the diet of C. gariepinus can promote blood cholesterol transport to the liver and thereby preventing the incidence of atherosclerosis, thus it significance in the production of fish for human consumption. Paradoxically to the increase in the HDL-C/LDL-C ratio, the dietary inclusion of the garlic-ginger mixture induced hyperlipidic effects in the treated fish, although the triglycerides and cholesterol levels obtained were within the normal range of 1-6 g/l for triglyceridemia and 1-4 g/l for cholesterolemia. (Fremont *et al*., 1981). During vitellogenesis, lipids stored as energy reservoirs in different tissues are mobilized and transferred to the liver for metabolism and transport to the gonads (Craig *et al*., 2000). In parallel with this mobilization, an increase in gonad weight was observed in females, reflecting their maturation state due to the accumulation of lipid particles in the ovaries. In the present study, serum hyperlipidemia (hypertriglyceridemia and hypercholesterolemia) observed in the experimental fish when compared to control was followed with significant increase in females gonadosomatic indices. These results suggest a possible involvement of the bioactive molecules of garlic and ginger in the modulation of the function of the reproductive organs of female African catfish.

CONCLUSION

This study demonstrated that the use of the garlic-ginger mixture as a feed additive had beneficial effects on the growth performance, feed nutrients utilization and retention, organosomatic indices and the serum biochemical profile of *C. gariepinus* juveniles. The inclusion of a mixture of these two spices as feed additives therefore seems very promising for improving the production of African catfish *Clarias gariepinus*.

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