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Growth Performance of *Clarias gariepinus* Fingerlings Fed Fermented *Canavalia ensiformis* Seed Meals

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ABSTRACT

Background and Objective: Fish farms' output and profits have been facing a greater challenge with a hike in the price of feed ingredients hence, this study was designed to evaluate the growth performance of *Clarias gariepinus* fingerlings fed fermented *Canavalia ensiformis* seed meals. **Materials and Methods:** The proximate analysis of the diets was done using the Association of Official Analytical Chemists methods. Fermented *Canavalia ensiformis* meals were used to formulate five different 40.0% isoproteic experimental diets each at 0, 25, 50, 75 and 100% and coded FCe, which were fed at 5% body weight to fingerlings of *Clarias gariepinus* fingerlings. One hundred and fifty *Clarias gariepinus* (0.96 g±0.03 and 3.93 cm±0.28) were randomly assigned to five treatments of ten fingerlings, replicated three times in a Completely Randomised Design (CRD) in 35 L plastic circular tanks through a semi-flow-through system for 84 days. Weights and lengths were measured biweekly and used to calculate the growth and nutrient utilization parameters. Nutrients, digestibility, haematology and economic evaluation of the fish fed the diets were also determined. Data collected were analysed using a one-way analysis of variance. **Results:** There were significant differences ($p<0.001$) in the specific growth rate as the inclusion levels of the tested meals increased. The mean water quality parameters were within the tolerable limit for aquaculture. Nutrient digestibility and haematology were significantly ($p<0.001$) reduced in the treatment diets. Inclusion of the tested meals in the diets resulted in the reduction of feed cost kg^{-1}, cost of feed intake/fish and feed cost/weight gain with increased inclusion levels. **Conclusion:** *Clarias gariepinus* fingerlings can feed on up to 25% inclusion-level fermented *Canavalia ensiformis* diet without adverse effects on the growth performance.

KEYWORDS

Fermented, *Canavalia ensiformis*, haematology, digestibility, economic evaluation, growth performance, *Clarias gariepinus*

INTRODUCTION

The recent increase in aquaculture development in Nigeria and the world at large is accompanied by the need to be incorporating plant materials in fish feed[1], to dispense total reliance on conventional legumes that have successfully replaced fishmeal. Soybean that could replace not less than 50% of the fishmeal in

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the diet of fish species is not so much available in the market and commands a high price because of their competitive use as food by man and feed ingredients by other livestock feed producers[2]. There is, therefore, a need to shift priority to searching for alternative plant protein feed ingredients of little or no use and cheaper in terms of cost to replace these conventional legumes. The search for alternative feedstuff to the current conventional feed ingredients requires that the digestibility of the alternative feed ingredients be favourably compared to the conventional feed ingredients[3].

Legumes have been recognized to be the second most valuable plant source for human and animal nutrition and the third largest family among flowering plants, consisting of approximately 650 genera and 20000 species[4,5]. Kalidass *et al.*[6] reported that legume seeds are important sources of nutrients and can serve as high-quality dietary protein to meet the nutrient requirements of fishes[7,8].

The wild legumes, which have tremendous potential for commercial exploitation but remain ignored, form a good scope in this context[9]. Inbasekar[10] reported that common proteinaceous edible legumes (soybean, cowpea and others) are available in the market and in most cases, production rates are compared with consumption (as food and feed) has remained unmet and an ever-increasing demand has been witnessed by food and feed industries. Also, switching most of the world's population to a protein-rich vegetarian-based diet from animal-based protein has created an unwarranted scarcity to plant protein resources. In this regard, legumes have been highlighted as an effective substitute for animal[11].

Canavalia ensiformis is an annual or weak perennial legume with climbing or bushy growth forms. It is woody with a long taproot. The 8 in (20 cm) long and 4 in (10 cm) wide leaves have three egg-shaped leaflets that are wedge-shaped at the base and taper towards the tip. The 1 in (2.5 cm) long flowers are rose-coloured, purplish, or white with a red base. It has a 12 in (30 cm) long, 1.5 in (3.8 cm) wide, sword-shaped seed pod. Seeds are white, red, brown and smooth with a brown seed scar that is about one-third the length of the seed. Its roots have nodules which fix nitrogen[12,13]. The genus *Canavalia* comprising of 48 species of these underutilized legumes. They are widely distributed and indigenous to the tropics[12], rarely eaten by man[14] and their nutritional potential has been well studied in the monogastric and poultry industry[15,16]. Nutritional trial in fish includes the works of the some researchers[12,17-20].

Alonso *et al.*[21] define Anti-Nutritional Factors (ANFs) as innate components of a food/feed ingredient that have a limiting effect on the food/feed intake, digestion and nutrient absorption. Possibly the most limiting factor for the use of plant feed ingredients as nutrient sources for fish are ANFs inherent to them. Francis *et al.*[22] reported that ANFs in legumes can be divided into several groups based on their chemical and physical properties such as non-protein amino acids, quinolizidine alkaloids, cyanogenic glycosides and pyrimidine glycosides, isoflavones, tannins, oligosaccharides, saponins, phytates, lectins or protease inhibition. Their elimination can be achieved either by a selection of plant genotypes with low levels of such factors or through post-harvest processing (germination, cooking, boiling, leaching/soaking, toasting and fermentation).

This study intends to use fermentation methods to eliminate the anti-nutrients in *Canavalia ensiformis* seed meals and its inclusion level effects on the growth of *Clarias gariepinus*.

MATERIALS AND METHODS

Study area: The research work commenced in January 2022. The feeding trial was conducted at the Fisheries Research Farm of the Department of Fisheries, Moddibo Adama University of Technology (MAUTECH), Yola. Adamawa State is located at latitude 9.14°N, longitude 12.38°E and an altitude of 185.9 m. Girei is located on latitude 9.22°N, longitude 12.33°E and altitude of 245 m. It has an average annual rainfall of about 759 mm with a maximum temperature of 39.7°C. The rainy season run from May through October, while the dry season commences in November and ends in April. The driest months of the year are January and February when the relative humidity drops to 13%[23,24].

Table 1: Percentage compositions of ingredients with fermented *Canavalia ensiformis* meal

	Inclusion levels				
Ingredients (g/100 g)	Control (0%)	FCe (25%)	FCe (50%)	FCe (75%)	FCe (100%)
Fishmeal (68%)	32.00	32.00	32.00	32.00	32.00
Glycine max meal (46%)	33.00	24.75	16.50	8.26	0.00
Fermented *Canavalia ensiformis* (34.7%)	0.0	10.93	21.87	32.81	43.75
Yellow maize (10)	30.00	27.32	24.63	19.29	19.25
Vitamin/mineral premix	1.0	1.0	1.0	1.0	1.0
Lysine	0.5	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5	0.5
Palm oil	1.0	1.0	1.0	1.0	1.0
Cassava starch	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5
Common salt	0.5	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0	100.0
Calculated crude protein	39.94	39.67	39.40	38.87	38.87
Gross energy (KJ/100 g)	1868.60	1818.80	1769.70	1766.40	1732.90
GE:CP	46.78	45.84	44.91	45.13	44.58

FCeM: Fermented *Canavalia ensiformis* meal, GE:CP: Gross energy:Crude protein, Calculated gross energy (KJ/100 g) = Protein×23.6 KJ/100 g+Lipid×39.5 KJ/100 g+NFE×17.2 KJ/100 g (Blaxter, 1989), Vitamin-mineral premix provides per kg the following: 12.000.000 IU Vitamin A, 2.000.000 IU Vitamin D3, 10 g Vitamin E, 2 g Vitamin K3, 1 g Vitamin B1, 5 g Vitamin B2, 1.5 g Vitamin B6, 10 g Vitamin B12, 30 g Nicotinic acid, 10 g Pantothenic acid, 1 g Folic acid, 50 g Biotin, 250 g Choline chloride 50%, 30 g Iron, 10 g copper, 50 g Zinc, 60 g Manganese, 1 g Iodine, 0.1 g Selenium and Cobalt 0.1 g

Canavalia ensiformis fruits were collected from Girei and their surroundings in Adamawa State. They were identified using a field handbook by Larese *et al.*[25] a plant taxonomist at the Forestry and Wildlife Department of Modibbo Adama University of Technology, Yola (MAUTECH). Raw seeds were moistened with water, kept in a container with a cover to ferment for 72 hrs under laboratory conditions, oven-dried at 50°C then milled and tagged Fermented Seed Meal (FSM).

Determination of nutrient compositions: The seed meals were analyzed for nutrient compositions[26].

Feed formulation: A basal diet of 40% crude protein was formulated from the commercial ingredients (fish meal, *Glycine max* meal, yellow maize, vitamin, minerals, palm oil, dicalcium phosphate and starch) and *Glycine max* was toasted. The dry ingredients were milled with a grinding machine to very fine particle size and sieved. The ingredients were weighed and mixed to homogeneity and pelleted with a pelleting machine with a 0.2 mm diameter size. The pellets were air-dried at room temperature and stored in a refrigerator until the commencement of the feeding. Fermented *Canavalia ensiformis* were included in the experimental diets to replace *Glycine max* meal as a plant protein source at inclusion levels of 0 (control), 25, 50, 75 and 100% as shown in Table 1.

Collection and acclimatization of experimental fish: One hundred and fifty fingerlings of *Clarias gariepinus* were obtained from Aqua Guide Farm in Federal Housing Estate, Girei LGA, Adamawa State, Nigeria and were used for the feeding trials. The fish were kept acclimatizing to the farm conditions and fed with the experimental diets for 12 weeks.

Experimental design: A completely randomized design was used, where one hundred and fifty fingerlings were randomly allocated to five experimental groups (ten fingerlings of *Clarias gariepinus* per bowl and replicated).

Data collections

Growth performance and nutrient utilization: The initial weight and length of fish in each treatment were taken at 2 weeks intervals. The feed rations fed were adjusted based on the new weight. The weight and length recorded were used to determine the growth performance of the fish and the feed supplied was also used to determine the nutrient utilization parameters following the methods of Udo and Umoren[27].

Growth performance: At the end of 12 weeks, the growth rates, condition factor, survival rate and nutrient utilization were computed and analyzed.

Weight gain (g): The total and mean weight gains were calculated for each replicate and treatment as follows[27]:

$$\text{Weight gain/fish (g/fish)} = (W_f - W_I)$$

$$\text{Mean weekly weight gain} \left(\text{g/week}\right) = \frac{W_f - W_I}{n}$$

Where:
W_f = Final weight of fish at the end of the experiment
W_I = Initial weight of fish at the beginning of the experiment
n = Number of weeks

Relative Growth Rate (RGR): This is the percentage ratio of the weight gain to the initial body weight and was determined as follows:

$$\text{RGR} \left(\%\right) = \frac{W_f - W_I}{W_I} \times 100$$

Specific Growth Rate (SGR %/day): This is the percentage of daily weight gain and was computed according to the formula below:

$$\text{SGR} \left(\%/\text{day}\right) = \frac{\text{Log } W_f - \text{Log } W_I}{t} \times 100$$

Where:
Log W_f = Logarithm of the fish final weight
Log W_I = Logarithm of the fish's initial weight
t = Experimental period in days

Condition factor (K): This expresses the health status of fish as a result of the experimental treatment and was computed at the beginning and end of the experiment (K1 and K2) using Fulton's condition factor formula as expressed by Udo and Umoren[27] as:

$$K = \frac{100W}{L^3}$$

Where:
W = Weight of fish
L = Length of fish

Survival (%):

$$SR\ (\%) = \frac{N_f}{N_i} \times 100$$

Where:

N_i = Number of cultured fish stocked at the beginning of the experiment

N_f = Number of fish alive at the end of the experiment

Feed utilization

Feed Conversion Ratio (FCR): This is a numerical value used to measure the utilization of feed for growth. The feed conversion ratio was calculated following Udo and Umoren[27] as:

$$FCR = \frac{\text{Feed intake}}{\text{Weight gain (g)}}$$

Feed Intake (FI): This will be taken as the addition of the amount of feed supplied during the experimental period.

Protein Intake (PI): This is the numerical value of the quantity of protein present in the feed that was fed to the fish during the experimental period and was determined as following Getso *et al.*[28]:

$$\text{Protein intake} = \text{Feed intake} \times \text{Crude protein}$$

Protein Efficiency Ratio (PER): This index uses growth as a measure of the nutritive value of dietary protein:

$$PER = \frac{\text{Mean weight gain (g)}}{\text{Mean protein intake (g of protein in 100 g of diet/fish)}}$$

Water quality parameters: Water quality parameters such as temperature, pH, dissolved oxygen and ammonia concentration were taken biweekly before feeding using the NIFFR (National institute for freshwater fisheries research, new bussa, Niger State, Nigeria) innovative multitec kit and ammonia by titration with sulfuric acid.

Determination of nutrient digestibility coefficient: Indirect methods of digestibility were applied by using chromium III oxide incorporated into experimental diets as an inert indicator of digestion[12]. Faecal samples were collected twice a week at regular intervals by slightly pressing the anal region of the fish after seven hours of feeding. The samples that were collected are weighed, ground in a mortar, seal in polythene bags, labelled (according to each dietary treatment code) and kept in a refrigerator before proximate analysis. The indices were calculated as:

Apparent Digestibility of dry matter (ADdm):

$$ADdm\ (\%) = \frac{1 - \text{Dietary chromic oxide}}{\text{Feacal chromic oxide}} \times \frac{\text{Feacal dry matter}}{\text{Dietary dry matter}} \times 100$$

Apparent Digestibility of protein (ADp):

$$\text{ADp}\ (\%) = \frac{1-\text{Dietary chromic oxide}}{\text{Feacal chromic oxide}} \times \frac{\text{Feacal protein}}{\text{Dietary protein}} \times 100$$

Apparent Digestibility of energy (ADe):

$$\text{ADe}\ (\%) = \frac{1-\text{Dietary chromic oxide}}{\text{Feacal chromic oxide}} \times \frac{\text{Feacal protein}}{\text{Dietary energy}} \times 100$$

Haematological examination

Collection of blood sample: At the end of the feeding trials, three samples of live fish from each treatment were removed. About 10 mL of blood was collected from the caudal peduncle of each fish using a separate heparinized 10 mL disposable syringe, kept into properly labelled sterilized bottles containing EDTA (Ethylenediaminetetraactatic acid) as anticoagulant and transported to the laboratory for analysis according to Sveier *et al.*[29] methods.

Haematocrit (PCV): Heparinized capillary tubes were 75% filled with blood samples by suction pressure and one end was sealed with plasticine. The tubes were centrifuged for 5 min in a haematocrit centrifuge at 3000 r.p.m. The Packed Cell Volumes (PCV) were read by the use of a haematocrit reader. The results were expressed in percentages.

Haemoglobin concentration (Hb): The cyanmethemoglobin method by Sveier *et al.*[29] was used. 0.02 mL of well-mixed blood was added to 4 mL of modified Drabkins solution (a mixture of 250 mg potassium ferricyanide, 200 mg potassium cyanide and 50 mg of potassium dihydrogen phosphate) and the volume was diluted to 1 L with distilled water. The mixture was allowed to stand for 35 min and the haemoglobin concentration (g dL^{-1}) was read photometrically by comparing it with the cyanmethemoglobin standard with the yellow-green filler at 625 nm.

Leucocyte count (WBC): The haemocytometer was used for LC determination with a 0.8 cm objective of the microscope and large squares (area = 1 mm^2, depth = 0.1 mm) the volume was 0.1 mm^3 and the dilution factor was 20. Four squares were used and the total counts per mm^3 were:

$$20\times1\times\text{L cells}/0.4 = 50\times\text{L cells}$$

Where:
L = Number of leucocytes that were counted

Erythrocytes (RBC): These were determined in heparinized blood diluted by Hayman solution at a ratio of 1:200. Neubauer improved haemocytometer placed on a compound microscope stage was used to count/estimate the erythrocyte population. The number of cells counted, R (average of two fields) was multiplied by the dilution factor and the volume of 1/4000 mm^3 (area = 1/4000 mm^3, depth = 1/10 mm^3) and counting was done in 80 squares with the total volume of 1/50 mm^3 the dilution factors is 200:

$$200\times500\times\text{R cells} = 10{,}000\times\text{R}$$

Determination of Mean Corpuscular Haemoglobin (MCH): The Mean Corpuscular Haemoglobin (MCH) was calculated using the formula:

$$\text{MCH}\ (\text{pg}) = \frac{\text{Hb}\times10}{\text{RBC}}$$

Determination of Mean Corpuscular Volume (MCV): The Mean Corpuscular Volume (MCV) was determined as follow:

$$\text{MCV}\ (\text{fL}) = \frac{\text{Packed cell volume} \times 10}{\text{RBC}}$$

Determination of Mean Corpuscular Haemoglobin Concentration (MCHC): The Mean Corpuscular Haemoglobin Concentration (MCHC) was calculated using the formula:

$$\text{MCHC}\ (\text{g dL}^{-1}) = \frac{\text{Hb} \times 100}{\text{PCV}}$$

Economic analysis: The economic analyses were computed to estimate the cost of feed required to raise a kilogram of fish using the various experimental diets. The major assumption is that all other operating costs for commercial fish production will remain the same for all diets. Thus, the cost of feed was the only economic criterion in this case. The cost was based on the current prices of the feed ingredients at the time of purchase. The economic evaluations of preparing the diets were calculated from the method of Witeska *et al.*[30] and Agbo *et al.*[31]:

Investment Cost Analysis (ICA) = Cost of feeding (₦)+Cost of fingerlings stocked (₦)

Profit Index (PI) =Net profit value (₦)/Cost of feeding (₦)

Incidence of Cost (r) = Cost of feeding (₦)/Weight of fish produce (g)

Benefit-Cost Ratio (BCR) = Net profit value (₦)/Investment cost analysis (₦)

Statistical analysis: Data collected were subjected to descriptive statistics, graphical representations and one-way analysis of variance ANOVA. Significant differences between treatment means were compared using the least significant difference at a 5% probability level using IBM SPSS statistics 19.

RESULTS

Proximate compositions of fermented *Canavalia ensiformis* (FCe) diets: The highest crude protein value was from the control diet (40.36%) and the lowest was from the 100% FCe diet (39.40%). The highest lipid content was from the control diet (7.01%) and the lowest from the 25% FCe diet (6.75%). The highest fibre value was from the 100% FCe diet (5.76%) and the lowest was from the control diet (5.43%). The highest ash content was from the 100% FCe diet (6.60%) and the lowest was from the control diet (6.52%). The highest nitrogen-free extract was from the 100% FCe diet (32.77%) and the lowest was from the control diet (32.18%). The highest dry matter content was from the 75% FCe diet (91.51%) and the lowest was from the 50% FCe diet (91.40%). The highest gross energy value was from the control diet (417.71 kcal g^{-1}) and the lowest was from the 100% FCe diet (414.48 kcal g^{-1}) in Table 2.

Growth performance of *Clarias gariepinus* fed FCe diets: Growth parameters measured, decreased as fermented *Canavalia ensiformis* meal inclusion levels increased. The highest and lowest Mean Weight Gains (MWG) were recorded from the control and 100% FCe diets (6.78 and 2.64 g/fish) respectively (Table 3). There were significant differences ($p<0.001$) between the MWG of the diets. The relative growth rate (RGR) ranged from 162-674%/fish. The highest value was from the control diet and the lowest value was from FCe 100% diet. There was a significant difference ($p<0.001$) among the diets. Specific Growth Rate (SGR) ranged from 0.46-1.07%/day. The lowest value was recorded from FCe 75% diet and the highest was from the control diet. The highest survival was from control, FCe 25 and 50% diets (80.0%) and the lowest from 75 and 100% FCe diets (30.0 and 20.0%), respectively. There were significant

Table 2: Proximate compositions of fermented *Canavalia ensiformis* diets on dry matter basis

Nutrients	FCe (0%)	FCe (25%)	FCe (50%)	FCe (75%)	FCe (100%)
Protein %	40.36	40.25	40.15	39.45	39.40
Fat %	7.01	6.75	6.84	6.95	6.97
Fibre %	5.43	5.66	5.72	5.75	5.76
Ash %	6.52	6.55	6.55	6.57	6.60
NFE %	32.18	32.24	32.14	32.79	32.77
Dry matter %	91.5	91.45	91.40	91.51	91.50
Calculated analysis					
Gross energy (kcal g^{-1})	417.71	414.98	414.84	414.66	414.48
Digestible energy (kcal g^{-1})	273.81	271.43	271.57	271.09	271.02
Metabolizable energy (kcal g^{-1})	3202.52	3180.52	3180.56	3186.64	3185.70

FCe: Fermented *Canavalia ensiformis*

Table 3: Growth parameters and survival rate of *Clarias gariepinus* fingerlings fed femented *Canavalia ensiformis* diets

Parameters	Control (0%)	FCe (25%)	FCe (50%)	FCe (75%)	FCe (100%)	SEM
Total initial weight (g)	9.6±0.52	9.6±0.52	9.6±0.52	9.66±0.57	9.6±0.52	0.31^{ns}
Total final weight (g)	61.86±6.11^{a}	28.53±2.57^{b}	24.0±2.88^{b}	11.2±1.44^{c}	9.9±0.65^{c}	1.91***
Mean initial weight (g/fish)	0.96±0.05	0.93±0.05	0.96±0.05	0.96±0.05	0.96±0.05	0.03^{ns}
Mean final weight (g/fish)	7.73±0.76^{a}	3.56±0.32^{b}	3.0±0.36^{bc}	2.8±0.36b^{bc}	2.5±0.20^{c}	0.25***
Mean weight gain (g/fish)	6.77±0.71^{a}	2.63±0.27^{b}	2.04±0.31^{bc}	1.84±0.31^{bc}	1.54±0.15^{c}	0.25***
Mean weekly weight gain (g/fish/week)	0.56±0.71^{a}	0.21±0.27^{b}	0.17±0.31^{bc}	0.15±0.31^{bc}	0.12±0.15^{c}	0.02***
Mean initial length (cm/fish)	4.06±0.51	4.06±0.51	3.83±0.35	4.1±0.36	3.96±0.15	0.23^{ns}
Mean final length (cm/fish)	7.73±0.80^{a}	6.23±0.55^{b}	6.16±0.25^{b}	5.66±0.20^{b}	5.66±0.35^{b}	0.27***
Relative growth rate (%/fish)	673.0±0.71^{a}	282.7±0.27^{b}	212.5±0.31^{bc}	191.6±0.31^{bc}	160.0±0.15^{c}	34.43***
Specific growth rate (%/day)	1.06±0.71^{a}	0.69±0.27^{b}	0.57±0.31^{c}	0.45±0.31^{c}	0.48±0.15^{c}	0.03***
K1	1.43	1.38	1.7	1.39	1.54	0.25^{ns}
K2	1.67	1.47	1.28	1.46	1.5	0.21^{ns}
Survival (%)	80^{a}	80^{a}	80^{a}	30^{b}	20^{c}	2.12***

Mean±Std on the same row with different superscripts are significantly different ($p<0.001$), ***SEM: Standard error of the mean, ($p<0.001$), ns: Not significant ($p>0.05$) and FCe: Fermented *Canavalia ensiformis*

differences ($p<0.001$) between the highest and lowest survival of fish-fed FCe diets. To compare the initial and final condition factors K1 and K2, the highest K1 was from fish fed 50% FCe diet (1.70) and the lowest was from fish fed 25% FCe diets (1.38) while the highest K2 was from fish fed the control diet (1.67) and the lowest was from fish fed FCe 50% diet (1.28). There was no significant difference ($p>0.05$) between K1 and K2.

Feed intake and nutrient utilization of *Clarias gariepinus* fed fermented *Canavalia ensiformis* (FCe) diets: The highest Mean Feed Intake (MFI) was obtained from fish fed a control diet (14.07 g/fish) and the lowest was from fish fed FCe 100% diet (3.4 g/fish), there was a significant difference ($p<0.001$) in the MFI of the fish fed the diets. The Voluntary Feed Intake (VFI) ranged between 1.16-2.92 g/fish. The highest value was from the fish fed the control diet and the lowest was from the fish-fed FCe 100% diet (Table 4). There was a significant difference ($p<0.001$) in the VFI of the fish fed the diets The highest Feed Acceptability Index (FAI) was from the control diet (0.228%) and the lowest was from the fish-fed FCe 100% diet (0.086%). There were significant differences ($p<0.001$) between the FAI of the control diet and FCe diets. The highest Feed Conversion Ratio (FCR) was in the fish-fed FCe 50% diet (3.27) and the lowest was from the fish-fed control diet (2.07). There was a significant difference ($p<0.001$) between the FCR of the control diet and FCe diets. The protein intake ranged from 133.9-567.8 g/100 g diet/fish. The highest value was obtained from fish fed the control diet and the lowest was from fish fed FCe 100% diet. There was a significant difference.

Mean water quality parameters of fish fed fermented *Canavalia ensiformis* diets: The biweekly mean water quality parameters in the holding facilities during the feeding period are presented in figures (Fig. 1-4). The highest temperature (27.8°C) was recorded from 0% control and 25% FCe diets in week 2

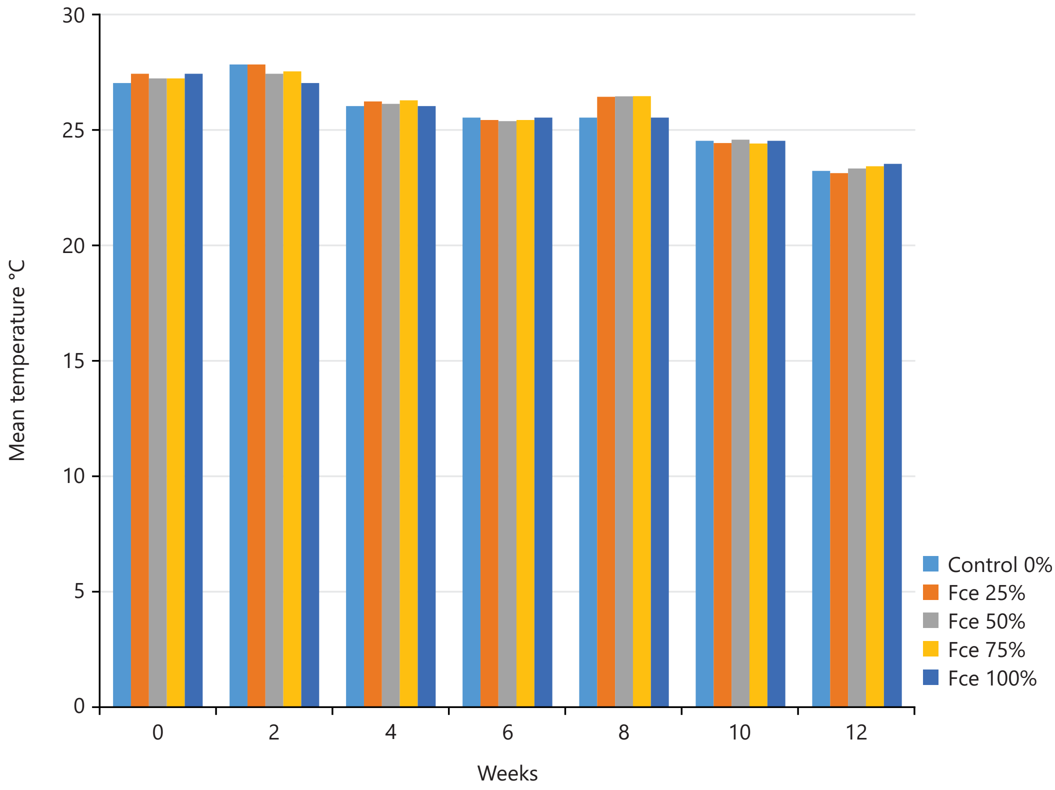

Fig. 1: Biweekly temperature (°C) of water used to raise *Clarias gariepinus* fed FCe diets

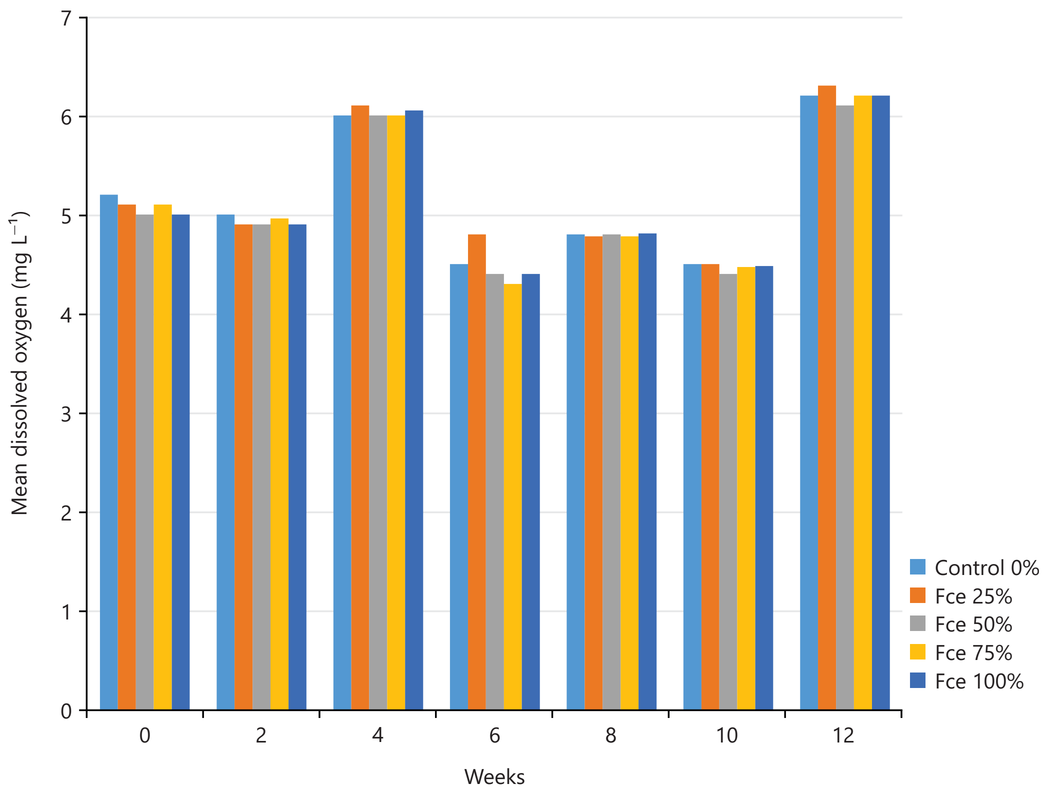

Fig. 2: Biweekly dissolved oxygen (mg L^{-1}) of water used to raise *Clarias gariepinus* fed FCe diets

while the lowest (23.1°C) was from the 25% FCe diet in week 12. The highest dissolved oxygen (6.3 mg L^{-1}) was recorded from the 25% FCe diet in week 12 while the lowest (4.3 mg L^{-1}) was from the 75% FCe diet in week 6. The highest pH (7.3) was recorded from the 75% FCe diet in week 4 while the lowest (4.8) from

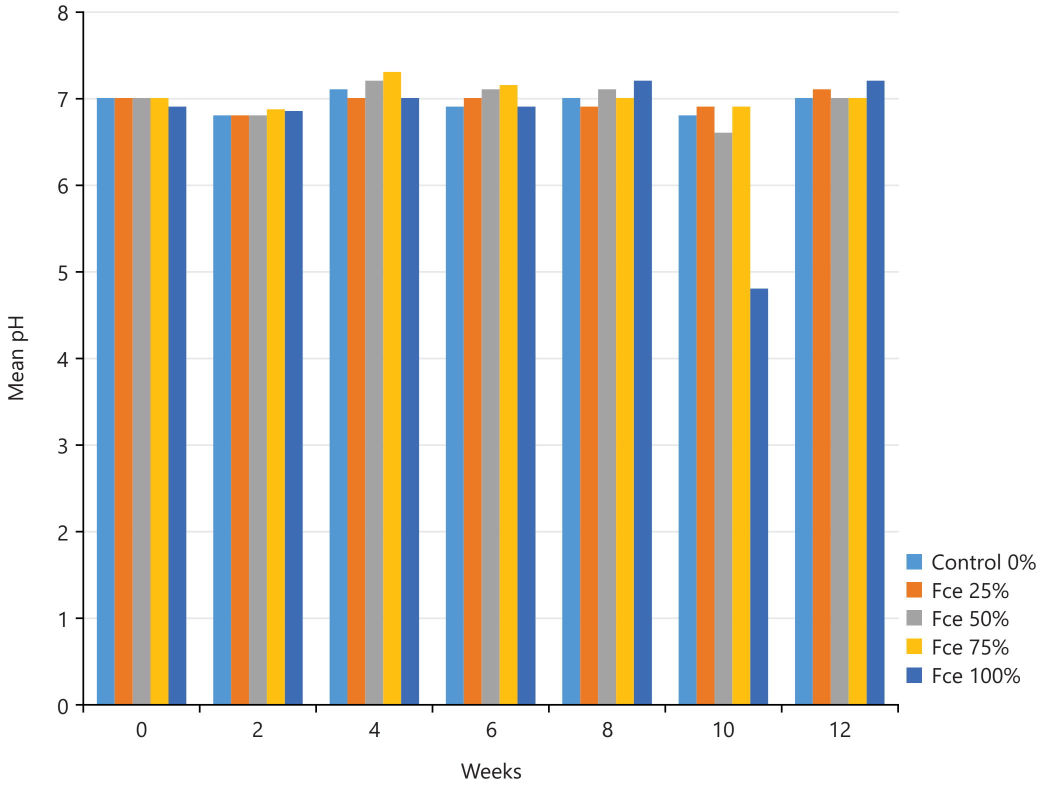

Fig. 3: Biweekly pH of water used to raise *Clarias gariepinus* fed FCe diets

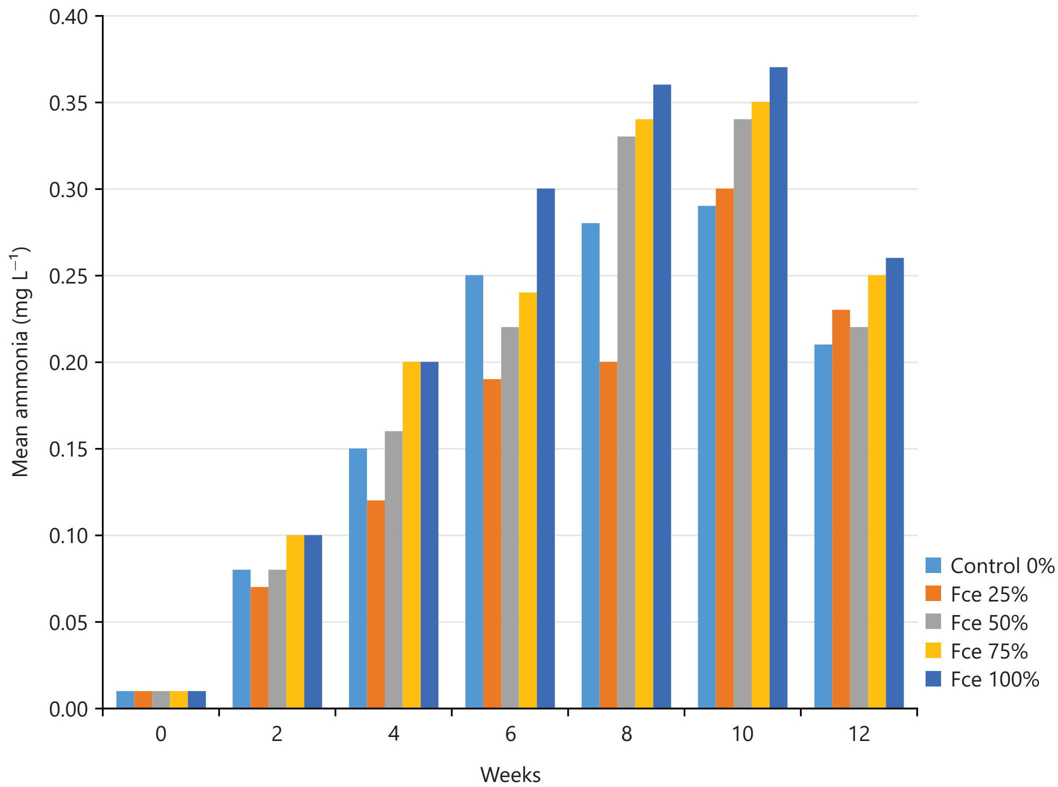

Fig. 4: Biweekly ammonia (mg L^{-1}) of water used to raise *Clarias gariepinus* fed FCe diets

100% FCe in week 10 and the highest ammonia (0.37 mg L^{-1}) was from the 100% FCe diet in week 10 while the lowest (0.01 mg L^{-1}) was from the five (0, 25, 50, 75 and 100%) FCe diets in week 0. The results of the water quality parameters were significantly ($p<0.001$) different across the treatment groups.

Table 4: Feed intake and nutrient utilization indices of *Clarias gariepinus* fingerlings fed fermented *Canavalia ensiformis* diets

Parameters	Control (0%)	FCe (25%)	FCe (50%)	FCe (75%)	FCe (100%)	SEM
Total feed intake (g)	1181.88^a	642.6^b	561.12^c	338.52^d	285.6^e	2.19***
Mean feed intake (g/fish)	14.07±0.17^a	7.65±0.11^b	6.68±0.14^c	4.03±0.13^d	3.4±0.17^e	0.02***
Biweekly feed intake (g/week)	196.98^a	107.1^b	93.52^c	56.42^d	47.6^e	0.31***
Voluntary feed intake (g/fish)	2.92±0.14^a	2.02±0.26^a	2.01±0.34^a	1.27±0.31^b	1.16±0.68^b	0.09***
Feed acceptability index (%)	0.228±0.02^a	0.153±0.01^b	0.142±0.01^c	0.092±0.01^d	0.086±0.01^e	0.01***
Feed conversion ratio	2.07±0.23^b	2.9±0.40^a	3.27±0.45^a	2.19±0.41^b	2.2±1.13^b	0.00***
Protein intake (g/100g diet/fish)	567.8±0.01^a	307.9±0.01^b	268.2±0.01^c	158.9±0.01^d	133.9±0.01^e	1.03***
Protein efficiency ratio	0.13^a	0.052^b	0.042^b	0.038^b	0.036^b	0.00***

Mean±Std on the same row with different superscripts are significantly different (p<0.001), ***SEM: Standard error of the mean and FCe: Fermented *Canavalia ensiformis*

Table 5: Nutrient digestibility coefficients of *Clarias gariepinus* fed FCe diets

Indices (%)	Control (0%)	FCe (25%)	FCe (50%)	FCe (75%)	FCe (100%)	SEM
Dry matter digestibility	88.8±0.1^a	87.1±0.1^b	85.5±0.1^c	83.9±0.1^d	82.3±0.1^e	0.05***
Protein digestibility	74.5±0.1^a	70.0±1.0^b	65.0±1.0^c	60.7±0.1^d	57.8±0.1^e	0.36***
Lipid digestibility	41.56±0.01^a	40.66±0.00^b	39.61±0.01^c	35.84±0.00^e	38.88±0.01^d	0.01***
Energy digestibility	70.7±0.1^a	69.0±1.00^b	68.0±1.00^{bc}	67.1±0.1^{cd}	66.3±0.1^d	0.36***

Mean±Std on the same row with different superscripts are significantly different (p<0.001), ***SEM: Standard error of the mean and FCe: Fermented *Canavalia ensiformis*

Table 6: Some haematological indices of *Clarias gariepinus* Fed FCe diets

Indices	Control (0%)	FCe (25%)	FCe (50%)	FCe (75%)	FCe (100%)	SEM
PCV (%)	35.4±0.51^a	34.05±0.81^b	32.65±1.05^c	31.46±0.47^{cd}	30.35±0.36^d	0.00***
RBC (×10^6)	1.40±0.41^{bc}	1.38±0.39^c	1.30±0.31^d	1.25±0.26^{ab}	1.14±0.15^a	0.85***
WBC (×10^3)	20.5±1.25^{bc}	19.35±1.02^c	16.75±0.76^d	21.83±0.84^a	22.94±0.95^a	0.01***
Hb (g dL^{-1})	10.6±0.82^a	9.85±1.3^{ab}	9.23±0.44^{abc}	8.75±0.76^{bc}	8.09±0.85^c	0.04***
MCV (dL)	252.85±1.08^b	246.73±0.85^d	251.15±0.16^c	251.68±0.69^{bc}	266.22±0.23^a	0.01***
MCH (Pg)	75.71±0.83^a	71.37±0.82^b	71.0±1.00^b	70.0±1.00^b	70.96±0.97^b	0.01***
MCHC (g dL^{-1})	29.94±0.95^a	28.92±1.03^{ab}	28.26±0.81^{abc}	27.81±0.82^{bc}	26.65±1.05^c	0.02***

Mean±Std on the same row with different superscripts are significantly different (p<0.01), (p<0.001), ***SEM: Standard error of the mean, FCe: Fermented *Canavalia ensiformis*, PVC: Packed cell volume, RBC: Red blood cell, WBC: White blood cell, Hb: Haemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin and MCHC: Mean corpuscular haemoglobin concentration

Nutrient digestibility coefficients of *Clarias gariepinus* fed FCe diets: Table 5 showed that the highest dry matter value was from the control diet (88.8%) and the lowest was from the 100% diet (82.3%) diet. The highest protein value was from the control diet (74.5%) and the lowest was from the 100% diet (57.8%). The highest lipid value was from the control diet (41.56%) and the lowest value was from the 75% diet (35.84%). The highest energy value was from the control diet (70.7%) and the lowest value was from the 100% diet (66.3%). There was a significant difference (p<0.001) in nutrient digestibility.

Some haematological indices of *Clarias gariepinus* fed FCe diets: Table 6 showed that the highest PCV value was from the control diet (35.4%) and the lowest from the 100% diet (30.35%). The highest RBC value was from the control diet (1.40) and the lowest was from 100% (1.14). The highest WBC value was from the 100% diet (22.94) and the lowest from the 50% diet (16.75). The highest Hb value was from the control diet (10.6 g dL^{-1}) and the lowest from the 100% diet (8.09 g dL^{-1}). The highest MCV value was from the 100% diet (266.22 dL) and the lowest from the 25% diet (246.73 dL).

An economic evaluation of fermented *Canavalia ensiformis* (FCe) diets fed to *Clarias gariepinus* for six months: The result of the production cost of FCe diets (Table 7) showed that the highest profit index was from FCe 75% diet (3.0) and the lowest was from the control and FCe 25% diets (2.8). The highest net profit was from the control diet (1142.5) and the lowest was from FCe 100% diet (838.6). The highest benefit-cost ratio was from FCe 25% diet (7.2) and the lowest was from the control and FCe 100% diets (6.5), there was a significant difference (p<0.001) in all the parameters across the treatment groups.

Table 7: Economic evaluation of *Clarias gariepinus* fingerlings fed fermented *Canavalia ensiformis* diets for six months

Indices	Control (0%)	FCe (25%)	FCe (50%)	FCe (75%)	FCe (100%)	SEM
Cost of feed (₦)	479.5[a]	443.5[b]	407.5[c]	363.5[d]	336.5[e]	0.18***
Cost of feeding (₦)	67.5[a]	33.9[b]	27.2[c]	14.6[d]	11.4[e]	0.12***
Estimated investment cost (₦)	207.5[a]	173.9[b]	167.2[c]	154.6[d]	151.4[e]	0.18***
Net profit value (₦)	1350[a]	1260[b]	1170[c]	1080[d]	990[e]	0.58***
profit index (₦)	2.8[b]	2.8[b]	2.9[ab]	3.0[a]	2.9[ab]	0.06***
Incidence of cost	120.5[c]	161.4[a]	160[b]	97.3[d]	95[e]	0.36***
Benefit cost ratio	6.5[c]	7.2[a]	7.0[b]	7.0[b]	6.5[c]	0.05***
Net profit (₦)	1142.5[a]	1086.1[b]	1002.8[c]	925.4[d]	838.6[e]	0.05***

Values on the same row with different superscripts are significantly different ($p<0.001$), ***SEM: Standard error of the mean and FCe: Fermented *Canavalia ensiformis*

DISCUSSION

In applied research and practice, weight gain and specific growth rate are usually considered the most important measurement of productivity of diet[31,32] and a reliable indication of a marketable product. The fact that growth was recorded from all experimental diets, indicated that the fish was able to convert the protein in the feed to muscles as reported by Sogbesan and Ugwumba[33]. The growth performance and nutrient utilization of fish-fed FCe diets were significantly different ($p<0.001$) from the control. The control treatment had higher values than the FCe treatments. In terms of FCe treatments, better performances were recorded from 25% and followed by 50% FCe treatment and agree with the report of Sogbesan *et al.*[31] on fermented Sunflower meal diet[34]. This study disagrees with[35] those who reported better performance in (D3) fish fed a 20% fermented sicklepod seed meal diet. In this study, the reason for the difference in acceptability of the diets among treatments agrees with earlier reports by researchers[36-38]. The researchers suggested that when alternative protein sources especially plant protein sources are used in the fish diet, the palatability and attractiveness of the diets are usually affected. As suggested by Ahmad[39] proper utilization of dietary protein is dependent on the good quality or amino acid balance of the protein sources. Nguyen *et al.*[40] stated that the Weight Gain (WG) of young fish is a reliable indicator of nutritional efficiency. This present study showed that fermentation and inclusion levels both affected the weight gained. This agrees with the findings of some studies[40-44], that one of the major limitations of using alternative plant protein sources in fish feeding is the changes in palatability and the presence of anti-nutrition factors in the ingredient. The reduction in feed intake due to the unpalatability may lead to deterioration in growth performance and feed utilization. A reduction in growth and nutrient utilization in fish fed 75 and 100% FCe diets was observed, this reduction observed could be attributed not only to the dietary amino acid profile of the ingredient but also to the presence of anti-nutritional factors. This assertion is in line with the findings of some studies[45-47] that higher inclusion levels of most plant protein source base meals resulted in poor growth and nutrient utilization. The best Feed Conversion Ratio (FCR) was recorded in the control diet, followed by the 25% FCe diet which is an indication of an optimum level of utilization of the diet by the *Clarias gariepinus* fingerlings, this corresponds with Wang *et al.*[48], who stated that the lower the FCR, the better the feed utilization by the fish and observations made by scientists[49-51] in related studies on feeding trials. The Protein Efficiency Ratio (PER) is known to be regulated by the non-protein energy input of the diet and is a good measure of the protein-sparing effect of lipids and/or carbohydrates[52,53]. The general well-being of the fish-fed fermented *Canavalia ensiformis* diets is expressed by the condition factor (K). The values were above 1.0 and agreed with[54]. Survival rate was higher in control, 25 and 50% treatments while lower in 75 and 100% treatments and this might be due to the presence of antinutrients in the diets or other extraneous factors which agreed with Jamabo *et al.*[55], who reported that mortality might not be due to the antinutrients in the diets alone but also to any other extraneous factors such as stress resulting from handling.

These water quality parameters ranges for all the treatment diets were within the tolerable limit in aquaculture[54,56-58] recommended dissolved oxygen levels of between 4-8 mg L^{-1} in the pond and the values observed during the experimental period fall within this range. Lawson[59] reported that when the

DO level is consistently between 1.5-5 mg L^{-1}, fish will be alive, but feed intake will reduce. The growth rate will also reduce and high Feed Conversion Ratios (FCR) will be recorded. When DO levels are lower than 1.5 mg L^{-1}, fish will be stressed and they will die. The periods of achieving desired weights in fish will be lengthened and ultimate loss on investment will occur. The range in the average temperature recorded during the experimental period was probably because all the treatments were outdoors and faced with environmental factor variations. Anjulo *et al.*[60] reported that for African Catfish, an acceptable temperature range is between 26-32°C. When the water temperature in the ponds consistently stays between 16 and 26°C, feed intake reduces and the fish growth rate also drags tremendously. A farmer will record high FCR and the fish will also be stressed. Prolonged stress can open up the fish to opportunistic infections. When fish are consistently exposed to temperatures below 15°C, fish growth will ultimately stop and death is just around the corner. Low temperature negatively affects the rates at which wastes are converted into water. However, when the water temperature is above 32°C, the resultant effect on the African Catfish is not good at all. This is because oxygen is not readily soluble in very warm water. High temperatures in ponds will stress the fish and eventually lead to death[60]. Anjulo *et al.*[60] reported that pH is the level of the hydrogen ion present in the water. For the fish in the pond, the acceptable pH value is between 6.5-7.5. When it is below 4, fish will die due to water acidity. When pH is constantly between 4-6, fish will be alive, but due to stress, will experience slow growth. Feed intake will be highly staggered and reduced. The FCR will also be very high. In fact, for the observant fish farmer, low pH in pond water is an indication of high CO_2, (carbon dioxide) in the water. High pH values of between 9-11 in pond water will also retard fish growth. Fish will ultimately die when pH levels rise above 11. Low pH aids in higher proportions of ionized ammonia which is less toxic to fish. The reverse is the case with high pH in water.

There is a slight increase in the digestibility coefficient of fishes fed fermented *Canavalia ensiformis* (FCe) at a 25% level than those fed toasted *Canavalia ensiformis* (TCe) diets, which agrees with the report of Fagbenro *et al.*[12] that the likely thermostable antinutrients in the jack bean seeds formed a good proportion of the solubilized and removed nitrogenous compounds, which might be partly responsible for the improvement in the nutritive values of processed jack bean seeds. The high dry matter digestibility by *Clarias gariepinus* may be due to the crude fibre content of the diets. This finding is similar to the report of Ologbobo[61] that dry matter digestibility could be affected by the fibre content of the diet. The reduction in crude protein digestibility may be due to the presence of the anti-nutrients. Tannins have been implicated in reducing protein digestibility[62]. The relatively high apparent digestibility coefficient of energy recorded in this study is similar to the values recorded by scientists[63,64] of the same seed meal fed to *Oreochromis niloticus*. Osuigwe *et al.*[65] and Bhagya *et al.*[66] revealed that cooking improved gross energy digestibility.

The decrease in haemoglobin, haematocrit and RBC observed with increasing dietary fermented *Canavalia* seed meal as compared with the control diet could be a result of the anti-nutritional factors still present in FCe diets at higher inclusion levels which agrees with the report of Gboshe and Ukorebi[67]. Some of these anti-nutritional factors are known to cause some negative effects on some haematological parameters. The Con-A causes agglutination of red blood cells in monogastric[68], while saponins are known to cause erythrocyte haemolysis and reduction of blood[69]. Probably the increasing presence of anti-nutritional factors in increasing dietary *Canavalia* caused the inferior haematological parameters observed in *C. gariepinus* fed such diets. This is in line with the findings of Jimoh *et al.*[70] that nutritional toxicity is associated with anaemia. Adogu *et al.*[71] equally observed that gossypol an anti-nutritional factor found in some legumes severely reduced blood PCV and Hb concentration in rainbow trout. When viewed from the perspective of diet processing type, it was observed that *C. gariepinus* fed the control diet had PCV, RBC count, WBC count and Hb concentration that were higher and significantly ($p<0.001$) different from the values of those fed fermented *Canavalia* diets which were, in turn, higher than those fed toasted *Canavalia* diets. According to Seena *et al.*[72] RBC greater than 1x106/mm^3 is considered high and is indicative of the high oxygen-carrying capacity of the blood which is characteristic of fishes capable of aerial respiration and with high activity. The better performance of *C. gariepinus*-fed fermented *Canavalia* diets to those fed toasted *Canavalia* diets is an indication that fermentation significantly improved the quality of FCe meals[67]. The improvement may be due to among other factors inactivation of the anti-nutritional factors present in *Canavalia* as earlier reported by the works of Megbowon *et al.*[73] and the transformation of some of the component nutrients to non-toxic more readily digestible absorbable forms Gboshe and Ukorebi[67] and Charo-Karisa *et al.*[74] . It is of importance to note that despite the reduction in the levels of haematological values observed, they were still within the normal ranges reported for *C. gariepinus*[75].

The economic evaluation of feeding *Clarias gariepinus* fingerlings with the experimental diets showed that FCe 25% had the highest benefit: Cost ratio. The positive benefit: Cost ratio recorded in all the diets, showed that *Clarias* fingerlings can be economically reared on all diets. However, the result further revealed that the substitution of fermented *Canavalia ensiformis* seed meal for soybean meal lowered the cost of diet production, which is an indication of a more cost-efficient and cheaper non-convectional ingredient relative to the soybean meal by Hassaan *et al.*[34] and Charo-Karisa *et al.*[74]. This is similar to the report that Non-Conventional Feed Resources (NCFRs) are very cheap by-products or wastes from agriculture, farm-made feeds and processing industries[34]. It implies that fermented *Canavalia ensiformis* seed diets at a 25% inclusion level can be fed to *Clarias gariepinus* fingerlings with better growth performance, haematology, digestibility and economic evaluations.

CONCLUSION

Optimal growth and weight gain in this study were obtained at an inclusion level of 25% of fermented *Canavalia ensiformis* meal. It is concluded that fermented *Canavalia ensiformis* meal can be used as a growth-promoting agent in the diet of *Clarias gariepinus* with better haematology and economic evaluation.

SIGNIFICANCE STATEMENT

This study discovered that Fermented *Canavalia ensiformis* diets can be beneficial for *Clarias gariepinus* fingerling's growth. This study will help the researchers to uncover the critical areas of inclusion levels that many researchers were not able to explore. Thus a new theory on *Canavalia ensiformis* may be arrived at.

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