Applied Tropical Agriculture
Volume 22, No. 2, 156-165, 2017.

Bioproductive Effects of *Clarias gariepinus* Fingerlings Fed Guava (*Psidium guajava*) Leaves and Drumstick (*Moringa oleifera*) Leaves Extracts Supplemented Diets

Olusola, S.E.* and Olorunfemi, B.V.

Department of Biological Sciences (Fisheries and Aquaculture Programme), Ondo State University of Science and Technology, Okitipupa, Ondo State
*Corresponding author: belloolus@yahoo.com

ABSTRACT

Feeding trials were conducted in experimental tanks to assess guava (Psidium guajava) leaves (GL) and drumstick (Moringa oleifera) leaves (DL) on nutrient utilization and growth performance of Clarias gariepinus fingerlings. Nine experimental diets composed of control (0%), DL2 (1%), DL3 (2%), DL4 (3%), GL5 (1%), GL6 (2%), GL7 (3%), Oxytetracycline (OXY)8 (15mg/kg) and OXY9 (30mg/kg) were formulated and replicated twice at 40% crude protein. Twenty fish per treatment (mean weight 2.59 ± 0.02) were fed twice daily at 3% body weight for 8 weeks. Mean Weight Gain (MWG), Nitrogen Metabolism (NM) and Specific Growth Rate (SGR) were determined. Temperature, dissolved oxygen and pH were measured using standard methods. Data were analysed using descriptive statistics and ANOVA at p=0.05. Fish fed on GL and DL based diets had higher growth rates than those fed the control diets but C. gariepinus fed OXY 8 had significantly higher mean weight gain (2.44±0.04g), nitrogen metabolism (116.83±0.05) and specific growth rate (0.52±0.01g) followed by DL2 of 2.41±0.63g, 116.37±0.04, 0.51±0.99g respectively than the control. The values for temperature, dissolved oxygen and pH among the treatments were within the range for fish culture in the tropics. Fish fed with guava leaves and drumstick leaves extracts diets had improved mean weight gain, specific growth rate and nitrogen metabolism at 1% inclusion level. The inclusion of guava and drumstick leaves extracts in organic aquaculture would lead to an increase in fish productivity.

Key words: *Clarias gariepinus*, *Psidium guajava*, *Moringa oleifera*, fish growth, nutrient utilisation

INTRODUCTION

Fishing from oceans, lakes and rivers has been a major source of food, a provider of employment and other economic benefits for humanity (FAO, 2010). However, with increased knowledge and dynamic development of Fisheries and Aquaculture, it was realized that living aquatic resources, although renewable, are not infinite and need to be properly managed, if their contribution to the nutritional, economic and social well-being of the growing world's population is to be sustained (FAO, 2010).

World Fisheries have become a vital sector of the food industry. Many countries are taking advantage of this new opportunity by investing in modern fishing fleets and processing factories in response to growing international demand for fish and fishery products (FAO, 2010). Aquaculture, the fastest growing food producing sector in which fish meal is a primary protein source in fish diets, has emerged as important component in food security. In Aquaculture, feeding of culture fish is one of the most important factors that must be considered (Olele *et al.,* 2013). Nutrition plays an important role in the maintenance of health and marketable product. Therefore, uses of functional feed are novel to the aquaculture industry (Bello *et al.,* 2012a). Fish like other animals have a requirement for essential nutrients in order to grow properly (Olele *et al.,* 2013). Fish depend on protein and minerals supplied through feed and pond environment for fast and healthy growth. Feed formulations accounts for more than 50% of the total production costs in modern intensive aquaculture (Bello *et al.,* 2012a). Increasing feed efficiency, especially by improving the metabolic assimilation of dietary nutrients, is of high priority in contemporary animal production (Bello *et al.,* 2012a).

The use of plant immuno-stimulants seems to be attractive alternative to enhance growth and control disease infection. Immuno-stimulants are chemical compound that stimulate the non-specific immune system when given alone or the specific immune mechanism when given with antigen, thereby making the animal more resistant to microbial and parasitic infections (Cuesta *et al.*, 2005). Immuno-stimulants can be grouped under chemical agents, bacterial

preparations, polysaccharides, animals or plants extracts, nutritional factors and cytokines. Immuno-stimulants are more widely and successfully applied to improve fish welfare, health and production, it facilitates function of phagocytic cells, increase their bactericidal activities and stimulate natural killer cells, complement system, lysozyme activity and antibody response in fish and shellfish which confer enhanced protection from infectious diseases (Bello *et al.,* 2012b).

Psidium guajava and *Moringa oleifera* leaves as plant immuno-stimulants can be used as growth promoters and for health management, but information on mechanism of action of these plants in fish farming is not adequately documented. Hence, this study therefore aimed at investigating the bioproductive effects of *C. gariepinus* fed with *P. guajava* and *M. oleifera* leaves extracts.

MATERIALS AND METHODS

Experimental System

The experiment was carried using eighteen (18) plastic experimental bowls for eight (8) weeks in the Fisheries and Aquaculture Laboratory of Ondo State University of Science and Technology (OSUSTECH), Okitipupa. The water level in each bowl was maintained at volume of thirty-five (35) litres throughout the experimental period. Water in each bowl was replaced every three (3) days throughout the period of the experiment to maintain relatively uniform physiochemical parameters and also to prevent fouling that may result from food residues. The source of water was from OSUSTECH water station (borehole). The water temperature of the experimental bowls was measured using mercury-in-glass thermometer while the pH and dissolved oxygen values were measured using pH metre (Jenway 3015 pH meter, 0.01 accuracy) after standardizing the metre.

Experimental Procedures and Feeding Trials

Each treatment had two replicates, with each replicate containing 20 fish with mean initial body weight of 2.59±0.01g, which were selected from 400 uniform-sized fingerlings fish. The fish were acclimated for seven (7) days in bowl before the experiment. The experiment lasted for eight (8) weeks during which the fish was fed at 3% body weight daily. The diet per day was divided into two: 1.5% given in the morning between 8.00 - 9.00 and 1.5% given in the evening by 5pm. Measurement of the weight changes was performed fortnightly and the feeding rate adjusted fortnightly according to the new body weight.

Plant Collection, Preparation and Extraction of Plant Material

Plant collection

The *P. guajava* and *M. oleifera* leaves used in the study was obtained in Teaching and Research Farm, Ondo State University of Science and Technology, Okitipupa and was identified by an expert in the Department of Biological Sciences, Ondo State University of Science and Technology, Okitipupa.

Drum stick (*Moringa oleifera*) leaves extraction

The extraction was carried out using the method of Ajaiyeoba and Fadare (2006). The air-dried *M. oleifera* leaves were ground with a hammer mill and 200g of fine powder of *M. oleifera* were soaked in 1000ml of 95% ethanol for 48 hours. The *M. oleifera* were properly mixed with ethanol, filtered using sterile muslin cloth after which the extract was obtained, air-dried and stored at 25^0C until required.

Guava (*Psidium guajava*) leaves extraction

The extraction was carried out using the method of Ajaiyeoba and Fadare (2006). The air-dried guava leaves were ground with a hammer mill and 200g of fine guava letaves powder were soaked in 1000ml of 80% methanol for 48 hours. The guava leaves powder was properly mixed with methanol and filtered using sterile muslin cloth after which the extract was obtained, air-dried and stored at 25^0C until required.

PREPARATION OF EXPERIMENTAL DIETS

Nine (9) experimental diets were prepared by incorporating *M. oleifera* leaves and *P. guajava* leaves extracts at the different inclusion levels: Control (0%), DL2 (1%) DL3 (2%), DL4 (3%), GL5 (1%), GL6 (2%), GL7 (3%), OXY8 (15mg/kg) and OXY9 (30mg/kg). Feed ingredients such as fishmeal, soybean, yellow maize, starch, wheat bran, vitamin-mineral premix, bone meal and cod liver oil were purchased from a popular feed mill in Osogbo, Osun State.

Diet Formulation

After preparation, feed ingredients were mixed together to formulate 40% crude protein diet (Table 1). Each diet mixture that was treated separately was extruded through ¼ mm die mincer of Hobalt A-200T pelleting machine to form a noodle like strand which was mechanically broken into suitable size for the *C. gariepinus* juveniles. The pelleted diets were sun dried, packed in labelled polythene bags and stored in a cool and dry place.

Biological Evaluation

The following computations were carried out as described by Olaifa and Bello (2011):

Weight gain = $Final\ body\ weight - Initial\ body$

Weight gain (%) = $\frac{Final\ body\ weight - Initial\ body\ weight}{Initial\ body\ weight} x\ 100$…… (1)

Increase in standard length (CM) = $L_2 - L_1$ ….(2)
Where L_2 = Final standard length, L_1 = Initial standard length

Specific growth rate (SGR) = $\frac{(Loge\ final\ body\ weight - Loge\ initial\ body\ weight)}{Time\ (days)} x\ 100$…(3)

Feed conversion ratio (FCR) = $\frac{Dry\ weight\ of\ feed\ fed\ (g)}{Fish\ weight\ gain\ (g)}$...(4)

Protein efficiency ratio (PER) = $\frac{Body\ weight\ gain\ (g)}{Crude\ protein\ fed}$….(5)

Protein productive value (PPV) = $\frac{(Final\ fish\ protein - Initial\ body\ protein)}{Crude\ protein\ intake} x\ 100$

Survival rate (%) = $\frac{Initial\ number\ of\ fish\ stocked - Mortality}{Initial\ number\ of\ fish\ stocked} x\ 100$….. (6)

Condition factor (k) = 100 W/L^3 ……..(7)
Where W = Weight of fish (g), L = Standard length (cm)

Protein intake = $\frac{Feed\ intake\ x\ percentage\ protein\ in\ diet}{100}$ … (8)
100

Nitrogen metabolism = $\frac{(0.549)(a+b)h}{2}$
Where, a = initial mean weight of fish, b = final mean weight of fish, h = experimental periods in days (Nwanna, 2003).

Phytochemical Analyses

Detection of Saponins

1. **FROTH TEST**: The extract of guava and drumstick leaves were diluted separately with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.
2. **FOAM TEST**: Extracts of guava and drumstick leaves of 0.5 g was shaken with 2 ml of water. If foam produced persists for ten minutes, it indicates the presence of saponins.

Detection of Phenols

FERRIC CHLORIDE TEST: Extracts of guava and drumstick leaves were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of Tannins

Extracts of guava and drumstick leaves of 0.1 g was taken up in 10 ml distilled water, and filtered. Then a few drops of ferric chloride ($FeCl_2$) reagent were added to 1 ml of the filtrate. The mixture was observed for the formation of blue, blue-black, green or green-black colouration or precipitate.

Detection of Flavonoids

ALKALINE REAGENT TEST: Extracts of guava and drumstick leaves were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Table 1: Ingredients composition of the experiment diets (g/100g diet)

Ingredients/Treatments	Control 0%	DL (-1%)	DL (-2%)	DL (-3%)	GL (-1%)	GL (-2%)	GL (-3%)	Control (Chloramphenicol)	
								15mg/kg	30mg/kg
Fish meal	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8
Soybean	39.59	39.59	39.59	39.59	39.59	39.59	39.59	39.59	39.39
Yellow Maize	16.31	16.31	16.31	16.31	16.31	16.31	16.31	16.31	16.31
Wheat Bran	16.31	15.31	14.31	13.31	15.31	14.31	13.31	14.1	13.31
Starch	1	1	1	1	1	1	1	1	1
Cod liver oil	3	3	3	3	3	3	3	3	3
Bone meal	2	2	2	2	2	2	2	2	2
*Vit-min premix	2	2	2	2	2	2	2	2	2
Moringa leaves	-	1	2	3	-	-	-	-	-
Guava leaves	-	-	-	-	1	2	3	-	-
Chloramphenicol	-	-	-	-	-	-	-	1.5	3
	100	100	100.00.	100	100	100	100	100	100

*Vit-min premix (vitamin and minerals premix) each 2.5kg of premix contains; vitamin A,12.5 million international unit (MIU); D3, 2.5 MIU; E, 40g; K3, 2g;B1, 3g; B2, 5.5g; B6, 5g; B12, 0.25g; Niacin 55g; Calcium pantothenate 11.5g; Choline chloride, 500g; folic acid, 1g; Biotin, 0.08g; Manganese,120g; Iron,100g; Zinc,80g; Copper, 8.5g ; Iodine, 1.5g ; Cobalt, 0.3g; Selenium, 0.12g ; Anti- oxidant,120g.
DL: Drumstick leaves; GL: Guava leaves

Detection of Glucosinolates

Extracts of guava and drumstick leaves of 0.1 g was dissolved in 5 ml of chloroform followed by filtration as described by Adeoye and Oyedapo (2004). Concentrated tetraoxosulphate (IV) acid (Sulphuric acid) was carefully layered at the bottom of the tube without disturbing the solution. It was observed for the formation of a sharp brown ring at the chloroform/sulphuric acid interface.

Test for Triterpenes and Steroids

THE SALKOWSKI TEST: Extracts of guava and drumstick leaves of 1 g was warmed in 5 ml of chloroform for 30 minutes. The chloroform solution was then treated with a small volume of concentrated tetraoxosulphate (iv) acid (H_2SO_4) and shaken. The red colour produced within a few minutes indicated a positive reaction.

Detection of Proteins and Amino acids

XANTHOPROTEIC TEST: The extracts of guava and drumstick leaves were treated with few drops of concentrated Nitric acid. Formation of yellow colour indicated the presence of proteins.

Investigation of Proximate Composition

Experimental diets and fish carcasses were analyzed for proximate composition before and after the experiment. Six and four fishes were taken before and after the experiment respectively and analyzed for their proximate composition according to the methods of Association of Official Analytical Chemists (AOAC, 2005).

Statistical Analysis

Water quality parameters, growth performance and nutrient utilization indices resulting from the experiment were subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences version 15). Duncan new multiple range test was used to separate means of significant treatment (p=0.05).

RESULTS

Phytochemical Analysis of Drumstick and Guava Leaves

The result showed the presence of tannin, saponins, flavonoids, glucosinolates, phenol, amino acids and polysterols. Phenol and amino acids were present in small quantity in both *M. oleifera* and *P. guajava* leaves extracts. The flavonoids contents were present moderately in both plants. Small quantity of tannins was observed in *P. guajava* and absent in *M. oleifera* while polysterols were absent in both plants (Table 2).

Table 2: Phytochemical analysis of drumstick and guava leaves

Phytochemical	Drumstick leaves	Guava leaves
Tannin	-	+
Saponins	+	+++
Flavonoids	++	++
Glucosinolates	+	-
Phenol	+	+
Amino Acids	+	+
Polysterols	-	-

Key: - = Absent += Small Quantity ++ = Moderate Quantity +++ = Present in Abundant

Proximate Analysis of the Experimental Diets

The proximate compositions of the control diets were lower than the value obtained in other diets treated value with *M. oleifera* and *P. guajava* leaves extracts. The value of proximate composition of experimental diets was better in the treated diets compared to the control diets. The highest crude protein was recorded in diet 4 while the lowest was recorded in diet 5. The highest moisture was recorded in diet 6 and the lowest in diet 4. The highest ether extracts were recorded in diet 8 and the lowest recorded in diet 7. The highest ash content was recorded in diet 5 and the lowest in diet 4 while the highest NFE was recorded in diet 4 and the lowest in diet 5. The values of moisture, crude protein, ether extracts, ash and nitrogen free extracts were significantly different ($p<0.05$) among the treatments (Table 3).

Proximate Composition of fish before and after the experiment

Generally, moisture content was highest in the fish fed treated diets compared to the control after the experiment and the moisture taken before the experiment, there were significant differences ($P< 0.05$) among the treatments before and after the experiment. The crude protein level of the fish increased significantly ($p<0.05$) during the experiment, with the highest value recorded in DL2 compared to the value of control. Higher ether extract contents were observed in control compared to the value obtained in treated groups after the feeding trial. The value recorded before the experiment was higher than the values obtained among all the treatments after the experiment. There was a general decline in the value recorded in the treated groups than the value recorded in the control. There were significant differences ($p<0.05$) among the treatments. The ash content recorded in the treated groups were higher than that of the control with highest value in DL2 and lowest in the control, there were significant differences ($p< 0.05$) between the treatments (Table 4).

Table 3: Proximate composition of the experimental diets

Parameters	Control (0%)	DL (-1%)	DL 3 (-2%)	DL 4 (-3%)	GL (-1%)	GL (-2%)	GL 7 (-3%)	OXY 8 (15mg/kg)	OXY 9 (30mg/kg)
Moisture	8.36±0.02^{d}	8.02±0.03^{c}	8.54±0.01^{e}	7.04±0.03^{a}	8.42±0.06^{d}	8.99±0.03^{h}	8.79±0.03^{g}	8.62±0.03^{f}	7.24±0.04^{b}
Crude Protein	40.06±0.03^{abc}	40.08±0.06^{abc}	40.07±0.00^{abc}	40.12±0.03^{c}	40.02±0.02^{a}	40.09±0.03^{bc}	40.05±0.02^{ab}	40.09±0.00^{bc}	40.10±0.03^{bc}
Ether Extracts	6.12±0.04^{c}	6.24±0.06^{d}	6.16±0.03^{c}	6.32±0.05^{e}	6.42±0.03^{f}	5.77±0.04^{b}	5.61±0.08^{a}	6.46±0.03^{f}	6.23±0.05^{d}
Ash	10.46±0.01^{f}	10.84±0.03^{g}	10.34±0.01^{e}	9.12±0.06^{a}	11.62±0.03^{i}	11.30±0.04^{h}	9.82±0.00^{c}	9.94±0.02^{d}	9.35±0.07^{b}
NFE	35.00±0.05^{e}	34.82±0.05^{c}	34.89±0.02^{d}	37.40±0.09^{h}	33.52±0.01^{a}	33.85±0.06^{b}	35.73±0.07^{f}	34.89±0.04^{d}	37.08±0.07^{g}

Key: The above values are means of duplicate data, mean values in each row with similar superscripts are not significantly different ($p > 0.05$)
NOTE: GL: Guava Leaves, DL: Drumstick Leaves, NFE: Nitrogen Free Extract (100 – summation of crude protein, moisture, ash content and ether extract)
OXY: Oxy-tetracycline treatment

Table 4: Proximate Composition of fish before and after the experiment

Parameters	Before	Control (0%)	DL (-1%)	DL 3 (-2%)	DL 4 (-3%)	GL (-1%)	GL (-2%)	GL 7 (-3%)	OXY 8 (15mg/kg)	OXY 9 (30mg/kg)
Moisture	5.84±0.02^{bc}	5.92±0.01^{d}	5.67±0.03^{a}	5.78±0.04^{b}	5.69±0.00^{a}	5.85±0.03^{c}	5.98±0.01^{def}	6.00±0.10^{ef}	6.02±0.50^{f}	5.95±0.10^{de}
Crude Protein	58.78±0.01^{a}	63.75±0.09^{b}	68.71±0.08^{h}	66.85±0.15^{d}	66.81±0.18^{d}	67.15±0.08^{e}	67.89±0.03^{g}	67.58±0.10^{f}	65.98±0.14^{c}	67.58±0.31^{f}
Ether Extract	8.75±0.06^{f}	8.10±0.03^{e}	7.89±0.03^{cd}	7.95±0.04^{d}	7.69±0.01^{b}	7.89±0.04^{cd}	7.84±0.10^{c}	7.92±0.20^{d}	7.58±0.10^{a}	7.58±0.01^{a}
Ash	9.10±0.02^{a}	10.09±0.15^{b}	11.83±0.05^{i}	11.53±0.09^{h}	10.95±0.11^{d}	11.03±0.06^{e}	11.10±0.14^{f}	10.85±0.13^{c}	11.32±0.05^{g}	11.08±0.08^{ef}
NFE	17.53±0.11^{j}	12.14±0.14^{i}	5.90±0.09^{a}	7.89±0.16^{e}	8.86±0.18^{g}	8.08±0.12^{f}	7.19±0.12^{b}	7.65±0.25^{c}	9.10±0.35^{h}	7.81±0.25^{d}

Key: The above values are means of duplicate data, mean values in each row with similar superscripts are not significantly different ($p > 0.05$)

Table 5: Mean water quality parameters of the drumstick leaves, guava leaves and oxytetracycline treatment of the experimental bowls

Treatment	Parameters	Week 2	Week 4	Week 6	Week 8
Control	Temperature	29.00±0.00^{a}	30.00±0.00^{b}	29.00±0.00^{a}	29.50±0.50^{a}
	pH	5.97±0.01^{a}	6.03±0.01^{a}	5.70±0.20^{a}	5.95±0.45^{a}
	Dissolved oxygen	6.37±0.01^{a}	6.62±0.03^{b}	6.38±0.05^{a}	6.63±0.02^{b}
DL 2	Temperature	30.00±0.00^{a}	29.5±0.50^{a}	29.55±0.50^{ab}	29.00±0.10^{ab}
	pH	5.84±0.04^{a}	6.01±0.03^{a}	5.75±0.20^{a}	5.80±0.00^{a}
	Dissolved oxygen	6.57±0.01^{a}	6.75±0.02^{b}	6.81±0.04^{b}	6.82±0.04^{b}
DL 3	Temperature	29.50±0.50^{a}	30.00±0.00^{a}	29.50±0.50^{a}	30.00±0.00^{a}
	pH	5.84±0.02^{a}	6.01±0.01^{a}	6.04±0.02^{a}	6.00±0.00^{a}
	Dissolved oxygen	6.63±0.02^{b}	6.88±0.01^{a}	6.82±0.05^{a}	6.87±0.05^{a}
DL 4	Temperature	29.50±0.50^{a}	29.50±0.50^{a}	30.00±0.00^{a}	29.00±0.00^{a}
	pH	5.84±0.02^{a}	6.19±0.11^{b}	5.70±0.20^{a}	5.70±0.02^{a}
	Dissolved oxygen	6.77±0.05^{b}	6.79±0.01^{ab}	6.85±0.50^{a}	6.91±0.02^{a}
GL 5	Temperature	29.50±0.50^{a}	29.50±0.50^{a}	29.00±0.00^{ab}	28.50±0.50^{ab}
	pH	5.87±0.05^{a}	6.07±0.02^{a}	6.03±0.01^{a}	5.85±0.05^{a}
	Dissolved oxygen	6.62±0.01^{b}	6.82±0.03^{a}	6.84±0.01^{a}	6.65±0.02^{b}
GL 6	Temperature	29.50±0.50^{a}	29.50±0.50^{a}	30.00±0.00^{b}	28.00±0.00 ^{a}
	pH	5.89±0.08^{a}	6.03±0.02^{a}	6.03±0.03^{a}	5.85±0.35^{a}
	Dissolved oxygen	6.45±0.01^{a}	6.50±0.03^{a}	6.60±0.05^{a}	6.55±0.02^{a}
GL 7	Temperature	29.50±0.50^{a}	29.50±0.50^{a}	28.50±0.50^{a}	30.00±0.00^{c}
	pH	5.87±0.09^{a}	6.01±0.01^{a}	6.03±0.00^{a}	6.05±0.05^{a}
	Dissolved oxygen	6.63±0.07^{a}	6.72±0.02^{a}	6.68±0.02^{a}	6.69±0.04^{a}
OXY 8	Temperature	30.00±0.00^{a}	29.50±0.50^{a}	29.58±0.50^{ab}	29.00±0.00^{abc}
	pH	5.91±0.09^{a}	6.01±0.03^{a}	6.01±0.47^{a}	5.85±0.05^{a}
	Dissolved oxygen	6.88±0.02^{ab}	6.91±0.04^{a}	7.08±0.01^{a}	7.07±0.02^{a}
OXY 9	Temperature	30.00±0.00^{a}	29.50±0.50^{a}	28.50±0.50^{a}	29.00±0.00^{abc}
	pH	5.86±0.04^{a}	6.02±0.02^{a}	6.01±0.01^{a}	6.00±0.40^{a}
	Dissolved oxygen	6.75±0.01^{b}	6.78±0.02^{b}	6.82±0.00^{b}	7.08±0.05^{a}

Key: The mean values in each row with similar superscripts are not significantly different ($p > 0.05$)

Water Quality Parameters

The water quality parameters (temperature, pH and dissolved oxygen) of the experimental tank, temperature and pH were closely related. The highest temperature was recorded in diet 2, diet 3, diet 4 and diet 8 and while lowest record was found in diet 5. The highest pH was recorded in diet 7 and the lowest in the control diet (Table 5).

Growth Performance and Nutrient Utilisation of *Clarias gariepinus* fed Treated Diets

The growth performances and feed utilization in terms of body weight gain, feed conversion ratio, protein efficiency ratio, specific growth rate and Nitrogen metabolism was better in the treated groups and were significant differences ($p<0.05$) among the treatments than the control as presented in Table 6.

Table 6: Growth performances and nutrients utilization of *C. gariepinus* fed treated diets for 8 weeks

Parameters	Control	DL 2	DL 3	DL 4	GL 5	GL 6	GL 7	OXY 8	OXY 9
Initial body weights (g)	2.59±0.01^{a}	2.58±0.00^{a}	2.59±0.00^{a}	2.59±0.01^{a}	2.59±0.00^{a}	2.59±0.01^{a}	2.59±0.01^{a}	2.58±0.02^{a}	2.58±0.01^{a}
Final body weights (g)	4.34±2.08^{a}	4.99±1.28^{b}	4.82±1.03^{b}	4.42±0.76^{a}	4.44±0.16^{a}	4.32±1.41^{a}	4.78±1.70^{b}	5.02±0.05^{bc}	4.85±0.80^{b}
Body weight gain (g)	1.75±1.04^{ab}	2.41±0.63^{b}	2.23±0.52^{ab}	1.84±0.38^{ab}	1.85±0.08^{ab}	1.73±0.70^{ab}	2.19±1.71^{ab}	2.44±0.04^{ab}	2.27±0.79^{ab}
Body weight gain (%)	67.57±1.04^{a}	93.41±0.84^{abc}	86.10.±0.06^{ab}	71.04±0.39^{a}	71.43±0.04^{a}	66.80±0.76^{a}	84.56±0.82^{ab}	94.57±0.04^{abc}	87.98±0.45^{ab}
Food conversion ratio	0.57±0.34^{a}	0.28±0.08^{a}	0.55±0.23^{a}	0.47±0.07^{a}	0.63±0.06^{a}	0.46±0.19^{a}	0.38±0.15^{a}	0.81±0.04^{a}	1.71±0.11^{b}
Protein efficiency ratio	0.03±0.01^{a}	0.04±0.00^{a}	0.04±0.01^{a}	0.03±0.01^{a}	0.03±0.00^{a}	0.03±0.01^{a}	0.04±0.01^{a}	0.04±0.00^{a}	0.04±0.01^{a}
Protein intake (g)	0.26±0.00^{a}	0.25±0.02^{a}	0.22±0.01^{a}	0.24±0.01^{a}	0.21±0.00^{a}	0.26±0.02^{a}	0.28±0.04^{a}	0.24±0.03^{a}	0.28±0.03^{a}
Nitrogen metabolism	106.53±16.06^{ab}	116.37±4.31^{b}	113.91±1.25^{b}	107.76±5.90^{ab}	108.07±1.20^{ab}	106.22±10.85^{ab}	113.29±13.00^{b}	116.83±0.25^{b}	114.21±2.20^{b}
Specific growth rate	0.40±0.01^{b}	0.51±0.09^{ab}	0.48±0.02^{ab}	0.41±0.08^{b}	0.42±0.02^{b}	0.40±0.00^{b}	0.48±0.01^{ab}	0.52±0.01^{ab}	0.49±0.02^{ab}
Condition factor									
A. Initial	0.22±0.02	0.30±0.04	0.16±0.04	0.23±0.02	0.11±0.00	0.22±0.05	0.28±0.03	0.09±0.03	0.05±0.02
B. final	0.72±0.01	0.99±0.02	0.50±0.03	0.75±0.02	0.35±0.00	0.71±0.05	0.90±0.02	0.31±0.01	0.17±0.00
C. Difference	0.50±0.01^{ab}	0.69±0.02^{b}	0.34±0.01^{a}	0.52±0.00^{ab}	0.24±0.01^{a}	0.49±0.00^{ab}	0.62±0.01^{b}	0.22±0.02^{a}	0.12±0.02^{a}

Key: The above values are means of duplicate data, mean values in each row with similar superscripts are not significantly different ($p > 0.05$)

DISCUSSION

The phytochemical screening of drumstick and guava leaves obtained in this work was similar to the results obtained by Akinyeye *et al.* (2014) and Kumari, (2013) who reported the presence of phytochemical (e.g. tannins, saponins, alkaloid and phenols) in *M. oleifera* and *P. guajava* leaves Also, the report of Azubuogu (2012) confirmed the presence of these phytochemical.

The results of the experimental diets revealed that the crude protein values recorded in the present study were in line with the values (40.20% and 40.04%) reported by Bello *et al*., (2012a) and Degnani *et al*. (1989), who opined that the protein required for the growth of *C. gariepinus* fingerlings was about 40%. The proximate composition of the experimental diets used in this study support the growth of *C. gariepinus* juvenile as reported by Bello *et al.* (2012a) and Okechi, 2004 that for maximum growth, fry, fingerlings and juveniles must have a diet in which nearly half of the digestible ingredients consist of balanced proteins.

The proximate composition of *C. gariepinus* juveniles before and after experiments is presented in Table 4. The crude protein level of the fish increased significantly ($p<0.05$) during the experiment with the highest value recorded in treated group, DL 2 (68.71 ± 0.08%) compared to the value 63.75 ± 0.09% of control and 58.78 ± 0.01% of fish before the experiment. The reason being that fish fed *M. oleifera* and *P. guajava* leaves extracts - based diets showed increased growth response and high protein deposition compared to control and the value recorded before experiment might be that 'free' amino acid was better utilized or growth promoting constituents present in *M. oleifera* and *P. guajava* leaves and amino acid profile in the combine ingredients might have formed a better balanced diet for the juvenile catfish, *Clarias gariepinus* or enhancement of hormones and repatriating agents that alter the physiology and bio- metabolites in the fish. The plant phytochemical such as tannin, flavonoids, and saponins were beneficial in promoting growth and to stimulate the immune response in fish (Jadhav *et al*., 2006; Kumar *et al*., 2007).

The results indicated that the diets supported the growth of fish as increased body protein levels were recorded in all the treatments. This also showed that the protein requirement for the African catfish was met for body maintenance and growth. The reason for this might be as a result of presence of growth promoting constituents in *M. oleifera* and *P. guajava* leaves. The higher body protein deposition and increased weight gain is indicative of the adequacy of the protein content and higher protein intake. This result agrees with the findings of Fagbenro *et al*. (1992) of higher body deposition and weight gain at 40% crude protein for *Heterobranchus bidosalis* fingerlings fed compound diets.

The ash content recorded was significantly increased in all the diet treatments compared to the control. There was significant difference ($p<0.05$) between the treated groups and control. This result agrees with the report of Dada *et al*. (2001) who reported that the ash content of fingerlings after 84 days of feeding were significant ($P< 0.05$) higher than the initial ash content.

The temperature, dissolved oxygen and pH measured during this study were within recommended limits for warm water fishes (Boyd, 1981; Bello *et al*., 2012a). Hogendoorn *et al*. (1983) reported that the optimum temperature for the growth of small *C. gariepinus* (0.5 – 5g) is 30^0C while it is 25^0C for large (25g) ones, the result of temperature obtained during the study is within this range. This result is in agreement with the report of Okechi (2004) who reported 25 – 30^0C as optimum temperature for culture fish. The *M. oleifera* and *P. guajava* leaves can be used in aquaculture as they did not alter the water quality.

The result of the experiment showed that the drumstick and guava leaves extracts – based diets increased the body weight of fish compared with the control diet. This increase in the body weight of fish fed on drumstick and guava leaves extracts supplemented diets could be attributed to the improved digestive activity by enhancing the synthesis of vitamins, cofactors, enzymatic activity and hormones in the fish. The highest growth performance was observed in fish fed on 15mg/kg Oxy-tetracycline (5.02±0.05g) followed by DL 2 (4.99±1.25g).

The fish fed supplemented diets had better weight gain than those fed control diet, which could be as result of the presence of growth stimulants or constituents in the *M. oleifera* and *P. guajava* leaves as reported by Akinyeye *et al.* (2014) and Kumari (2013). These phytochemical properties could contribute to improving the digestion and nutrient absorption with a subsequent increase in the fish-weight. This result is in agreement with the report of Bello *et al.* (2012a) who obtained high growth performance in *C. gariepinus* with 1.5% walnut leaves and onion bulb extracts as well as that of Shalaby *et al.* (2006), who obtained the highest growth performance in *O. niloticus* with 30g/kg garlic diet.

The highest specific growth rate value of 0.52±0.01 was recorded in OXY 8 diet, followed by DL 2 (0.51±0.09), both of which had better growth rate than the control (0.40±0.01) diet, although there were no significant differences ($p>0.05$) between the treatments. This result agrees with the work of Shalaby *et al*. (2006) who reported better growth rate in treated groups than the control. Feed conversion ratio (FCR) is used to assess feed utilization and absorption. FCR was highest in DL 2 (0.28±0.08) and the lowest (1.71±0.11) in OX9. The result obtained showed drumstick and guava leaves extracts supplemented diets were better utilized by the *C. gariepinus* than the control diets. There were no significant differences in feed conversion ratio ($p>0.05$) between the treatments, excepts OXY9 that was significantly higher.

The result of the experiment showed that the group of fish fed DL 2, DL 3, GL 7, OXY 8 AND OXY 9 diets recorded the highest value of protein efficiency ratio while the highest nitrogen metabolism of 116.83±0.25g was recorded in OXY 8 . The best growth performance was observed in OXY 8 (15 mg/kg diet) followed by DL 2 (1.0%) of drumstick leaves extracts while the lowest was recorded in the control. Feed Efficiency Ratio (FER) and Protein Efficiency Ratio (PER) are used as quality indicators for fish diet and amino acid balance. These parameters are used to assess protein utilization and turnover. These results are also in agreement with those obtained by Shalaby *et al.* (2006) who recorded increase in FCR, FER and PER on *O. niloticus* with 30g garlic/kg diet compared to the control which had the lowest value. The robustness and general well – being of the fish fed different graded levels of *M. oleifera* and *P. guajava* leaves extracts – based diets as expressed by the condition factor (k) was best in DL 2 (1.0% inclusion) with a gain of 0.69 ± 0.02 from the initial body status while the lowest gain of robustness (0.12 ±0.02) was recorded in OXY 9 (30 mg/kg diet). There were no significant differences in the condition factors ($p>0.05$) between the treatments.

CONCLUSION

Diets with *M. oleifera* and *P. guajava* leaves extracts had nutritional properties that enhance growth. These leaves are found in abundance, and thus can be obtained at little or no cost. This makes them relatively cheap natural nutritional products that can be used in aquaculture industry as feed supplement to enhance productivity. The use of *M. oleifera* and *P. guajava* leaves are safe because they are biodegradable and has little or no side effect as the excess will serve as food and nourishment in the body unlike the synthetic or chemical supplement used in fish feed. Therefore, inclusion of 1% *M. oleifera* in the diet of fish will affect the growth and productivity in fish farming.

REFERENCES

Adeoye, B. A. and Oyedapo, O. O. (2004): Toxicity of erythrophleum stem-bark: role of alkaloids fraction. *African Journal of Traditional Complementary and Alternative Medicine* (CAM) 1:45 – 54

Ajaiyeoba, E. O. and Fadare, D. A. (2006): Antimicrobial potential of extracts and fractions of the African walnut (*Tetracarpidium conophorum*). *African Journal of Biotechnology*, 5(22): 2322-2325.

Akinyeye, A. J., Solanke, E. O. and Adebiyi, I. O. (2014): Phytochemical and antimicrobial evaluation of leaf and seed of *Moringa oleifera* extracts, *International Journal of Research in Medical and Health Sciences*, 4 (6): 1-10.

Association of Official Analytical Chemist, AOAC (2005): Official methods of analysis (W. Horwit Zeachtor) 18th edition A.O.A.C Washington, DC: 129-146

Azubuogu, C. U. (2012). Phytochemical analysis on *Moringa oleifera* and *Azadrichta indica* leaves, unpublished dissertation, Caritas University Enugu Nigeria, Pp 21- 31.

Bello, O. S., Olaifa, F. E., Emikpe, B. O. and Ogunbanwo, S. T. (2012a): Effect of Walnut (*Tetracarpidium conophorum*) leaf and Onion (*Allium cepa*) Bulb residues on the tissue bacteriological changes of *Clarias gariepinus* juveniles, *Bulletin of Animal Heath and Production in African,* 60 (2): 205-212.

Bello, O. S., Olaifa, F. E, and Emikpe, B. O. (2012b): The Effect of Walnut (*Tetracarpidium conophorum*) leaf and Onion (*Allium cepa*) Bulb residues on growth performance and nutrient utilization of *Clarias gariepinus* juveniles. *Journal of Agricultural Sciences*, 4(12): 205-213.

Boyd, C. B (1981): Water quality management in pond culture, research and development, Agricultural Experimental Station University of Auburn Alabana USA, pp 35 – 45.

Cuesta, A, Rodrı, A, Esteban, M. A and Meseguer, J. (2005): *In vivo* effects of propolis, a honeybee product, on gilthead sea bream innate immune responses, *Fish and Shellfish Immunology,* 18:71–80

Degnani, G., Yigal, B., Levanon, D. (1989): Effects of different dietary protein level and temperature on feed utilization and growth of African catfish *Clarias gariepinus* (Burchell, 1822), *Aquaculture*, 38, 55-74.

Fagbenro, O. A, Balogun, O. M and Anyanwu, C. N. (1992): Optimal dietary protein level for *Heterobranchus bidosalis* fingerlings fed compound diets. *The Israeli journal of Aquaculture*- Bamidgeh, 44(3): 87 – 92

Food and Agriculture Organization (FAO), (2010): FAO Technical Guidelines for Responsible Fisheries, Food and Agriculture Organization of the United Nations, Rome. Pp. 11-68.

Hogendoorn, O.T., Jamssen, J. A., Koops, W. J., Michael, M. A., Van-Tiews, P. H., Van-Hee, J. P. (1983): Growth and Production of the African Catfish *Clarias lazara* In Effects of body weight temperature and feeding level in intensive tank culture, *Aquaculture,* 34: 265-285. http://dx.doi.org/10.1016/0044-8486 (83) 90208-9

Jadhav, V.S, Khan S. I, Girkar M. M and Gitte, M. J. (2006): The role of immunostimulants in fish and shrimp aquaculture. Aquatic animal health,

Aquaculture Asia Magazine, July – September 2006: 24 – 27

Kumar, J. S. S and Anantharaja K. (2007): Herbal health care in aquaculture – the Indian experience. *Aquaculture*, INFOFISH International: 12 – 16

Kumari, N., Gautam, S., Ashutosh, C. (2013): *Psidium guajava,* A Fruit or Medicine – An Overview, *The Pharma Innovation – Journal***,** 2 (8), 63 -67.

Nwanna, L. C. (2003): Nutritional value and digestibility of shrimp head waste meal by African catfish, *Clarias gariepinus*, *Pakistan Journal of Nutrition*, 2(6), 339 – 345. http://dx.doi.org/10.3923/pjn.2003.3399.345.

Okechi, J. K (2004): Profitability assessment: A case study of African catfish (*Clarias gariepinus*) farming in the Lake Victoria Basin, Kenya, Kenya Marine and Fisheries Research Institute (KMFRI), Kisumu Research Centre, final project, Pp 1- 56.

Olaifa, F.E and Bello, O. S. (2011): Effect of Differently Processed African Yam Beans (*Sphenostylis stenocarpa Harms)* on Performance of African Catfish (*Clarias gariepinus*) Juveniles. *Israeli Journal of Aquaculture –Bamidgeh* IIC: 63: 595 – 602.

Olele, N. F, Onyema, M. I., and Odiko, A. E. (2013): Growth performance, survival rate and nutrient profile of *Clarias gariepinus* fingerlings fed rations of soybean as alternative protein source*, Academic Journal of Interdisciplinary Studies*, 2(10):193-202.

Shalaby A. M., Khattab Y. A., Abdel Rahman A. M. (2006): Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile Tilapia *(Oreochromis niloticus*). *Journal of Venomous Animal Toxins including Tropical Diseases,* 12 (2): 172-201.

*****