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The Effect of Walnut (*Tetracarpidium conophorum*) Leaf and Onion (*Allium cepa*) Bulb Residues on the Growth Performance and Nutrient Utilization of *Clarias gariepinus* Juveniles

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Abstract

Feeding trial were conducted in experimental tanks (50 x 34 x 27 cm) to assess the growth responses and nutrient utilization of Walnut Leaf (WL) and Onion Bulb (OB) residues in *Clarias gariepinus.* Nine experimental diets: control (0%), OB2 (0.5%), OB3 (1.0%), OB4 (1.5%), OB5 (2.0%), WL6 (0.5%), WL7 (1.0%), WL8 (1.5%) and WL9 (2.0%) were formulated and replicated thrice at 40% crude protein. Fish (mean weight 7.39±0.02 g and length 10.37±1.24 cm) were fed twice daily at 3% body weight for 12 weeks. Mean Weight Gain (MWG), Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) were measured. Temperature, dissolved oxygen and pH were determined using standard methods and economic analysis was investigated. Data were analyzed using descriptive statistics and ANOVA at p=0.05.

Results showed that the Fish on OB and WL residue- based diets had higher growth than those on control diet but *C. gariepinus* fed WL8 had significantly higher MWG, SGR and FCR of 53.81±1.20 g, 1.09±0.11 g and 2.16±0.01 respectively of all treatments. The values of temperature, dissolved oxygen and pH were closely related and were still within the range for fish culture in the tropics. WL8 had higher profit index (1.40) and incidence of cost (0.032) and the least in OB5 (1.18) (0.039) respectively. These suggest that walnut leaf residue at 1.5% inclusion could be a potential, less expensive and promising dietary supplementation than onion bulb residue and control that would positively affect growth and water quality of *Clarias gariepinus* in aquaculture.

Keywords: *Clarias gariepinus*, onion bulb, Walnut leaf, performance, water quality

1. Introduction

Fish is one of the cheapest and promising sources of animal protein; people can easily digest 93.2% and 93.7% of fish protein and fat, respectively (Onusiriuka, 2002). Fish depend on protein and minerals supplied through feed and from the pond environment for fast and healthy growth. Feed formulations accounts for more than 50% of the total production costs in modern intensive aquaculture (Ibrahem et al., 2010). Increasing feed efficiency, especially by improving the metabolic assimilation of dietary nutrients, is of high priority in contemporary animal production (Ibrahem et al., 2010). The concept of functional feed is novel to the aquaculture industry. It represents an emerging new paradigm to develop diets that extend beyond satisfying basic nutritional requirements of the cultured organism (Ibrahem et al., 2010). Artificial feeds supplemented with antibiotics were used to prevent the spread of diseases and improve food conversion ratio (FCR) (Reilly & Käferstein, 1997).

Scientists have researched on growth improvement in aquaculture, which have led to the uses of synthetic antibiotics. Many antibiotics are commonly used in aquaculture to promote growth and health in Carp, Trout and Nile Tilapia (Essa et al., 1995). Recently, due to uncontrolled and repeated use of antibiotics in aquaculture to promote growth for a long time has been incriminated carcinogenic, considering the potential threat on human and animal health, current growth management therefore, looking for less harmful approaches and more environmentally-friendly treatments become of premium importance (Ibrahem et al., 2010).

This problem has led to the use of natural products such as onion (*Allium cepa*) bulb and walnut (*Tetracarpidium conophorum*) leaf that has the same effect as synthetic antibiotics. Onion bulb and walnut leaf as plant immunostimulants can be used as a growth promoter and health management in African catfish, *Clarias gariepinus* in which it could increases body weight gain, feed intake and feed efficiency. Onion bulb and walnut leaf as herbal therapeutics are fully biological products that have almost 90% utilization by culture organism (Kumar & Anantharaja, 2007). It does not cause any damage to the physiological system and organelles of the organism (Kumar & Anantharaja, 2007).

The present study aims to assess the effects of onion (*A. cepa*) bulb and walnut (*T. conophorum*) leaf on the growth performance and nutrient utilization of *C. gariepinus* juveniles.

2. Materials and Methods

2.1 Experimental System

The experiment was carried out in twenty seven plastic experimental tanks (50 x 34 x 27 cm) for 12 weeks in the Fisheries Laboratory of the University of Ibadan, Ibadan. The water level in each tank was maintained at volume of 35litres throughout the experimental period. Water in each tank 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 University of Ibadan (U.I) water station and each experimental tank was well aerated using air stone and aerator pumps (Lawson, 1995). The dissolved oxygen content of the experimental tanks was measured using dissolved oxygen metre (Jenway 3015 pH metre, 0.01 accuracy). The water temperature of the experimental tanks was measured by mercury-in-glass thermometer. While the pH value will be measured by using pH metre (Jenway 3015 pH metre, 0.01 accuracy) after standardizing the metre.

2.2 Experimental Procedure & Feeding Trials

Each treatment have three replicates, 20 fish per replicate with mean initial body weight of 7.39±0.02 g and uniform- sized fish was selected from 700 juveniles. Weighed and distributed in experimental tank. The fish was acclimated for fourteen days in glass aquaria before the experiment. The experiment last for 12 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 by 8.00-9.00 am and 1.5% in the evening by 5.00 pm. Measurement of the weight changes was performed forthnightly and the feeding rate adjusted forthnightly according to the new body weight.

2.3 Preparation and Extraction Of Plant Materials

2.3.1 Onion Extraction

The onions were washed with clean sterile distilled water and allowed to air dry at ambient temperature (25^{o}C) for one hour. The dry outer covering of the onion were manually peeled off, washed and extracted as described by Azu and Onyeagba (2007). 200 g of the fresh onion bulbs were blended into fine powder and soaked in 100ml of 95% ethanol for 24 hrs. The pulp obtained was left in a clean, sterile glass container and shaken vigorously to allow for proper extraction and it was filtered using a sterile muslin cloth after which the extract was obtained, air-dried and stored (4^{o}C) until required.

2.3.2 Walnut Leaves Extraction

The extraction was as described by Ajaiyeoba and Fadare (2006). The air-dried walnut leaves were ground with a hammer mill and 200g of fine powder of walnut leaves were soaked in 100ml of 80% methanol for 72 hours. Walnut leaves were properly mixed with methanol, filtered using a sterile muslin cloth after which the extract was obtained, air-dried and stored at (25^{o}C) until required.

2.4 Diet Formulation

After preparation feed ingredients was mixed together to formulate 40% crude protein diet. Each diet mixture treated separately will be extruded through a 1/4mm die mincer of Hobart A-200T pelleting machine to form a noodle like strand which was mechanically broken into suitable sizes for the *Clarias gariepinus* juveniles. The pelleted diets were sun dried, packed in labeled polythene bags and stored in a cool dry place to prevent mycotoxin formation (Table 1).

Table 1. Gross composition of experimental diets

INGREDIENTS	Control	OB2	OB3	OB4	OB5	WL6	WL7	WL8	WL9
Fishmeal	21.25	21.25	21.25	21.25	21.25	21.25	21.25	21.25	21.25
Soybean	42.49	42.49	42.49	42.49	42.49	42.49	42.49	42.49	42.49
Maize	28.26	28.19	28.13	28.06	27.99	28.19	28.13	28.06	27.99
Vit-min*	2.00	1.50	1.00	0.50	-	1.50	1.00	0.50	-
Starch	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vegetable oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Chromium oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DCP	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Onion	-	0.50	1.00	1.50	2.00	-	-	-	-
Walnut leaves	-	-	-	-	-	0.50	1.00	1.50	2.00
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

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

2.5 Biological Evaluation and Analytical Methods

2.5.1 Biological Evaluation

$$Weight\ gain = final\ body\ weight - initial\ body\ weight$$

$$Weight\ gain\ (\%) = \frac{(final\ body\ weight - initial\ body\ weight)}{Initial\ body\ weight} X100$$

$$Increase\ in\ standard\ length\ (CM) = L_2 - L_1$$

Where: L_2 *= Final standard length;* L_1 *= Initial standard length*

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

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

$$Protein\ efficiency\ ratio\ (PER) = \frac{Wet\ body\ weight\ gain\ (g)}{Crude\ protein\ fed}$$

$$Protein\ productive\ value\ (PPV) = \frac{(Final\ fish\ body\ protein - initial\ body\ protein)}{Crude\ protein\ intake} X100$$

$$Survival\ rate\ (\%) = \frac{Initial\ Number\ of\ Fish\ Stocked - Mortality}{Initial\ number\ of\ fish\ stocked} X100$$

$$Condition\ factor\ (K) = 100\ W/L^3$$

Where: W =Weight of fish (g); L = Standard length (cm)

$$Protein\ intake = \frac{Feed\ intake\ x\ percentage\ protein\ in\ diet}{100}$$

$$Nitrogen\ metabolism = \frac{(0.549)\ (a + b)\ h}{2}$$

Where, a = initial mean weight of fish; b= final mean weight of fish; h = experimental periods in days (Nwanna, 2003).

2.6 Analytical Methods

Experimental diets, fish faeces and fish carcasses was 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 [A.O.A.C] (1990).

2.7 Statistical Analysis

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 2006 version 15.0). Duncan multiple range test was used to compare differences among individual means.

3. Results

3.1 Proximate Composition of Experimental Diets

The result of the proximate composition of the experimental diets was presented on Table 2.

Table 2. Proximate composition of experimental diets (DM)

	Control	OB2	OB3	OB4	OB5	WL6	WL7	WL8	WL9
Moisture	6.80±0.02[a]	6.98±0.02[c]	7.55±0.01[d]	7.78±0.01[e]	6.90±0.03[b]	8.10±0.02[g]	7.98±0.04[f]	7.50±0.06[d]	6.78±0.01[a]
Crude protein	40.08±0.24[ab]	40.08±0.27[ab]	40.10±0.11[b]	40.08±0.09[ab]	40.04±0.18[a]	40.10±0.05[b]	40.18±0.01[c]	40.20±0.08[c]	40.13±0.24[b]
Ether extract	15.38±0.10[c]	16.62±0.01[h]	16.40±0.06[f]	15.10±0.02[b]	14.92±0.00[a]	16.20±0.12[e]	16.00±0.03[d]	15.98±0.05[d]	16.50±0.06[g]
Ash	14.80±0.02[a]	15.10±0.03[b]	15.40±0.01[c]	16.10±0.50[ef]	15.98±0.04d	16.02±0.01[d]	16.08±0.11[e]	16.15±0.08[f]	15.99±0.10[d]
NFE	22.94±0.17[e]	21.22±0.18[bcd]	20.55±0.10[cd]	20.94±0.32[bc]	22.16±0.13[de]	19.58±0.11[a]	19.76±0.09[a]	20.17±0.15[ab]	20.60±0.21[abc]

Key: Mean followed by the same letter is not significantly different ($p > 0.05$).

3.2 Proximate Composition of Experimental Fish (before and after Experiment)

The result of the proximate composition of the experimental fish before and after experiment was presented on Table 3.

Table 3. Proximate composition of the fish before and after the experiment

	BEFORE	Control	OB2	OB 3	OB 4	OB 5	WL 6	WL 7	WL 8	WL 9
Moisture	73.37±0.02	77.22±1.64[i]	75.94±0.90[e]	76.81±0.16[h]	76.41±0.02[f]	75.81±0.24[d]	75.74±0.33[c]	75.57±0.62[b]	76.58±0.59[g]	75.46±0.33[a]
Crude protein	47.46±0.01	70.32±0.04[a]	71.15±0.08[f]	70.77±0.04[e]	70.46±0.05[c]	70.76±0.07[e]	71.14±0.04[f]	70.38±0.06[b]	70.70±0.02[d]	71.25±0.04[g]
Ether extract	2.87±0.02	4.89±0.00[e]	4.95±0.04[e]	3.65±0.02[bc]	3.90±0.11[d]	3.45±0.03[a]	3.67±0.07[bc]	3.62±0.09[b]	3.61±0.00[b]	3.71±0.05[c]
Ash	12.75±0.08	15.50±0.03[a]	18.65±0.02[c]	17.95±0.01[b]	19.20±0.10[f]	18.99±0.06[e]	18.89±0.12[d]	18.65±0.06[c]	19.00±0.15[e]	18.85±0.13[d]
NFE	36.92±0.06	9.29±0.04[i]	5.25±0.07[a]	7.63±0.01[h]	6.44±0.13[d]	6.80±0.08[e]	6.30±0.12[c]	7.35±0.11[g]	6.89±0.09[f]	6.18±0.11[b]

Key: Mean followed by the same letter is not significantly different ($p>0.05$).

3.3 Water Quality Parameters of Experimental Tanks

The mean values of water quality parameters of the experimental tanks were presented on Table 4.

3.4 Growth Performances and Nutrients Utilization of Clarias Gariepinus Fed Onion Bulb and Walnut Leaves

The growth performance and feed utilization in terms of body weight gain, feed conversion ratio, protein efficiency ratio, protein productive value and specific growth rate was presented on Table 5.

3.5 Economic Analysis of the Experimental Diets Fed Clarias Gariepinus for 84 Days

The cost of producing 1kg of fish diet and the cost of producing 1 kg weight of fish at the given rate of growth is presented in Table 6.

Table 4 A. Mean bi-weekly water parameters of the onion treatment of the experimental tanks

Treatments	Parameters	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	Mean
Control	Temp (℃)	25.07±0.05	25.50±0.01	26.70±0.01	26.80±0.05	26.82±0.01	26.87±0.00	26.29±0.79
	DO (mg/l)	6.58±0.01	6.72±0.03	6.60±0.05	6.70±0.02	6.71±0.10	6.76±0.01	6.68±0.07
	PH	7.80±0.01	7.20±0.10	7.93±0.07	7.20±0.01	8.10±0.09	8.20±0.12	7.74±0.44
OB2	Temp (℃)	25.27±0.10	26.17±0.02	26.80±0.12	27.10±0.00	27.50±0.11	27.80±0.02	26.77±0.93
	DO (mg/l)	6.68±0.01	6.80±0.02	6.78±0.04	6.81±0.04	6.75±0.06	6.81±0.01	6.77±0.05
	PH	7.80±0.02	7.50±0.02	7.95±0.08	7.55±0.01	8.10±0.02	8.10±0.01	7.83±0.26
OB3	Temp (℃)	25.33±0.15	26.53±0.05	26.90±0.01	27.70±0.11	27.70±0.02	27.90±0.06	27.01±0.98
	DO (mg/l)	6.75±0.02	6.89±0.01	6.80±0.05	6.96±0.05	6.87±0.02	6.97±0.03	6.87±0.09
	PH	7.84±0.10	7.78±0.10	7.97±0.01	7.90±0.10	8.15±0.01	8.20±0.10	7.97±0.12
OB4	Temp (℃)	25.50±0.00	25.67±0.20	26.97±0.04	27.80±0.01	27.80±0.01	28.01±0.06	26.96±1.12
	DO (mg/l)	6.86±0.05	6.91±0.01	6.90±0.50	7.09±0.02	7.02±0.03	7.05±0.20	6.97±0.09
	PH	7.84±0.01	7.90±0.01	7.95±0.01	7.95±0.03	8.50±0.04	8.55±0.01	8.12±0.32
OB5	Temp (℃)	25.10±0.02	25.60±0.01	26.90±0.00	26.80±0.02	26.89±0.11	26.95±0.05	26.37±0.81
	DO (mg/l)	6.72±0.01	6.79±0.03	6.72±0.01	6.78±0.02	6.82±0.07	6.89±0.06	6.79±0.06
	PH	7.87±0.01	7.39±0.00	7.95±0.08	7.30±0.02	8.16±0.07	8.22±0.05	7.82±0.39

Table 4 B. Mean bi-weekly water parameters of the walnut leaf treatment of the experimental tanks

Treatments	Parameters	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	Mean
Control	Temp (℃)	25.07±0.05	25.50±0.01	26.70±0.01	26.80±0.05	26.82±0.01	26.87±0.00	26.29±0.79
	DO (mg/l)	6.58±0.01	6.72±0.03	6.60±0.05	6.70±0.02	6.71±0.10	6.76±0.01	6.68±0.07
	PH	7.80±0.01	7.20±0.10	7.93±0.07	7.20±0.01	8.10±0.09	8.20±0.12	7.74±0.44
WL6	Temp (℃)	25.17±0.06	26.07±0.04	26.70±0.05	27.02±0.00	27.35±0.01	27.70±0.01	26.67±0.92
	DO (mg/l)	6.60±0.07	6.70±0.02	6.70±0.02	6.71±0.04	6.69±0.01	6.71±0.09	6.69±0.04
	PH	7.75±0.06	7.41±0.04	7.85±0.01	7.43±0.09	8.03±0.02	8.05±0.03	7.75±0.28
WL7	Temp (℃)	26.03±0.05	26.53±0.75	27.20±0.01	27.70±0.01	27.70±0.03	28.90±0.01	27.34±1.00
	DO (mg/l)	6.90±0.02	6.97±0.04	7.10±0.01	7.05±0.02	7.07±0.02	7.27±0.01	7.06±0.13
	PH	7.84±0.01	7.98±0.01	7.99±0.03	7.99±0.08	8.10±0.05	8.20±0.01	8.02±0.12
WL8	Temp (℃)	25.55±0.03	25.70±0.20	27.00±0.02	27.85±0.02	27.83±0.01	28.10±0.03	26.79±1.25
	DO (mg/l)	6.90±0.01	6.95±0.02	6.92±0.00	7.13±0.05	7.07±0.08	7.09±0.10	7.01±0.10
	PH	7.88±0.02	7.94±0.01	7.97±0.04	7.98±0.02	8.53±0.02	8.90±0.05	8.20±0.42
WL9	Temp (℃)	25.10±0.02	25.52±0.01	26.73±0.02	26.84±0.01	26.85±0.05	26.90±0.10	26.32±0.80
	DO (mg/l)	6.62±0.01	6.75±0.05	6.65±0.04	6.75±0.02	6.75±0.07	6.80±0.01	6.72±0.07
	PH	7.82±0.03	7.25±0.03	7.96±0.01	7.25±0.03	8.14±0.01	8.25±0.02	7.78±0.44

Table 5. Growth performances and nutrients utilization of Clarias gariepinus fed the experimental diets for 12 weeks

Parameters	Control	OB2	OB3	OB4	OB5	WL6	WL7	WL8	WL9
Initial body weight(g)	7.39±0.29a	7.41±0.58a	7.38±0.20a	7.39±0.29a	7.39±0.29a	7.41±0.58a	7.39±0.29a	7.40±0.50a	7.39±0.29a
Final body weight (g)	52.83± 2.18a	55.17± 4.68a	55.67± 1.28a	56.70± 4.61a	53.22± 0.04a	53.70± 4.10a	56.80±5.28a	61.21±11.20a	53.08±7.96a
Body weight gain (g)	45.44±2.18a	47.76±4.68ab	48.08±0.92ab	49.31±4.61ab	45.83±0.04a	46.29±4.18ab	49.41±05.24ab	53.81±11.20b	45.69±7.96a
Body weight gain (%)	614.93±0.19a	644.58±0.25a	651.45±0.40a	667.30±0.50a	620.16±0.49a	624.65±0.26a	668.56±0.02a	727.16±1.40a	618.27±0.30a
Food conversion ratio	17.61±0.83a	16.76±1.74a	17.05±0.32a	16.19±1.54a	16.79±0.01a	16.51±1.50a	15.87±4.25a	15.02±2.96a	16.37±3.06a
Protein efficiency ratio	1.13±0.05a	1.19±0.12a	1.20±0.03a	1.23±0.12a	1.14±0.01a	1.15±0.11a	1.23±0.38a	1.34±0.28a	1.14±0.20a
Protein productive value	57.00±0.11a	58.95±0.19e	58.02±0.09d	57.38±0.12b	58.20±0.18d	58.98±0.00e	57.04±0.14a	57.80±0.06c	59.30±0.09f
Protein intake (g)	480.72±0.87ab	478.95±4.55ab	492.83±6.45ab	506.81±0.22b	462.26±0.85ab	457.04±2.54ab	446.66±0.62ab	467.43±1.46ab	440.43±1.42a
Survival rate (%)	100b	100b	100b	100b	90a	90a	90a	100b	90a
Nitrogen metabolism	1218±0.20a	1272.19±0.91ab	1278.72±0.17ab	1307.47±0.19ab	1227.15±0.83a	1238.14±0.39ab	1309.77±0.75ab	1582.01±0.34b	1223.92±0.47a
Specific growth rate	1.02±0.02a	1.04±0.05a	1.05±0.01a	1.05±0.04a	1.02±0.00a	1.02±0.04a	1.04±0.13a	1.09±0.11a	1.02±0.08a
Condition factor:									
A Initial	0.59±0.01	0.60±0.00	0.59±0.01	0.59±0.01	0.59±0.01	0.60±0.00	0.59±0.01	0.60±0.00	0.59±0.00
B Final	3.30±1.29	3.91±0.47	3.05±0.47	2.94±0.44	3.16±0.001	2.59±0.39	2.84±0.61	3.34±0.74	3.18±0.37
C Difference	2.71±0.65a	2.59±0.24a	2.46±0.24a	2.35±0.23a	2.57±0.11a	1.99±0.19a	2.25±0.32a	2.74±0.37a	2.59±0.18a

Key: Mean followed by the same letter is not significantly different (p > 0.05).

Table 6. Economic analysis of differently graded level of onion bulb and walnut leaves fed Clarias gariepinus juveniles

INGREDIENTS	Control	OB2	OB3	OB4	OB5	WL6	WL7	WL8	WL9
Fishmeal	95.63	95.63	95.63	95.63	95.63	95.63	95.63	95.63	95.63
Soybean	30.59	30.59	30.59	30.59	30.59	30.59	30.59	30.59	30.59
Maize	25.43	25.43	25.43	25.43	25.43	25.43	25.43	25.43	25.43
Vitamin-minerals premix	9.00	6.75	4.50	2.25	-	6.75	4.50	2.25	-
Starch	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vegetable oil	4.32	4.32	4.32	4.32	4.32	4.32	4.32	4.32	4.32
Chromium oxide	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
DCP	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Salt	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Onion	-	3.90	7.80	11.70	15.60	-	-	-	-
Walnut leaves	-	-	-	-	-	2.30	4.60	6.90	9.20
Total amount (cost/kg feed) (₦)	174.17	175.82	177.47	179.12	180.77	174.22	174.27	174.32	174.37
Cost/kg flesh gain (₦)	211.32	220.68	222.68	226.80	212.88	214.80	227.20	244.80	212.32
Profit index	1.21	1.26	1.24	1.27	1.18	1.23	1.30	1.40	1.22
Incidence of cost	0.038	0.037	0.037	0.036	0.039	0.038	0.035	0.032	0.038

*cost of feedstuffs at the prevailing markets prices in Nigeria (March, 2010): 1 US $ = N 140.00.

4. Discussion

In this study, experimental diets were formulated with differently graded levels of walnut leaf and onion bulb residues for *C. gariepinus* juveniles and the following values were recorded, for moisture the highest value were recorded in diet 6 while the least in diet 9, the highest value of crude protein was recorded in diet 8 and least in diet4. The values of crude protein recorded in all treatments of this study were aligned with report of Degnani et al. (1989) that gross protein requirement for *C. gariepinus* juveniles were 40%, 38-42% crude protein respectively for warm water fish. The value of ether extract recorded was highest in diet 2 and least in diet 5. The highest ash content was recorded in diet 8 and the least in control while the highest nitrogen free extract (NFE) was recorded in control and the least in diet 6. The proximate composition of the experimental diets of this study supports the growth of *C. gariepinus* juveniles and it's agree with the report of Eyo (1995) that for grow at maximum rate, fry and juveniles must have a diet in which nearly half of the digestible ingredients consist of balance protein.

The result of proximate composition of fish *Clarias gariepinus* juveniles before and after experiments fed on experimental diets was presented in table 3. The fish fed diet WL 9 had the highest numerical value and the least in control. Also, the values obtained after the experiment showed that the treated groups were significant difference (P<0.05) than the control There were differences in the crude protein content of the fish composition between the initial and the end of the experimental period. The crude protein level of the fish increased significantly with highest value recorded in treated group, WL 9 (71.25 ± 0.04) compared to the value 70.32 ± 0.04 of control and 47.46 ± 0.01 recorded before the experiment and there were significant difference (P<0.05) among the treatments. The reason being that fish fed onion bulb and walnut leaf- based diets showed a significant (P<0.05) growth response and high protein level compared to control and the value recorded before experiment might be that 'free' amino acid was better utilized or growth stimulant constituents present in onion bulb and walnut leaf and amino acid profile in the combine ingredients might have formed a better balanced diet for the juvenile catfish, *Clarias gariepinus*.

The result indicated that the diets supported the growth of fish as increased body protein level was recorded. 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 walnut leaf and onion bulb. The higher body protein deposition and increased weight gain is an indicative of the adequacy of the protein content and higher protein intake. This result agree with the findings of Fagbenro et al. (1992) that reported higher body deposition and weight gain at 40% crude protein for *Heterobranchus bidosalis* fingerlings fed compound diets. Also, the result of the study support the report of Dada et al. (2001) who reported higher crude protein content after 84 days of feeding at different dietary levels than crude protein before feeding.

The water quality parameters of the experimental tanks, temperature, dissolved oxygen and pH were closely related. The highest temperature was recorded in WL7 and WL8 while the least value was recorded in control. The water quality parameters; temperature, dissolved oxygen and pH measured during the experiment (Table 4.) were within recommended limits for warm water fishes (Boyd, 1981). Also, Hogendoorn et al. (1983) reported that the optimum temperature for the growth of small *C. gariepinus* between (0.5-5 g) was 30℃ and for large (25 g) was 25℃. The result obtained during the experiment was still within the range. From the result obtained walnut leaf and onion bulb residues could be used in aquaculture as they did not alter the water quality.

The initial mean body weight and initial length of *C. gariepinus* are 7.39±0.02 g and 10.37±1.24 cm respectively. At the end of the experiment there was general increase in weight gain (Figure 1). From the results of this study on growth parameters, the highest growth performance was observed in fish fed on 1.5% walnut leaves (61.21±11.20 g) and 1.5% onion bulb (56.70±04.61 g). The treated groups had a better growth than the control diet. There were no significant different (P<0.05) in the final weight gain among the fish fed on diet 0.5%, 1.0%, 1.5% onion bulb and 0.5%, 1.5%, 2.0% for those fed on walnut leaves. The higher values obtained in the treated groups could be due to the presence of growth stimulants or constituents in onion bulb (flavonoids and thiosulfinates) and walnut leaves (alkaloids and tannins). These properties could contribute to improving the digestion and nutrient absorption with a subsequent increase in the fish-weight. This result agree with those obtained by Shalaby et al. (2006) who obtained highest growth performance in *Oreochromis niloticus* with 30 g garlic /kg diet, also Diab et al. (2002) obtained the highest growth performance in *O. niloticus* with 2.5% garlic /kg diet. Abou-Zeid (2002) showed that Allium sativum supplementation positively affected *O. niloticus* biomass and specific growth rate (SGR).

Specific growth rate and condition factor are a reflector of the health status in fish (Ibrahem et al., 2010). Specific growth rate (SGR) from the results revealed that WL 8 (1.09±0.11 g) had better growth rate compared to the control, although they there were no significant differences among the treatments (p>0.05). This results agree with Azza and Abd-El-Rhman (2009) who found that the specific growth rate was not significantly affected by the dietary intake of propolis at 0.1 and 10 g propolis kg^{-1} diet.

Feed Conversion Ratio (FCR) is used to assess feed utilization and absorption (conversion of feed to flesh). FCR was best (2.16±0.02) with WL 8 (1.5% inclusion) and least recorded in control (2.64±0.03), there were no significant difference (P>0.05) among the treatments. The result revealed that diet containing walnut leaves at 1.5% inclusion (WL 8) was better utilized by the *C. gariepinus* juveniles.

The result of the experiment also, showed that WL 8 recorded the highest value of protein efficiency ratio and nitrogen metabolism of 1.34±0.28 g and 1582.01±0.34 g respectively. Based on these parameters, the best performance is WL 8 (1.5%) followed by WL 7 (1.0%) of walnut leaves, followed by OB4 (1.5%) of onion bulb 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. So, 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 30 g garlic/kg diet compared to the control which had the least value and Khattab et al. (2004) who found that the dietary of Biogen® increased feed intake, FCR, PER, and body composition (crude protein, ether extract, ash, and moisture) in fish. Also, Azza and Abd-El-Rhman (2009) revealed that Propolis-ethanolic-extract and crude propolis (1%) were added to artificial basal diet with (30% crude protein) to evaluate their efficacy on the fish growth-performance, the propolis –ethanolic extract showed increase and highest values in FCR, FER and PER compare to control.

However, the survival rate in control, OB2-OB4 and WL8 showed a similar trend (100% survival) while the least value of 90% was recorded in OB5, WL6, WL7 and WL9. The robustness and general well-being of the fish fed different graded levels of onion bulb and walnut leaf residues-based diets as expressed by the condition factor (k) was best in WL8 (1.5% inclusion) with a gain of 2.74±0.37 from the initial body status while the least gain of robustness (1.99±0.19) was recorded in WL6 (0.5% inclusion). They were no significant difference (P> 0.05) among the treatments. All the fish were however in better condition at the end of the experiment than they were at the beginning (see Table 5). Ibrahem et al. (2010) reported that the body weight gain, specific growth rate, condition factor and survival rate were significantly higher (P<0.05) in fishes fed basal diet supplemented with insulin and vitamin c than the control which is in accord with the present study.

The presence of vitamins such as vitamin C in walnut leaf and onion bulb could be a possibility of the growth factor or antioxidant property that enhance growth in the treated groups compared to the control. The uses of walnut leaf and onion bulb residue in fish feed could be a novel approach to aid productivity in aquaculture industry.

In determine the cost of diets, control diet had the lowest cost of ₦174.17 for producing 1 kg of fish feed among the treated groups OB5 had the highest cost of producing 1 kg of fish feed (₦ 180.77) while the lowest cost of fish feed was recorded in WL6 (₦ 174.22). The highest cost of producing 1kg of fish flesh was observed in diet WL8 with ₦ 244.80 followed by diet WL7 with ₦ 227.20. The findings of the study shows that WL8 had the highest profit index and incidence of cost (1.40) (0.032), followed by WL7 (1.30) (0.035) and the least was obtained in OB5 (1.18) (0.039) respectively, the reason for these observation may be due to high inclusion rate which increase the cost of producing the feed such as (OBR5 at 2.0% inclusion). It is therefore recommended that walnut leaf residue, WL8 (1.5% inclusions) in the diet of *C. gariepinus* should be absorbed by farmers, aquaculturists and other stakeholders since inclusion of 1.5% of walnut leaf residue promote growth and had highest profit index and lowest incidence of cost.

5. Conclusion

Diets with onion bulb and walnut leaf residues had nutritional possible use in fish farming and inclusion of 1.5% of the latter in fish feed enhanced growth. The relative abundance and availability of walnut leaf and onion bulb makes them a cheap natural nutritional and antimicrobial natural product to be explored in aquaculture. Therefore, future research should be carried out on interaction/synergetic effects of walnut leaf and onion bulb on *C. gariepinus*.

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