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Growth Response and Nutrient Utilization of *Clarias gariepinus* on Feeds Supplemented with African Oil Bean (*Pentaclethra macrophylla Benth*) Seed Residues

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ABSTRACT

An 8-week feeding trial was conducted in circular plastic tanks (50 × 34 × 27 cm^3) to assess the performance of *Clarias gariepinus* juveniles on feeds supplemented with *Pentaclethra macrophylla* seed residue. Five diets were formulated at 35% crude protein content with 0, 15.58, 31.16, 46.74 and 62.32% *P. macrophylla* seed residue as partial replacement for soybean meal. Each treatment was replicated thrice with 15 fish per replicate (mean initial body weight and standard length 8.32 ± 0.06 g and 12.01 ± 0.01 cm, respectively). Fish were fed twice daily at 3% of body weight Changes in body weights were recorded weekly. Fish on control diet performed better than those on *P. macrophylla* seed residue-containing diets though no significant differences were observed between the proximate composition of all experimental diets and the control. Significant ($P < 0.05$) increases were observed in the packed cell volume, haemoglobin, white blood cells, mean corpuscular volume, platelets, Mean corpuscular haemoglobin, lymphocytes, heterocytes and eosinophylls during the experiment and between diets. The histology of the liver, kidney, brain, small intestine, gill and heart of fish on all treatments were also recorded. It was concluded that further studies should be carried out to further process *P. macrophylla* seed residues for inclusion in fish diets.

Keywords: mineral element, performance, physico-chemical, proximate

INTRODUCTION

Pentaclethra macrophylla is a perennial multi-purpose tree from Africa found naturally in the humid lowlands of West Africa (Aju and Okwulehie 2005). It is a leguminous tree (family: Leguminosae; sub-family: Mimosoideae) and valuable for its soil improvement potentials in South Eastern Nigeria (Akindahunsi 2004). It has been cultivated in Nigeria since 1937 (Ladipo and Boland 1995) growing to about 21 m in height and 6 m in girth. Its pod generally contains about eight flat, glossy, brown edible seeds (Keay *et al.* 1989) and the bark is greyish to dark reddish brown. Its compound leaves have stout angular petioles. The tree flowers between March and April with smaller flushes in June and November. Fruits are available at most periods of the year because the large woody pods are persistent. Fruits split open explosively with valves curling up. Common uses of *P. macrophylla* include food, salt substitute, edible oil, seed craft, dye, fencing and palings, charcoal, carving bowls, medicine (convulsion, itching, lactogenicity, wound, diarrhea, seed, wood and ornamental (Enujiugha and Agbede 2000; Asoegwu *et al*. 2006; Ugbogu and Akukwue 2009; Agbogidi 2010). The major use of African oil bean seeds is for the preparation and fermentation of a local snack and condiment called "ugba" or "ukana" eaten in South Eastern and South-South geo-political areas of Nigeria (Nwamarah and Madueke 2010).

African oil bean seeds contain more than 52% oil in its cotyledons (Enujiugha and Ayodele-Oni 2003), with polyunsaturated fatty acids linolenic and oleic acids making up greater than 88% of the fatty acids in the oil (Achinewhu 1982; Enujiugha 2003). Several authors have reported on some aspects of *P. macrophylla* seeds and seed oil (Ajayi *et al*. 2002; Enujiugha and Akanji 2002; Odoemalam 2005; Ajayi *et al*. 2008, 2011; Amadi *et al*. 2011). This study was carried out to assess the performance of *Clarias gariepinus* juveniles on diets containing graded levels of *P. macrophylla* seed residues remaining after the extraction of the seed oil.

MATERIALS AND METHODS

The experiment was carried out using fifteen plastic tanks (50 × 34 × 27 cm^3) for 8 weeks in the Department of Wildlife and Fisheries Laboratory of the University of Ibadan, Nigeria. The water level in each tank was maintained at a depth of 0.30 m throughout the experiment and replaced every three days to maintain relatively uniform physicochemical parameters and prevent fouling from feed residues. The source of water was from University of Ibadan water station. Each tank was well aerated using air stones and aerator pumps (Lawson 1995). The dissolved oxygen content and pH of the water were measured using dissolved oxygen metre (Jenway 3015 pH metre, 0.0 accuracy; Genway, Staffordshire, UK) after standardizing the metre and water temperature by a mercury-in-glass thermometer (producer Paragon Scientific Ltd, Birkenhead, Wirral, UK).

There were five dietary treatments each having three replicates, with 15 fish/replicate and mean initial body weight and standard length of 8.32 ± 0.06 g and of 12.01 ± 0.01 cm, respectively. The fish were weighed, distributed into experimental tanks and allowed to acclimatize for 14 days before the experiment. The experiment lasted for 8 weeks during which the fish were fed at 3% body weight (in two equal portions of 1.5%) twice daily. Weight changes were recorded weekly and feeding rates adjusted to the new body weights.

P. macrophylla seeds were purchased from a market in

Received: 1 January, 2012. Accepted: 15 June, 2012.

Original Research Paper

Table 1 Gross composition of experimental diets.

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal	15.58	15.58	15.58	15.58	15.58
P. macrophylla	-	15.58	31.16	46.74	62.32
Soybeans	62.32	46.74	31.16	15.58	-
Wheat	7.06	7.06	7.06	7.06	7.06
Maize	7.06	7.06	7.06	7.06	7.06
Vitamin premix	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00
Starch	1.00	1.00	1.00	1.00	1.00
Vegetable oil	3.00	3.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00	100.00

Ibadan, Oyo State, Nigeria. The seeds were shelled by cracking to remove the kernels inside. The kernels were ground to powder in a Hammer mill and stored in an airtight sample bottle in a refrigerator (4°C) until needed for analysis. Seed oils were extracted in the Department of Chemistry, University of Ibadan using the continuous Soxhlet extraction technique with petroleum ether (40–60°C) for 8 h (Ajayi *et al*. 2006). The residue obtained was air-dried for about a week before being used for this study.

Other feed ingredients were bought, ground and mixed together to formulate a 35% crude protein diet. Each diet mixture was treated separately and extruded through a ¼ mm die mincer of Hobart A-200T pelleting machine (Hobart GmbH, Rben-Bosch, Offenbug, Germany) to form noodle-like strands, which were mechanically broken into suitable sizes for the *C. gariepinus* juveniles. The pellets were sun-dried, packed in labeled polythene bags and stored in a cool dry place to prevent fungal growth. *P. macrophylla* served as full or partial replacement for soybean meal. The gross composition of the experimental feeds is shown on **Table 1** and diet 1 served as the control with no *P. macrophylla* residue supplementation.

Biological evaluation

Weight gain = final body weight - initial body weight

$$\text{Weight gain (\%)} = \frac{\text{(final body weight - initial body weight)}}{\text{initial body weight}} \times 100$$

$$\text{Increase in standard length (cm)} = L_2 - L_1$$

where: L_2 = final standard length; L_1 = initial standard length.

$$\text{Specific growth rate (SGR)} = \frac{(\log_e \text{final body weight} - \log_e \text{initial body weight})}{\text{time (days)}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{dry weight of feed fed (g)}}{\text{fish weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{wet body weight gain (g)}}{\text{crude protein fed}}$$

$$\text{Protein productive value (PPV)} = \frac{\text{(final fish body protein - initial body protein)}}{\text{crude protein intake}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{initial number of fish stocked} - \text{mortality}}{\text{initial number of fish stocked}} \times 100$$

$$\text{Protein intake} = \frac{\text{feed intake} \times \text{percentage protein in diet}}{100}$$

Statistical analysis

P. macrophylla residue, experimental diets and fish carcasses were analyzed for proximate composition before and after the experiment using the methods of the Association of Official and Analytical Chemists (1990). One-way analysis of variance (ANOVA) was used to analyze the data obtained during the trial using Statistical Package for Social Sciences (SPSS version 15). Correlation and regression analyses were used to investigate relationships between weight and length (**Table 8**).

Table 2 Proximate composition of *Pentaclethra macrophylla* residue used in the experiment.

Component	*P. macrophylla*
Moisture	10.93 ± 0.02
Crude protein	32.01 ± 0.35
Ether extract	7.22 ± 0.06
Crude fibre	16.21 ± 0.05
Ash	13.41 ± 0.04
NFE	20.02 ± 0.02

RESULTS AND DISCUSSION

The proximate composition of *P. macrophylla* residues when compared with results obtained by Odoemelam (2005) for whole seeds of *P. macrophylla* showed that the residues (**Table 2**) contained higher ash (minerals) moisture and carbohydrate (nitrogen free extractives, N.F.E.) but lower crude protein and ether extract than whole seeds. These differences could be due to the extraction of oil and locations where the *P. macrophylla* was grown.

Fish on the control diet showed the best growth performance indices ($P < 0.05$) above all the diets containing different quantities of *P. macrophylla* residue (**Table 4**) though no significant differences were observed in the proximate composition of all the experimental diets and the control (**Table 3**). The highest percentage mortality ($P < 0.05$) was recorded on treatment 5 (62.32% *P. macrophylla* residue and 0% soy bean meal). Raw and unfermented *P. macrophylla* have been shown to contain anti-nutrients like saponins, tannins, flavonoids, oxalates, phytates, cyanogenic glycoside, terpenoids, reducing compounds and alkaloids (Ajayi *et al*. 2011; Amadi *et al*. 2011). Traditionally, *P. macrophylla* seeds are used as fish poison (Asoegwu *et al.* 2006) and the extraction of oil from raw seeds may not have been adequate to remove the anti-nutrients in the cake used for fish feed production which may have been left in the residues. Cooking and fermentation are the two methods of processing the seeds that have been shown to reduce the anti-nutrients (Nwamarah and Madueke 2010; Amadi *et al.* 2011). Except for diet 3 (*P. macrophylla* 31.16% and soybean meal 31.16%) all other *P. macrophylla* residue-containing diets recorded various percentage mortalities. There were general increases ($P < 0.05$) in crude protein, ether extract, crude fibre and ash in fish on all treatments (**Table 5**) during the experiment indicating growth but lower NFE in fish during the experiment than before the experiment. Significant differences ($P < 0.05$) were also recorded in CP, NFE and moisture content on all treatments. Significant and positive correlations were observed between length increments and weights of fish in all treatments (**Figs. 1, 2**).

There were significant ($P < 0.05$) increases in PCV, Hb, WBC, platelets, MCV, MHC, lymphocytes, heterocytes and eosinophylls during the experiment and between diets. Lymphocytes decreased significantly ($P < 0.05$) in fish on diets 3 and 4 while RBC also decreased in treatment 5 during the experiment which could be due to poor adjustment to the feed (**Table 6**). The decreases in these haematological parameters could also be due to stress and changes in dietary protein intake during the experiment. These findings were supported by other workers (Peter *et al.* 1991; Harms *et al.* 1996). The water quality parameters recorded during this study (**Table 7**) fell within the recommended ranges for fish in warm water environments (Boyd 1981).

Gross appearance and microscopic appearance of the liver, kidney, brain, small intestine, gill and heart were examined. The histopathology of the fish showed that the livers of the *C. gariepinus* juveniles had brown colouration and showed moderate sinusoidal and vascular congestion; widespread vascular degeneration of hepatocytes were

Table 3 Proximate composition of experimental diets (%).

	Diet 1 (control)	Diet 2	Diet 3	Diet 4	Diet 5
Moisture	9.56 ± 0.01^{a}	9.63 ± 0.02^{a}	9.68 ± 0.05^{a}	9.85 ± 0.02^{a}	9.86 ± 0.08^{a}
Crude protein	35.25 ± 0.06^{a}	35.70 ± 0.01^{a}	35.70 ± 0.03^{a}	36.40 ± 0.07^{a}	35.87 ± 0.01^{a}
Ether extract	3.51 ± 0.02^{a}	3.63 ± 0.03^{a}	3.64 ± 0.04^{a}	3.67 ± 0.05^{a}	3.71 ± 0.75^{a}
Crude fibre	2.26 ± 0.05^{a}	2.31 ± 0.01^{a}	2.53 ± 0.04^{a}	2.56 ± 0.10^{a}	2.59 ± 0.02^{a}
Ash	11.16 ± 0.08^{a}	11.21 ± 0.04^{a}	11.28 ± 0.02^{a}	11.63 ± 0.03^{a}	11.79 ± 0.09^{a}
NFE	38.26 ± 0.75^{b}	37.52 ± 0.08^{b}	37.17 ± 0.03^{b}	35.89 ± 0.02^{a}	36.18 ± 0.01^{ab}

Means followed by the same letter superscripts within a row are not significantly different ($P > 0.05$)

Table 4 Growth performance and nutrient utilization of *Clarias gariepinus* fed diets containing *P. macrophylla* residue for 8 weeks.

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Initial body weight (g)	8.26 ± 0.02^{a}	8.31 ± 0.17^{a}	8.33 ± 0.16^{a}	8.32 ± 0.22^{a}	8.26 ± 0.02^{a}
Final body weight (g)	21.60 ± 4.90^{d}	12.46 ± 0.06^{ab}	15.75 ± 0.86^{bc}	14.82 ± 0.85^{b}	10.63 ± 1.64^{a}
Body weight gain (g)	13.34 ± 4.92^{c}	4.15 ± 0.05^{ab}	7.42 ± 0.88^{b}	6.50 ± 0.63^{ab}	2.37 ± 1.68^{a}
Body weight gain (%)	161.50 ± 0.10^{e}	49.94 ± 0.12^{b}	85.65 ± 0.06^{d}	78.13 ± 0.03^{c}	27.97 ± 0.52^{a}
Initial length (cm)	12.00 ± 0.07^{a}	12.01 ± 0.01^{a}	12.01 ± 0.05^{a}	12.01 ± 0.02^{a}	12.00 ± 0.03^{a}
Final length (cm)	18.53 ± 0.02^{c}	15.07 ± 0.13^{ab}	16.07 ± 0.04^{b}	15.50 ± 0.30^{ab}	14.40 ± 0.05^{a}
Length increment (cm)	6.53 ± 0.09^{c}	3.06 ± 0.03^{ab}	4.06 ± 0.02^{b}	3.49 ± 0.04a^{b}	2.40 ± 0.07^{a}
Food conversion ratio	7.88 ± 1.92^{a}	16.93 ± 2.27^{c}	11.64 ± 0.96^{b}	12.21 ± 0.76^{b}	17.15 ± 0.61^{c}
Protein efficiency ratio	0.38 ± 0.14^{b}	0.12 ± 0.02^{a}	0.20 ± 0.03^{a}	0.18 ± 0.02^{a}	0.07 ± 0.05^{a}
Protein productive value	0.67 ± 0.03^{a}	0.07 ± 0.00^{ab}	0.07 ± 0.03^{b}	0.72 ± 0.01^{b}	0.72 ± 0.00^{b}
Protein intake (g)	35.06 ± 5.06^{b}	24.85 ± 1.01^{a}	29.80 ± 1.06^{a}	28.79 ± 0.90^{a}	24.82 ± 2.16^{a}
Survival rate (%)	100^{c}	91.11^{b}	100^{c}	91.11^{b}	86.67^{a}
Specific growth rate	0.73 ± 0.17^{c}	0.32 ± 0.04^{ab}	0.48 ± 0.04^{b}	0.45 ± 0.02^{b}	0.19 ± 0.12^{a}
Condition factor:					
A Initial	±0.00	±0.00	±0.00	±0.00	±0.00
B Final	±0.03	±0.04	±0.06	±0.03	±0.001
C Difference	±0.02^{a}	±0.02^{a}	±0.03^{a}	±0.02^{a}	±0.00^{a}

Means followed by the same letter superscripts within a row are not significantly different ($P > 0.05$)

Table 5 Proximate composition of experimental fish before and after the experiment.

	Before	Diet 1 (control)	Diet 2	Diet 3	Diet 4	Diet 5
Moisture	10.82 ± 0.09	4.20 ± 0.16^{a}	4.15 ± 0.30^{a}	7.50 ± 0.06^{b}	4.07 ± 0.17^{a}	4.23 ± 0.10^{a}
Crude protein	35.50 ± 0.10	50.34 ± 0.83^{a}	51.68 ± 0.20^{ab}	51.45 ± 0.35^{ab}	52.85 ± 0.35^{b}	53.09 ± 0.27^{b}
Ether extract	6.54 ± 0.05	9.72 ± 0.78^{a}	10.30 ± 0.06^{a}	9.84 ± 0.055^{a}	9.77 ± 0.55^{a}	9.83 ± 0.66^{a}
Crude fibre	0.10 ± 0.03	1.70 ± 0.13^{a}	1.60 ± 0.16^{a}	1.63 ± 0.08^{a}	1.76 ± 0.10^{a}	1.83 ± 0.18^{a}
Ash	11.44 ± 0.20	19.08 ± 0.08^{a}	19.30 ± 0.07^{a}	19.08 ± 0.35^{a}	18.82 ± 0.46^{a}	18.36 ± 0.95^{a}
NFE	35.60 ± 0.01	14.96 ± 0.02^{c}	12.97 ± 0.10^{b}	10.50 ± 0.06^{a}	12.73 ± 0.50^{b}	12.64 ± 0.10^{b}

Means followed by the same letter superscripts within a row are not significantly different ($P > 0.05$)

Table 6 Mean haematological parameters of *Clarias gariepinus* juveniles fed diets supplemented with *P. macrophylla* residues.

Parameters	Before experiment	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
PCV (%)	20.61 ± 0.49	32.00 ± 0.88^{bc}	30.00 ± 0.03^{b}	34.00 ± 0.01^{c}	40.00 ± 0.04^{d}	27.00 ± 0.70^{a}
Hb (g/dl)	7.08 ± 0.15	10.40 ± 0.10^{ab}	9.80 ± 0.07^{ab}	11.20 ± 0.90^{b}	13.50 ± 0.70^{c}	8.70 ± 0.05^{a}
RBC × 10^{12}/L	2.60 ± 0.37	3.33 ± 0.11^{a}	3.29 ± 0.14^{a}	3.36 ± 0.01^{a}	3.42 ± 0.08^{a}	2.51 ± 0.60^{a}
WBC × 10^{9}/L	10.500 ± 1.07	13.000 ± 0.25^{a}	18.850 ± 1.45^{c}	14.120 ± 3.10^{ab}	18.900 ± 5.20^{c}	15.100 ± 6.50^{b}
Platelets × 10^{9}/L	99.500 ± 2.01	246.000 ± 0.8^{e}	128.000 ± 0.12^{b}	210.000 ± 2.34^{d}	205.000 ± 1.70^{c}	104.000 ± 5.19^{a}
MCV (Fl)	850.15 ± 0.03	960.96 ± 0.34^{b}	911.85 ± 0.78^{a}	1011.90 ± 1.05^{c}	1169.59 ± 0.55^{e}	1075.70 ± 5.23^{d}
MCH (Pg)	2.50 ± 3.01	3.17 ± 0.56^{a}	2.98 ± 0.01^{a}	3.33 ± 0.50^{a}	3.95 ± 1.50^{a}	3.47 ± 1.00^{a}
MCHC (%)	0.29 ± 1.02	0.33 ± 0.02^{ab}	0.33 ± 0.05^{ab}	0.33 ± 0.07^{ab}	0.34 ± 0.00^{ab}	0.32 ± 0.01^{a}
Lym (%)	40.00 ± 0.23	68.00 ± 0.23^{d}	52.00 ± 0.90^{c}	25.00 ± 0.25^{a}	36.00 ± 0.50^{b}	75.00 ± 0.15^{e}
Mono (%)	1.50 ± 0.00	0.00 ± 0.00^{a}	3.00 ± 0.02^{b}	3.00 ± 0.25^{b}	2.00 ± 0.03^{b}	2.00 ± 0.06^{b}
Hetero	30.00 ± 1.10	35.00 ± 0.30^{b}	43.00 ± 0.02^{c}	68.00 ± 0.50^{e}	62.00 ± 0.08^{d}	22.00 ± 0.16^{a}
Eosin	1.00 ± 0.05	2.00 ± 0.10^{b}	2.00 ± 0.01^{b}	4.00 ± 0.05^{c}	0.00 ± 0.00^{a}	1.00 ± 0.01^{ab}

RBC = red blood cells; MCH = mean coruscular haemoglobin; WBC = white blood cells; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; LYM = lymphocytes; Eosin = eosinophils; Mono = monocytes; Hetero = heterocytes
Means followed by the same letter superscripts within a row are not significantly different ($P > 0.05$)

Table 7 Mean weekly water parameters during the experiment.

Parameters	0	1	2	3	4	5	6	7	8	Mean
Temperature	24.00	25.00	26.0	26.02	26.03	26.0	25.0	25.4	25.3	25.42 ± 0.63
DO	6.40	6.50	6.50	6.60	6.60	6.40	5.90	6.30	6.40	6.43 ± 0.21
PH	6.90	6.80	6.90	6.90	7.20	7.10	7.10	7.50	7.20	7.10 ± 0.22

observed in fish on diets 4 and 5. Liver receives about 30% of the total cardiac output so it is susceptible to chemicals present in systemic circulation (Bridges 1990). Also, liver is considered the most important target organ from a toxicological point of view because of its role in detoxification, biotransformation and excretion of xenobiotics (Hassanein *et al*. 1999).

The heart showed no pathological changes during the feeding trials and this may be due to the fact that the heart is a very resilient organ and is highly regenerative in nature such that the process of acclimation was more rapid in this organ than all the other organs. The kidneys were reddish brown, pulpy and bloody when broken open but no visible lesions were found among all the treatments. The brains of the fish were whitish and soft and no visible lesions were observed in fish on all diets. This was similar to the findings

Table 8 Linear equation, coefficients of determination (r) relating dependent variables to independent variables.

Treatment	X	Y	Prediction equation	R	R2
Control	Weight	Length	Y=8.89+0.43X	0.9905	0.9812
Treatment 2	Weight	Length	Y=6.44+0.74X	0.9409	0.8853
Treatment 3	Weight	Length	Y=7.67+0.56X	0.9885	0.9772
Treatment 4	Weight	Length	Y=8.95+0.46X	0.9513	0.9051
Treatment 5	Weight	Length	Y=6.46+0.73X	0.9455	0.8939

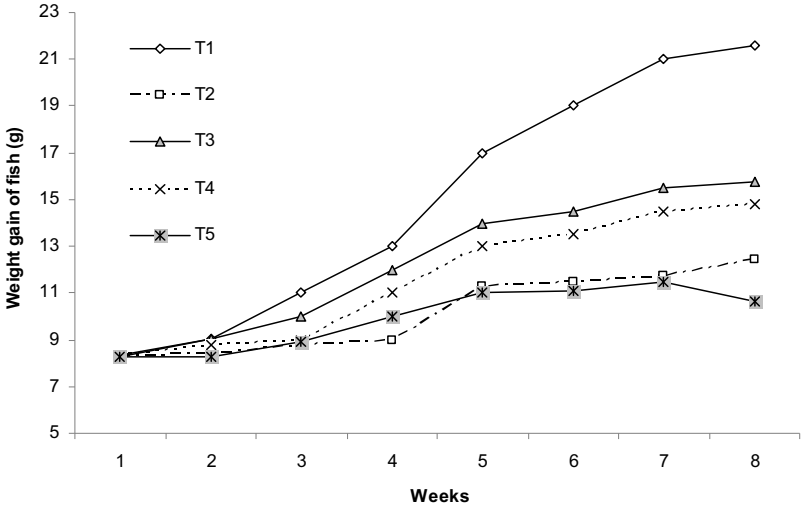

Fig. 1 Weekly weight gain of *Clarias gariepinus* fed experimental diets for 56 days.

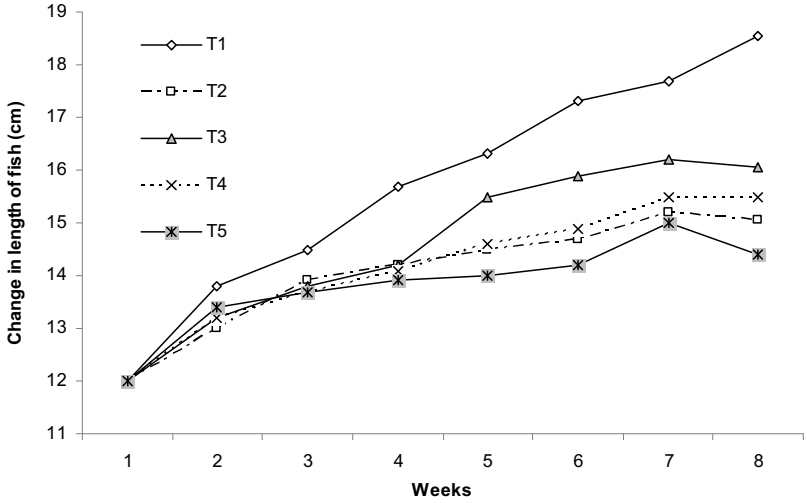

Fig. 2 Weekly length increment of *Clarias gariepinus* fed experimental diets for 8 weeks.

of Adeogun (1994). However, pathological changes were observed in the gills of the fish which showed torn tissues. Moderate congestion of vessels in lamina propria and villi, severe widespread villous mating (fusion) and goblet cell hyperplasia were observed in the small intestine of *C. gariepinus* juveniles during the experiment. Environmental stress may cause changes in cellular function that alter the physiology of organ systems in the fish as reported by van Vuren *et al.* (1994).

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