Continental J. Fisheries and Aquatic Science 7 (1): 32 - 41, 2013 ISSN: 2141 – 4246
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Printed in Nigeria doi:10.5707/cjfas.2013.7.1.32.41

THE GROWTH AND BODY COMPOSITION OF *Clarias gapiepinus* Juvenile FED COMBINED DIFFERENT SOURCES OF LIPID

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ABSTRACT
A study of the growth and body composition of *Clarias gariepinus* juveniles 8.51 ± 2.29g fed combined different sources of lipid was carried out on a feeding trial that lasted for 56 days. Three diets containing varying protein levels (50, 40, 30%) with isolipid levels of 35.76, 47.61 and 59.45% respectively at different levels of inclusion of different sources of lipid which included Fish oil, palm oil and groundnut oil were formulated while a catfish commercial feed (multi-feed) was used control. The results indicated significant differences ($p<0.05$) in the growth parameters. Diet 2, containing 50% crude protein and 47.61% combined lipids exhibited significantly high ($p<0.05$) mean weight gain ($66.69^{a}\pm17.44$g), specific growth rate ($3.80^{a}\pm0.01$), protein efficiency ratio ($6.90^{a}\pm0.01$) as well as low feed conversion ratio ($0.29^{a}\pm0.01$). Diet 4, containing 30% crude protein and 45% combined lipids of 59.45% gave a significantly low ($p<0.05$) growth parameters. It is recommended that the inclusion of appropriate levels of combined lipid sources in the diets of *Clarias gariepinus* juveniles would spare protein for growth, thereby reducing its inclusion level in a diet.

KEYWORDS: *Clarias gariepinus* juveniles, Combined different source of lipid,

Received for Publication: 22/10/12 Accepted for Publication: 01/02/13
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INTRODUCTION

Aquaculture plays an important role in many countries by providing better nutrition as a result of high quality animal protein, higher income, better employment opportunities, and also earning foreign exchange. Presently, fish accounts for more than or approximately 50% of the total animal protein consumed in most countries of the world. Protein is the single most expensive ingredient in fish diets. The fact that high levels of dietary protein may lead to the consumption of protein for energy purposes, has led to the investigation of the use of non-protein energy sources in fish diets (Shiau and Peng (1993) and Erfanullah and Jafri, 1995). It has been reported that fish do not have a carbohydrate requirement (NRC, 1993; Orire and Sadiku, 2011). However, several studies have been carried out on the carbohydrate-lipid ratio in fish diet to spare protein (Nematipour *et al.*, 1992 and Ali *et al.*, 2001).

Proteins are the major organic materials in most fish tissue and form an important component of the diet. One of the major requirements of fish culture is the efficient transformation ofdietary protein into tissue protein (Webster and Lim, 2002). However, protein is essential for normal tissue function, for the maintenance and the renewal of fish body protein and for growth. Due to the cost of the protein, the feed will be more cost effective if all the protein is used for tissue repair and growth and little stabilized for energy (Gauquelina *et al.*, 2007) from a practical point of view, the ideal situation should tend to maximize the use of dietary proteins for growth, minimizing the use of proteins for functional protein synthesis, gluconeogenesis, lipogenesis and energy (Jamabo and Alfred, 2008).

If adequate protein is not provided in the diet, there is a rapid reduction or cessation of growth and a loss of weight due to withdrawal of protein from less vital tissues to maintain the functions of more vital tissues. On the other hand, if too much protein is supplied in the diet, only part of it will be used to make new proteins and the remainder will be catabolized to produce energy (Alatise *et al.*, 2006; Orire and Sadiku, 2011). Although the utilization of protein

for basal energy metabolism is a well-established phenomenon, conventional energy-yielding nutrients like fats and carbohydrates can reduce the oxidation of protein to satisfy the energy needs of the fish and thereby improve the utilization of dietary protein (Kim *et al.*, 2004).

Lipids are an extremely diverse group of compounds, many of which functions as important sources of metabolic energy. Among the various types of lipids, it is the simple, glycerol based fats and oils that are of most interest in terms of general nutrition (*Du et al.,* 2008). Lipids normally occur in food stuffs and in the fat deposits of most animals in the form of triglycerides, which are esters of fatty acids and glycerol (Kissling *et al.,* 2001). Thus, dietary lipids provide a source of indispensable nutrients, the essential fatty acids. In addition, they also act as carriers of certain non-fat nutrients, notable the fat soluble vitamins A, D, E and K. They are also an important source of energy (Storebakken, 2002). Lipid contains more energy per unit weight than any biological compound. Example, One gram of lipid contains about twice as much total energy as either one gram of carbohydrate or one gram of protein (Gullaine *et al.,* 2001). Dietary lipids, mainly in the form of triglycerides, are hydrolyzed to free fatty acids and glycerol by pancreatic lipase, aided by the saponifying and emulsifying action of bile acids in the digestive tract. Absorption generally occurs primarily in the anterior ileum including the caecum. (Subhadra *et al.,* 2006). Lipids are transported in the blood streams either as lipoprotein complexes called Very Low-Density Lipoproteins(VLDLs) or as very small droplets called Chylomicrons. The triglycerol components of VLDLs and Chylomicrons are hydrolyzed to free fatty acids and glycerol in the target tissues (generally adipose tissue and skeletal muscle) outside of the cell by an enzyme called Lipo-Protein Lipase (LPL). The other source of long chain fatty acid is synthesized (Lipogenesis) from acetyl-coA derived from carbohydrate (glucose), mainly in adipose tissue and the liver (Mourente *et al.,* 2005).

Digestibility is the quantification of the digestive processes which gives relative measures of the extent to which ingested food and its nutrient components are digested and absorbed by the animal (Burel *et al.,* 2000). Proteins in most feed–stuffs that are properly processed are highly digestible to fish. As with other animals, fish control the feed intake to meet their energy requirements (Kaushik and Mendale, 1994; Borda *et al.,* 2006). This research seeks to establish sparing effects of combined lipid of various sources on the growth and body composition of *Clarias gariepinus* juveniles.

MATERIALS AND METHODS

Experimental Procedure

The research work was carried out at the Department of Water Resources, Aquaculture and Fisheries Technology, Step-B Laboratory, Federal University of Technology Minna, Gidan kwano, Minna while 240 pieces of the experimental fishes; *Clarias gariepinus* were obtained from Tagwai Hatchery farm, Niger State, Nigeria.

The fishes were acclimatized for about 1 week in a brood stock holding facility. During the period of acclimatization, they were carefully monitored, fed on commercial catfish feed (0.5 Multi-feed), their water quality was monitored daily by siphoning of uneaten feed and waste while dead fishes were also removed.

Experimental Setup

The experimental design was a complete randomized design (CRD) where fishes and their treatments were allotted randomly. The fishes were weighed and distributed into twelve plastic rounded bowls of 45cm x 30cm x 30cm each at the stocking rate of 20 fish per bowl, filling each tank to 20 liters capacity with clean fresh water. The plastic bowls were fitted with pipes, with water flowing in a re-circulatory system from an overhead tank. The dirty water passed through filtering materials to recycle for the wellbeing of fishes. A constant volume of 20 liters of water were maintained in the tanks/ bowls every day and covered with fine nets to prevent the fish from jumping out.

Experimental Diets

Feedstuffs comprised fishmeal, fish oil, groundnut oil and palm oil were purchased at Minna market, Niger state. While Multi-feed (control) and vitamin premix were obtained from Makholo Stores. Three formulated diets were produced, containing varying inclusion levels of crude proteins at 50, 40, and 30% with inclusion levels of lipid in a combined form of fish oil, palm oil and groundnut oil at 10%, 15% and 20% respectively (Orire,2010).Quadratic equation of two unknown (X + Y = Z)was used to formulate the experimental diet.

Where X= fish meal (protein source)
Y= lipid (energy source)
Z= Bulk (Quantity)
(Halver, 1989)

All ingredients were mixed together in the right proportion of formulation and then refrigerated.

TABLE 1: Ingredients used for the Experimental Diets and their Proximate Composition

FEED STUFFS	DIET 1 (control)*	DIET 2	DIET 3	DIET 4
Fish meal		59.24	47.39	35.55
Fish oil		7.95	10.58	13.21
Palm oil		11.92	15.87	19.82
Groundnut oil		15.89	21.16	26.42
Premix		5.0	5.0	5.0
Proximate composition of diets				
% Crude protein	50.00	51.47	39.89	33.03
% Lipid	12	30.60	39.64	52.41
% Crude fibre	2.5	1.84	1.52	1.23
% Ash	8.5	7.57	9.23	8.19
% Moisture content	32.00	8.39	9.17	4.67

*Diet1: Composition is as indicated by the manufacturer (Multi feed)
Diet 2: CP 50:35.76 Lipid ratio
Diet 3: CP 40:47.61 Lipid ratio
Diet 4: CP 30: 59.45 Lipid ratio

Experimental Practices

The experimental practice was divided into routine and non-routine management. The routine management for the feeding trial was the feeding of the fishes daily, monitoring water quality weekly, picking dead fish, cleaning the system regularly and daily recycling of water to optimize dissolved oxygen concentration. While the checking of the system for leakages and malfunctioning constituted the non-routine management. Fishes were acclimated for one week and were fed during these periods on a commercial catfish diet (Multi-feed). They were weighed with an electronic sensitive weighing balance (MP 300 citizen) at the commencement of the experiment, by- weekly as well as at the end of experiment in all the experiments.

Experimental Analysis

This comprised both chemical analysis and biological evaluation as detailed below.

Chemical Analysis

General chemical analysis was carried out on the water, feed stuffs, diets and carcass for the approximate analysis for moisture, protein, lipid, ash, and crude fiber, using standard procedures (AOAC, 2000)

Water Quality Parameter Monitoring

During the period of the experiment, the water quality was monitored weekly on temperature, dissolved oxygen concentration, conductivity and hydrogen ion concentration (pH) (AOAC, 2000) as shown in Table 2

Temperature

Water temperature records for each aquarium tank was taken weekly between 8 am and 9 am using mercury in glass thermometer ($-10.0 - 110^0$) graduated in degree centigrade (^0C). The temperatures were taken by dipping the thermometer into the water inside the plastic container for about 3 minutes before taking the reading (AOAC, 2000).

Dissolved Oxygen (Do)

The dissolved oxygen concentration was determined using Wrinkle method (AOAC, 2000). Dissolved oxygen bottle was used to get water from the plastic bowls and while it was still in the water, it was corked and taken to the laboratory for analysis. Two drops of Manganese sulphate (Reagent 1) and alkaline iodide solution (Reagent 2) was added to the water contained in the dissolved oxygen bottle. Then you allow it to settle. Add 1ml of concentrated sulphuric acid when it separates and shake. Get a conical flask and pipette 10ml into the conical flask. Put 2 – 3 drops of starch solution into the conical flask. Then titrate with sodium thiosulphate (5ml).

$$\text{Dissolved oxygen} = \frac{\text{volume of } Na_2SO_3 \text{ X N X 8 X 100}}{\text{Volume of sample taken (ml)}}$$

N = Normality of sodium thiosulphate = 0.025

Hydrogen Ion Concentration (pH)

The pH was determined using electronic pH meter mode KENTEIL 7045 / 36 with reference electrode. The meter was standardized according to the manufacturer instruction before measuring any sample. The electrode was immersed in buffer solution and electrodes were thoroughly washed before measuring the pH of each sample. The electrode was dipped into the water sample inside the container, and system was allowed to stabilize before the reading was taken.

TABLE 2 Mean Water Quality Parameters of *Clarias gariepinus* for The Feeding trials

	PARAMETERS			
TREATMENTS	Temperature (^{0}C)	pH	Conductivity (micromoles/cm)	Dissolved oxygen(mg/L)
Diet 1	27.0–29.3	7.00-7.78	1.99-2.70	5.1-12.3
Diet 2	28.0–30.0	6.77-7.60	1.76-4.60	3.4-9.0
Diet 3	27.3–30.3	6.74-7.44	1.76-3.14	3.0-11.0
Diet 4	25.7–28.0	6.82-7.48	1.66-3.41	5.5-10.0

Moisture

This is gravimetric measurement of moisture in the feed stuffs, diets and carcass. It is expressed as a percentage of the initial sample weight. A representative sample was dried to a constant weight in an oven at 110^{0}C. One gram of sample was pre-weighed (W_1) on a foil tray and placed in an oven (Gallenkamp) at 60^{0}C for 24 hours. The sample was removed from the oven, cooled in a desiccators, and re-weighed (W_2). Moisture percentage was calculated according to the formula:

$$\text{Moisture (\%)} = W_1 - W_2 / W_1 \text{ X } 100$$

Crude Protein (CP)

Crude protein was determined by the Kjeldahl method (2000), as described below:-0.5g – 1.0g of the sample was digested with 20ml of concentrated sulphuric acid and a selenium digestion tablet. It was heated on a heating mantle until the solution became clear. The ammonia in the digest was released when reacted with 10ml of 40% sodium hydroxide during distillation which was trapped in 2% boric acid mixed with methyl red indicator. 50-75ml of the distillate was collected and titrated against standard 0.1ml hydrochloric acid. A digest treated the same way was used as the blank titre. Percentage crude protein valve was calculated using the titre valve for the blank and test samples as follows:

$$\text{\% crude protein} = \frac{\text{sample titre – blank titre x 0.1 x 0.014 x 6.25 x100}}{\text{Weight of sample (g)}}$$

Where,
0.1 = Normality of hydrochloric acid
0.14 =Molecular weight of hydrogen
6.25=Nitrogen factor; since protein is assumed to be 16% nitrogen

Crude Lipid
The method applied was that of solvent extraction using a soxhlet extractor. Crude lipid in diets was determined by extraction with petroleum ether in feedstuffs and fish carcasses. The extract will be collected in a cup and when the process is completed, the solvent is evaporated and the remaining crude lipid is dried and weighed. One gram of sample was weighed into an extraction thimble and covered with absorbent cotton.50ml of solvent was added to a pre-weighed cup, which contained 5 glass balls. Both thimbles and cup were attached to the extraction unit. Extraction unit temperature was fixed at 115^0C for petroleum ether as a solvent at 40 – 60^0C. The sample was subjected to boiling in solvent for 30 minutes followed by rinsing for 1 – 1.5 hours. Solvent was evaporated from the cup to the condensing column. Extracted lipid in the cup was placed in an oven at 110^0C for 1 hour and after cooling, the crude lipid was calculated using the following formular(AOAC, 2000):

Crude lipid (%) = (extracted lipid/sample weight) x 100

Ash
Ash content was determined as total inorganic matter by incineration of the sample at 600^0C. 1g of sample was weighed into a pre-weighed porcelain crucible and incinerated (Gallenkamp Muffle Furnace) for 6 hours at 600^0C. The crucible was removed from the muffle furnace, cooled in desiccators and weighed. Ash content was calculated according to the following formula(AOAC,2000):

Ash (%) = (ash weight / sample weight) x 100

Biological Evaluation
Biological parameters were measured according to Maynald *et al.*, 1979, Bondi (1987) and Halver (1989), as described below; the biological evaluation will be carried out on;

Specific Growth Rate according to Brawn 1957 was measured

$$SGR = \frac{LnMFW - LnMIW\ (g)}{Time/days} \times 100$$

Where W_1 is the initial weight of fish (g) at time T_1 (day)and (W_2) is the final weight of fish (g) at time T_2 (day) and Ln as natural logarithm.

Food Conversion Ratio (FCR)

$$FCR = \frac{\text{Mean Dry Weight of food fed}}{\text{Mean Weight of Fish}} \text{ i.e. } \frac{\text{Wt. of food fed}}{\text{Wt. gain of fish}}$$

FCR = How much fish feed is needed to produce 1kg of fish flesh (meal)

Protein Efficiency Ratio
PER is defined as fish weight gain per gram of crude protein fed. PER gives an indication of the efficiency of protein utilization and is calculated as follows

$$PER = \frac{\text{Mean Weight Gain of fish}}{\text{Mean Weight of protein fed}}$$

Protein fed = % C.P. level x quantity of feed intake

Statistical Analysis
Result of carcass composition, the evaluation of biological parameters and all other data that were obtained, was subject to one way analysis of variance (ANOVA) using turkey's test(Steel and Torrie, 1980). All statistical analyses were executed using the software Minitab Release.

RESULTS
From the results in Table 3, there were significant differences ($p<0.05$) in the mean weight gain of the fishes fed various diets. Diet 2 recorded significantly high ($P<0.05$) mean weight gain (66.69g). It was significantly different ($p<0.05$) from diet 3 with a mean weight gain (38.03g). There was no significant difference ($p>0.05$) between diet 1(control diet) and diet 4 with a mean weight gain (15.02g) and (14.31g) respectively.

The Specific Growth Rate (SGR) also differed significantly ($p<0.05$) among the diets. Diet 2 recorded significantly high ($p<0.05$) SGR value of 3.80. It differed significantly ($p<0.05$) from diets 3, 1 and 4 with SGR values of 2.95, 1.98, 0.98 respectively.

The Feed Conversion Ratio (FCR) of the diets differed significantly from each other. However, diet 4 recorded the highest FCR value of 2.81 which was significantly higher ($p<0.05$) than other diets. Diet 2 recorded the lowest FCR value of 0.29.

Protein Efficiency Ratio (PER) differed significantly ($p<0.05$) from each other. Diet 2 recorded significantly ($p<0.05$) high PER value of 6.90, which differed significantly from other diets. This was followed by diet 3 and 1 with PER value of 4.74 and 2.45 respectively. Diet 4 recorded the lowest PER value of 1.19.

From the results in Table 4, the values on Body composition consisting of Crude protein, Crude ash, Crude lipid and moisture, there was significant differences ($p<0.05$) observed amongst the experimental fish. The body Crude protein for Diet 2, containing 50% crude protein and 45% lipid (10% fish oil, 15% palm oil, and 20% groundnut oil) was significantly higher ($p<0.05$) (53.08) than initial carcass crude protein (47.06), with significant difference ($p<0.0$) from the control diet (55.75). However, there was corresponding increase in the body lipid content for diet 3 and 4, containing 40% crude protein (40.87) and 30% crude protein (48.01) which was significantly higher ($p<0.05$) than other diets.

There was significant difference ($p<0.05$) in the crude ash content of the experimental fish. Diet 1 (9.23) recorded the highest body ash content, which was significantly higher than other diets. Moreover, all diets were of high ash values than the initial carcass crude ash (3.12).

There was no significant difference ($p>0.05$) between the carcass moisture content of diet 1 (8.17) and diet 2 (8.13) and between diet 3 (7.70) and the initial carcass composition (7.55). However, diet 4, recorded the lowest moisture content.

DISCUSSION
The results from the study revealed the possibility of utilizing different lipid sources (palm oil, groundnut oil, and fish oil) in an appropriate inclusion levels in the diets of *Clarias gariepinus* juvenile for growth enhancement. Diet 2 recorded the best performance in terms of growth parameters, which was in agreement with study carried out by Lim *et al.*, (2001) that up to 8% of crude palm oil can be included in diets for the African catfish with improved performance, protein retention and fillet vitamin E concentration of the fish. Also, Pie *et al.*, 2004 reported an improvement in Carp fed dietary lipid.

The fish on the experimental diet 4 which was at highest lipid inclusion level, showed low growth performance level when compared with Diet 2, which was similar to the work of Aderolu *et al.*, 2011 in the utilization of palm oil and shea-butter in Catfish diet. The present study showed that increase in dietary lipid level was associated with decline in feed intake. This indicates that there was either palatability problem or feed intake depression. This agreed with the report of Ellis and Reigh (1991) and Aderolu *et al.*, 2011, that at high dietary lipid level, growth rate may

decrease due to reduced ability to digest and absorb high lipid, reduction in feed intake and/or fatty acid imbalance in feed.

The observation from the results shows that, there was no palatability problem except for Diet 4 and that their utilizations were adequate. This is similar to the work of Aderolu and Akinremi (2009) in the utilization of coconut oil and peanut oil in Catfish.

From the experiment, diet 2 exhibited better growth performance than the control diet (Commercial catfish feed-Multi-feed) which was contrary to Ojukannaiye, 2006. This can be attributed to the presence of sufficient amount and appropriate combination of fatty acids of various lipid sources (fish oil, palm oil and groundnut oil). This combination provided desired energy in diet that resulted in protein sparing for growth (Figure 1). At the same time, there was a limit at which *Clarias gariepinus* juveniles can tolerate lipid in its diets as shown observed in diet 4. The high-level performance of diet 2 could also be because of the inclusion levels of the various lipids used, which was within the fish acceptable limit which was in agreement with the reports of Lim *et al.,* (2001) and Orire (2010).

Carcass analysis showed a significant difference ($p<0.05$) between the control diet and others in terms of crude lipid, crude ash, crude protein and moisture. In terms of crude protein value, diet 2, which had crude protein level (53.08), was slightly lower than that of control diet (55.75). And diet 2 also had better ratio of growth which was in agreement with Aderolu *et al*.,2011, that although the control diet had a higher crude protein value, the experimental diet performed better in terms of growth and nutrient utilization than the control.

However, with the lowering of crude protein to 30%, there was a resultant effect in body lipid increment which was an indication of poor utilization of lipid. This was in agreement with Orire, 2010; Weatherley and Gill, 1987 who reported poor utilization of lipid as probably due to poor feed intake, which prevented the fish from ingesting enough nutrients to meet the carcass nutrient requirements for the development of tissues.

From the experiment, fish oil, palm oil, and vegetable oil could be used in their appropriate inclusion level as energy sources in the diet of *Clarias gariepinus* juveniles up to 35.76% combined lipid inclusion level without negative effect on the growth of fish.

CONCLUSIONS

Based on the findings in this study, it could be inferred that the lipid sources such as palm oil, fish oil, and Groundnut oil, could be successfully used in the diets for African catfish, without negatively affecting growth and feed utilization efficiency, provided it is within the appropriate level.

ACKNOWLEDGEMENT

The authors sincerely appreciated the STEP-B Grant of Innovation of Tomorrow which enabled smooth execution of the research work.

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Table 3: Mean growth parameters for *Clarias gariepinus* juveniles fed with variouslipid/protein ratios for 8 weeks.

Growth Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SD
Mean Initial weight gain (g)	7.39^{a}±3.37	9.03^{a}±0.32	9.19^{a}±1.67	8.41^{a}±2.58	2.29
Mean Final Weight gain (g)	22.40^{b}±15.68	75.72^{a}±17.40	47.22^{ab}±22.65	22.71^{b}±9.31	16.94
Mean weight gain (g)	15.02^{b}±12.31	66.69^{a}±17.44	38.03^{ab}±21.01	14.31^{b}±8.474	15.57
Mean feed fed (g)	12.50^{c}±0.01	20.05^{a}±0.01	19.30^{a}±0.01	17.40^{b}±0.01	0.01
Specific growth rate (%/Day)	1.98^{c}±0.01	3.80^{a}±0.01	2.95^{b}±0.01	0.98^{d}±0.01	0.01
Feed Conversion Rate	0.82^{b}±0.02	0.29^{a}±0.01	0.53^{ab}±0.01	2.81^{c}±0.01	0.01
Protein Efficiency Ratio	2.45^{c}±0.01	6.90^{a}±0.01	4.740^{b}±0.01	1.19^{d}±0.01	0.01

Means on the same row carrying letters(s) with different superscript(s) are significantly different from each other ($p<0.05$).

Table 4: Body composition of Experimental fishes fed Combined Different Sources of Lipid

Parameters	Initial carcass	Final carcass				
		D1	D2	D3	D4	Pooled SD
Crude protein	$47.06^{b\pm}$0.01	$55.75^{a\pm}$0.01	$53.08^{ab\pm}$0.01	$35.30^{c\pm}$0.01	$30.31^{d\pm}$0.01	0.01
Crude lipid	$18.35^{c\pm}$0.01	$22.94^{d\pm}$0.01	$28.76^{c\pm}$0.01	$40.83^{b\pm}$0.07	$48.01^{a\pm}$0.01	0.03
Ash	$3.12^{d\pm}$0.01	$9.23^{a\pm}$0.01	$8.93^{ab\pm}$0.02	$8.55^{b\pm}$0.01	$7.00^{c\pm}$0.01	0.01
Moisture	$7.55^{b\pm}$0.01	$8.17^{a\pm}$0.01	$8.13^{a\pm}$0.01	$7.70^{b\pm}$0.01	$5.90^{c\pm}$0.01	0.01

Diet 1: CP 50: 12 Lipid, Diet 2: CP 50:35.76 Lipid ratio, Diet 3: CP 40:47.61 Lipid ratio, Diet 4: CP 30: 59.45 Lipid ratio

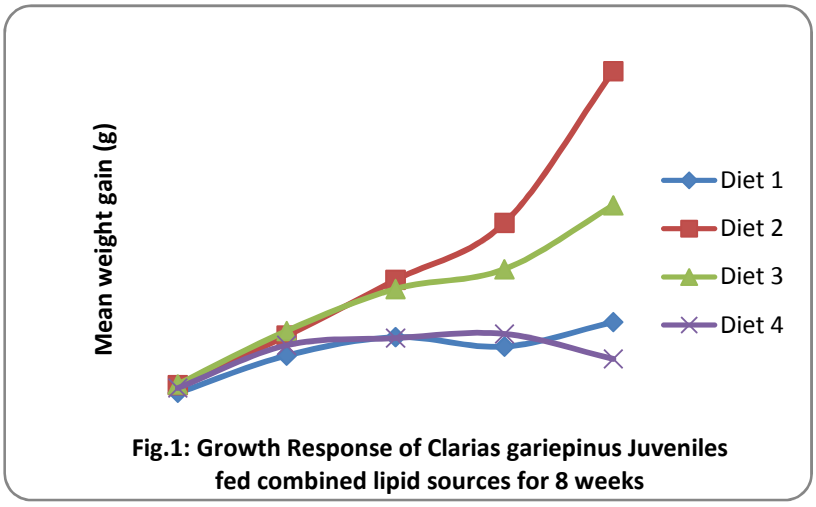

Fig.1: Growth Response of Clarias gariepinus Juveniles fed combined lipid sources for 8 weeks