GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL: 12 NO. 4 2006 : 491 - 497
 ISSN 1118 - 0579

DIGESTIBLE NUTRIENT REQUIREMENT OF THE AFRICAN GIANT MUDFISH *HETEROBRANCHUS LONGIFILIS*

S. O. OVIE, S. O. E. SADIKU and S. I. OVIE

(Received 9 June 2005; Revision Accepted 14 February, 2006)

ABSTRACT

Heterobranchus longifilis fingerlings (3.12 ± 0.01g) were fed nine practical diets with protein /energy combination ranging from 34.26 to 45.84% crude protein and 450 and 500 kcal/100g gross energy for 56 days. Diets were formulated with locally available ingredients such as groundnut cake, toasted soybean (*Glycine max* Linn Merrill), fishmeal (*Pellonulla afzeluisi*) and guinea corn (*Sorghum sp*). All nine diets fed to *H. longifilis* resulted in the fish growing positively. The rearing was carried out in a mini flow- through system consisting of 27troughs of 26.4 litres water capacity. The digestible protein requirement of 27.04% for the fish was obtained when weight gain was regressed with digestible protein using polynomial third order solid curve. The digestible lipid requirement of 15.05% was obtained when weight gain was regressed with digestible lipid in a polynomial third order solid curve. The ratio of digestible protein to digestible lipid of 2.475 was obtained from a polynomial third order solid curve of weight gain against the ratio of digestible protein to digestible lipid.

KEYWORD: weight gain, digestible protein, digestible lipid, nutrition

INTRODUCTION

Digestibility measures the proportion of nutrients assumed to have been absorbed by the gut mucosa by quantifying the nutrients ingested and those voided in the faeces (Fagbenro, 2001).

The proportions of nutrients in the diet have an effect on digestibility. Page and Andrew (1973) indicated that decreased protein digestibility occurred in channel catfish when the diet had high levels of carbohydrates. High level of fibre in diet (10%) can inhibit lipid digestibility (Kurzinger *et al.* 1986). It is important when developing experimental diets to ensure that the addition of an ingredient does not alter digestibility values and other dietary components. (Appleford and Anderson 1997). Appleford and Anderson (1997) stressed that it is necessary to determine the digestibility of dietary ingredients because when they included 15% tuna oil to a range of diet the digestibility became lower than 10% inclusion levels.

Kurzinger *et al.* (1986) found that lipid content was significantly different for faeces remaining in the static aquarium water for 10 minutes and 24 hours. Anderson (1988) noticed that digestibility of higher lipid diets decreased and concluded that leaching did not have confounding effect on the determination of lipid digestibility. Encarnacao and Bureau (2000) stated that soybean, corn gluten meal, bone meal, feather meal, poultry by- product meal are very useful in formulating cost- effective diets provided their digestible composition and requirements of the fish are well- defined. Various studies have demonstrated that as meal size increases absorption efficiency decreases (Solomon and Brafield 1972). In order for a particular diet to be adopted by farmers there should be assurance that the nutrients are highly digestible to affect growth and healthy rearing.

According to Fagbenro (2001), digestibility estimation can be done directly or indirectly. According to Maynard *et al.* (1979) and Bondi (1987), Cr_2O_3 is biochemically inert. Two levels (0.5% and 1.0%) are commonly utilized in digestibility studies but chromic oxide is the most widely used.

Fagbenro (2001) citing Furukawa and Tsukahara (1966); Austreng *et al.* (1979); De Silva *et al.* (1990) reported the use of endogenous markers. This study sets out to use endogenous marker, the siliceous materials in the diets to determine the digestible protein, digestible lipid and the ratio of the digestible protein and digestible lipid of *H. longifilis*.

MATERIALS AND METHODS

Practical diets and experimental design

H. longifilis fingerlings (3.12 ± 0.01g) produced by induced breeding in the genetic laboratory of National Institute for Freshwater Fisheries Research, New Bussa, were acclimatized before being distributed: 15 fingerlings per 26 litres of water in 27 separate plastic troughs in a mini flow through system .The mini flow- through system was supplied with bio- filtered water from an impounded water reservoir using an overhead tank. Nine diets were tested in triplicate plastic troughs: diet I 34.26/500 kcal, diet II 35.20/450 kcal, diet III 36.27/450 kcal, diet IV 37.08/450 kcal, diet V 40.58/500 kcal, diet VI 41.53/450 kcal, diet VII 41.53/500 kcal, diet VIII 43.36/500 kcal and diet IX 45.84/500 kcal. These nine diets were the only combinations derived from the equation method of feed formulation. The protein/energy levels in the diets, the composition and proximate analysis of the test diets are presented in Table 1.

The faecal matter for each diet treatment was collected every 3 hours after feeding and siphoned into Whatman filter paper held in a funnel. Proximate analysis for the faecal matter is presented in Table 2 showing acid insoluble ash for digestibility studies.

Rearing, chemical and statistical analysis

In all the treatments feeding was done at 5% of the body weight of fish for daily ration. Each ration was divided into three equal portions, which were distributed at 8.00 hours, 13.00 hours, and 18.00 hours for 56 days. Sampling was done by bulk weighing of the fish fortnightly. Feed quantities were adjusted according to the mean weight of the fish per trough. The troughs were cleaned an hour after each feeding and complete exchange of water was done on sampling days. Since the experimental set-up is a mini-flow-through system there was a continuous flow of water in and out the troughs throughout the experimental period except when there was occasional erratic power supplies. At the beginning and end of the experiment 5 fingerlings were collected from each treatment for proximate carcass composition using established method of AOAC (1990). Some water quality parameters like water temperature, pH,

S. O. Ovie, National Institute for Freshwater Fisheries Research, P. M. B. 6006 New Bussa, Niger State, Nigeria.
S. O. E. Sadiku, Federal University of Technology, P.M.B. 65 Minna, Niger State, Nigeria.
S. I. Ovie, National Institute for Freshwater Fisheries Research, P. M. B. 6006 New Bussa, Niger State, Nigeria.

Table 1: Ingredients and proximate composition of diets containing varying ratios of protein energy fed to *H. longifilis* (g/100g)

Diets / Ingredients	I (g)	II(g)	III(g)	IV(g)	V(g)	VI(g)	VII(g)	VIII(g)	IX(g)
Soybean (Toasted)	22.91	1.92	1.40	21.79	22.35	0.29	0.85	21.24	20.68
Groundnut Cake	22.91	1.92	1.40	21.79	22.35	0.29	0.85	21.24	20.68
Fish meal (P. afzeluisi)	22.62	45.72	50.40	32.17	27.40	60.04	55.27	36.94	41.72
Guinea corn (*Sorghorm sp.*)	27.57	46.36	42.70	20.25	23.91	35.39	39.04	16.59	41.72
Premix (vitamin and mineral)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Binder (cassava starch)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Proximate composition of diets (% as fed)									
Moisture	6.40	4.50	6.37	6.50	6.71	6.61	6.89	6.82	6.70
Protein	34.26	35.20	36.27	37.08	40.58	41.53	41.53	43.36	45.84
Lipid	20.18	13.21	13.00	14.00	22.02	12.50	19.63	19.64	20.95
Ash	5.69	5.88	7.43	7.31	6.66	5.58	5.73	7.01	7.64
Crude fibre	10.53	3.77	6.00	28.00	7.34	24.04	10.28	17.86	13.33
Acid Insoluble Ash	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Energy Kcal/100g	500	450	450	450	500	450	500	500	500
Protein Energy ratio P/E	0.076	0.078	0.08	0.082	0.081	0.092	0.083	0.087	0.09

BIOMIX VITAMIN AND MINERAL PREMIX (mg)

Folic acid mg 800 4.00	Vitamin B12 mg 10.00 0.05	Biotin mg 160.00 0.80
Chloride mg 120 600	Inositol mg 40 200	Panthothenic acid mg 10,000.00 60.00
Betaine mg 40 200	Cobalt mg 400 2.00	Niacin mg 30,000 150.0
Iron mg 8000 40.00	Iodine mg 1000 40.00	
Manganese mg 6000 30.00	Copper mg 800 4.00	
Zinc mg 800 40.00	Selenium mg 40.00 0.20	
Methionine mg 20,000 100.00	Antioxidant mg 20,000.00 100.00	
Vitamin C mg 4000 20,000.00	Vitamin D3 mg 400,000.00 2000.00	
Vitamin B1 mg 4000.00 20.00	Vitamin K3 mg 16000.00 8.00	
Vitamin B6 mg 2,400.00 12.00	Vitamin B3 mg 6000.00 30.00	

Table 2: Percentage proximate composition of faecal material collected from *H. longifilis* fed varying ratios of protein energy

Diets fed/protein and energy ratios	Crude protein	Lipid	Ash	Acid insoluble ash
I 34.26/500	22.50^{a}	25.00^{d}	50.00^{d}	37.80^{dc}
II 35.20/450	29.70^{b}	16.70^{bc}	16.70^{a}	14.70^{a}
III 36.27/450	34.20^{c}	12.50^{b}	50.00^{d}	37.50^{dc}
IV 37.08/450	34.20^{c}	13.30^{b}	40.00^{c}	28.57^{cd}
V 40.58/500	40.50^{d}	22.20^{cd}	44.20^{cd}	33.33^{d}
VI 41.53/500	36.45^{cd}	20.00^{c}	26.70^{b}	26.67^{c}
VII 41.53/450	54.00^{c}	6.10^{a}	42.40^{c}	33.33^{d}
VIII 43.36/500	37.80^{cd}	20.00^{c}	26.70^{b}	15.38^{ab}
IX 45.84/500	40.50^{d}	16.70^{b}	41.70^{c}	33.33^{d}

Figures in the same column having the same superscript are not significantly different dissolved oxygen and conductivity were routinely taken using standard methods (APHA 1980)

Results of weight gain, specific growth rate, feed conversion, protein efficiency ratio and proximate carcass and faecal matter composition were pooled for each treatment, computed and analyzed using One-way Analysis of Variance (ANOVA) followed by Tukey's least significant different test and correlation coefficient for comparison among means. Polynomial regression third order solid curve of mean weight gain plotted against digestible nutrient requirement of *H. longifilis* was drawn using the computer packages of STATGRAPHICS (version 3.0) and MINITAB (version 10.1), computational method using the quadratic equation.

RESULTS

H. longifilis responded positively in terms of weight gain to all nine treatment (Table 3). The percentage survival in the experiment ranged from 51.11 to 91.11% (Table 3). There was significant difference ($P < 0.05$) between the percentage survival of the fish fed varying ratios of protein and energy during the study. Table 2 shows the proximate composition of faecal matter of *H. longifilis* fed the varying protein and energy practical diets. Table 2 shows that there was significant difference ($P < 0.05$) between the crude protein content of the faecal matter of the fish fed the varying diets.

Table 3: Growth of *H. longifilis* in a mini-flow through system using varying ratios of protein and energy combinations for 56 days

DIET	MIW (g)	MFW (g)	MWG (g)	SGR (%)	FCR	PER	ANPU	PS
I	3.11 ± 0.01^{a}	20.75 ± 16^{d}	17.64 ± 0.17^{c}	3.12 ± 0.27^{a}	2.46 ± 0.06^{a}	4.41 ± 0.04^{d}	1.99 ± 0.10^{a}	51.11 ± 2.22^{a}
II	3.11 ± 0.01^{a}	12.85 ± 0.92^{a}	9.74 ± 0.43	2.68 ± 0.17^{a}	2.26 ± 0.05^{a}	2.83 ± 0.27^{bc}	1.90 ± 0.10^{a}	80.00 ± 7.70^{ab}
III	3.11 ± 0.01^{a}	13.05 ± 0.59^{a}	10.74 ± 0.69^{a}	2.66 ± 0.07^{a}	2.42 ± 0.04^{a}	2.45 ± 0.16^{ab}	6.45 ± 0.10^{e}	77.78 ± 2.22^{ab}
IV	3.12 ± 0.01^{a}	11.88 ± 0.77^{a}	8.76 ± 0.77^{a}	2.59 ± 0.22^{a}	2.23 ± 0.04^{a}	1.95 ± 0.17^{ab}	4.56 ± 0.10^{c}	84.45 ± 9.69^{b}
V	3.13 ± 0.01^{a}	17.50 ± 0.52^{c}	14.37 ± 0.52^{bc}	3.07 ± 0.57^{a}	2.26 ± 0.02^{a}	3.38 ± 0.13^{c}	2.95 ± 0.05^{b}	62.22 ± 2.22^{ab}
VI	3.11 ± 0.01^{a}	12.76 ± 0.98^{a}	9.65 ± 0.98^{a}	2.67 ± 0.17^{a}	2.20 ± 0.08^{a}	2.04 ± 0.21^{ab}	5.63 ± 0.10^{d}	80.00 ± 7.70^{ab}
VII	3.11 ± 0.01^{a}	12.14 ± 0.60^{a}	9.03 ± 0.60^{a}	2.42 ± 0.09^{a}	2.13 ± 0.06^{a}	2.01 ± 0.13^{ab}	6.72 ± 0.10^{e}	91.11 ± 4.44^{b}
VIII	3.13 ± 0.003^{a}	11.85 ± 0.89^{a}	8.72 ± 1.19^{a}	2.51 ± 0.17^{a}	2.17 ± 0.04^{a}	1.83 ± 0.19^{a}	3.21 ± 0.01^{b}	86.67 ± 7.70^{b}
IX	3.11 ± 0.00^{a}	14.10 ± 0.40^{b}	10.99 ± 0.10^{ab}	2.77 ± 0.09^{a}	2.19 ± 0.10^{a}	2.27 ± 0.18^{ab}	1.58 ± 0.02^{a}	73.33 ± 3.85^{ab}
+ SEM	0.01 ± 0.003	2.83 ± 0.22	2.85 ± 0.255	0.22 ± 0.14	0.11 ± 0.02	0.80 ± 0.061	1.89 ± 0.04	11.85 ± 2.75

Figures in the same column having the same superscript are not significantly different

MIW (g) = Mean Initial Weight
SGR (%) = Specific Growth Rate
MFW (g) = Mean Final Weight
FCR = Food Conversion Ratio
MWG (g) = Mean Weight Gain
PER = Protein Efficiency Ratio
ANPU (g) = Apparent Net Protein Utilization
PS = Percentage Survival
MWG = MFW − MIW = Wt − Wo
SGR = 100*(lnWt − lnWo)
FCR = dry weight of feed/ total wet weight gain by fish
PER = total weight gain by fish/ protein intake

There was significant difference ($P < 0.05$) in the lipid content of the faecal matter (Table 2) from the fish fed various diets except for diets IV and IX. The acid insoluble ash content also showed a trend of variation in which faecal matter of fish fed diets I & III were not significantly different. Diets V, VII and IX were not significantly different ($P > 0.05$) at various levels although the latter group were significantly different from the former and other diets in the experiment ($P < 0.05$) (Table 2). The apparent digestibility coefficient, digestible protein and weight gains in the various treatments are presented in Table 4. There was no significant variation in the SGR and FCR fed the varying ratios of protein and energy ($P>0.05$). There was no significant variation for the PER of fish fed diets III, IV, VI, VII and IX ($P>0.05$) although these varied significantly from the PER of other diets in the experiment ($P<0.05$). There was no significant variation in the ANPU of fish fed diets I, II and IX ($P>0.05$). Also there was no significant variation in the ANPU of fish fed diets III, IV and VII ($P>0.05$). The ANPU of this latter group varied significantly from the former group and the ANPU of other diets in the experiment ($P<0.05$). The PS also varied

Table 4: Apparent digestibility coefficient, Digestible protein and mean weight gain of *H. longifilis* fed varying protein / energy ratios levels

Diet	% ADC	% CP	%DP	MWG
I 34.26/500	77.14	34.26	26.43	10.10
II 35.20/450	70.49	35.20	24.81	10.25
III 36.27/450	66.99	36.27	24.30	11.77
IV 37.08/450	67.87	37.08	25.17	13.64
V 40.58/500	65.07	40.58	26.41	13.14
VI 41.53/500	69.28	41.53	29.06	9.63
VII 41.53/450	54.49	41.53	22.63	13.40
VIII 43.36/500	69.49	43.36	30.13	11.94
IX 45.84/500	69.25	45.84	31.74	13.34

ADC - Apparent Digestibility Coefficient
CP - Crude Protein
DP - Digestible Protein
MWG - Mean Weight Gain

significantly. The digestible protein requirement as derived from the polynomial regression curve and calculation of quadratic equation was 27.04% (Fig. 1). The apparent digestibility coefficient of lipid fed, digestible lipid and weight gain is presented in Table 5. The digestible lipid requirement obtained when weight gain was regressed with digestible lipid levels of the diets using polynomial third order curve and quadratic equation was 15.05% (Fig. 2). Digestible protein and Digestible lipid, mean weight gain and the ratio of digestible protein/ digestible lipid are presented in Table 7.

The ratio of digestible protein to digestible lipid obtained when mean weight gain was regressed with the ratios using polynomial third order curve was 2.475 (Fig.3). Statistically it is possible for the solid curves of this nature to be slightly skewed in their presentation. The solving of the regression equation of the curve to derive the point at which dy/dx is equal to zero gives a more accurate answer for X- max (Hayman 1979; Popoola et al 1990). In the three graphs (Figures 1,2 and 3) skewness has created some discrepancies and so approximate values are obtained. According to Hayman (1979) the calculated values from the quadratic equation are more accurate and so 27.04%,

Table 5: Apparent digestibility coefficient, Digestible lipid and mean weight gain of *H. longifilis* fed varying protein/energy ratio

Diet	%.ADC LP	% LP	% DLP	MWG
I 34.26/500	60.44	20.18	12.20	10.10
II 35.20/450	69.26	19.10	13.23	10.25
III 36.27/450	78.13	21.00	16.41	11.77
IV 37.08/450	76.82	20.10	15.37	13.64
V 40.58/500	64.68	22.00	14.23	13.14
VI 41.53/450	82.92	20.00	16.58	10.9
VII 41.53/500	68.18	19.63	13.38	9.63
VIII 43.36/500	68.18	22.00	15.00	11.94
IX 45.84/500	73.62	22.02	16.21	14.29

ADC LP - Apparent digestibility coefficient for lipid
LP - Lipid
DLP - Digestible lipid
MWG - Mean weight gain

$$\text{ADC (\%)} = 100 - \frac{100 \times \text{Acid insoluble ash in diet} \times \text{nutrient in faeces}}{\text{Acid insoluble ash in faeces} \times \text{nutrient in diet}}$$

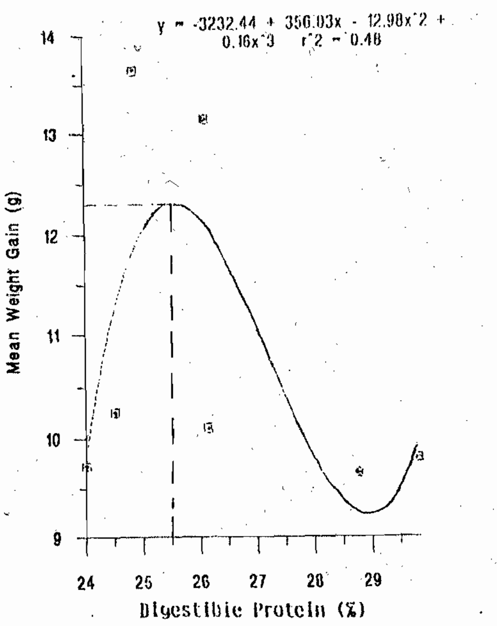

Fig. 1: Digestible protein requirement of *Heterobranchus longifilis* as derived

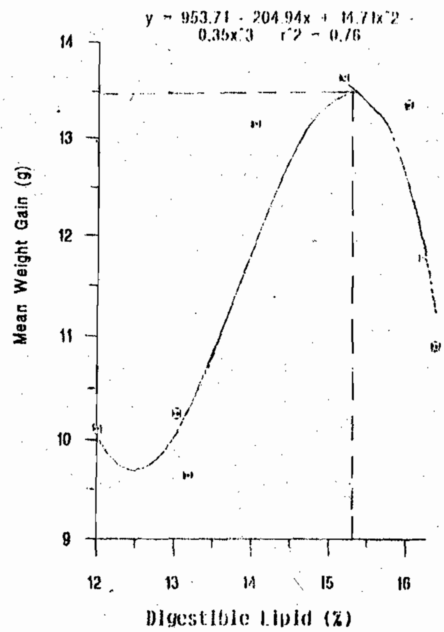

Fig. 2.: Digestible lipid requirement of *Heterobranchus longifilis* as derived

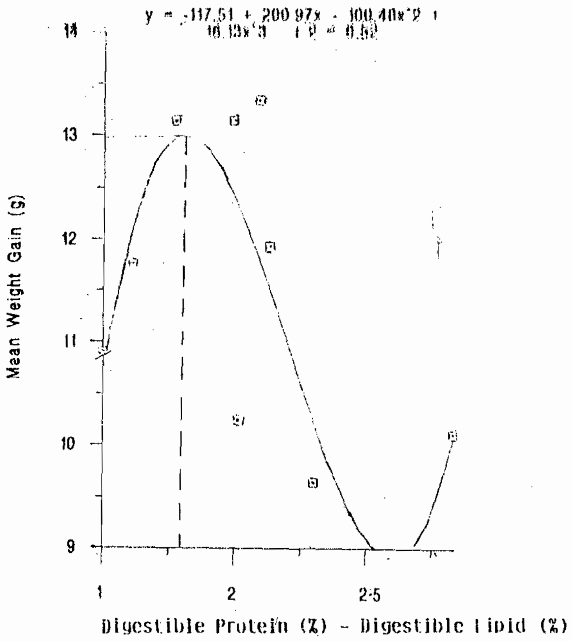

Fig. 3: Digestible protein - digestible lipid requirement of *Heterobranchus longifilis* as derived

Table 6: Digestible protein and digestible lipid of *H. longifilis* fed practical diets of varying protein / energy ratios.

Diets	% ADC* Protein	% ADC Lipid	Digestible Protein (DP)	Lipid	Digestible Lipid (DLP)
I 34.26/500	77.14	60.44	26.43	20.18	12.20
II 35.20/450	70.49	69.26	24.81	19.10	13.23
III 36.27/450	66.99	78.13	24.30	21.10	16.41
IV 37.08/450	67.87	76.83	25.17	20.10	15.37
V 40.58/500	65.07	64.68	26.41	22.00	14.23
VI 41.53/450	54.49	82.29	22.63	20.00	16.58
VII 41.53/500	69.28	68.18	29.06	19.63	13.38
VIII 43.36/500	69.49	68.18	30.13	22.00	15.10
IX 45.84/500	69.25	73.62	31.74	22.02	6.21

*ADC - Apparent Digestibility Coefficients

Table 7: Digestible protein/digestible lipid ratio of *H. longifilis* fed varying protein/energy ratios in practical feed

Diets	Digestible Protein (DP)	Digestible Lipid (DLP)	Mean Weight Gain	DP: DLP
I 34.26/500	26.43	12.20	10.10	2.70
II 35.20/450	24.81	13.33	10.25	1.88
III 36.27/450	24.30	16.41	11.77	1.48
IV 37.08/450	25.7	16.37	13.64	1.64
V 40.58/500	*26.41	14.23	13.14	1.86
VI 41.53/450	22.63	16.58	10.90	1.37
VII 41.53/500	29.06	13.38	9.63	2.17
VIII 43.36/500	30.13	15.10	11.94	2.0
IX 45.84/500	31.74	16.21	13.34	1.96

15.05%, and 2.475 are the digestible protein, digestible lipid and ratio of digestible protein to digestible lipid respectively.

DISCUSSION

H. longifilis is a fish of high aquaculture potential at commercial scale especially because of its fast growth and the high quality of its flesh. Enhancement of its diet utilization for growth will lower operational costs.

The digestible protein requirement derived in this study is 27.04% for *H. longifilis* fingerlings. This derived level must have been due to the proportion of nutrients in the diet (Page and Andrew, 1973). They showed that decreased protein digestibility occurred in channel catfish when the diet had high levels of carbohydrates.

De Silva *et al.* (1990) and Nandeesha *et al.* (1991) observed that the digestibility of protein from test ingredients decrease with level of inclusion of protein. In this study this was also the trend for diets with protein level between 35.20% to 40.58% thereafter there was no such relationship.

Digestible lipid requirement for *H. longifilis* is 15.05%. Schherbina (1973) recommended 2.5 to 3.0% fat for common carp yearly because lower levels of dietary lipids caused the fish to secrete endogenous fat into the intestine. Thus endogenous fat is lost when apparent digestibility values are calculated, it is not included. The trend earlier described by Nandeesha *et al.* (1991) was also noticed with all diets having 500 kcal/100g except for diet VII. Appleford and Anderson (1997) observed that lipid digestibility decreased with an increase in lipid inclusion from 10 to 15 %. Kurzinger *et al.* (1986) reported increase in lipid digestibility for faeces collected over 24hrs when compared with faeces collected over 10hrs although there was similarity for faeces collected over 6 and 10hrs. Anderson (1988) reported that lipid content was not significantly different for faeces remaining in static aquarium water for 10 minutes and 24 hours. In this study faecal matter was collected within 3hrs of its being voided since the time of collection of faeces does not have any effect on the result of digestibility there may not have been alteration of the digestible nutrient content in this experiment. As earlier observed by Anderson (1988) the longer the feed passes through the digestive tract the more nutrients are absorbed, collection of faeces only once during the experimental period is not enough to draw conclusion on how much more nutrients will be absorbed.

The ratio of digestible protein to digestible lipid for *H. longifilis* was 2.475. This ratio when utilized in the preparation of practical diet for the fish will go a long way to spare protein in the diet for growth while the lipid will be adequate for energy generation. Appleford and Anderson (1997) stated that it is important to ensure that the addition of an ingredient does not alter the digestibility value of other dietary components in the development of diets. Studies on *Acipenser baeri* (Medale *et al.* 1991) showed no effect of lipid on protein digestibility. Energy and protein are the two most important components of a diet. Failure to include adequate quantities of protein and energy in diet results in reduced growth, whereas excessive quantities of energy result in fat deposition or reduced feed consumption (NRC 1993). Fish generally are known to have low energy requirements than other animals. It is preferable for their energy needs to be met with the lipid and carbohydrate components of the feed. The use of protein in fish diets to generate energy is therefore undesirable. Kaushik and Medale (1994) estimated the relative cost of the use of protein to meet energy needs in fish and found it to be higher.

In conclusion the result shows that when 27.04% digestible protein and 15.05% digestible lipid or the ratio of 2.475 are considered in the formulation of practical diets for *H. longifilis*. The weight gain or growth response will be favorable. Although, the protein requirement of this fish has been studied (Eyo, 1995; Ovie, 2003) other aspects of nutrition such as the protein/energy requirement and digestible nutrient requirements should assist in the formulation of a feed that will boost its culture commercially. This study will go a long way to spare protein for energy production, reduce excesses in these nutrients, provide adequate energy and affect growth positively in the rearing of the fish.

REFERENCES

AOAC 1990. Official methods of analysis (15th edition) Association of Official Analytical Chemists, Virginia, 1298pp.

A.P.H.A 1980. Standard methods for analysis of water and waste water. The American Public Health Association. Washington D.C. 1270pp.

Anderson, T.A. 1988. The effect of feeding frequency on utilization of algal nutrients by the marine herbivore, the luderick, *Girella tricuspidata* (Quay and Gaimaid). Journal of Fish Biology, 37: 911 - 921

Appleford P. and Anderson T. A. 1997. Apparent digestibility of tuna oil for common carp, *Cyprinus carpio*- effect of inclusion level and adaptation time. Aquaculture 148: 143-151

Austreng, E., Skrede, A. and Eldegard, A. 1979. Effect of dietary fat source on the 'digestibility of fat and fatty acids in rainbow trout and mink. Acta. agric Scand. 29: 119 – 129.

Bondi, A. A. 1987. Animal nutrition. John Wiley and Sons, New York. 540pp.

De Silva, S. S. and Perera, M. K. 1983. Digestibility of an aquatic macrophyte by the Cichlid *Etiopus suratensis* (Bloch) with observations on the relative merits of three indigenous components as markers and daily changes in protein digestibility. Journal of Fish Biology. 23: 675 – 684.

De Silva, S.S. and Perera, M.K. 1984. Digestibility in *Sarotherodon niloticus* fry: effect of dietary protein level and salinity with further observations on variability in daily digestibility. Aquaculture, 38: 293 - 306

De Silva, S. S., Shim, K. F. and Ong, A. K., 1990. An evaluation of the method used in digestibility estimations of a dietary ingredient and comparisons on external and internal markers, and time of faeces collection in digestibility studies in fish *Oreochromis aureus* (Steindachner) Reprod. Nutr. Dev. 30: 215 – 226.

Encarnacao, P. and Bureau D. P. 2000. Essential amino acid requirements of fish: a matter of controversy. Fish Nutrition Research Laboratory and Department of Animal and Poultry Science University of Guelph, Guelph, Canada.www/uoguelph.ca/fishnutrition.10p

Eyo A.A.1995. Dietary protein requirement of *Heterobranchus longifilis*. National Institute for Freshwater Fisheries Research (N.I.F.F.R) Annual Report. 118 - 125p.

Fagbenro, O. A. 2001. Feedstuff digestibility in culturable freshwater fish species in Nigeria. Proceedings of the First National Symposium on Fish Nutrition and Fish Feed Technology held at the Nigerian Institute for Oceanography and Marine Research (NIOMR), Lagos 26th – 29th October, 1999. Fisheries Society of Nigeria,pp. 26 – 37.

Furukawa, A. and Tsukahara, H. 1966. On the acid digestion for the determination of chromic oxide as the index substance in the study of feed. *Bull. of Jpn. Soc. For Fish.* 32: 502 – 506.

Hayman Margaret 1979. Essential Mathematics. Macmillan education. p138.

Kurzinger, von H., Schwarz, F. J. and Kirchgessner, M., 1986. Measurement of digestibility in carp (*Cyprinus carpio* L.) J. Anim physiol. Anim . Nurt., 56: 208 – 217.

Kaushik, S. J. and Medale, F. 1994. Energy requirements, utilization, and dietary supply to Salmonids. Aquaculture. 124: 81-97.

Medale, F., Blanc, D. and Kaushik, S.J., 1991. Studies on the nutrition of Siberian Sturgeon Acipencer baeri II. Utilizaton of dietary non - protein energy by sturgeon. Aquaculture, 93: 143 - 154.

Maynard, L. A., Loosli, J. K., Hintz, H. F. and Warner, R. G.1979. Animal Nutrition, 7th ed. McGraw – Hill, New York, 620pp.

Nandeesha, M. C., Srikanth, G.K., Kesavanath, P. and Das, S.K. 1991. Protein and fat digestibility of five feed ingredients by an Indian major carp, *Catla catla* (Ham.). Asian Fish Soc.Spec. Publ., 5: 75 – 81.

NRC (National Research Council) 1993. Nutrient Requirements of Fish. National Academy Press, Washington, D.C. 114pp.

Ovie, S.O. 2003. Macro- nutrient requirements of the giant African mudfish *H. longifilis* (Val. 1840) Ph.D. Thesis Federal University of Technology, Minna, Niger State, Nigeria. 216p.

Page , J. W. and Andrews, J.W. 1973. Interaction of dietary levels of protein and energy on channel catfish (*Ictalurus punctatus*). Journal Nutrition. 102: 1339-1346.

Popoola O., Ejike M.C., Osondu C.N. and Adeniyan A.1990. Effective Mathematics for Secondary Scchools, Book One. Evans Brothers (Nigeria Publishers) ltd. p70.

Schherbina, M. A.1973. A study of the digestive processes in the carp (*Cyprinus carpio* L.) Communication I. Absorption of crude fat in the intestine from synthetic diets. *Journal of Icthiology*. 13: 104 – 111.

Solomon, D. J. and Brafield, A.G. 1972. The energetics of feeding metabolism, and growth of perch (*Perca fluviatilis* L.). Journal of Animal Ecology. 41: 699-718.

Tacon, A. G. J. and Rodrigues, A. M. P. 1984. Comparison of chromic oxide crude fibre , polyethylene and acid insoluble ash as dietary markers for estimation of apparent digestibility coefficients in rainbow trout. Aquaculture, 43: 391 – 395.