SECTION C:
AQUACULTURE PRACTICES AND FISH PRODUCTION

EFFECTS OF LIPID ON GROWTH AND FEED UTILIZATION OF *HETEROBRANCHUS LONGIFILIS* FINGERLINGS.

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Abstract

A study was carried out to examine the effect of lipid level on growth and feed utilization of *Heterobranchus longifilis*. Fingerlings of *H. longifilis* (28 days old (0.7 ± 0.05g) were fed three formulated diets with three levels of lipid (7.00%, 12.5% and 18.0% of dry matter) at one level of protein (35.0% crude protein dry matter (DM) basis) for eight weeks. Weight gain (g) and specific growth rate (SGR, % day^{-1}) values indicated that fish fed diets with 7.0% and 12.5% lipid exhibited higher growth performance. Lowest growth was recorded for fish fed diet with 18.0% lipid. Feed intake (FI, g $fish^{-1}$) was also significantly ($P < 0.05$) affected by dietary lipids and tended to decrease with increasing lipid levels. Fish that showed the highest FI were those that were fed the 7% and 12.5% lipid diets. Feed conversion ratio (FCR) values indicated that diets containing 12.5% and 18% lipid were more efficiently utilized. No significant differences in muscle composition were observed among fish fed the different diets. There was a linear relationship ($P<0.05$) between dietary lipid level and liver lipid. Hepatosomatic index (HIS) increased with dietary lipid level. The results indicated that *H. longifilis* fingerlings performed best with the diets containing 7.0% and 12.5% lipid when protein concentration was 35.0% and, reduced growth and increased body fat were evident when dietary energy increased from 19.06 to 20.54 kJ g^{-1}.

Key words: Dietary lipids, growth, feed utilization, liver lipid, *Heterobranchus longifilis*

Introduction

In the last years there has been a trend in commercial fish feed formulations to increase dietary lipid levels to improve feed utilization for the optimization of production. Dietary protein is the most important factor affecting growth performance and feed cost (Lovell, 1989). Generally, increasing protein level in the diets can lead to improved fish production. Protein utilization for growth may be improved by partially replacing dietary protein with lipid to produce a protein-sparing effect. However, excessive energy in diets can lead to increased body lipid deposition and growth reduction of fish because of a lack of necessary nutrients for growth resulting from a reduction in feed consumption (Daniels and Robinson, 1986). On the contrary, insufficient non-protein energy in diets causes protein waste as the proportion of dietary protein used for energy increases, and ammonia excreted after amino acids are metabolized can reduce water quality (Phillips, 1972; Shyong *et al.*, 1998). Therefore, it is important to improve dietary protein utilization for body protein synthesis rather than for energy purposes. A protein-sparing effect associated with increasing dietary energy level has been reported for several species of fish (Cho and Kaushik, 1990; Peres and Oliva-Teles, 2001, 2002; Boujard *et al.*, 2004). Higher energy levels generally come from increased dietary lipid as lipid is an energy-dense nutrient and readily metabolised by fish (NRC, 1993).

The use of the liver as an indicator organ of the nutritional and physiological status in fish is well-known (Hibiya, 1982; Storch and Juario, 1983; Segner and Juario, 1986). In *Clarias gariepinus* larvae, Verreth *et al.* (1994) showed that lipid volume in the liver of larvae fed high HUFA-enriched *Artemia* was higher than in livers of larvae fed low HUFA-enriched *Artemia*, probably due to different digestibilities. Also, these authors concluded that feeding level can result in an accumulation of lipid in the liver and be the most decisive parameter for larval growth and metabolic performance of the liver than feed type. *Heterobranchus longifilis* (Valenciennes, 1840) is an economically important food fish. It has High yield potentials, rapid growth and high fecundity among other qualities that makes it suitable for commercial culture. Although the dietary crude protein requirement for *H. longifilis* has already been studied by Fagbenro *et., al* (1992), no information about the protein-sparing effect of dietary lipids is available for this species. The present study, therefore, was conducted to evaluate the effect of lipid levels in a practical diet on growth and body lipid deposition of *H. longifilis* fingerlings.

Materials and Methods

Heterobranchus longifilis (Valenciennes, 1840) fingerlings were obtained from National Institute of Freshwater Fisheries Research Hatchery. They were acclimated for two (2) weeks prior to the start of the experiment during which time they were fed the diet containing 7% lipid. Feeding was stopped 48 hours to the commencement of the feeding trial. The fingerlings with initial average weight of 0.7 ± 0.05g were sorted, grouped, weighed and were randomly assigned into groups of twenty per 30-l circular plastic bowls in a static experimental system. Each dietary treatment had three replications and the experiment was conducted for 8 weeks.

Experimental diets were formulated using practical ingredients, with increasing amounts of cod liver oil incorporated to provide levels of 7, 12.5 and 18% lipid in isonitrogenous diets. The various ingredients were milled, weighed, mixed and pelleted by passing the cold dough through a meat mincer with 2mm die after which it was sun dried. The dry pellets were packed in polythene bags, sealed and stored at 20°C until used. The composition and proximate analysis of the experimental diets are given in Table 1. The experimental diets were fed to triplicate groups of fish for eight weeks at feeding rate of 5% of body weight per day, sub divided into two equal feeding at 09:00, and 15:00 daily. Growth and feed efficiency were monitored weekly by collectively weighing each group of fish and feeding rate adjusted accordingly. An initial sample of fish was sacrificed prior to the start of the experiment and subjected to proximate analysis (AOAC, 1995). A final carcass sample of three fish per replicate was treated similarly.

Water quality parameters were monitored in the course of the feeding trial and average values for temperature, dissolved oxygen, hydrogen ion concentration (pH) and conductivity were 28 ± 2.5°C, 5.8 ± 1.1mg/l, 7.2 ± 0.4 units and 220 ± 2.2 µmhos cm^{-3} , respectively

The data were subjected to one-way ANOVA to test the effects of dietary lipid levels. Where significant ($P < 0.05$) differences were detected, Duncan's (1955) multiple range tests was used to rank the means. The data are presented as means S.D. of three replicates. All statistical analyses were carried out using the SPSS Version 10.0 .

Results

Weight gain, SGR, and FI values indicated that fish fed diets containing 7% or 12.5% g/100g lipid (diet 7L and 12.5L) were significantly higher than those observed in other treatment ($P < 0.05$). Fish fed the 18% lipid diet had the lowest growth ($P < 0.05$). The total FI (g) and SGR were also significantly ($P < 0.05$) affected by dietary lipid levels and tended to decrease with increasing lipid levels. The fish that showed the maximum FI were those fed with the 7L and 12.5L diets (Table 2). The FCR values indicate that the 12.5L and 18L diets were the most efficiently utilized. The fish fed the 18L diet showed the highest PER.

The effect of dietary lipid levels on muscle and liver composition are presented in Table 3. Dietary lipid level had no significant effect ($P > 0.05$) on lipid, protein and dry matter contents of muscle. Lipid content of liver increased with increasing dietary lipid levels. Protein content showed a declining trend with increasing dietary lipid level ($P < 0.05$). The concentration of protein in the liver varied significantly ($P < 0.05$) in relation to the dietary treatment; with the greatest value corresponding to the fish fed the 7L (20.13%), and followed by 12.5L (17.30%). The lowest protein in the liver was observed among fish fed 18L diet. The HIS also increased with the lipid levels in the experimental diets ($p < 0.05$, Table 3).

Discussions

Increasing lipid level of fish feeds has been shown to be an effective approach to improving feed efficiency and protein utilization, and decreasing N waste outputs and feed costs. Moreover, the nutritional strategy for protein sparing effect is to increase adequate amount of lipid in fish diet to reduce protein inclusion without compromising growth (Sargent *et al.*, 2002; Ai *et al.*, 2004).

In this study, effects of increasing dietary lipid level were observed on growth, SGR, FCR and PER of *H .longifilis* fingerlings. The growth response data indicated that the maximum growth was obtained at 7% and 12.5% L. These findings are similar to results reported for other fish species, like

red drum, black rockfish, red sea bream and Asian seabass, which have shown that lipid level improved growth of fish (Takeuchi *et al.*, 1991; Craig *et al.*, 1999; Lee *et al.*, 2002; Williams *et al.*, 2003). However, increased dietary lipid to more than 12.5% in 35% protein diet did not improve weight gain, SGR and PER of *H .longifilis* fingerlings. Some authors have reported that high dietary lipid level might depress growth in some species (Espinós *et al.*, 2003; Pei *et al.*, 2004; Du *et al.*, 2005). The growth reduction at high lipid levels could be due to the limited ability to digest and absorb high amounts of lipid, a reduction in feed intake, excess lipid accumulation in liver and other visceral organs, or creation of dietary or metabolic imbalances (Luo *et al.*, 2005). At the end of the trial, FCR was significantly lower ($P<0.05$) for fish fed the diet with the highest lipid level (18%) than fish fed the other diets. Dietary lipids had a significant effect on FCR. Although, the higher performance among fingerling *H. longifilis*, in terms of growth was observed at diets 7L and 12.5L, the best FCR values were achieved with the diets containing the highest lipid levels (12.5 and 18 g/100 g). In this study, FI of *H. longifilis* was regulated by the diet lipid level supplied by the diets ($P<0.05$). Other studies have reported that the amount of digestible energy (DE) regulated the amount of feed ingested by the fish (Lupatsch *et al.*, 2001; Peres and Oliva-Teles, 2001) and that growth decreased with increasing dietary energy content (Ellis and Reigh, 1991).

The fact that in this experiment, the diets 12.5L and 18L had the higher FCR values suggests that nutrient dense feed could be an effective tool to improve feed utilization and minimize waste outputs. It appears that the fish that were fed 18L diets did not grow as well as the others because the higher amount of lipid reduced consumption, and therefore decreased growth. The fish with the higher growth consumed more feed and consumed a higher amount of lipid than the fish fed the high lipid diet.

Because protein retention is generally regulated by non-protein energy input of the diet, PER is a good measure of the "protein sparing effect" of lipid (Lie *et al.*, 1988). Fish fed on 7% resulted in significant difference in PER (2.55 ± 0.06) compared with the rest of the treatments. Many studies in other fish species have demonstrated the result of a protein sparing effect of lipid (Morales and Oliva-Teles, 1995; Vergara *et al.*, 1996; Van der Meer *et al.*, 1997; Dias *et al.*, 1998; Peres and Oliva-Teles, 1999, 2001, 2002; Boujard *et al.*, 2004).

No significant differences in muscle dry matter, protein or lipid content were observed in *H. longifilis* offered the experimental diets. However, there was a strong relationship between the dietary lipid levels and the levels of lipid in the liver. This agrees with many other studies that have shown that dietary lipid levels correlate strongly with liver lipid content but not with muscle composition (Peres and Oliva-Teles, 2001; Nanton *et al.*, 2001). The HSI also increased with increasing lipid levels in test diets. The accumulation of fat in the liver of *H. longifilis* fingerlings fed the diets 12.5L and 18L suggests that *H. longifilis* have a limited ability to metabolize lipids.

In conclusion, *H. longifilis* fingerlings (28 days old, 0.7 ± 0.05 g) performed best with the diets containing 7% and 12.5% lipid when protein concentration was 35%. Reduced growth and increased body fat were evident when dietary energy increased from 19.06 to 20.54 kJ g^{-1}.

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Table 1 Composition and proximate analysis of the experimental diets.

	Diets		
	7L	12.5L	18L
Ingredients (percentage dry weight)			
Fish meal	29.00	29.00	29.00
Soybean meal	27.00	27.00	27.00
Cod liver oil	3.02	8.52	14.02
Maize	36.78	31.28	25.78
Cassava starch	2.00	2.00	2.00
Vitamin/mineral mixture[a]	2.00	2.00	2.00
Salt (NaCl)	0.20	0.20	0.20
Proximate analysis (percentage dry weight)			
Moisture	6.33	6.69	6.31
Crude protein	35.10	35.1	34.98
Crude lipid	7.10	11.95	17.97
Ash	9.82	9.12	8.52
Crude fibre	1.42	1.39	1.37
Nitrogen free extract[b]	40.23	35.54	30.85
Gross energy (MJ/kg)[c]	17.92	19.06	20.54

[a] supplied the following (per kg of diet): calcium, 4500mg; phosphorus, 4200mg; potassium, 1700mg; magnesium, 400mg; iron, 30mg; zinc, 30mg; manganese, 20mg; copper, 5mg; iodine, 1mg; selenium, 0.25mg; vitamin A, 5000IU; vitamin D, 2000IU; DL--tocopherol acetate, 100mg; menadione, 15mg; thiamine hydrochloride, 5mg; riboflavin, 10mg; pyridoxine hydrochloride, 10mg. Panthothenic acid, 35mg; nicotinic acid, 50mg; biotin, 0.5mg; folic acid, 2mg; ascorbic acid, 200mg; inositol, 250mg; choline, 400mg; vitamin B_{12}, 0.1mg and ethoxyquin, 60mg. [b]calculated by difference (100 crude protein crude lipid ash crude fibre).
[c]Based on 23.4 MJ/kg protein, 39.2 MJ/kg lipid and 17.2 MJ/kg NFE

Table 2 Growth performance and feed efficiency of *Heterobranchus longifilis* fingerlings fed the experimental diets[a].

	Diets					
	7 L		12.5 L		18 L	
Initial body weight (g)	0.70	0.01	0.70	0.01	0.69	0.02
Final body weight (g)	6.960	0.47[b]	6.87	0.22[b]	5.95	0.16[a]
Weight gain	6.26	0.21[b]	6.15	0.32[b]	5.26	0.06[a]
Specific growth rate [b]	4.10	0.09[b]	4.07	0.01[b]	3.85	0.37[a]
Feed intake	6.99	1.54[c]	6.59	1.68[b]	5.37	0.20[a]
Feed conversion ratio [c]	1.12	0.06[b]	1.07	0.08[ab]	1.02	0.01[a]
Protein efficiency ratio [d]	2.55	0.06[a]	2.64	0.19[ab]	2.80	0.21[b]

[a] Figures in the same line with different superscript letters are significantly different ($P < 0.05$). Mean standard deviation.
[b] SGR: [(ln final body weight ln initial body weight)] / (number of days) X 100.
[c] FCR: wet weight gain / dry feed intake.
[d] PER: wet weight gain / crude protein intake.

Table 3 Muscle and liver composition (dry weight) and hepatosomatic index (HIS) of *Heterobranchus longifilis* fingerlings fed experimental diets for eight weeks[a].

	Diets					
	7 L		12.5 L		18 L	
Muscle						
Dry matter (g/100g)	19.97	0.19	19.80	0.82	19.70	1.50
Protein (g/100g)	40.63	0.34	40.06	0.21	39.70	0.54
Lipids (g/100g)	6.04	0.04	6.03	0.09	6.04	0.18
Liver						
HIS [b]	2.17	0.03[a]	2.67	0.17[b]	2.83	0.12
Dry matter (g/100g)	50.13	0.20[a]	52.17	0.12[c]	50.97	0.14[b]
Protein (g/100g)	20.13	1.11[b]	17.30	0.07[a]	16.17	0.30[a]
Lipids (g/100g)	11.17	0.10[a]	11.39	0.11[b]	13.69	0.02[c]

[a] Figures in the same line with different superscript letters are significantly different ($P < 0.05$). Mean standard deviation.

[b] Hepatosomatic index: (liver weight / body weight) X 100.