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Effects of Energetic Diets on Growth, Blood Chemistry, and Liver Pathology of African Catfish, *Clarias gariepinus* (Burchell 1822)

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Abstract

The effects of isonitrogenous diets (350 g crude protein/kg diet) with different energy levels (10.85, 11.82, 12.73, 13.69, and 15.06 MJ dietary energy/kg feed) on growth, feed utilization, blood chemistry, and liver histopathology of African catfish, *Clarias gariepinus*, were investigated to determine the optimum diet for this species. The diet containing 12.73 MJ digestible energy/kg feed resulted in the best growth, blood parameters, and liver histology. Fish that consumed the 10.85 and 11.82 diets had similar weight gains, feed, and protein utilization as fish fed diets containing 13.69 or 15.06 MJ (p>0.05) but fish fed diets containing 13.69 or 15.06 MJ/kg had signs of hepatic lipidosis.

Introduction

African catfish (*Clarias gariepinus*, Claridae family) is suitable for culture in a variety of environments including cage, raceway, and pond, in monoculture or polyculture subtropic systems (Huisman and Richter, 1987). They can utilize feed constituents such as dry brewery wastes, rice bran, ground maize, cotton seed cake, sesame cake, and blood meal. In intensive culture, they require formulated feed to meet their macro and micro nutrient requirements. A diet with an optimum balance between protein and energy results in a higher growth rate and better economic results. However, while high energy diets that include lipids may improve growth and protein utilization, they may also cause problems such as rancidity, off-flavor, and deterioration of meat quality due to oxidation (Scaife et al., 2000).

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Most earlier studies on African catfish diets examined their effects on growth performance, feed utilization, and chemical composition of the fillet (Henken et al., 1986; Lim et al., 2001; Giri et al., 2003). The aims of this study were to examine blood biochemistry parameters and determine the optimum diet for obtaining the best growth and feed utilization in relation to blood parameters and liver pathology.

Materials and Methods

Two hundred juvenile African catfish (10-15 g) were divided into five diet groups with four replicates of 10 fish, each. After a 15-day acclimization, the fish were stocked in 96-l aquaria and fed ad libitum (at 9:00 and 17:00) for 180 days. Water was totally exchanged daily in all aquaria before the first feeding by siphoning. Aeration was supplied continuously. Water temperature, dissolved oxygen, pH, and total alkalinity varied 25.5-26.5°C, 5.5-6.6 mg/l, 7.65-8.01, and 250-256 mg/l $CaCO_3$, respectively.

Five isonitrogenous (35% crude protein) pelleted diets with different energy levels were prepared (Table 1). For each diet, the major ingredients were ground (<500 μ) and mixed. Warm water (40°C) and the lipid source were added to the blend. The resultant dough was

Table 1. Ingredients and chemical composition (g/kg diet on dry matter basis) of experimental diets.

	Diet				
	I	*II*	*III*	*IV*	*V*
Ingredient					
Fish meal	250	250	260	270	280
Soybean meal	250	270	280	300	320
Corn meal	190	180	190	190	150
Barley meal	100	80	60	10	10
Wheat bran	50	30	10	10	0
Cotton seed cake	130	120	95	70	40
Soy-acid oil	0	40	85	130	180
Premix*	10	10	10	10	10
Corn starch	10	10	0	0	0
DCP	10	10	10	10	10
Chemical composition					
Crude protein	356	358	352	357	353
Crude lipid	36	70	110	154	205
Nitrogen free extract	422.4	401.2	367.5	309.2	281.1
Digestible energy (MJ/kg diet)	10.85	11.82	12.73	13.69	15.06

* Premix (per kg diet): vitamin A, 5,000,000 IU; vitamin D, 1,250,000 IU; vitamin E 12,500 mg; vitamin K_3, 1250 mg; vitamin B_1, 750 mg; vitamin B_2, 2000 mg; niacin, 15,000 mg; vitamin B_5 5000 mg; vitamin B_6, 1750 mg; vitamin B_{12}, 8 mg; folic acid, 375 mg; biotin, 25 mg; vitamin C, 50,000 mg; choline choline, 225,000 mg; carophyll red, 12,500 mg; carophyll yellow, 2500 mg; Mn, 50,000 mg; Fe, 50,000 mg; Zn, 50,000 mg; Cu, 10,000 mg; Co, 150 mg; I, 800 mg; Se, 150 mg.

passed through a 2-mm diameter die in a food grinder. The pellets were dried at 45°C and stored at 4.0±1.0°C until use. Digestible energy (DE) values were estimated using values adopted for channel catfish of 14.6 MJ/kg protein, 33.9 MJ/kg lipid, and 10.5 MJ/kg carbohydrate (NRC, 1993).

The proximate compositions of the diets were analyzed according to AOAC (1997) procedures as follows: moisture was determined by oven-drying at 105°C for 24 h, crude protein (N x 6.25) by the Kjeldahl method, and crude ash by combustion in a muffle furnace at 550°C for 16 h. Total lipid concentrations were determined by the chloroform-methanol extraction method described by Bligh and Dyer (1959). After 180 days, growth parameters were calculated as follows: daily feed intake = total feed consumed/180; daily energy intake = total energy consumed/180; daily protein intake = total protein consumed/180; feed conversion ratio = feed intake/weight gain; protein efficiency ratio = weight gain/protein intake; apparent net protein retention = ([final wet wt in g x final wet body protein in %] - [initial wet wt in g x initial wet body protein in %]/protein intake in g) x 100; apparent net energy retention = ([final wet weight in g x final wet body energy in %] - [initial wet weight in g x initial wet body energy in %]/energy intake in g) x 100.

At the end of the trial, the fish in each treatment were pooled and five were randomly selected to determine serum parameters. About 3 ml blood was drawn from the caudal vein and samples were immediately transferred to individual silicone-coated EDTA free Vacutainer Tubes (Becton Dickinson). Blood samples were promptly centrifuged at 2500 rpm for 5 min, and the serum was removed with a disposable transfer pipette (Shakoori et al., 1996; Wood et al., 1996). Blood serum was analyzed with an autoanalyzer (Roche P-800).

Hepatopancreas specimens were manually fixed in 4% buffered formaldehyde for histology and embedded in paraffin wax. Sections (5 µ) were mounted on glass slides, stained with Mayers hematoxylin and eosin, and examined and photographed under light trinocular microscopy (Olympus CH40; Genc et al., 2005).

Growth performance and serum parameters were statistically analyzed with one-way ANOVA and Duncan multiple range tests (SPSS for windows, 10.01). Differences were considered significant at 5% (p<0.05).

Results

Fish fed the diet containing 12.73 MJ digestible energy/kg (diet III) resulted in the best weight gain, feed conversion ratio, protein efficiency ratio, and protein and energy retention (Table 2). Daily feed and protein intakes decreased as the dietary energy level increased while daily energy intake did not significantly differ.

Glucose concentrations significantly differed among groups (Table 3). Liver enzyme and bilirubin levels decreased as dietary energy increased while lactate dehydrogenase increased. Blood lipids (cholesterol and triglycerides), total serum protein, albumin, and globulin increased with the dietary energy. Blood urea nitrogen and creatinine levels did not differ while uric acid, sodium, potassium, chloride, and calcium rose and phosphorus dropped as the energy level rose.

In post-mortem examinations, liver hypertrophy and pale yellowish color were observed in fish fed diets IV and V (Fig. 1).

Discussion

Increasing the dietary energy level improved growth performance and feed utilization up to the level of 12.73 MJ DE/kg diet (diet III). Although the final weight of fish fed the diet with the lowest energy level (diet I) did not significantly differ from that of the fish fed diet III, diet III resulted in the best weight gain, feed conversion and efficiency, and protein and energy retention. Enhancement of feed and protein efficiency with higher dietary energy levels was reported for other cultured fish species (Meske and Becker, 1981; Akyurt and Erdogan, 1994; Helland-Grisdale and Helland, 1997; Keembiyehetty and Wilson, 1998; Santina et al., 1999; Thoman et al., 1999; Lee et al., 2002). Although energy intake and retention did not statistically differ among

Table 2. Growth performance, feed intake, and utilization parameters of African catfish fed experimental diets with different energy levels for 180 days (means of four replicate groups of ten fish, each).

	Diet				
	I	*II*	*III*	*IV*	*V*
Initial body wt (g)	14.32±0.16^{a}	14.27±0.17^{a}	14.12±0.23^{a}	14.22±0.14^{a}	14.15±0.11^{a}
Final body wt (g)	160.92±13.73^{a}	139.80±17.16^{ab}	166.38±12.52^{a}	134.96±19.91^{ab}	104.84±8.03^{b}
Daily feed intake (g)	12.04±0.93^{a}	10.08±0.53^{b}	9.75±0.36^{bc}	8.01±0.75^{cd}	7.53±0.30^{d}
Feed conversion ratio	1.72±0.05^{a}	1.62±0.17^{ab}	1.34±0.06^{b}	1.53±0.12^{ab}	1.67±0.11^{ab}
Protein efficiency ratio	1.85±0.04^{a}	2.19±0.18^{ab}	2.47±0.08^{b}	2.26±0.15^{b}	1.83±0.11^{a}
Protein retention (%)	31.42±0.88^{a}	32.65±3.05^{a}	39.34±1.56^{b}	35.99±2.76^{ab}	31.31±2.11^{a}
Energy retention (%)	16.58±0.51^{a}	16.77±1.75^{a}	19.76±0.85^{a}	16.44±1.44^{a}	16.02±1.06^{a}

Values in rows with different superscripts significantly differ (p<0.05).

treatments, the highest energy retention was obtained with fish fed diet III, supporting the common opinion that feed intake is regulated by available dietary energy (Lee and Putnam, 1973). Based on our results, the diet with a P:E ratio of 27.65 (352 g crude protein and 12.73 MJ/kg diet) was adequate for growth in African catfish fingerlings.

Due to differences in the chemical compositions of the diets, glucose ranged 153-208 mg/dl, similar to results of other researchers who reported that serum glucose ranges 25-350 mg/dl depending on fish species (Nikolsky, 1963; Tewari et al., 1987; Kaminska et al., 1988; Folmar et al., 1992; Jeney et al., 1995; Shakoori et al., 1996). Transaminases (aspartate aminotransferase [AST], alanine aminotransferase [ALT], and alkaline phosphatase [ALP]) are important enzymes for monitoring the health status of fish (Racicot et al., 1975). Lactate dehydrogenase (LDH) is most often measured to evaluate the presence of tissue damage, especially liver pathology. The higher LDH concentrations in fish fed diets IV and V might have been caused by liver degeneration. Steffens et al. (1999) claimed that plasma activities of creatinekinase, AST, ALT, and LDH did not differ among fish fed diets with different lipid levels. Our results, however, indicate that the dietary energy level influenced all enzyme activities, especially in fish fed diets IV and V. This may have been due to inhibited liver function. Decreased levels of bilirubin are due to inefficient liver function, excessive fat digestion, and possibly a diet low in nitrogen bearing foods. This is true for fish that consume a higher amount of dietary energy (especially those fed diet IV or V), resulting from a lower intake of protein due to earlier satiation.

Serum lipids (cholesterol and triglycerids) dramatically increased with the dietary energy level, as established by Rehulka and Parova (2000). The increased total protein, albumin, and globulin concentrations in fish fed diet IV or V may be an indicator in liver pathology. The level of uric acid biosynthesis is an indicator of the need for amino acids and the nutritive value of feeds (Koudela et al., 1983; Rehulka and Minarik, 2003). It seems, therefore, that

Table 3. Blood serum of African catfish fed diets with different energy contents for six months (means±SEM of four replicates of five fish, each).

Parameter	*Diet*				
	I	*II*	*III*	*IV*	*V*
Glucose (mg/dl)	208±1.02^{a}	195±0.71^{b}	183±1.07^{c}	170±0.86^{d}	153±1.00^{e}
AST (U/l)*	226.4±0.66^{a}	219.8±0.28^{b}	207.9±0.16^{c}	185.8±0.69^{d}	184.6±0.29^{d}
ALT (U/l)*	40.2±0.14^{a}	37.3±0.13^{b}	31.2±0.12^{c}	23.1±0.11^{d}	21.4±0.15^{e}
ALP (U/l)*	411.8±0.66^{a}	409.8±0.37^{b}	407.6±0.24^{c}	403.2±0.20^{d}	400.8±0.20^{e}
LDH (mg/dl)	845.6±1.72^{a}	996±1.41^{b}	1030±1.41^{c}	1105±1.72^{d}	1234±2.00^{e}
Direct bilirubin (mg/dl)	0.28±0.02^{a}	0.26±0.02^{ab}	0.24±0.02^{ab}	0.22±0.02^{ab}	0.20±0.03^{b}
Total bilirubin (mg/dl)	0.056±0.002^{a}	0.054±0.002^{ab}	0.048±0.002^{bc}	0.046±0.002^{c}	0.044±0.002^{c}
Cholesterol (mg/dl)	124±3.08^{a}	146.8±2.35^{b}	176.6±1.50^{c}	196.8±1.24^{d}	205.6±1.21^{e}
Triglycerides (mg/dl)	55.4±0.92^{a}	72.8±1.15^{b}	89.6±1.07^{c}	104±0.71^{d}	116.2±1.15^{e}
Total protein (g/dl)	4.84±0.02^{a}	4.90±0.00^{b}	4.92±0.08^{b}	4.96±0.02^{b}	5.06±0.02^{c}
Albumin (g/dl)	2.48±0.02^{a}	2.50±0.00^{ab}	2.56±0.02^{bc}	2.66±0.02^{d}	2.76±0.02^{e}
Globulin (g/dl)	2.80±0.03^{a}	2.88±0.02^{b}	2.96±0.02^{c}	3.06±0.02^{d}	3.22±0.02^{e}
BUN (mg/dl)	2.5±0.00^{a}	2.5±0.00^{a}	2.5±0.00^{a}	2.5±0.00^{a}	2.5±0.00^{a}
Creatinine (mg/dl)	0.274±0.002^{a}	0.272±0.002^{a}	0.278±0.002^{a}	0.276±0.002^{a}	0.278±0.002^{a}
Uric acid (mg/dl)	1.278±0.002^{a}	1.286±0.002^{b}	1.294±0.002^{c}	1.316±0.002^{d}	1.326±0.002^{e}
Sodium (mg/dl)	205±0.71^{a}	211.4±1.16^{b}	231.4±0.92^{c}	237±0.71^{d}	242.6±0.51^{e}
Potassium (mg/dl)	6.46±0.02^{b}	6.68±0.02^{c}	7.02±0.02^{d}	7.06±0.02^{d}	7.16±0.02^{e}
Chloride (nmol/l)	149.4±0.51^{a}	157±0.71^{b}	174±0.71^{c}	182.8±0.49^{d}	190.2±0.58^{e}
Calcium (mg/dl)	8.06±0.02^{a}	8.28±0.02^{b}	8.62±0.02^{c}	8.66±0.02^{cd}	8.70±0.03^{d}
Phosphorus (mg/dl)	14.4±0.24^{a}	13.8±0.20^{a}	13.0±0.31^{b}	10.4±0.24^{c}	9.6±0.24^{d}

Values in a row with different superscripts significantly different (p<0.05).

* Liver enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)

LDH = lactate dehydrogenase

BUN = blood urea nitrogen

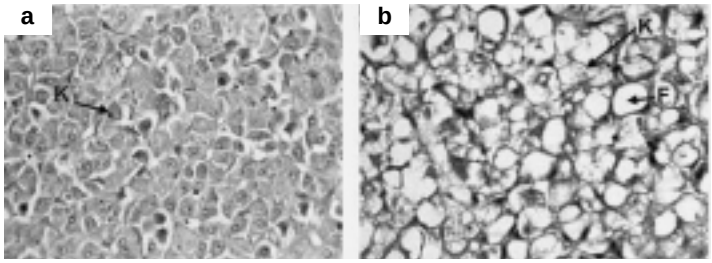

Fig. 1. Sections of hepatic tissue from African catfish (*Clarias gariepinus*) fed diets with different energy levels: (a) normal liver structure typical of fish fed diets containing 10.85, 11.82, or 12.73 MJ dietary energy/kg feed; (b) hepatic lipidosis seen as extensive lipoid infiltration and diffused macrovesicular and microvesicular fatty changes in hepatic cells, typical of fish fed diets containing 13.69 or 15.06 MJ dietary energy/kg feed. K: hepatic cell; F: lipid droplet (H&E, x 400).

diets IV and V had a lower nutritive value than diets I, II, and III, probably caused by imbalances in nutrient contents. Blood urea nitrogen and creatinine levels did not differ among experimental groups. This could be attributed to the similar protein contents of the diets.

In conclusion, our findings indicate that the preferable energy content of diets for African catfish is 12-13 MJ digestible energy/kg feed for optimum growth, blood parameters, and healthy liver.

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