Growth performance and body composition of giant trahira fingerlings fed diets with different protein and energy levels

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Abstract – The objective of this work was to determine the proper levels of protein and energy in diets of *Hoplias lacerdae* fingerlings. The dietary crude protein (CP) and gross energy (GE) levels for fingerlings of giant trahira were evaluated in a completely randomized 4x3 factorial design with 35, 39, 43 and 47% CP and 4,100, 4,300 and 4,500 kcal kg^{-1} of GE, and four replicates. The survival rate was 99.22%, and a linear improvement on the performance parameters was detected after increasing diet crude protein levels. Feed conversion ratio decreased with increasing levels of dietary protein and energy in the diets. A significant interaction between crude protein and gross energy was observed over body protein and mineral matter. Body lipid has increased linearly as gross energy in the diet increased. The retention of crude protein and energy showed a linear increasing with rising of crude protein levels in the diet. Crude protein level at 47% provides the best performance and energy retention, independently of the gross energy levels in the diet.

Index terms: *Hoplias lacerdae*, carnivorous fish, nutrition.

Crescimento e composição corporal de alevinos de trairão alimentados com diferentes níveis de proteína e energia nas dietas

Resumo – O objetivo deste trabalho foi determinar os níveis apropriados de proteína e energia em dietas para alevinos de trairão (*Hoplias lacerdae*). Os níveis de proteína bruta (PB) e energia bruta (EB) foram avaliados em delineamento inteiramente casualizado em esquema fatorial 4x3, com 35, 39, 43 e 47% PB e 4.100, 4.300 e 4.500 kcal kg^{-1} de EB, e quatro repetições. A taxa de sobrevivência foi de 99,22%, e um aumento linear nos índices de desempenho foi detectado após aumento dos níveis de proteína bruta na dieta. A conversão alimentar decresceu com o aumento dos níveis de proteína e energia na dieta. Foi observada interação significativa entre a proteína e energia bruta sobre a proteína corporal e matéria mineral. O lipídeo corporal aumentou linearmente com o aumento da energia bruta das dietas. A retenção de proteína e energia bruta mostrou aumento linear com os níveis crescentes de proteína bruta nas dietas. Dietas com 47% proteína bruta proporcionam o melhor desempenho e a melhor retenção de energia, independentemente de seus níveis de energia bruta.

Termos para indexação: *Hoplias lacerdae*, peixe carnívoro, nutrição.

Introduction

Protein is an essential nutrient for animal growth and development, and represents the most expensive portion in aquaculture diets (Lovell, 1989; Ai et al., 2004; Cho et al., 2005; Miller et al., 2005). Therefore, the amount of protein in fish diets should fit the minimum requirements to assure the nutritional demands of amino acids and the optimum fish growth (National Research Council, 1993). Amino acid-deficient diets or diets with improper amino acid profile might decrease fish growth and feed efficiency, and affects the immune system, what leads to weight losses, since the available proteins would be mobilized to certain tissues in order to maintain vital functions (National Research Council, 1993; Wilson, 2002).

In general, carnivorous species demand high protein levels in the diet (Kim & Lee, 2005), which can vary from 40 to 50% (De Silva et al., 2002; Deng et al., 2006). Protein requirement can be influenced by the relationship between energy and protein in the diet. Moreover, fishes fed low energy:protein diets use protein as an energy source, increasing feed intake to supply their energetic demands and, consequently, increasing feed conversion (National Research Council, 1993; Wilson, 2002; Piedras et al., 2004). In addition, diets with high protein levels are more expensive (Lovell, 1989), and produce effluents that can lead to a greater environmental impact of the production systems (Schulz et al., 2008; Ono et al., 2008).

The low protein:energy ratio in diet may lead to a decrease of feed intake, consequently decreasing the ingestion of proteins and other nutrients essential for fish growth (Lovell, 1989; Cho et al., 2005). This fact leads to body fat accumulation (National Research Council, 1993) and reduces carcass yield and shelf life of the product (Sampaio et al., 2000). Therefore, the utilization of lipids as energy source might direct protein use to growth, since it won't be necessary to provide energy (National Research Council, 1993; Martino et al., 2002a, 2002b). This process is known as protein sparing effect (Ai et al., 2004). Thus, adequate protein:energy ratio is essential to the formulation of industrialized feeds for giant trahira, in order to obtain good productive, economic and environmental results.

Amongst the carnivorous fishes, the giant trahira *Hoplias lacerdae* has a remarkable potential for aquaculture. This species can be easily adapted to captivity, presenting high rusticity, fast weight gain and low energy demand due to its sedentary behavior, besides representing a suitable fish for sport fisheries with a high-quality meat (Luz et al*.,* 2002; Luz & Portella, 2005).

However, there are few studies investigating diet protein:energy ratios for Brazilian native species, especially carnivore ones such as giant trahira.

The objective of this work was to determine the proper levels of protein and energy in diets of *Hoplias lacerdae* fingerlings.

Materials and Methods

The experiment was carried out in the Laboratory of Fish Nutrition, in the aquaculture facility of the Departmento de Biologia Animal, at Universidade Federal de Viçosa, Viçosa, MG, Brazil, in 2007, during 60 days.

A completely randomized experimental design, in a 4x3 factorial arrangement (n=4), was performed using 35, 39, 43 and 47% crude protein (CP) and 4,100, 4,300, 4,500 kcal kg^{-1} of gross energy levels (GE).

The chemical composition of ingredients was selected according to Rostagno et al. (2005) for the experimental diets (Table 1). After formulation, the experimental diets were pelletized in a commercial electric grinder, dried in a chamber at 55°C for 24 hours, ground in manual mill and seized in order to obtain pellets of 2-mm diameter. The chemical composition of experimental diets was analyzed according to standard methods of AOAC International (1990).

A total of 384 giant trahira fingerlings with mean weight of 1.85±0.07 g and standard length of 4.74±0.05 cm, were used in the experiment. These animals were confined for 12 hours, and subsequently distributed into 48 aquariums (35x30x14 cm) with 7 L of water, and under a 12-hours photoperiod. Fish were daily fed to satiation at 8, 12 and 16 h.

All aquariums were equipped with a biological filter of constant aeration and a heat-control system adjusted to 26±1.5ºC, connected to 10 W heaters per aquarium, and they were daily monitored. A black net was used to cover the aquariums, in order to prevent fish escape.

The aquariums were cleaned three times a week, by aspiration of the feces with a siphon, and by replacing 50% of the aquarium water volume. The water used in the periodical changes was stored in a 500-L box, with aeration and controlled temperature.

At the end of the experiment, the survival rate, the performance parameters, body composition and nutrient retention were evaluated, using the following expressions: survival rate, S (%) = (final number of fish specimens/initial number of fish specimens) x 100; length gain, LG (cm) = (final length - initial length); weight gain, WG (g) = (final weight - initial weight); feed intake, FI (% of initial weight per day) = [(total feed intake/initial weight)/60 days] x 100; feed conversion ratio, FCR = (amount of consumed food/ weight gain); specific growth ratio, SGR (% day^{-1}) = 100 x [(ln final weight - ln initial weight)/60 days]; protein efficiency ratio, PER = (weight gain /total protein intake); protein productive value, PPV (%) = 100 x [(final body protein x final weight) - (initial corporal protein x initial weight)]/ total protein intake; and energy productive value, EPV (%) = 100 x [(final body gross x final weight) - (initial body gross x initial weight)]/total gross energy intake.

At the beginning of the experiment, eight specimens were euthanized, following the bioethical resolution number 714, using benzocaine, and kept at -80ºC for chemical composition analysis. At the end of the experiment, all specimens were euthanized, and samples of the experimental diets were also chemically analyzed, according to AOAC International (1990).

Sample fish were lyophilized, pre-degreased and crushed in a ball mill. Dry matter of fish and diet was determined after drying at 105°C, and subsequently incinerated in a muffle at 600°C. Evaluations were also made for: protein content, by micro Kjeldahl method; ether extract, by extraction with petroleum ether; crude fiber, by digestion with sulfuric acid and sodium hydroxide (feed samples only); and gross energy, by bomb calorimetry.

Data were evaluated by analysis of variance at 5% probability, in order to study the effect of interaction between levels of protein and energy in the diets. Regression equations were adjusted according to the protein and energy levels, using the SAEG program for statistical analysis (Universidade Federal de Viçosa, 2007), in case of significance.

Results and Discussion

No significant interaction between levels of dietary protein and energy on the survival ratio was observed, and protein and energy levels did not affect this ratio. Once the trahira is a carnivorous fish with cannibalistic behavior from the early life history (Baras & Jobling, 2002), the high survival ratio (99.22%) and the absence of cannibalism in the present study might be related to the efficient feed conditioning of these animals made before the experiment (Luz et al., 2000, 2002; Salaro et al., 2003), and to the maintenance of specimens of similar size in the experimental aquariums.

There was no significant interaction of dietary protein and energy levels on weight gain, length gain, specific growth rate, feed intake, feed conversion ratio and protein efficiency ratio. Diet protein levels did not

Table 1. Formulation and chemical composition of experimental diets (original matter basis) of *Hoplias lacerdae* fingerlings, according to crude protein (%) and gross energy (kcal kg^{-1}) levels.

Ingredients (%)	4,100				4,300				4,500			
	35	39	43	47	35	39	43	47	35	39	43	47
Soybean meal	12.00	8.00	4.00	0.50	12.00	8.00	4.00	0.50	16.00	15.80	12.00	1.00
Corn gluten	1.00	4.00	7.00	9.00	1.60	4.70	8.15	9.80	2.10	5.00	8.65	14.50
Fish meal	40.00	45.00	48.00	52.00	40.00	45.00	48.00	52.00	40.00	45.00	48.00	51.50
Meat meal-45	4.00	8.00	14.00	20.00	4.00	8.00	14.00	20.00	0.50	0.50	6.00	13.50
Corn meal	24.72	16.59	9.02	2.20	24.71	16.58	9.00	2.20	22.95	13.31	6.00	0.00
Wheat meal	15.00	15.00	14.50	12.57	11.00	11.00	10.00	8.57	9.00	11.00	10.00	9.47
Cellulose	1.00	1.40	1.80	2.30	1.40	1.70	2.15	2.50	1.20	1.40	1.80	2.50
L – lysine	0.50	0.33	0.18	0.00	0.51	0.34	0.20	0.00	0.48	0.30	0.15	0.10
DL – methionine	0.25	0.15	0.07	0.00	0.25	0.15	0.07	0.00	0.24	0.16	0.07	0.00
Soybean oil	0.00	0.00	0.00	0.00	3.00	3.00	3.00	3.00	6.00	6.00	6.00	6.00
Dicalcium phosphate	0.70	0.70	0.60	0.60	0.70	0.70	0.60	0.60	0.70	0.70	0.50	0.60
Vitamin C(1)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Common salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin/mineral Premix(2)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
BHT (3)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Composition												
Crude protein (%)	35.31	39.28	42.97	46.7	35.06	39.09	42.97	46.57	35.16	39.17	43.06	46.36
Gross energy (kcal kg^{-1})	4,092	4,128	4,178	4,183	4,296	4,306	4,323	4,340	4,509	4,480	4,568	4,582
Crude lipid (%)	4.38	4.92	5.58	6.25	7.23	7.78	8.45	9.12	9.74	9.87	10.5	11.43
Starch(%)	57.73	52.15	47.85	44.07	55.19	50.51	44.43	41.03	52.46	47.51	42.35	39.01
Crude fiber (%)	2.58	3.65	3.60	2.98	2.52	2.62	4.15	3.28	2.64	3.45	4.09	3.20

(1)Ascorbil-2-monophosphate with 35% activity. (2)Guaranteed levels: vitamin A, 16,000 UI; vitamin D, 4,500 UI; vitamin E, 250 mg; vitamin K, 30 mg; vitamin B_1, 32 mg; vitamin B_2, 32 mg; vitamin B_{12}. 32 mcg; vitamin B_6. 32mg; vitamin C, zero; panthotenic acid. 80 mg; niacin, 170 mg; biotin, 10 mg; folic acid, 10 mg; choline, 2,000 mg; cobalt, 0.5 mg; copper, 20 mg; iron, 150 mg; iodide, 1 mg; manganese, 50 mg; selenium, 1 mg; zinc, 150 mg; antioxidative additive, 150 mg. (3)Butylated hydroxytoluene, antioxidant.

affect feed intake and protein efficiency ratio. Weight gain, length gain and specific growth rate showed a linear increasing with the rise of dietary protein, as well as a linear reduction in the feed conversion ratio according to protein and energy levels in the diet (Table 2).

The values of feed conversion observed in the present study might be regarded as excellent, for carnivorous species, as showed by Martino et al. (2002a) for juveniles of surubim (*Pseudoplatystoma corruscans*). Such result can be related to the development stage of the analysed fish, once younger individuals showed higher growth rates and, consequently, a higher efficiency in the utilization of the dietary nutrients. Results observed in the feed conversion rate with giant trahira fingerlings, in the present work, are similar to those reported in fingerlings of peacock bass (*Cichla* sp.) (Sampaio et al., 2000), in which feed conversion ratio improved with increasing dietary protein levels. Feed conversion ratio data of the present study also corroborate others with carnivorous species, such as surubim (*Pseudoplatystoma corruscans*) (Martino et al., 2002a), malabar groupers (*Epinephelus malabaricus*) (Tuan & Williams, 2007), perch (*Sander lucioperca*) (Schulz et al., 2008) and fingerlings of rainbow trout (*Onchorhynchus mykiss*) (Eliason et al., 2007), for which the best feed conversion rates were observed when the energy and lipids were increased in diet.

Table 2. Weight gain, length gain, specific growth rate, food conversion rate, protein efficiency rate, feed intake, and survival ratio of *Hoplias lacerdae* fingerlings expressed by adjusted regression equations according to the crude protein (%) and gross energy (kcal kg^{-1}) levels in the diets.

Factor of variation	Adjusted equations of performance	R^2 (%)
	Weight gain (g)	
Crude protein	$\hat{y}$ = - 1.66 + 0.13x	98.20
Gross energy	ns	-
	Length gain (cm)	
Crude protein	$\hat{y}$ = - 0.11 + 0.05x	95.50
Gross energy	ns	96.70
	Food conversion rate	
Crude protein	$\hat{y}$ = 3.03 - 0.04x	86.80
Goss energy	$\hat{y}$ = 3.26 - 0.0004x	85.60
	Specific growth rate (% day^{-1})	
Crude protein	$\hat{y}$ = 0.09 + 0.05x	98.60
Gross energy	ns	-
	Protein efficiency rate	
Crude protein	ns	-
Gross energy	ns	-
	Feed intake (g)	
Crude protein	ns	-
Gross energy	ns	-
	Survival ratio (%)	
Crude protein	ns	-
Gross energy	ns	-

nsNon-significant according to F test, at 5% probability.

The best performance parameters of giant trahira fingerlings was detected with 47% CP diets, independently of the dietary energy levels. These diets resulted in faster weight and length gains and higher specific growth rates. However, the feed conversion ratio decreased with increasing dietary energy levels, showing a sparing protein effect. In other words, fingerlings feeds with high energy concentration (4,500 kcal kg^{-1} of GE) avoided the use of a large proportion of dietary protein as energy source, and this protein was spared for muscle deposition.

Probably, the energy levels tested in the experimental diets were insufficient to promote sparing protein effect in other performance variables. However weight gain for all treatments was low. The high protein demand observed in giant trahira fingerlings might be related to their development stage, since younger animals usually require more protein, and might also be related to the species feed habit. Alternatively, such demand can also be high because proteins represent the main energy source in the metabolic routes in early development of fish (Hepher, 1988; Meyer & Fracalossi, 2004), especially in carnivorous species.

Possibly, the low growth performance observed in giant trahira fingerlings fed diets containing the lowest protein levels also occurred due to the diet high carbohydrate:lipid ratio. The use of low levels of dietary protein, associated to diets with high carbohydrate:lipid ratio, usually results in a decrease in productive performance in carnivorous fishes (Tibbetts et al., 2005). Such low efficiency in the carbohydrate utilization might be related to the difficulty of carnivorous species in hydrolyzing carbohydrates, since they have a reduced amilolytic activity in their digestive tract, and might also be related to the complexity of the physico-chemical structure of carbohydrates in the prepared food (Wilson, 1994).

Furthermore, it is well known that lipids are important as energy source for fishes (Martino et al., 2002a), especially carnivorous ones. Lipids metabolized by carnivorous fishes more easily and are important to promote good growth ratios and development because they are effectively used for sparing protein.

Neither protein nor energy levels influenced feed intake in the diet. Probably, the energetic level in the treatments and the inclusion of 0, 3, and 6% of soybean oil in the diets were not enough to decrease feed intake, since fishes, as well as birds and mammals, feed to supply their energetic requirements only (National Research Council, 1993; Sampaio et al., 2000). These results corroborate reported ones on fingerlings of rainbow trout (Eliason et al., 2007). Nonetheless, studies in surubim juveniles (*Pseudoplatystoma corruscans*) (Martino et al., 2002a) and malabar groupers (*Epinephelus malabaricus*) (Tuan & Williams, 2007) reported a reduction in the feed intake, as long as the levels of dietary energy have been increased.

A significant interaction between the dietary protein and energy levels was detected for mineral matter and corporal protein (Table 3).

Neither significant interaction between protein and energy levels nor a significant effect of the dietary protein level was observed on the body lipid content. Nonetheless, corporal lipids showed a linear increasing with the rise of dietary energy levels (Table 3). Body lipid contents increased with increasing energy levels in the diet. These results indicate that the highest lipid levels in the diet reduced the utilization efficiency of such non protein energetic source for growth, leading to a higher accumulation of body fat in the tissues (Schulz et al., 2008). Such increasing on the body fat is undesirable, once it reduces carcass yields, meat quality and the shelf life of the final product (Sampaio et al., 2000; Ono et al., 2008).

Interaction effects between protein and energy levels in the diet were not observed on dry matter. However, a linear increasing in dry matter was detected when both dietary protein and energy levels were increased (Table 3). No significant interaction effects between protein and energy levels in the diet or their levels individually were detected on the protein retention ratio. However, there was better retention of protein with the highest dietary protein levels (Table 3). The protein retention also tended to increase with higher dietary energy levels ($p<0.12$). Some carnivorous species show higher protein efficiency rates when the levels of dietary protein are low and when the levels of lipids or energy in the diet are increased, due to the sparing protein effect (Catacutan & Coloso, 1995; Eliason et al., 2007; Schulz et al., 2008). Nonetheless, the protein sparing effect was so evident in fingerlings of giant trahira.

As for the retention of gross energy, there were no significant interaction effects between dietary protein and energy levels. However, a linear increasing in the retention of gross energy was present when dietary protein levels were increased (Table 3). Moreover, the feed intake was similar in all the treatments. However, in juvenile spotted surubim (*Pseudoplatystoma*

Table 3. Body protein, mineral matter, body lipid, dry matter mass, moisture content, protein productive value and energy productive value of *Hoplias lacerdae* fingerlings expressed by adjusted regression equations according to crude protein (%) and gross energy (kcal kg^{-1}) levels in the diets.

Factor of variation	Adjusted equations of body composition	R^2 (%)
	Body protein (%)	
Crude protein		
35	$\hat{y} = -611.03 + 0.292x - 0.000034x^2$	100.00
39	$\hat{y} = -0.82 + 0.004x$	99.61
43	$\hat{y} = 4.98 + 0.0027x$	86.19
47	$\hat{y} = -199.49 + 0.102x - 0.000012x^2$	100.00
Gross energy		
4,100	$\hat{y} = 32.20 - 0.93x + 0.13x^2$	97.90
4,300	ns	-
4,500	$\hat{y} = -53.27 + 3.35x - 0.0398x^2$	91.23
	Mineral matter (%)	
Crude protein		
35	$\hat{y} = 105.74 + 0.001x$	81.10
39	ns	-
43	ns	-
47	ns	-
Gross energy		
4,100	ns	-
4,300	$\hat{y} = 3.69 + 0.02x$	81.60
4,500	$\hat{y} = 1.50 + 0.07x$	87.90
	Body lipid (%)	
Crude protein	$\hat{y} = -4.25 + 0.0025x$	96.20
Gross energy	ns	-
	Dry matter mass (%)	
Crude protein	$\hat{y} = 20.22 + 0.13x$	98.90
Gross energy	$\hat{y} = 9.00 + 0.039x$	96.70
	Moisture content (%)	
Crude protein	$\hat{y} = 77.57 - 0.14x$	97.30
Goss energy	$\hat{y} = 93.82 - 0.051x$	99.90
	Protein productive value (%)	
Crude protein	$\hat{y} = 15.27 + 0.40x$	58.00
Gross energy	ns	-
	Energy productive value (%)	
Crude protein	$\hat{y} = -3.29 + 0.61x$	97.30
Gross energy	ns	-

nsNon-significant according to F test, at 5% probability.

corruscans), the retention of crude energy was directly proportional to the levels of dietary lipids (Martino et al., 2002a).

Conclusion

The highest level of dietary protein yields the best performance indexes, as well as the best energy retention in trahira fingerlings, independently of the dietary energy levels.

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