HYBRID SORUBIM VISCERA PROTEIN CONCENTRATE IN THE DIETS FOR BARRED SORUBIM*

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ABSTRACT

The current study evaluated the growth performance and the haematological profile of barred sorubim fed with levels of hybrid sorubim viscera protein concentrate (0.0; 5.0; 10.0 e 15.0%) in isoprotein and isoenergetic diets. Eighty fish with an average initial weight of 39.8 ± 0.28 g and an average initial length of 19.92 ± 0.25 cm were distributed in 16 aquaria of 60 L, with recirculation system and temperature control, during 35 days. The experimental design was completely randomized with four treatments and four replications. For the growth performance assay were analyzed the follows variables: weight gain (WG), feed consumption (FC), apparent feed conversion ratio (AFCR) and protein efficiency ratio (PER). Furthermore, the hemogram were assessed on the treated fish with levels of protein concentrate. It was observed no significant difference (P>0.05) for all the parameters evaluated. It was concluded that the protein concentrate from hybrid sorubim viscera can be included up to 15% in diets for barred sorubim juveniles without negative response on the performance and health.

Key words: Carnivorous fish; coproduct; hemogram; protein; *Pseudoplatystoma corruscans; P. reticulatum*

CONCENTRADO PROTEICO DE VÍSCERAS DE SURUBIM HÍBRIDO EM DIETAS PARA CACHARA

RESUMO

O presente trabalho avaliou o desempenho produtivo e a influência nos parâmetros hematológicos de cacharas alimentadas com diferentes níveis de inclusão de concentrado proteico de vísceras de surubim híbrido na dieta (0,0; 5,0; 10,0 e 15,0%) em rações isoproteicas e isoenergéticas. Foram utilizados 80 peixes com peso médio inicial de 39,8 ± 0,28 g e comprimento médio inicial de 19,92 ± 0,25 cm, os quais foram distribuídos em 16 aquários de 60 L, em sistema de recirculação com biofiltro e controle de temperatura, durante 35 dias. O delineamento experimental foi inteiramente casualizado com quatro tratamentos e quatro repetições. Para avaliação do desempenho zootécnico foram analisadas as seguintes variáveis: ganho de peso (GP), consumo de ração (CR), conversão alimentar aparente (CAA) e taxa de eficiência proteica (TEP). Além disso, procedeu-se o hemograma completo dos peixes alimentados com as diferentes concentrações de concentrado proteico. Observou-se que nenhuma das variáveis analisadas diferiu estatisticamente (P>0,05). Pode-se concluir que o concentrado proteico de vísceras de surubim híbrido pode ser incluído em até 15% em rações para juvenis de cacharas, sem que haja interferência negativa no desempenho e saúde dos animais.

Palavras chave: Peixes carnívoros; coproduto; hemograma; proteína; *Pseudoplatystoma corruscans; P. reticulatum*

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INTRODUCTION

The production of sorubim catfish is in undergoing expansion in Brazil, mainly in the Brazilian Central-West region, because of its attractive commercial, as light color filet and absence of intramuscular bones, fast growth and efficient feed conversion ratio (INOUE *et al.*, 2009). According to recent data published by the Ministry of Fisheries and Aquaculture of Brazil, the production of surubim increased 94% between 2006 and 2009, from 1,004 t in 2006 to 2,126 t in 2009 (BRASIL, 2010).

Among the genus *Pseudoplatystoma*, the main species commercially produced are: spotted sorubim (*Pseudoplatystoma corruscans* - Spix & Agassiz, 1829), barred sorubim (*Pseudoplatystoma reticulatum* - Eigenmann & Eigenmann, 1889), besides the interspecific hybrid (*P. reticulatum* x *P. corruscans*), which currently represents the largest volume of commercial production.

Despite the representative increase on the sorubim catfish culture in recent years, there are still many gaps in the technology production. Pioneering studies have been conducted for the genetic improvement of this species (RESENDE, 2009). However, there is limited scientific and technical information for several areas. According to GONÇALVES and CARNEIRO (2003), nutrition and feeding of sorubim represent a challenge for its farming, mainly by the lack of knowledge on its nutritional requirements and on the digestibility of the various feedstuffs.

Fishmeal is the most widely protein source used in fish diets due its high quality and protein content, besides acting as palatalizing (FURUYA *et al.*, 2000). This feedstuff is obtained mainly from the processing of marine fish resources that are found decreased in recent years (STARKEY, 1994; TACON, 1998; MILLAMENA, 2002). The intensified demand, coupled with the reduction of fishmeal world production, has provided an increase of your price, and therefore, is necessary to identify alternative sources to replace this feedstuff by others that are economically viable (PORTZ, 2001).

Fish offal is composed basically of skin, head, spine, viscera, fins, scales and fragments of muscles obtained from the toilet process (TEJADA, 1992; OETTERER, 1994) and can be a potential alternative protein source. The improvement and growth of the fish processing plants in Brazil, increase the production of mechanically deboned meat (MDM), therefore, the viscera tend to become the main coproduct with potential use in animal feeding.

Viscera from fresh water fish represent 5-11% of the fish weight, depending on the species. Its averaged chemical composition is 67% water, 10% protein, 14% fat and 3% ash (MAHENDRAKAR, 1995). The processing of this offal to produce protein concentrate (PC) may represent an important alternative for replacement of fishmeal in fish diets, especially carnivorous species, as barred sorubim. The PC consists of fish meat submitted to successive washings with the purpose to remove the water-soluble proteins, fat, blood and odoriferous substances, resulting in an odorless white paste (OETTERER 2002; VIDAL, 2007).

Thus, the current study aimed to evaluate the inclusion of levels of protein concentrate obtained from viscera of hybrid sorubim in the diets of barred sorubim and to verify possible influences on the growth performance and haematological profile.

MATERIAL AND METHODS

The hybrid sorubim viscera were removed from the fish at vacuum (without manual contact) in a federal inspected fish processing plant, and kept at -18 °C for the protein concentrate (PC) production. Immediately, this material was transported under refrigerated conditions to the Laboratory of Bioengineering, Universidade Federal da Grande Dourados, Dourados, MS, where it was grounded into a meat grinder to obtain a homogeneous mass.

Viscera were washed in 3 cycles utilizing in each cycle a washing solution:viscera ratio of 4:1 (v/w), temperature of 7 °C, for 10 min. In each washing cycle, the stirring was kept constant at 220 rpm using a mechanical agitator (Marconi model MA-259, Piracicaba, Brazil). It was utilized 0.25% $NaHCO_3$ solution for the first and second washings and 0.3% NaCl solution for the last one. After each washing cycle, samples were centrifuged at 7 °C (Sigma model 6-15, Osterode, Germany). The first and second centrifugations were carried out at 3,000 x g for 15 min, while the third one at 7,000 x g for 25 min. The supernatant containing fat and water-soluble proteins was removed. Proximal composition of PC was 18.48% of dry matter, 88.74% of crude protein and 4.71% of ether extract. The final slurry was sieved through a 1 mm-mesh metal screen to remove connective tissues, packaged in 5-layer nylon propylene bags, overnight stored at -18 °C.

Four diets were prepared with the following inclusion levels of PC: 0.0, 5.0, 10.0 and 15.0% (Table 1) to be isoprotein and isoenergetic with 42% crude protein and 4,200 kcal kg^{-1} crude energy, respectively. All ingredients were ground until they reach a particle size of 0.5 mm, and then mixed. After this process, water was added at 55 °C, except in the treatment with 15% of PC. Then, the mixtures were processed in an experimental pelletizer and dried in an oven with forced ventilation at 55 ºC for 12 hours.

Table 1. Percentage and chemical-bromatological composition of the experimental diets.

Ingredients (g)	**Levels of sorubim viscera protein concentrate**			
	0%	**5%**	**10%**	**15%**
Soybean meal	40.00	40.00	40.00	40.00
Fish meal	41.50	32.50	23.50	14.60
Protein concentrate	-	5.00	10.00	15.00
Corn	5.00	6.60	10.30	14.30
Wheat middlings	8.00	10.00	10.00	10.00
L-lysine	0.27	0.45	0.64	0.80
DL – methionine	0.15	0.20	0.24	0.30
Soybean oil	3.56	3.73	3.80	3.48
Dicalcium phosphate	0.50	0.50	0.50	0.50
Premix vit/min [1]	1.00	1.00	1.00	1.00
BHT [2]	0.02	0.02	0.02	0.02
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**
Calculated values [3] (%)				
Crude protein	42.0	42.0	42.0	42.0
Gross energy	4200	4205	4215	4203
Ether extract	10.43	9.97	9.43	8.52
Lysine	2.80	2.80	2.80	2.80
Methionine	1.00	1.00	1.00	1.00
Calcium	2.71	2.19	1.66	1.14
Phosphorus	1.57	1.35	1.12	0.90

[1] *Vitamin and mineral premix (M. Cassab) Composition/kg of product: Vit. A = 1,200,000 UI; vit. D3 = 200,000 UI; vit. E = 12,000 mg; vit. K3 = 2,400 mg; vit. B1 = 4,800 mg; vit. B2 = 4,800 mg; vit. B6 = 4,000 mg; vit. B12 = 4,800 mg; folic acid = 1,200 mg; calcium pantotenate = 12,000 mg; vit. C = 48,000 mg; biotin = 48 mg; choline = 65,000 mg; nicotinic acid = 24,000 mg; Fe = 10,000 mg; Cu = 600 mg; Mn = 4,000 mg; Zn = 6,000 mg; I = 20 mg; Co = 2 mg and Se = 20 mg*

[2] *BHT = (antioxidant) = Butyl hydroxy toluene;*

[3] *According to proximal composition of PC evaluated, ESPE et al. (1999) and ROSTAGNO et al. (2011).*

Growth performance assay was conducted in the Laboratory of Fish Culture, Embrapa Agropecuária Oeste, Dourados, MS. Two hundred juveniles of barred sorubim were obtained from commercial hatchery, Terenos, MS. They were acclimated under laboratory conditions (100 L tank) and trained to receive commercial feed for carnivorous fish during 45 days. After this period, 80 fish with an average initial weight of 39.8 ± 0.28 g and average initial length of 19.92 ± 0.25 cm were randomly selected and distributed in 16 aquaria of 60 L each, in a water recirculation system with mechanical and biological filter and controlled temperature, during 35 days.

The fish were fed *ad libitum* twice daily (8h00 a.m. and 4h30 p.m.), until apparent satiation. Dissolved oxygen and water temperature were measured daily. Cleanings management was periodically achieved by siphoning and renewing 20% of the total volume of the system.

After growth performance trial, all fish, previously anesthetized with 50 mg L^{-1} clove oil were weighted. It were evaluated weight gain (WG), feed consumption (FC), apparent feed conversion ratio (AFCR) and protein efficiency ratio (PER).

For haematological analysis, eight animals per treatment were randomly captured. The blood collected was performed by means of caudal puncturing, as recommended by ISHIKAWA *et al*. (2010). Immediately, blood smears were carried out in duplicates, which were then subjected to panchromic staining with the May-Grünwald-Giemsa-Wright combination. These blood smears were used for global and differential leukocyte counting, as well as for thrombocyte counting by the indirect method (TAVARES-DIAS and MORAES, 2006).

The remaining blood was placed in polypropylene tubes (1.5 mL), maintained between 5-7 °C until processing. From these blood samples was determined the percentage of hematocrits by the microhaematocrit method (GOLDENFARB *et al*., 1971), haemoglobin rate by the technique of cyanmetahaemoglobin (COLLIER, 1944) and erythrocyte counting after blood dilution (1:200) in formalin-citrate solution, with counts conducted in a haemocytometer. It was also calculated the haematimetric indexes of WINTROBE (1934), comprised by the Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC).

It was used a completely randomized design with four treatments and four replications. Data were analyzed using the statistical one-way analysis of variance (ANOVA) and Tukey´s test (P<0.05), both using the statistical program SISVAR 5.3 (FERREIRA, 2008).

RESULTS

It was recorded average values for temperature of 27.23 ± 1.22 °C and dissolved oxygen of 4.74 ± 1.01 mg L^{-1} during the experiment period. The temperature and oxygen remained close to that recommended by MARQUES *et al*. (1992) for genus *Pseudoplatystoma*.

There was no significant effect (P>0.05) for PC inclusion on the growth performance parameters WG, FC, AFCR and PER (Table 2).

Table 2. Average values ± standard deviation of the growth performance of barred sorubim fed with levels of hybrid sorubim viscera protein concentrate.

Variables	**Levels of viscera protein concentrate**			
	0%	**5%**	**10%**	**15%**
WG (g)	26.05 ± 3.03	26.31 ± 3.59	26.26 ± 0.13	28.80 ± 1.48
FC (g)	51.10 ± 6.34	57.17 ± 2.57	52.10 ± 11.39	58.59 ± 0.71
AFCR	1.99 ± 0.42	2.21 ± 0.21	1.87 ± 0.40	2.04 ± 0.13
PER (%)	1.24 ± 0.27	1.09 ± 0.10	1.30 ± 0.28	1.17 ± 0.07

WG = weight gain. FC = feed consumption. AFCR = apparent feed conversion ratio. PER = protein efficiency ratio.

The values obtained in the erythrogram and thromboleukogram of barred sorubim fed levels of protein concentrate are listed in Tables 3 and 4, respectively. There was no significant difference (P>0.05) at the levels of replacement of fishmeal by fish protein concentrate on these parameters.

Table 3. Erytrogram of barred sorubim fed with levels of hybrid sorubim viscera protein concentrate.

Parameters	Levels of viscera protein concentrate			
	0%	5%	10%	15%
Hematocrit (%)	19.6 ± 4.9	21.1 ± 7.5	18.0 ± 4.7	19.4 ± 2.8
Erythrocyte (x 10^6 μL^{-1})	1.9 ± 0.3	1.6 ± 0.3	1.8 ± 0.5	1.8 ± 0.3
Hemoglobin (g dL^{-1})	2.4 ± 0.2	2.0 ± 0.5	1.9 ± 0.4	2.3 ± 0.7
MCV (fL)	108.5 ± 33.1	136.7 ± 61.8	109.7 ± 44.0	111.3 ± 27.2
MCHC (g dL^{-1})	11.0 ± 6.0	10.1 ± 4.0	11.6 ± 5.4	10.8 ± 5.7

MCV = Mean Corpuscular Volume. MCHC = Mean Corpuscular Haemoglobin Concentration

Table 4. Absolute values of thrombocytes and leukocytes of barred sorubim fed with levels of hybrid sorubim viscera protein concentrate.

Cells (x 10^3 μL^{-1})	Levels of viscera protein concentrate			
	0%	5%	10%	15%
Thrombocytes	42.8 ± 26.9	30.0 ± 10.8	43.8 ± 30.0	43.1 ± 12.0
Leukocytes	69.1 ± 21.3	56.9 ± 18.7	58.9 ± 21.0	63.8 ± 11.6
Monocytes	0.1 ± 0.1	0.2 ± 0.3	0.1 ± 0.3	0.2 ± 0.3
Lymphocytes	66.2 ± 20.0	57.9 ± 15.7	57.1 ± 21.4	58.7 ± 10.2
Basophils	0.4 ± 0.5	0.2 ± 0.4	0.8 ± 0.8	0.4 ± 0.3
Eosinophils	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.2	0.3 ± 0.3
Neutrophils	1.8 ± 1.6	1.5 ± 1.1	1.3 ± 1.1	2.3 ± 1.4
GL-PAS*	0.5 ± 0.4	0.3 ± 0.5	0.3 ± 0.4	0.5 ± 0.7

**GL-PAS: Granular leukocyte PAS positive.*

DISCUSSION

Although different species might use the feed differently, similar results were found by FEIDEN *et al*. (2010), when evaluating diets with organic fish meal (residue from tilapia filleting industry at level of 25% in the diet) and commercial diets for juvenile silver catfish (*Rhamdia voullezi*). They found no significant effect (P>0.05) on weight gain and for apparent feed conversion ratio among the treatments.

Data reported by BOSCOLO *et al*. (2005) corroborate with those obtained in this study. They found that the fish meal originated from tilapia filleting waste (viscera, head, fins and skin with scales) can be added up to 20% into diets of tilapias in sex reversal phase, without compromising growth performance. These authors attribute this effect to the isonutrient diets, and also by the fast processing of the industrial filleting offal, resulting in products with higher quality when compared to the raw materials obtained from extractive fishing. This fact can also be observed in the present work, once that the diets were isoprotein (42% crude protein) and isocaloric (4,200 kcal kg^{-1} crude energy), and the sorubim viscera were obtained from a local fish processing plant and quickly processed.

Likewise, KOTZAMANIS *et al*. (2001) evaluated the effect of trout offal derived from a filleting line of fish processing plant on gilthead bream (*Sparus aurata* L.) feeding and observed that the replacement of up to 20% of fishmeal by fish offal can be used in diets for this species, without compromising feed consumption and performance. Likewise, ESPE *et al*. (2012) evaluating the partial replacement (0, 3.75, 7.5, 11.25 or 15%) of fishmeal by blue whiting (*Micromesisticus poutasou*) hydrolyzed protein concentrate in diets for Atlantic salmon (*Salmo salar*) found no significant effect on voluntary feed consumption, performance and protein efficiency ratio among the treatments.

On the other hand, ESPE *et al*. (1999), in a study with the use of hydrolyzed protein concentrate from whole herring (*Clupea harengus*) or herring filleting residue for Atlantic salmon feeding in replacement of fishmeal (proportions of 0 to 40% and 0% to 30%, respectively) found significantly higher growth rate in partial replacement up to 10% of fishmeal. Levels above 15% did not differ from the control treatment. Fish fed with whole fish protein concentrate had an increase in consumption and consequent decrease in feed conversion ratio, while that protein concentrate obtained from the filleting residue, the animals reduced consumption, but there was no effect on feed conversion ratio. In addition, HEVRØY *et al.* (2005), evaluating the fish protein hydrolysate for Atlantic salmon feeding, observed that the increase of the inclusion of this coproduct in diets for fish yielded negative effect on the protein efficiency ratio and feed conversion ratio.

GONÇALVES *et al.* (1989), evaluating levels (10, 15 and 20%) of sardine and blue whiting silages for eel *(Anguilla rostrata)* fry, found higher growth and lower feed conversion ratio of fish fed with silage when compared to control (experimental diet based on fishmeal and meat and bone meal). Later, for juvenile silver catfish (*Rhamdia quelen*), fed diets containing 0 to 50% of silage from characin (*Cyphocharax voga*) waste during 75 days, ENKE *et al.* (2009) observed a significant difference in the final weight, indicating that the best results were observed between 25 and 37.5% of silage flour, demonstrating that its inclusion in the diet influences the palatability, digestibility and attractiveness of the feed.

The use of the clinical hematology in nutrition studies allows to diagnostic certain deleterious effects determined by some toxic compounds of ingredients on the fish health. TAVARES-DIAS *et al*. (2009) report that the erythrogram supports the identification of anemiant processes, while the leukogram in the diagnostic of infectious processes and other states of homeostatic imbalance.

Pronounced haematological changes were observed in fish fed with protein plant sources (OSUIGWE *et al*., 2005). Although, this practice may represent an economical and sustainable alternative, however many species have antinutritional compounds that can affect the performance and health of the fish. Moreover, animal sources, *e.g.* fish protein concentrate do not exhibit these compounds.

The haematological profile observed was similar to those described for barred sorubim (RANZANI-PAIVA *et al*., 2005) and hybrid sorubim (TAVARES-DIAS *et al*., 2009) reared in ponds, however, MCHC and MCV were lower in present study. RANZANI-PAIVA *et al*. (2005) demonstrated that variations in these parameters can be related to the age, size and sex of animals.

In recent years, there has been an improvement of mechanization of fish processing plants in Brazil, *i.e.* an increasing on the production of mechanically separated meat (MSM). Thus, as observed for other species (poultry and swine), the viscera can become the primary coproduct of processing plants with potential application in animal feeding. Currently, the volume is not enough to production exclusively fish viscera meal.

Both fish viscera silage and fish viscera protein concentrate can maintain the nutritional quality of the raw material, or even add nutritional value to the product. The results obtained in this study demonstrated that the sorubim viscera protein concentrate did not affect the animal performance, as well as its haematological profile, demonstrating a rational use of this offal of difficult disposal as a potential fishmeal replacer.

CONCLUSION

The hybrid sorubim viscera protein concentrate can be utilized in diets for barred sorubim juveniles up to 15% without affecting the growth performance and health of the animals.

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