Juveniles of *Pseudoplatystoma fasciatum* fed with lyophilized bovine colostrum: IGF-I expression in muscle and intestine

Pauletti, P., Rodrigues, A.P.O., Cyrino, J.E.P. and Machado-Neto, R.

University of São Paulo - ESALQ/USP , Animal Science - Zootecnia, raul.machado@esalq.usp.br

INTRODUCTION

The farming of speckled catfish *Pseudoplatystoma fasciatum* (Linnaeus, 1766) is of economic importance through out Brazil. These fish support recreational fisheries and an emerging aquaculture industry possessing attractive market prices, but little information is available on its physiology and nutritional requirements.

The growth promoting effect of growth hormone (GH) is mediated by insulin-like growth factors-I and -II (IGF-I and IGF-II). The major site of IGF-I gene expression in bony fish is liver, but several extra hepatic sites also express the peptide. The production of IGF-I in various tissues suggest paracrine and autocrine actions, that are involved in organ growth in fish. Some studies provide evidence that autocrine/paracrine IGF-I can support normal post-natal growth and development. In relation to speckled catfish no information exists regarding the cellular sites of IGF-I synthesis.

OBJECTIVE

The objective of this study was evaluating effects of diets with partial replacement of protein source by defatted lyophilized bovine colostrum (LBC) on muscle and intestine IGF-I expression of juvenile speckled catfish fed *ad libitum* for 30 or 60 days.

MATERIALS and METHODS

Striped catfish juveniles conditioned to feed on dry diets were stocked in plastic cages housed in a closed water circulation system, continuously aerated and under controlled (28 ± 2°C) water temperature (Figures 1, 2 and 3). Fish (35.14 ± 2.23 g and 14.38±0.44 cm, n=3) were fed *ad libitum* in two daily meals for 30 or 60 days with five diets (45% crude protein; 4000 kcal kg^{-1}) with increasing levels of LBC (0, 5, 10, 15 and 20%) (n=3). At 30 and 60 days, all fish were weighed and the length determined for performance evaluation and seven fish from each treatment were randomly selected for evaluate IGF-I gene expression on muscle and intestine. Sampling routine included 24 h fasting and sacrifices by overexpositon to sedative, benzocaine-saturated medium (0.5 g L^{-1}). IGF-I gene expression in liver was detected by semi quantitative real-time assay. No specific primers (*Ictalurus punctatus*) were used to amplify the IGF-I mature region of *P. fasciatum*, having 18S ribossomal used as an active endogenous reference to normalize quantification of a mRNA target. A sample without cDNA template was used to verify amplification quality. The identity of the PCR products was confirmed by direct sequencing. All statistical analyses were performed using SAS, Version 6.12 software (SAS Institute Inc., Cary, NC, USA).

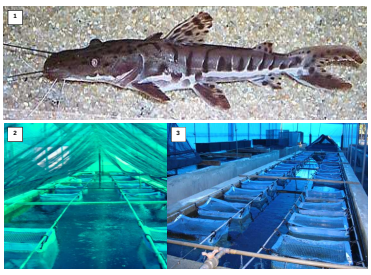

Figure 1 – *Pseudoplatystoma fasciatum* (1) covering of the "tunnel" tanks (2) distribution of the plastic cages (3)

RESULTS

The substitution of the animal protein source for milk protein did not influence the performance at 60 days, differences were observed in the weight gain, specific growth rate and food conversion of the juveniles at 30 days, probably related to an adaptation period to milk protein.

Table 1 - Fish performance at 30 and 60 days

Performance	Experimental Diets				
	control	5	10	15	20
Initial weight			35.14 ± 2.23		
Final weight (g) 30 days	63.98 ± 3.26	62.81 ± 3.80	56.77 ± 4.79	56.50 ± 6.88	62.75 ± 3.80
Final weight (g) 60 days	*102.35 ± 8.87*	*99.10 ± 6.11*	*101.13 ± 24.35*	*105.09 ± 8.19*	*103.86 ± 5.94*
WG[1] 30 days	69.65 ± 6.72^{a}	90.70 ± 10.07^{b}	61.03 ± 13.93^{a}	70.74 ± 12.59^{ac}	86.76 ± 11.77^{bc}
WG[1] 60 days	*194.31 ± 36.10*	*173.75 ± 11.77*	*227.61 ± 2.42*	*183.20 ± 2.82*	*220.12 ± 9.95*
EGR[2] 30 days	1.76 ± 0.13^{ac}	2.15 ± 0.17^{b}	1.58 ± 0.29^{c}	1.78 ± 0.25^{ac}	2.08 ± 0.21^{ab}
EGR[2] 60 days	*1.79 ± 0.21*	*1.67 ± 0.07*	*1.72 ± 0.45*	*1.73 ± 0.02*	*1.80 ± 0.24*
FCR[3] 30 days	0.74 ± 0.06^{ad}	0.64 ± 0.02^{bc}	0.77 ± 0.08^{d}	0.76 ± 0.09^{d}	0.64 ± 0.06^{ac}
FCR[3] 60 days	*0.85 ± 0.11*	*0.93 ± 0.06*	*0.90 ± 0.14*	*0.85 ± 0.04*	*0.85 ± 0.11*

[1]Weight gain (%); [2]specific growth ratio (%); [3]feed conversion ratio (kg kg^{-1}).
[abcd]Mean values with similar superscripts within rows are not significantly different (P<0.05).

Before using the ΔCt method –[ΔCt=Ct(target gene)−Ct(reference gene)] for quantification (comparative method), a validation experiment was performed to demonstrate that efficiencies of targets and reference gene were approximately similar.

Muscle and intestine IGF-I expression differed among periods and diets (P<0.05) (Tables 2 and 3). The highest expression of muscle IGF-I was observed at 60 days. The lowest expression was observed in control diet and diet with 5% of BC. Differently of the muscle, the lowest expression of intestine IGF-I was observed at 60 days and no differences were observed in response to the highest level of BC. In the intestine, at 30 and 60 days it was observed lower expression compared to muscle. The detection of IGF-I mRNAs in parenchymal cells supports the autocrine/paracrine sugested condition of this hormone in extrahepatic tissues.

Table 2. 18S ribossomal C_t and ΔC_t of IGF-I gene for muscle

Period	Experimental diets						Probability[1]		
	Control	5	10	15	20		Diet	Period	Diet X Period
			18S C_t						
30 days	18.72 ± 1.44	19.30 ± 0.92	18.87 ± 1.01	17.76 ± 0.93	17.07 ± 1.00	*18.34 ± 0.44*	NS	0.05	NS
60 days	16.11 ± 1.60	18.15± 1.67	17.02 ± 1.52	17.19 ± 0.56	15.36 ± 0.90	*16.77 ± 0.58*			
	17.42 ± 1.01	*18.72 ± 0.93*	*17.94 ± 0.91*	*17.48 ± 0.53*	*16.22 ± 0.69*				
			ΔC_t IGF-I gene						
30 days	28.64 ± 0.58	29.54 ± 0.69	31.06 ± 0.66	30.12 ± 0.69	31.39 ± 0.70	*30.15 ± 0.33*	0.05	0.05	NS
60 days	25.40 ± 0.73	25.48 ± 0.68	25.48 ± 0.68	28.59 ± 0.57	27.89 ± 0.99	*26.84 ± 0.38*			
	27.02 ± 0.63[a]	*27.51 ± 0.73[a]*	*28.93 ± 0.74[b]*	*29.35 ± 1.14[b]*	*29.64 ± 0.76[b]*				

**Mean values with similar superscripts within rows are not significantly different (P>0.05).
NS = no significant (P>0.05)
[1]Probability: diet, period – interaction diet vs. period

Table 3. 18S ribossomal C_t and ΔC_t of IGF-I gene for intestine

Period	Experimental diets						Probability[1]		
	Control	5	10	15	20		Diet	Period	Diet X Period
			18S C_t						
30 days	16.86 ± 0.52[ab]	18.46 ± 0.49[cd]	17.58 ± 0.57[abc]	15.95 ± 0.38[a][c][d]	15.50 ± 0.37[a][c]	*16.87± 0.27*	0.05	0.05	0.05
60 days	13.82 ± 0.40[a]	14.87 ± 0.36[a]	14.27 ± 0.61[a]	16.03 ± 0.31[a][c]	14.20 ± 0.37[a]	*14.64 ± 0.22*			
	15.34 ± 0.53	*16.67 ± 0.58*	*15.93 ± 0.61*	*15.99 ± 0.24*	*14.85 ± 0.32*				
			ΔC_t IGF-I gene						
30 days	36.50 ± 1.44	35.61 ± 1.15	34.73 ± 1.81	36.33 ± 1.29	38.79 ± 0.74	*36.39 ± 0.60*	0.05	0.05	NS
60 days	26.51 ± 0.48	28.59 ± 0.34	30.59 ± 0.99	28.99 ± 0.47	30.53 ± 1.24	*29.05 ± 0.42*			
	31.51 ± 1.56[a]	*32.09 ± 1.13[a]*	*32.66 ± 1.15[b]*	*32.66 ± 1.21[a]*	*34.65 ± 1.34[b]*				

**Mean values with similar superscripts within rows are not significantly different (P>0.05).
[ab]Mean values with similar superscripts within column are not significantly different (P>0.05).
NS – no significant (P>0.05)
[1]Probability: diet, period – interaction diet vs. period

CONCLUSIONS

Liophilized bovine colostrum can partially substitute the soybean meal providing a good performance in striped catfish juveniles. The presence of animal protein in the experimental diets is connected with variation of IGF-I expression.

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