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Time of feed transition and inclusion levels of exogenous protease in rations for piabanha-do-Pardo *Brycon* sp. hatchery

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ABSTRACT. Two experiments were conducted which consisted of determining the ideal time to make a feed transition from larvae of 'curimba' *Prochilodus hartii* to ration (from third, fifth and seventh days of life, with three days of co-feeding), and assess inclusion levels of exogenous protease (0, 0.02 and 0.2%) in the diet of larvae of piabanha-do-Pardo *Brycon* sp., in a completely randomized experimental design and duration of 15 and 17 days, respectively. Biomass, survival, total length, weight and specific growth rate were measured at the end of the experiments. Water quality parameters were measured every three days. The different transition periods and levels of exogenous protease did not affect water quality. The animals subjected to feed transition on the seventh day of life showed better results for length (23.1 mm), weight (110.9 mg) and SGR (25.5%), being similar in biomass and survival at the fifth day of life. Therefore, the transition can be made on the fifth day of life. The inclusion levels of exogenous protease in the commercial diet had no effect on performance.

Keywords: food additive, Pardo river basin, co-feeding, native fish species.

Momento para a transição alimentar e níveis de inclusão de protease exógena na ração da larvicultura de piabanha-do-Pardo *Brycon* sp.

RESUMO. Foram realizados dois experimentos, que consistiram em determinar o momento ideal de se realizar a transição alimentar de larva de curimba *Prochilodus hartii* para ração (a partir do terceiro, quinto e sétimo dia de vida, com três dias de coalimentação), e avaliar níveis de inclusão de protease exógena (0; 0,02 e 0,2%) na dieta de larvas de piabanha-do-Pardo *Brycon* sp., em delineamento inteiramente casualizado e duração de 15 e 17 dias, respectivamente. A biomassa, sobrevivência, comprimento total, peso médio e taxa de crescimento específico foram mensurados ao final dos experimentos. Já os parâmetros de qualidade de água foram aferidos a cada três dias. Os diferentes períodos de transição alimentar e níveis de protease exógena não interferiram na qualidade da água. Os animais submetidos à transição alimentar no sétimo dia de vida apresentaram melhores resultados para comprimento (23,1 mm), peso (110,9 mg) e TCE (25,5%), sendo similares em biomassa e sobrevivência aos do quinto dia de transição. Portanto, o início da transição pode ser realizada no quinto dia de vida. Os níveis de inclusão de protease exógena na ração comercial não influenciaram no desempenho dos animais.

Palavras-chave: aditivo alimentar, bacia do rio Pardo, coalimentação, espécie de peixe nativo.

Introduction

Induced reproduction and restocking programs with native species have proved ever more important and common for environmental preservation purposes in Brazilian rivers (KUBITZA et al., 2007).

Several authors have affirmed that hatchery is the most critical point in the production chain, out of all the different life stages of native fish species. The first life stages of fish are characterized by functional and morphological differentiation processes that include modifications in sensory organs, respiratory and enzyme systems, as well as in digestive physiology, all of which leads to changes in nutrient requirements (SEGNER et al., 1993) and high rates of cannibalism. As such, studies on larviculture have become increasingly necessary to find efficient solutions for the problems during that stage of production.

Diet stands out as the main factor responsible for eventual failure in larviculture, as organisms undergo a stage of structural and functional differentiation of the digestive system, which for most species changes from endogenous feeding (vitellus) to exogenous feeding (CESTAROLLI et al., 1997) consisting of live or inert organisms.

Prior to choosing which feeding management to undertake, some factors must be taken into account such as feeding behavior of the species, digestive system development, time of feed transition and properties of the provided diet (ROCHA et al., 2008), so that production of a given species can be successful.

The exclusive use of ration in the hatchery of Neotropical species, such as those belonging to genus *Brycon*, has not shown good results (LOPES et al., 1994), likely due to poor visual stimulus and low digestive capacity of the larvae (ZAVALA-CAMIN, 1996), making the use of live feed essential during that rearing stage. However, the use of live feed increases the cost of production, requires time, equipment and labor; therefore, the duration of live feeding must be reduced.

One frequently employed strategy for good zootechnical performance is gradual feed transition or co-feeding, which consists of initially providing live feed or meat and gradually replace it with an artificial diet (ADAMANTE et al., 2007).

The rations prepared for the initial stage of fish are characterized by the high protein level required during that stage (VEGA-ORELLANA et al., 2006), resulting in considerable water polluting potential. For that reason, as well as the high cost and limited digestive system of animals during that life stage, the use of feed additives such as free amino acids (NAZ; TÜRKMEN, 2009), hormones (KOLKOVSKI et al., 2000) and digestive enzymes (TESSER et al., 2006) has been progressively more tested with the objective of promoting greater animal survivability and growth as well as lower pollution of freshwater springs.

There are three basic reasons for using enzymes in animal feeding: 1 – eliminate anti-nutritional factors present in several feed ingredients; 2 – provide enzymes that animals are unable to synthesize at the required rates; 3 – supplement enzymes naturally produced by animals, particularly in young animals with immature digestive systems, in which endogenous enzyme production may be inadequate (BEDFORD; PARTRIDGE, 2001).

The most important enzyme studied in fish feeding is phytase, with proven benefits according to several researchers (BOCK et al., 2007; FURUYA et al., 2001, 2010). However, few studies mention the use of exogenous protease in the diets of native fish larvae.

The objective of this study was to determine the ideal time to undertake feed transition, from live to inert feed, and evaluate the inclusion of exogenous protease (RONOZYME ProAct®) at different levels in the diet of *Brycon* sp. larvae.

Material and methods

Two experiments were performed at the fish farming sector of the Machado Mineiro Hydroelectric Plant, belonging to CEMIG and located in the municipality of Águas Vermelhas (latitude 15°44' S; longitude 41°27' W), northern Minas Gerais State, Brazil. Both experiments used larvae from induced spawning, 24 h after hatching, at which time the larvae already had open mouths and ingested exogenous feed.

Experiment 1 – Gradual feed transition

The experiment was performed between January 14 and 29, 2010. 'Piabanha-do-Pardo' *Brycon* sp. larvae were used, averaging 2.3 ± 0.04 mg in weight and 7.79 ± 0.51 mm in total length. The animals were counted individually and randomly placed in one of 12 4-L tanks, at a stocking rate of 15 larvae L^{-1}, totaling 60 individuals $tank^{-1}$, with constant aeration and natural light cycle.

The larvae were subjected to the following feed transitions from *Prochilodus hartii* larvae to ration: starting on the 3rd day of life, starting on the 5th day of life, and starting on the 7th day of life.

An entirely randomized design was used, consisting of four replications per treatment.

Newly hatched larvae of 'curimba' *Prochilodus hartii* were used as live feed and offered up to two days after the start of the feed transition, at a rate of 12 larvae of curimba $piabanha^{-1}$ day^{-1} (720 larvae curimba $tank^{-1}$ day^{-1}), three times a day, at 8, 12 and 16h. The ration used (commercial powdered feed for fish larvae, containing: crude protein – minimum 55%; lipid – minimum 4%; crude fiber – maximum 6%; moisture – maximum 10%; mineral matter – maximum 18%; calcium – maximum 5%; and phosphorus – minimum 1.5%), was offered at a rate 25% of biomass throughout the entire experiment, at the same time as live feed, checking for excess feed, which was removed daily by syphoning, 40 minutes after the feeding period.

Experiment 2 – Exogenous protease in diet

The experiment was performed between February 16 and March 5, 2010. 'Piabanha-do-Pardo' *Brycon* sp. larvae were used, averaging 2.4 ± 0.02 mg in weight and 7.81 ± 0.46 mm in total length. The animals were counted individually and randomly placed in one of 15 4-L tanks, at a stocking rate of 15 larvae L^{-1}, totaling 60 individuals $tank^{-1}$, with constant aeration and natural light cycle.

Larvae were fed with the following inclusion levels of exogenous protease in the powdered feed: 0, 0.02 and 0.2%. The enzyme was weighed and included in the commercial feed, mixed in for 15 minutes.

An entirely randomized design was used, consisting of five replications per treatment.

Newly hatched larvae of 'curimba' *Prochilodus hartii* were used as live feed starting on the first day of exogenous feeding until the piabanha's eighth day of life, with feed transition starting on the 6th day of life at a rate of 12 larvae of curimba piabanha^{-1} day^{-1}, three times a day, at 8, 12 and 16h. The ration – the same as in the previous experiment – was offered at a rate of 25% of biomass.

Common protocol to both experiments

Water was syphoned daily from the tanks to remove feed leftovers and feces, and 30% of the water volume was replaced in each experimental unit.

Physical-chemical parameters water, temperature, dissolved oxygen, pH and electrical conductivity were monitored every three days prior to cleaning.

At the end of the experiments, the larvae were stunned, weighed and counted to determine biomass and survival rate, and then fixed in 10% formaldehyde to determine their weight (mg) in an analytical scale (0.1 mg precision) and total length (mm), measured using a pachymeter (0.02 mm precision), of 15 exemplars from each experimental unit. Using the results of mean initial weight (Pt_i) and final weight (Pt_f) for each replication, the specific growth rate was defined by the expression: $SGR = 100 (\ln Pt_f - \ln Pt_i)/ \Delta t$, considering Δt as the duration in days from the first to last day between the samplings.

Data analysis

Data on water quality, survival, weight, biomass, total length and specific growth rate were subjected to analysis of variance (ANOVA), and mean values were compared by Tukey's test at 5% probability.

Results and discussion

Cannibalism was observed in all treatments of both experiments, which makes it possible to affirm that this behavior is a natural characteristic of the studied species as well as others in genus *Brycon* (CECCARELLI; SENHORINI, 1996; LOPES et al., 1994; PEDREIRA et al., 2006, 2008; SACCOL-PEREIRA; NUÑER, 2003; SENHORINI et al., 2002).

Feed transition

Water quality parameters did not show significant differences between treatments (Table 1) and seem to be within the appropriate range for rearing the species in question, as they were similar to those observed in hatcheries of other species from genus *Brycon* (PEDREIRA et al., 2006, 2008; REYNALTE-TATAJE et al., 2004) and were observed in the same laboratory in which they were spawned, using water from the Pardo river.

Significant differences were observed between the times of feed transitions used in the experiment. The highest values for final weight, total length and specific growth rate (Table 2) were obtained when feed transition was carried out starting on the 7th day of life (6th day of the experiment), with the second best performance observed when feed transition was carried out starting on the 5th day of life; transition starting on 3rd day had the lowest performance. The longer period of live feed inclusion in diet favored animal growth and likely helped develop the digestive system of larvae, which in turn allowed for improved ration use.

The data corroborate the findings by Vega-Orellana et al. (2006), evaluating feed transition for larvae of 'dourado' *Salminus brasiliensis* that also used larvae of curimba *Prochilodus* sp. as live feed. Those authors concluded that the best performances are obtained when the transition is made on the 7th day of life; nevertheless, the same authors affirmed that the transition can be carried out on the 5th day of life, as long as it is done gradually.

Table 1. Mean values (± standard deviation) of the physical-chemical parameters of water, obtained during the experiment with larvae of piabanha-do-Pardo *Brycon* sp. subjected to different feed transition periods.

Transition starting on	Conductivity (μS cm^{-1})	pH	O_2 (mg L^{-1})	T (ºC)
3rd day	62.9 ± 0.86^a	7.0 ± 0.01^a	7.1 ± 0.11^a	24.8 ± 0.39^a
5th day	63.8 ± 0.79^a	6.9 ± 0.02^a	6.8 ± 0.36^a	24.9 ± 0.21^a
7th day	65.3 ± 2.65^a	6.9 ± 0.02^a	6.8 ± 0.43^a	25.2 ± 0.28^a

Means in the same column followed by different letters are different according to Tukey test ($p < 5\%$). O2 = Dissolved oxygen and T = Temperature.

Table 2. Mean values (± standard deviation) for final weight (Weight), total length (CT), survival (S), biomass (Bio) and specific growth rate (SGR) of larvae from piabanha-do-Pardo *Brycon* sp. subjected to different feed transition periods.

Transition starting on	Weight (mg)	CT (mm)	Bio (g)	S (%)	SGR
3rd day	54.3 ± 4.21^c	18.7 ± 0.48^c	1.6 ± 0.66^b	36.6 ± 15.1^b	20.7 ± 0.53^c
5th day	67.3 ± 1.82^b	20.5 ± 0.41^b	3.1 ± 0.26^a	53.3 ± 2.7^{ab}	22.2 ± 0.17^b
7th day	110.9 ± 6.8^a	23.1 ± 0.52^a	3.9 ± 0.27^a	55.4 ± 3.43^a	25.5 ± 0.41^a

Means in the same column followed by different letters are different according to Tukey test ($p < 5\%$).

The results of the present study clearly show the need to undertake feed transition; this was also evidenced in other native freshwater fish species such as 'trairão' *Hoplias lacerdae* (LUZ et al., 2002), dourado *Salminus brasiliensis* (VEGA-ORELLANA et al., 2006) and 'pirarucu' *Arapaima gigas* (CAVERO et al., 2003), as well as saltwater species such as flounder *Paralichthys orbignyanus* (ROCHA et al., 2008) and seabream *Sparus aurata* (KOLKOVSKI et al., 1997b). Feeding with live organisms such *Artemia* sp. nauplii, zooplankton and foraging larvae can increase digestive activity and consequently improve the ability of larvae to digest artificial diets (KOLKOVSKI et al., 1997a). However, Adamante et al. (2007), using nauplii of *Artemia* sp. in feed transition for catfish *Steindachneridion scripta*, reported no significant difference between the different feed transition periods.

The larvae of most fish species have relatively low enzymatic activity if compared to adult specimens, low hydrolysis and nutrient absorption capacity, making the supply of live feed essential during that life stage, as it assists the digestion process in three ways: 1 – donating digestive enzymes from the body itself; 2 – releasing enzymes that perform autolysis of the organism itself; 3 – activating zymogenes of the predator; and 4 – donating neuropeptides that favor digestive processes (KOLKOVSKI, 2001). To the author, these benefits are frequently omitted when formulated diets are used exclusively, as that type of feed contains around 60% to 90% dry matter, compared to only 10% in live feed. That can lead to insufficient digestibility, as it is much more difficult to break down hard dry particles than live organisms (KOLKOVSKI, 2001). Other authors have also observed the low efficiency of exclusive ration use to feed larvae of native species (CECCARELLI; SENHORINI, 1996; FEIDEN et al., 2006; LOPES et al., 1994; SIPAÚBA-TAVARES; ROCHA, 1994). That would explain why the treatment in which live feed was withdrawn first (transition starting on the 3rd day) showed a lower survival rate.

Ration intake was observed only in the 4th day of life of piabanha-do-Pardo larvae. The short duration of live feed availability likely hindered animal development. Those results are in agreement with those found by Diemer et al. (2009) in 'jundiá' hatchery. Nevertheless, survival and biomass were statistically similar when the transition – introduction of ration with live feed withdrawal after two days of co-feeding – started on the 5th and 7th day of life, making it possible to affirm that transition can start on the 5th day of life in piabanha-do-Pardo hatchery.

The tendency to replace live feed with ration as early as possible happens in order to simplify management and reduce costs, as according to Ruyet (1993) live feed production can represent nearly 80% of the total hatchery cost. Curimba larvae production to serve as live feed for piabanha-do-Pardo larvae required labor, time, materials used for spawning (hormone, hatcheries, among others) and energy. Maintaining nurseries and even the diet of curimba breeders throughout the year further increases the cost of production for that type of live feed.

Exogenous protease in diet

Water quality parameters did not show significant differences between treatments (Table 3), staying within the described range for tropical species (BOYD, 1990; REYNALTE-TATAJE et al., 2004) and observed in hatchery of other *Brycon* species (PEDREIRA et al., 2006, 2008; REYNALTE-TATAJE et al., 2004).

Of all the different levels of enzymes used, the analyses of final weight, total length, biomass, survival and specific growth rate did not show significant differences (Table 4) for larvae of piabanha-do-Pardo *Brycon* sp. fed with newly hatched larvae of curimba *Prochilodus hartii* up to the 9th day of life, with feed transition starting on the 7th day of life.

Table 3. Mean values (± standard deviation) of physical-chemical parameters of water obtained during the experiment with larvae of piabanha-do-Pardo *Brycon* sp. subjected to inclusion levels of exogenous protease in diet.

Protease levels (%)	Conductivity (μS cm^{-1})	pH	O_2 (mg L^{-1})	T (ºC)
0.00	75.0 ± 1.09^a	7.0 ± 0.02^a	7.1 ± 0.25^a	25.5 ± 0.26^a
0.02	75.9 ± 2.20^a	7.0 ± 0.06^a	6.9 ± 0.23^a	25.4 ± 0.32^a
0.2	76.7 ± 0.80^a	7.0 ± 0.03^a	6.9 ± 0.25^a	25.4 ± 0.16^a

Means in the same column followed by different letters are different according to Tukey test ($p < 5\%$). O_2 = Dissolved oxygen, T = Temperature.

Table 4. Mean values (± standard deviation) for weight, total length (CT), survival (S), biomass (Bio) and specific growth rate (SGR) for larvae of piabanha-do-Pardo *Brycon* sp. subjected to inclusion levels of exogenous protease in diet.

Protease levels (%)	Weight (mg)	CT (mm)	Bio (g)	S (%)	SGR
0.00	112.5 ± 4.49^a	23.0 ± 0.54^a	3.0 ± 0.54^a	50.1 ± 6.89^a	21.3 ± 0.22^a
0.02	108.7 ± 4.37^a	22.3 ± 0.68^a	2.8 ± 0.08^a	46.1 ± 2.07^a	21.1 ± 0.21^a
0.2	117.0 ± 5.86^a	23.4 ± 0.54^a	3.2 ± 0.12^a	50.0 ± 3.30^a	21.5 ± 0.28^a

Means in the same column followed by different letters are different according to Tukey test ($p < 5\%$).

These results corroborate those found by Nunes et al. (2006) who, while evaluating the effect of exogenous digestive enzymes (including a protease) in the feeding of juveniles of 'tambaqui' *Colossoma macropomum*, concluded that protease inclusion did not influence the zootechnical performance of the animals at any of the tested inclusion levels (0.0, 0.05, 0.1 and 0.2%).

In the case of piabanha-do-Pardo, protease inclusion did not significantly influence development, suggesting that this species is self-sufficient in digesting the protein fraction in the ration, as long as feed transition takes place starting on the 7th day of life. That is likely due to the rapid development of piabanha-do-Pardo, making it fit for partial ration assimilation during the first days of life. Precocious development of *Brycon* larvae has been described by several authors (CECCARELLI; SENHORINI, 1996; LEONARDO et al., 2008; PEDREIRA; SIPAÚBA-TAVARES, 2002; REYNALTE-TATAJE, et al., 2002, 2004; SENHORINI et al., 2002), with larvae of 'piracanjuba' *Brycon orbignyanus* capturing feed starting on the 3rd day of rearing and 4th day of life (PEDREIRA; SIPAÚBA-TAVARES, 2002).

Endogenous enzyme secretion occurs regardless of enzyme supplementation (SOARES et al., 2008). Larvae of piracanjuba *B. orbignyanus* have an enzymatic adaptation mechanism, varying the quantity and composition of digestive proteases according to the quality of feed and feeding regimen (GARCÍA-CARREÑO et al., 2002). The rapid development of larvae of piabanha-do-Pardo *Brycon* sp. and their respective enzymatic adaptation abilities could explain the similarities between the results found.

Nevertheless, different results from the ones obtained herein can be found. Tesser et al. (2006), while studying the supplementation of exogenous enzymes (swine pancreatin) in microdiets in larviculture of 'pacu' *Piaractus mesopotamicus*, observed an improvement in animal growth and survival. Another native species that responded positively to added exogenous enzymes in diet was 'pirarucu' *Arapaima gigas* during juvenile stage (CAVERO et al., 2003).

Traditionally, additives result in improved nutrient digestibility and animal growth (SILVA et al., 2007). Kolkovski et al. (1993) concluded that supplementation with 0.05% swine pancreatin in the microdiet of larvae from 'dourada' *Sparus aurata* resulted in 200% added growth in animals and increased feed assimilation by 30% compared to larvae fed without the additive. The use of enzyme complex has also been tested in diets of Nile tilapia *Oreochromis niloticus* juveniles, with favorable results in nutrient digestibility (OLIVEIRA et al., 2007), increasing ration intake and conversion of animals, although weight gain, survival and specific growth rates did not differ (SIGNOR et al., 2010).

Although no statistical difference was observed among treatments for final weight, total length, biomass and specific growth rate, it was observed that the inclusion of 0.2% protease led to a slight improvement in the cited variables, possibly because the additive used acted by supplementing the action of endogenous enzymes, releasing more tripeptides, dipeptides and free amino acids, favoring the absorption of these nutrients and thus animal performance, as suggested for fish (OLIVEIRA et al., 2007), birds (BARBOSA et al., 2008), swine (NERY et al., 2000) and ruminants (FAGUNDES et al., 2008).

Finally, it was observed that piabanha-do-Pardo features accelerated development, and that further studies to reduce the level and duration of live feed inclusion should be carried out, in order to optimize and reduce hatchery costs.

Conclusion

Larvae of piabanha-do-Pardo *Brycon* sp. accept artificial diets starting on the 4th day of life, 3rd day of experiment; therefore, it is recommended that feed transition be carried out, at least, starting on the 5th day of life, keeping live feeding for two more days, to only later provide exclusively artificial diet.

The inclusion of exogenous protease in commercial feed did not influence the zootechnical performance of larvae fed with larvae of curimba *Prochilodus hartii* up to the 9th day life, suggesting that this species features rapid development of the digestive tract and its enzymes.

Further studies on handling adjustments, time of feed transition, types of live feed and ration, protease inclusion levels and costs, should be carried out to continue the optimization of piabanha-do-Pardo larviculture.

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