Replacement of broiler liver by fish meal and soy protein concentrate in diets for silver catfish (*Rhamdia quelen*) post-larvae

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**ABSTRACT**

Muscular growth in fish is influenced by diet quality, mainly the protein sources and amino acids balance. A feeding trial was conducted for a period of 28 days. Muscle growth and histology of Silver catfish post-larvae was evaluated. The diets tested were partial and total replacement of broiler liver by fish meal and soybean protein concentrate (basal, 15FM, 30FM, 15SPC and 30SPC). The basal diet consisted mainly of fresh poultry liver plus sugarcane yeast. After the feeding test, the fish fed with 15FM diet presented higher growth (P<0.05) than the post-larvae fed with the other diets. The muscle development was performed in post-larvae-fed diets 15FM and 30SPC. Higher diameter and total number of fibres were found in fish fed diet 15FM diet (P<0.05). The replacement of 50% of the broiler liver by fishmeal provided a good diet for *Rhamdia quelen* post-larvae.

**KEYWORDS**

Histology; larviculture; muscle growth; native specie; nutrition

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Vegetable sources are widely used in diets for cultured fish species and are considered an alternative for fish meal (Espe et al. 2012; Yesilayer and Kaymak 2020). Soy derivatives have a relatively well-balanced protein content and amino acid profile (Gatlin et al. 2007; Kokou et al. 2017), which may be deficient in some essential amino acids, such as lysine and methionine (Zhao et al. 2017; Amer et al. 2020). The utilization of soybean derivatives in the diet of silver catfish post-larvae was tested (Plaia and Radünz Neto 1997; Fontinelli and Radunz Neto 2007). According to Zhang et al. (2019) among plant protein sources, soy protein concentrate (SPC) has lower anti-nutritional factors and higher protein content. Adequate proportion of soybean in fish diets can contribute to good development. However, excessive inclusion of soybean in the fish diet may inhibit growth.

In this study, partial and total replacement of broiler liver by fish meal and soybean protein concentrate was evaluated on growth and muscle histology of post-larvae of silver catfish Rhamdia quelen. In addition, it is expected to obtain a practical balanced diet and easily manufactured industrially, reducing risk of contamination.

Material and methods

Fish, facilities and diets

The experimental procedures were approved by the ethics committee on animal use/UFSM (062/2013). The silver catfish used in the study were obtained through breeding and commercial fish farming held in incubation (latitude 29°43′S, 53°42′W longitude). Spawning was induced using pituitary extract of carp (5 mg kg⁻¹ for females and 2.5 mg kg⁻¹ for males). The fish were kept in an incubator (Zoug) (60 L). The average incubation temperature used was 26.4°C and the average dissolved oxygen was 6.55 mg L⁻¹. For the feeding trial (28 days), they used 35 experimental units (240 post-larvae each), composed of two plastic containers, with a drain to control the volume of water (5 L). The inner container had a lateral screen protection to prevent the exit of post-larvae, with individual entrance and exit of water. A recirculation system with a biological filter composed of crushed stone was used to biofilter ammonia from the water.

The diets were elaborated (Table 1) and the dry ingredients mixed (particle size below 75 μm) (Trombetta et al. 2006). After homogenization, fresh liver and/or water was added (as needed) until the mixture provided sufficient moisture for pelletization in the meat grinder. Then, the samples were dried at 40°C for 24 h. Afterwards, the samples were crushed, sieved, and separated into portions that consisted of particles of 100–200 μm, 200–400 μm, 400–600 μm and 600–800 μm. These sizes are suitable for the mouth opening of post-larvae. The samples were then kept in a refrigerator at 4°C until feeding. The amino acids content of the experimental diets (Table 1) was extracted with hydrochloric acid (6 N) for 24 h (0.3 mg sample in 9 mL HCl) and then derivatized with phenylisothiocyanate. The derivatized samples were separated by high-performance liquid chromatography (HPLC, Model P4000-Thermo Fisher Scientific, Waltham, MA) in reverse phase with UV detection at 254 nm, based on the methodology described by White et al. (1986). The experimental design was completely random (five diets and seven replicates). The treatments correspond to partial and total replacement of broiler liver in the diets 15FM (fish meal), 15SPC (soy protein concentrate), 30FM (fish meal) and 30 SPC (soy protein concentrate). The standard treatment (control diet) was adapted by Coldebella et al. (2011).

Water quality

The daily temperature (24.6 ± 1.1°C) and dissolved oxygen (6.2 ± 0.6 mg L⁻¹) were measured by a YSI 550 digital oximeter. The pH (7.2 ± 0.24) – using a digital pH meter, (YSI®, Yellowsprings, U.S.A. – model 550A). The total alkalinity (37.3 ± 7.4 mg L⁻¹ CaCO₃), nitrite (0.03 ± 0.01 mg L⁻¹) and hardness (52 ± 11.25 mg L⁻¹ CaCO₃) were analysed weekly with Colorimetric kits. The flow of the experimental units was 0.20 L min⁻¹ in the

Table 1. Diet offered to silver catfish post-larvae for a period of 28 days of feeding.

| Ingredients (% feed)     | Standarda | 15SPC | 15FM | 30SPC | 30FM |
|--------------------------|-----------|-------|------|-------|------|
| Sugar cane yeast         | 37        | 36.7  | 36.75| 36.65 | 36.5 |
| SPC                      | 0         | 15    | 0    | 30    | 30   |
| Fish meal                | 0         | 0     | 0    | 0     | 0    |
| Broiler liverb           | 30        | 15    | 15   | 0     | 0    |
| Cooked egg yolk          | 20        | 20    | 20   | 20    | 20   |
| DBR                      | 8         | 8     | 8    | 8     | 8.3  |
| Soy lecithin             | 2         | 2     | 2    | 2     | 2    |
| Vitaminsc                | 2         | 2     | 2    | 2     | 2    |
| Mineralsd                | 1         | 1     | 1    | 1     | 1    |
| Taurine                  | 0         | 0     | 0.30 | 0.25  | 0.35 |
| Centesimal composition (%) and estimated energy | 87.31 | 91.94 | 92.7 | 90.17 | 92.73 |
| Dry massf                | 44.74     | 43.7  | 44.28| 43.79 | 45.05|
| Crude proteinf           | 3993      | 3869  | 3955 | 3750  | 3923 |
| Gross energy (kcal/kg)g  | 11.25     | 11.25 | 11.25| 11.25 | 11.25|
| Fatc                    | 19.45     | 17.93 | 19.48| 16.92 | 18.55|
| Mineral matterd         | 8.04      | 7.76  | 8.41 | 6.62  | 12   |
| NDFd                    | 5.08      | 8.75  | 5.13 | 11.16 | 10.53|
| Amino acid composition (%)h | 4.13 | 4.46  | 3.61 | 4.80  | 3.11 |
| Asparagine               | 5.02      | 5.74  | 4.64 | 6.48  | 4.28 |
| Glutamine                | 2.24      | 2.37  | 2.18 | 2.51  | 2.13 |
| Serina                   | 1.82      | 1.80  | 1.72 | 1.78  | 1.62 |
| Glycine                  | 0.92      | 0.93  | 1.66 | 0.94  | 2.39 |
| Histidine                | 0.05      | 0.33  | 0.42 | 0.35  | 0.49 |
| Taurine                  | 2.70      | 2.85  | 2.24 | 3.00  | 1.78 |
| Threonine                | 2.52      | 2.37  | 2.55 | 2.22  | 2.59 |
| Alanine                  | 1.70      | 1.77  | 1.92 | 1.84  | 2.15 |
| Proline                  | 1.43      | 1.54  | 1.41 | 1.65  | 1.38 |
| Tyrosine                 | 2.54      | 2.44  | 2.35 | 2.35  | 2.17 |
| Valine                   | 0.83      | 0.72  | 0.81 | 0.62  | 0.80 |
| Methionine               | 0.55      | 0.58  | 0.47 | 0.61  | 0.40 |
| Cystine                  | 1.99      | 2.00  | 1.89 | 2.02  | 1.78 |
| Isoleucine               | 3.33      | 3.27  | 3.07 | 3.22  | 2.82 |
| Phenylalanine            | 1.91      | 2.01  | 1.82 | 2.12  | 1.74 |
| Lysine                   | 2.09      | 2.99  | 2.97 | 3.00  | 2.96 |

Coldebella et al. (2011).
b% calculated on dry mass (DM), fresh poultry liver 25.9% DM.
Trombetta et al. (2006).
Diocalcium phosphate.
*Adapted from Chatzifotis et al. (2008).
*Analysed – Fisheries Laboratory – DZ/UFSM.
Calculated.
*Analysed – Campinas State University – UNICAMP (Laboratory of Protein Sources).
Treatments: Standard – liver plus yeast; 15FM and 30FM – replacement of 15% and 30% of poultry liver by fish meal (Rossato et al. 2014), 15SPC and 30SPC – replacement 15% and 30% of poultry liver by protein concentrate of soybean. SPC, soy protein concentrate; FM, fish meal; DBR, defatted rice bran; NDF, neutral detergent fibre. Amino acid profile of the diets, analysed by HPLC.
first week (Tronco et al. 2007) and gradually increased to 1 L min⁻¹ in the fourth experimental week. All parameters of water quality remained within the range suitable for silver catfish.

**Feeding management and samples**

The fish were fed every 2 h between 8:00 am and 8:00 pm (photoperiod 14 light/10 dark) in amounts that exceeded the fish intake capacity. The feeding rate was 1 g per day in the first experimental week, 2 g in the second week and 3 g in the two last weeks. At the beginning there was a little left and at the end of the trial period the whole diet was consumed. The experimental units were cleaned for the removal of dead post-larvae, faeces, and feed leavings twice a day. The biometric evaluation was performed in 10 post-larvae per experimental unit every seven days in order to monitor growth.

Samples were weighed on a digital scale (0.001 g), without return. The measurements of total length were performed with the aid of a digital caliper. All animals were counted for survival analysis. Fish were anesthetized with benzocaine (50 mg L⁻¹) (AVMA 2007), weighed and measured individually to obtain the following data:

- Weight of the whole fish (g); total length (TL): measurements from the end of the head to the end of the caudal fin (mm); Survival (at the end of each week (7 days) all post-larvae from each experimental unit was individually counted); condition factor: CF = (weight × 100)/(total length³); Specific growth rate (%/day): SGR = ((ln (final weight) – ln (initial weight))/days)×100; Daily weight gain (g): DWG = (final weight – initial weight)/days; Comparison of weight × survival (biomass).

The evaluation of muscular development was performed from samples of 10 post-larvae. Samples of 15FJ and 30SPC treatments were collected. These were chosen after statistical analysis, the treatment that presented higher and lower weight daily gain. The samples were fixed in Bouin solution for 12 h and then stored in 70% alcohol. The samples were cut into serial sections (4 μm) made transversely at the beginning of the dorsal fin. Histological slides of this anatomical region were made and evaluated in light photomicroscope (Zeiss, Primo Star model) magnification 60×. Then, with the obtained images, the area of the paravertebral musculature of each individual was measured.

The fibre diameter and muscle fibre counts were performed with a magnifying lens (Zeiss-Axion 4.8 vision). As a form of normalization of the data measurements of each image, the area of the spine whose value was the ratio denominator of the area of the musculature and area of the vertebrae. This procedure reduces the intrinsic error of the methodology used since different fish from different samples will be cut into different portions of the anatomical region chosen. The methodology used to obtain the diameter and number of fibres was adapted from Carani et al. (2008) and Alami-Durante et al. (2010). Using the light photomicroscope (Zeiss, Primo Star model), five sample areas were demarcated on each histological slide. Then, counting and measurement of the fibres were performed.

**Statistical analysis**

Initially, the data obtained were subjected to the normality test of Shapiro–Wilk and the homogeneity of variances (errors) were tested by Levene’s test. The data that presented P > 0.05 were considered of normal distribution. Subsequently, ANOVA and Tukey test were performed on the variables (P < 0.05) and (P < 0.01).

**Results**

The amino acid composition of the 15FM diet was considered the most adequate. After analysing the amino acids, we observed that the proportion found between the amino acids cystine and methionine was standard diet of 0.66%; 15SPC 0.80%; 15FM 0.58%; 30SPC 0.98%; 30FM 0.50%. The appropriate value for fish of this species is 0.60.

At the beginning of the experiment, the post-larvae presented weight of 1.37 mg ± 0.21 and length of 5.00 mm ± 0.01. After seven days of treatment, the fish fed with the 30SPC diet showed lower values of W, TL and DWG than the fish fed with the control diet (Table 2).

The overall performance at 14 days was lower for animals fed the 30SPC diet in relation to the animals fed the other diets. At 21 days, post-larvae of the 15FM treatment presented higher weight, total length, daily weight gain and survival rate compared to animals from treatments 30SPC and 30FM. At the end of the 28 days, the post-larvae of 15FM treatment presented all the parameters analysed superior to the other treatments.

The post-larvae fed the 30SPC diet did not survive until the end of the experimental period due to the different collections performed for the experimental analyses, higher mortality than the other treatments and cases of cannibalism. Usually cannibalism was observed near the evening and in the morning before the first feeding.

During the study period, the growth and consequent development of muscle fibres were observed, a numerical increase was observed (Table 3) and an increase in the diameter of the muscle fibres (Figure 1). Initially, the existence of a tangle of smaller fibres was observed. Subsequently, the diameter of the fibres present increased and new smaller fibres were detected (Table 3). The animals that consumed 15FM diet presented a greater muscle area (Figure 1(D)) and consequently a greater total number of fibres than those that consumed the 30SPC diet.

**Discussion**

The 15FJ diet presented excellent amino acid balance, with methionine:cystine ratio of 58%. Regarding the methionine:cystine ratio for catfish (Ictalurus punctatus) was 60% (Harding et al. 1977) and for Nile tilapia was 59.5% (Bomfim et al. 2008), similar to the best diet for Rhamdia quelen in the present study. According to Rotili et al. (2017) the methionine requirement for juvenile silver catfish was estimated at 34.42 and 35.85 g kg⁻¹ of CP or 12.74 and 13.26 g kg⁻¹ of the diet, respectively, with a content of 0.7 g kg⁻¹ cystine in the diet. Notably, the relationship between them was very close (98%). The reduced fish growth that consumed diets with soybean protein concentrate occurred due to the inadequate supplementation of some amino acids, mainly methionine and cystine.
A perfect interaction between amino acids in the diet is required to have some tissue formation (Saavedra et al. 2009). The diets showed a suitable percentage of taurine. The diet should contain at least 2 g kg$^{-1}$ taurine for optimal fish development (Chatzifotis et al. 2008). Taurine improves lipid metabolism since it participates in bile acid metabolism (El-Sayed 2014; Dehghani et al. 2020). It also participates in several processes that act to improve metabolism and increase the efficiency of muscle growth and deposition (Divakaran 2006; Wiriduge et al. 2020). Taurine supplementation may help reduce the use of ingredients such as fish meal (Saiz and Davis 2015; Adeshina and Abdel-Tawwab 2020). In this sense, more readily available and lower-price supplies can be used.

According to the growth assessment, the 15FM diet provided better results for $R.~quelen$ post-larvae. Similar results with the species were found by Coldebella et al. (2011). The combination of broiler liver with a fish meal (15%) provided amino acids and fatty acids necessary for fish development. Broiler liver is considered a valuable source of nutrients, especially in essential fatty acids (Cieslik et al. 2011). These acids are important on the eviscerated weight and survival (biomass); $S$, survival (%).

Table 3. Muscular development of post-larvae-fed diets composed of ingredients of animal and vegetable origin.

| Days/diets | 30SPC | 15FM | P |
|-----------|-------|------|---|
| Fibre diameter ($\mu m$) | | | |
| 0 | 28.02 ± 9.35 | – | – |
| 7 | 31.65 ± 9.35 | 51.78 ± 6.62 | < 0.0001 |
| 14 | 52.94 ± 9.01 | 66.63 ± 11.62 | 0.008 |
| 21 | 78.01 ± 12.74 | 99.09 ± 19.81 | 0.011 |
| Number of fibres (mm$^2$) | | | |
| 0 | 0.040 ± 0.015 | – | – |
| 7 | 0.037 ± 0.004 | 0.022 ± 0.006 | < 0.0001 |
| 14 | 0.036 ± 0.010 | 0.016 ± 0.007 | 0.0001 |
| 21 | 0.032 ± 0.005 | 0.017 ± 0.008 | 0.0002 |
| Total number of fibres | | | |
| 0 | 12.01 ± 3569 | – | – |
| 7 | 14.88 ± 1039 | 15.54 ± 2544 | 0.45 |
| 14 | 27.09 ± 6910 | 158.25 ± 191.738 | 0.04 |
| 21 | 27.70 ± 10.638 | 341.79 ± 328.476 | 0.007 |

Note: Values expressed as mean ± standard error of the mean. Means with different letters represent statistical difference by the Tukey test (P < 0.01).

$*$ indicate statistically significant differences (P < 0.001). Treatments: 15FM and 30FM – replacement of 15% and 30% of poultry liver by fish meal, respectively (Rossato et al. 2014); 15SPC and 30SPC replacement of 15% and 30% of poultry liver by soy protein concentrate. Variables: W, weight (mg); TL, total length (mm); CF, condition factor; SGR, specific growth rate; DWG (mg day$^{-1}$); daily weight gain; $W \times S$, Comparison of weight × survival (biomass); $S$, survival (%).
In the present study, the inclusion of 15% soybean protein concentrate resulted in reasonable growth and survival rates. This study is according to Chen et al. (2019) when concluding that the FM replacement with SPC should be less than 30%, as carnivorous fish or have limited capacity to use SPC as a protein source. In this context, the silver catfish is an omnivorous fish with a tendency to carnivore, the total replacement of broiler liver by SPC caused reduced growth, high mortality and cannibalism.

The use of balanced diets causes growth and/or development of the muscles more quickly and effectively. The increase of muscle fibres in the fish initially occurs due to hyperplasia (Kamaszewski et al. 2020). The diets affected the frequency of the muscle fibre diameters, mainly the growth by hyperplasia (Silva et al. 2017). Subsequently, fibre size increases (hypertrophy), despite there still being smaller fibres, which characterizes fish muscle growth. In this case, hyperplasia and hypertrophy are constant throughout life (Johnston et al. 2011; Hiebert and Anderson 2020).

The hypertrophy and/or hyperplasia processes are regulated by several signalling molecules, including the expression of myogenic and myostatin regulatory factors (Carani et al. 2013). For R. quelen fed 15FM diet, they were found diameter of the fibres of 99.09 μm. For Nile tilapia (Oreochromis niloticus), Dal Pai-Silva et al. (2003) found 15.35 μm in diameter in the fish weighing 0.04 g and 44.03 μm in 414 g. In a study with tilapia (Neu et al. 2017), it included isoleucine in the diet and found similar frequencies of muscle fibres in established classes of fibre diameters (< 20, between 20 and 50 μm, and > 50 μm).

In a study with pirarucu fingerlings (Arapaima gigas), Carani et al. (2008) described that in most fibres with a diameter below 30 μm, a greater increase in muscle mass could occur. And Lima et al. (2017) found the prevalence in the fingerling stage fibres with a diameter < 30 mm prevailed.

The results of this study are in accordance with the study of Dal Pai-Silva et al. (2005), where they concluded that the diameter of muscle fibres can vary from 10 to 100 μm. In a study with pacu (Piaractus mesopotamicus), 35 days after hatching, a high frequency of fibres ≥ 40 μm was observed in larvae (Leitão et al. 2011). In a study carried out with fingerlings and juveniles of pirarucu (Arapaima gigas), the presence of smaller muscle fibres was observed in the fingerlings compared to the fibres found in juveniles. From this, they concluded that the predominant muscle growth in fingerlings is hyperplasic and hypertrophic muscle growth is predominant in juveniles (Carani et al. 2008).

The higher muscle diameter and area observed in fish fed 15FM diet may have occurred due to the better amino acid balance and higher nutritional quality. According to Trejo-Escamilla et al. (2016), diets with high percentages of soy protein concentrate caused nutritional hypoproteinemia due to poor digestion and absorption of this ingredient. Total replacement of fish meal in the diet provides a significant reduction in the cross-sectional area of the muscle, which is mainly due to decreased muscle fibre size (Valente et al. 2016). The reduced growth and multiplication of muscle fibres for animals that consumed the 30CPS diet may have been influenced by the anti-nutrients present in small amounts in the soy protein concentrate (Booth and Pirozzi 2021).

The composition of the 15FM diet should be rich in essential fatty acids and amino acids so that the development of tissues and organs occurs properly and efficiently. Fishmeal contributes some essential amino acids that in the diet already tested for Coldebella et al. (2011) were not present. Thus, the combination of these ingredients provided an adequate diet for the maximum development of silver catfish post-larvae. These results show the importance of the nutritional quality of the diet for post-larvae from this species, reflecting the combination of quality protein sources.

**Conclusion**

The ingredients used in the 15FM diet provided the best combination of nutrients for the good growth of silver catfish post-larvae. The animals fed with the 15FM diet presented all parameters of growth and muscle development superior to the other treatments.

The inclusion of cooked liver can be tested to reduce the risk of contamination.

**Disclosure statement**

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