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Growth performance and intestinal histology of juvenile pirarucu fed with increasing levels of soybean meal

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ABSTRACT. The aim of this study was to evaluate the inclusion of different levels of soybean meal in pirarucu's diet. 200 pirarucu juveniles (152.15 ± 0.8 g) were distributed in 20 polyethylene tanks with a continuous water flow system. A completely randomized experimental design was used, with five treatments (n = 4) containing 0, 10, 20, 30 and 40% of soybean meal inclusion levels. Growth performance was measured as specific growth rate, feed conversion, weight gain and survival. Body composition, protein retention rate and histopathological changes in the intestine were analyzed. The different treatments did not affect weight gain, specific growth rate and survival. The body composition of the fish was affected by treatments for ash and ether extract and the IHS was also affected. The diet with 40% soybean meal compromised feed conversion rate. Additionally, the distal intestine of fish fed with the same diet showed a reduction in the height of mucosal folds and a loss of supranuclear vacuolation in enterocytes. Based on these results, the inclusion of up to 30% of soybean meal in the diet of juvenile pirarucu is possible without negative effects on performance and enteric morphology.

Keywords: nutrition; vegetable protein; fish meal; enteritis; histomorphometry.

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Introduction

The use of fish meal from capture fisheries as the main source of protein and amino acids in formulations for aquaculture diets has remained stable in the past 10 years. Since then, its use has been preferentially applied in starter diets, with decreasing inclusion in diets for fish in the grow-out, finishing and reproduction phases (Naylor et al., 2021; Glencross et al., 2023). The reduction of this dependence is connected to several technological advances in the aquaculture sector, such as improvements in feed processing and increasing use of meals from fish by-products and alternative plant-based protein sources (Naylor et al., 2021). Among the numerous sources of plant proteins, soybean meal stands out for being present in most commercial feed formulations used in aquaculture. This is due to its great availability and desirable nutritional characteristics, such as high protein content, constant composition, and good balance in amino acids (National Research Council [NRC], 2011). However, soybean meal has a low level of methionine and a wide variety of antinutrients that interfere in the digestion process and affect animal health and performance (Zhou, Ringø, Olsen, & Song, 2018). These undesirable characteristics of soybean meal are especially critical for carnivorous fish, which have greater sensitivity to antinutritional factors and limited ability to utilize the carbohydrates commonly present in vegetable sources (Buddington, Krogdahl, & Bakke-Mckellep, 1997; Ostaszewska, Dabrowski, Palacios, Olejniczak, & Wieczorek, 2005).

The pirarucu, *Arapaima gigas*, is considered one of the largest freshwater fish species in the world, reaching up to three meters in length and 200 kg in weight (Pino-Hernández et al., 2021). It stands out for aquaculture due to its rapid growth (up to 10 kg year^{-1}), rusticity, and high-quality meat, with mild flavor and boneless fillets (Ono, Halverson, & Kubitza, 2004; Pereira-Filho & Roubach, 2018). Furthermore, it supports high stocking densities and has obligatory air breathing behavior (Pereira-Filho & Roubach, 2018). Despite the characteristics above mentioned, its production is still low and has remained stable in the last four years (*Instituto Brasileiro de Geografia e Estatística* [IBGE], 2021) due to the lack of a consolidated consumer market and a series of technological limitations, such as the absence of species-specific feeds for the different growth phases.

Pirarucu farming, as well as other native fish species produced in Brazil, is based on the use of generalist diets formulated to attend only the fish feeding habits, resulting in low feed efficiency and productive performance. In the case of pirarucu, there are reports of mortalities during the grow-out phase associated with reduced feed intake, cachexia, and signs of enteritis after necropsy (Lima et al., 2017). Those signals are possibly related to the presence of antinutritional factors from soybean meal in the feed, the main source of vegetable protein used in Brazilian fish feed. Reduction in growth in response to increasing inclusion of soybean meal in diet was observed in juvenile pirarucu by Cerdeira et al. (2018). The authors also observed a reduction in diet digestibility and probable anemia in fish fed diet with 44% soybean meal inclusion. However, the effects of this ingredient on the intestinal morphology of pirarucu have not been investigated. Considering the carnivorous feeding habit of the species and the high levels of soybean meal in Brazilian diets, this work evaluated growth and aspects of intestinal morphology of juvenile pirarucu fed diets with increasing levels of soybean meal.

Materials and methods

Experimental design

The experiment complied with Brazilian legislation for the care and use of animals for educational and scientific purposes (Concea - protocol Ceua/Inpa 16/2017), and with the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (*Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* -AA4F2B0).

Juvenile pirarucu with an average weight of 152.2 ± 0.8 g were randomly distributed in 20 polyethylene tanks (500 L; 10 fish/tank) in a continuous water flow system. Fish were fed twice a day until apparent satiety (10:00 am and 3:30 pm) with diets formulated with 0, 10, 20, 30 and 40% inclusion of soybean meal (SB0, SB10, SB20, SB30 and SB40, respectively). The diets were evaluated in a completely randomized design with four replications per treatment for 58 days. Temperature (25.3 ± 0.1°C) and oxygen (5.9 ± 0.2 mg L^{-1}) were monitored daily, in the morning and afternoon, while pH (7.0 ± 0.0) and total ammonia (⩽ 0.25 mg L^{-1}) were monitored weekly.

Diet formulation and processing

The diets were formulated to be isonitrogenous and isoenergetic, according to the estimated requirement for essential amino acids (Rodrigues, Bicudo, Moro, Gominho-Rosa, & Gubiani, 2022b) and crude protein (Mattos et al., 2017) defined for pirarucu (Table 1). The ingredients were finely ground (Ø = 0.7 ± 0.2 mm), mixed and homogenized, and the mixture obtained was processed in an experimental extruder. Pellets of 3 to 4 mm were obtained, dried at 50°C for 24 hours and subsequently stored at 4°C until use. The digestible protein of the experimental diets was calculated based on the apparent digestibility coefficients of the ingredients defined for pirarucu (Cipriano et al., 2016; Rodrigues et al., 2022a; Ramos et al., 2022a).

Table 1. Ingredients and proximal composition of experimental diets.

Ingredients, %	Soybean meal inclusion levels (%)				
	0	10	20	30	40
Fish meal 54%	35.29	32.47	30.23	28.14	21.33
Soybean meal 46%	0.00	9.78	19.56	29.34	39.12
Corn gluten meal 60%	20.00	20.00	14.73	14.50	14.50
Feather meal 75%	5.00	5.00	5.00	3.43	1.50
Blood meal	5.00	5.00	5.00	5.00	5.00
Dry yeast	5.95	8.17	6.62	2.81	2.00
Broken rice	14.24	4.88	4.00	4.00	4.00
Fish oil	13.52	13.69	13.87	11.78	11.55
Premix Vitamin & Mineral[1]	1.00	1.00	1.00	1.00	1.00
Nutritional composition					
Crude energy (kcal kg^{-1})[2]	4.00	3.90	3.90	3.90	3.90
Crude protein (%)[3]	40.76	45.24	44.18	42.76	42.39
Ash (%)[3]	9.00	9.00	9.00	9.00	8.02
Crude fiber[3]	0.62	1.15	1.65	2.18	2.7
Digestible protein (%)[2]	27.53	30.60	29.76	29.83	29.92

[1]Guabi Nutrição e Saúde Animal S.A. mg kg^{-1}: manganês (26); zinco (140); ferro (100); cobre (14); cobalto (0.2); iodo (0.6); selênio (0.6). Vit. A (10.000 UI); Vit D3 (4.000 UI); Vit E (100); Vit K (5); Vit B1 (25); Vit B2 (25); Vit B6 (25); Vit B12 (30); niacina (100); ácido fólico (5); ácido pantotênico (50); biotina (0.8); colina (2000); inositol (50); Vit C (350). [2]Gross energy value was calculated based on combustion values of 23.7 protein, 39.5 fat, and 17.2 kJ g^{-1} carbohydrates (Molina-Poveda, 2016). [3]Analyzed according to Association of Official Analytical Chemists (AOAC, 1999).

Performance and body composition

Fish weight was recorded at the beginning and the end of the experimental period. Daily feed consumption was calculated by subtracting the uneaten feed in the container from that in the pre-weighed feed. The calculated performance variables were:

- Average weight gain (g): WG = (average final weight - average initial weight);
- Specific growth rate (% day^{-1}): SGR = [100 × (ln final body weight - ln initial body weight)/ experimental period];
- Feed conversion ratio: FCR = feed intake (g, as fed basis)/ weight gain (g, wet).

Body composition was assessed in 10 fish sampled at the beginning of the experiment and four fish per experimental unit sampled at the end of the experiment. Fish were euthanized in an overdose of eugenol, after fasting for 48 hours. The carcasses were analyzed according to the procedures defined by AOAC (1999). The apparent net protein utilization (Anpu) was calculated according to the formula:

- Anpu = [final body weight (g) × final body protein (g kg^{-1}) × initial body protein (g kg^{-1}intake (dry weight) (g) × 100.

Intestinal histology, hepatosomatic index and intestinal coefficient

At the end of the trial, eight fish per treatment were sampled for histological analysis of the intestine, hepatosomatic index (HSI) and intestinal coefficient (IC), after fasting for 48 hours. Fish were euthanized in an overdose of eugenol and subsequently dissected to collect the proximal and distal intestine (Rodrigues, Saturnino, & Fernandes, 2017). The tissues were fixed in 4% buffered formalin and subsequently processed for paraffin embedding. Non-sequential 5-μm longitudinal sections were stained with hematoxylin and eosin. The morphological aspect of the distal intestine, as well as the height of the branched intestinal folds (30 measurements per fish, at least) of both segments were analyzed under a light microscope with a digital camera and image analyzer system (Leica DM 2500, Heidelberg, Germany - Baeverfjord & Krogdahl, 1996).

Statistical analysis

Data obtained were analyzed by analysis of variance (ANOVA), followed by Tukey's test ($\alpha = 0.05$) to find possible differences between means. The assumptions of homoscedasticity and normality of residuals were checked. Variable values were expressed as mean ± standard deviation.

Results and discussion

The inclusion of increasing levels of soybean meal in the diet for juvenile pirarucu did not affect final weight, weight gain, specific growth rate and survival of juvenile pirarucu (Table 2; $p>0.05$). However, feed conversion was significantly higher for fish fed SB40 diet compared to the other treatments, which did not differ from each other (Table 2; $p < 0.05$).

Fish body composition varied only for ash and ether extract (Table 3; $p < 0.05$). SB30 and SB40 diets provided less body fat in pirarucu compared to SB0, SB10 and SB20 diets. S0 diet resulted in a higher concentration of ash in fish carcass in comparison to S40 diet. The ANPU did not vary with the increase in the inclusion of soybean meal in the diet (Table 3; $p < 0.05$).

Increasing levels of soybean meal in pirarucu diet affected the hepatosomatic index (Table 4; $p < 0.05$) but did not affect the height of the mucosal folds in the proximal intestine and the intestinal coefficient (Table 4; $p > 0.05$). In the distal intestine, however, there was a shortening of mucosal folds in fish fed with SB40 compared to those fed with SB0 (Table 4; $p < 0.05$). Supranuclear vacuoles were less evident or even absent in the enterocytes of the distal intestine of fish fed soybean meal (Figure 1). In the SB40 treatment, the frequency of fish with this condition was higher and fish with normal supranuclear vacuolation (abundant vacuoles) was not observed. In the SB0 treatment, most fish presented many supranuclear vacuoles (Figure 1).

Table 2. Performance of juvenile pirarucu fed diets with increasing levels of soybean meal for 58 days.

Soybean meal level (%)	Final weight (g)	Weight gain (g)	SGR (%)	Survival (%)	Feed conversion ratio
0	425.36 ± 46.15	274.01 ± 46.43	1.77 ± 0.19	70.00 ± 0.00	1.02 ± 0.18^{b}
10	446.07 ± 28.91	295.10 ± 27.83	1.87 ± 0.10	70.00 ± 0.00	1.12 ± 0.21^{b}
20	450.63 ± 64.13	298.15 ± 62.70	1.86 ± 0.23	67.50 ± 5.00	1.00 ± 0.12^{b}
30	463.71 ± 35.53	311.31 ± 35.19	1.91 ± 0.13	70.00 ± 0.00	1.06 ± 0.12^{b}
40	410.10 ± 18.05	257.47 ± 18.22	1.70 ± 0.08	63.33 ± 5.77	1.68 ± 0.12^{a}
p-value	0.4155	0.4011	0.3781	0.0951	0.0242

^{a,b}Different letters in the same column indicate differences between diets by the Tukey's test ($p < 0.05$).

Table 3. Body composition (wet basis) and Anpu of juvenile pirarucu fed diets with increasing levels of soybean meal for 58 days.

Soybean meal level (%)	Crude protein	Dry matter	Ash	Ether extract	ANPU
		%			
0	13.20 ± 0.44	26.35 ± 0.36	4.27 ± 0.23[a]	7.92 ± 0.39[b]	29.38 ± 2.34
10	13.49 ± 0.69	26.40 ± 1.53	4.06 ± 0.08[ab]	8.01 ± 0.45[b]	25.71 ± 5.28
20	13.66 ± 0.43	26.46 ± 0.89	3.95 ± 0.41[ab]	8.22 ± 0.20[b]	28.07 ± 5.45
30	13.72 ± 0.48	25.10 ± 0.64	3.90 ± 0.42[ab]	6.99 ± 0.31[a]	31.72 ± 2.24
40	14.13 ± 0.55	25.07 ± 0.51	3.54 ± 0.20[b]	6.95 ± 0.49[a]	27.65 ± 1.20
p-value	0.2027	0.0734	0.0453	0.0004	0.2995

[a,b]Different letters in the same column indicate differences between diets by the Tukey's test ($p < 0.05$).

Table 4. Hepato-somatic index (HSI), intestinal coefficient (IC) and height of the proximal and distal intestinal mucosal folds of juvenile pirarucu fed diets with increasing levels of soybean meal for 58 days.

Soybean meal level (%)	HSI	IC	Proximal intestine (µm)	Distal intestine (µm)
0	2.61 ± 0.09[a]	1.29 ± 0.04	1.077.92 ± 175.19	903.44 ± 123.26[a]
10	2.24 ± 0.04[ab]	1.30 ± 0.02	1.036.95 ± 126.66	798.89 ±113.37[ab]
20	2.04 ± 0.07[bc]	1.29 ± 0.01	1.049.76 ± 177.16	860.11 ±153.22[ab]
30	1.71 ± 0.16[c]	1.25 ± 0.09	1.180.61 ± 87.03	813.26 ±105.63[ab]
40	1.90 ± 0.33[bc]	1.23 ± 0.12	1.063.99 ± 118.50	685.11 ± 88.19[b]
p-value	< 0.0001	0.6244	0.3300	0.0472

[a,b]Different letters in the same column indicate differences between diets by the Tukey's test ($p < 0.05$).

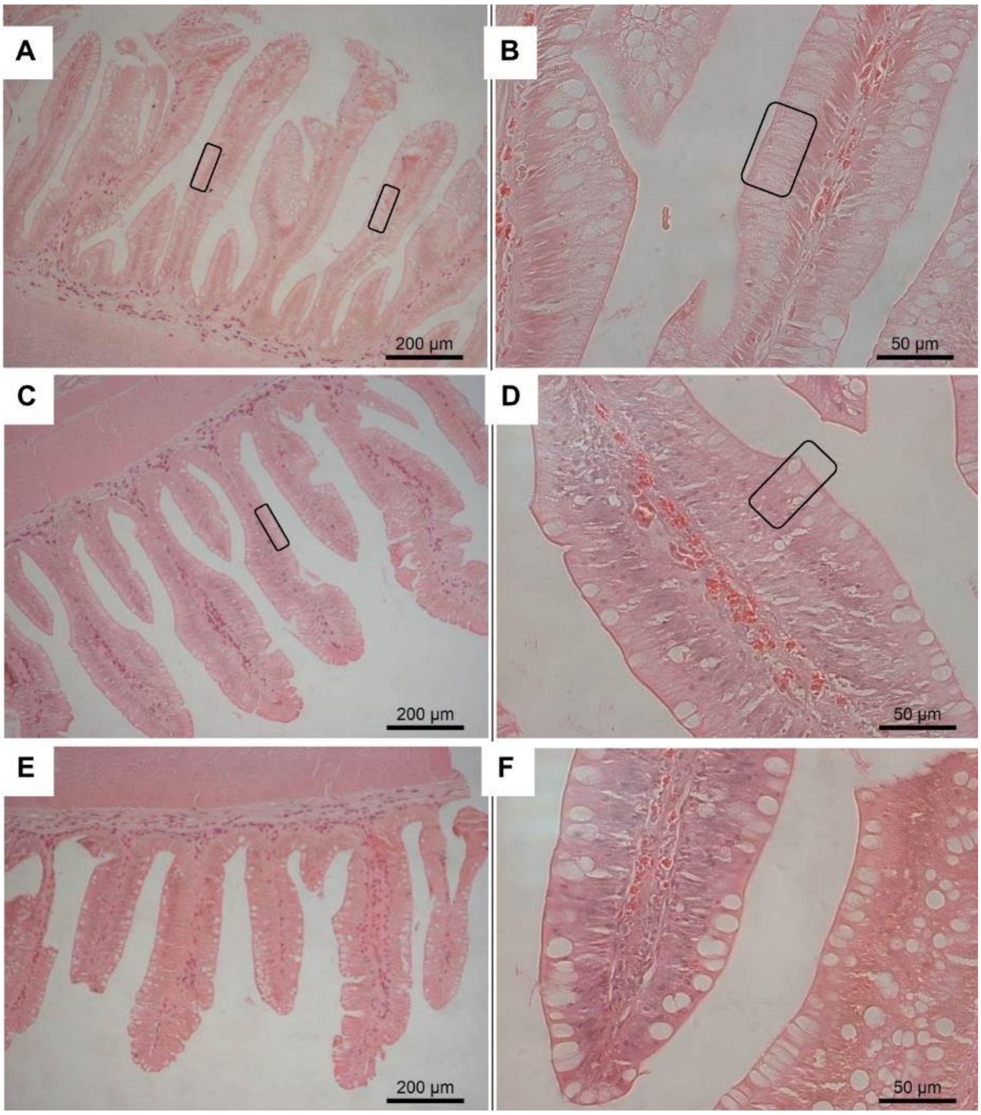

Figura 1. Distal intestine of juvenile pirarucu fed diets with 0 (A and B), 20 (C and D) and 40% (E and F) inclusion of soybean meal for 54 days. With the increase of soybean meal inclusion in the diet a reduction in the number of supranuclear vacuoles (rectangle) in the intestinal epithelium (C and D) is observed, up to their total absence (E and F). Hematoxylin-eosin staining at 100 and 400× magnification.

Unlike the results found in our study, the inclusion of soybean meal above 33% in pirarucu diet resulted in growth reduction (Cerdeira et al., 2018). In the same study, higher feed conversion and probable anemia were observed in fish fed with 44% inclusion of soybean meal, suggesting a maximum inclusion of 22% of soybean meal for juvenile pirarucu diet. This divergence can be attributed to the period fish were fed with soybean meal diets.; while in our study fish were fed for 58 days, Cerdeira et al. (2018) fed fish for 120 days, which submitted the animals to a longer exposure to the antinutrients of soybean meal. Another study, evaluating the total replacement of fish meal with soybean meal (20% inclusion in the diet) for juvenile pirarucu, found higher final weight in fish fed the control diet (20% inclusion of fishmeal), with no differences, however, in weight gain and specific growth rate (Corrêa et al., 2022). In this work, which lasted 64 days, pirarucu growth was similar to that obtained in our study.

For the carnivorous European catfish (*Silurus glanis*), the inclusion of 40% of soybean meal in diet impaired growth and feed conversion rate, being possible to replace 60% of fishmeal with soybean meal (30% inclusion in diet), without a decline in fish productive performance (Kumar et al., 2017). Hernández, Martínez, Jover, and García (2007) tested the inclusion of soybean meal for the sharpsnout seabream (*Diplodus puntazzo*) in two size classes (40 to 58 g and 175 to 216 g). Growth reduction at higher levels of soybean meal inclusion (above 40%) was only observed in fish of the larger size class. According to the authors the long-term supply of the tested diets could result in growth reduction, possibly due to histological changes in the digestive tract. In addition to these results, Hien, Be, Lee, and Bengtson (2015) found that the inclusion of soybean meal at up to 35% did not affect the growth of the snakehead (*Channa micropeltes*). The authors also observed a worsening in fish performance at the inclusion level of 43.5% soybean meal, even with the addition of phytase enzyme in the diet. For the Japanese seabass (*Lateolabrax japonicus*), a carnivorous fish species, it was observed a worsening in fish performance after 50% replacement of fish meal with soybean meal (30% of dietary soybean meal inclusion - Zhang et al., 2018). Likewise, replacing 100% of fishmeal with soybean meal (70% of dietary inclusion) in the Orange-spotted grouper (*Epinephelus coioides*) diet resulted in a significant worsening in weight gain, feed intake and feed efficiency, when compared to diets with 0 and 50% replacement (0 and 35% inclusion - Wang, Wang, Zhang, Zhang, & Song, 2017). Considering the studies aforementioned, the maximum inclusion level of soybean meal in diets for carnivorous fish species seems to be around 30-35%.

According to Krogdahl, Penn, Thorsen, Refstie, and Bakke (2010), the presence of antinutritional factors in plant protein sources directly influences the performance of fish fed with soy derivatives; however, this was not observed in the present study in relation to fish growth, except for feed conversion rate that was impaired in SB40 diet. According to Hernández et al. (2007), fish compensate the lower availability of nutrients in diets with high levels of soybean meal inclusion by increasing feed consumption.

Regarding the concentration of nutrients in the carcass and the use of protein, several authors report, for different fish species, no change in body protein and similar levels of use of dietary protein, although it is reported reductions in body fat and ash with the inclusion of soybean meal in diet (Tomás, De La Gándara, Garcáa-Gomez, Párez, & Jover, 2005; Hernández et al., 2007; Hien et al., 2015; Zhang et al., 2018; Zhao, Wang, Wang, & Ye, 2021; Rawles et al., 2022). According to Refstie et al. (2000), reduction in protein retention is more pronounced in older fish, being less evidenced in younger animals. Furthermore, intestinal morphological changes derived from the antinutritional factors in soy products provide a reduction in the absorption of nutrients throughout fish development, which may not be observed in young fish fed for short periods (Krogdahl, Bakke-McKellep, & Baeverfjord, 2003).

In our study, body fat was reduced with SB30 and SB40 diets. According to Hernández et al. (2007), this can occur due to less absorption of nutrients (protein and carbohydrates) caused by the presence of antinutrients that lowers enzymatic activity in fish digestive tract. Consequently, fish use most of the fat ingested as an immediate source of energy, leaving a smaller amount to be stored in the body. Another possibility, as noted by Zhang et al. (2018), is that the increase in dietary soybean meal inclusion causes a reduction in intestinal lipases and proteases, which leads to less digestion and absorption of fats by fish. Regarding body ash, it is possible that the presence of phytate in soybean meal leads to a lower availability of minerals to be digested and absorbed, as reported by Lanari, D'Agaro, and Turri (1998), Storebakken, Shearer, and Roem (1998) and Elangovan and Shim (2000).

A reduction in fish hepatosomatic index with the increase of soybean meal inclusion was also observed for *Diplodus puntazzo* (Hernández et al., 2007) and *Totoaba macdonaldi* (Fuentes-Quesada et al., 2018) and correlated with the reduction of fat vacuoles in the liver hepatocytes which possibly resulting in a smaller liver size and, consequently, a smaller HSI. This reduction in fat vacuoles is due to a lesser absorption and/or a greater use of dietary fat, similarly to what occurred for body fat.

The inflammatory process caused by the inclusion of soybean meal in diet causes important changes in the distal portion of the intestine, leading to atrophy and consequently, a reduction in the intestinal coefficient of fish (Fuentes-Quesada et al., 2018). This change was not observed in the present study, possibly because it is more evidenced in fish fed for longer periods with soybean meal.

The distal intestine performs an important immunological function in fish (Buddington et al., 1997), which explains the changes found in the mucosa of this intestinal segment for pirarucu. Morphological changes in the mucosa of the distal intestine induced by the inclusion of soybean meal in diet were first described for Atlantic salmon (*Salmo salar* L.) by Baeverfjord and Krogdahl (1996), who also observed a shortening of the intestinal folds as well as a loss of supranuclear vacuolation of enterocytes. This enteritis condition affects the integrity of the epithelial barrier, which together with the reduced nutrient digestion and absorption, may affect fish immunity (Krogdahl, Bakke-McKellep, & Baeverfjord, 2000; Gu, Bai, Zhang, & Krogdahl, 2016). More liquid and less consistent feces, common in cases of enteritis, were reported in studies evaluating the digestibility of soybean meal for pirarucu with diets containing 20% inclusion of this ingredient (Rodrigues, Moro, Santos, Freitas, & Fracalossi, 2019; Ramos, Fracalossi, Freitas, Santos, & Rodrigues, 2022b), corroborating the fish intestinal inflammation observed in this study.

Conclusion

Although the inclusion of soybean meal in the diet did not affect pirarucu growth in our study, previous studies indicate compromised production performance (Cerdeira et al., 2018; Corrêa et al., 2022) and loss of feces consistency (Rodrigues et al., 2019; Ramos et al., 2022b) with inclusion levels above 20%. However, changes in the mucosa of the distal intestine were more evident for fish fed the SB40 diet, indicating that the inclusion of up to 30% of soybean meal for juvenile pirarucu diet is possible without impairing fish growth and intestinal morphology.

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References

Association of Official Analytical Chemists [AOAC]. (1999). *Official methods of analysis* (16th ed.). Washington, DC: AOAC.

Baeverfjord, G., & Krogdahl, A. (1996). Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. *Journal of Fish Diseases, 19*(5), 375-387. DOI: https://doi.org/10.1046/j.1365-2761.1996.d01-92.x

Buddington, R. K., Krogdahl, Å., & Bakke-Mckellep, A. M. (1997). The intestines of carnivorous fish: structure and functions and the relations with diet. *Acta Physiologica Scandinavica, 638*, 67-80.

Cerdeira, K. A., Souza, K. J. N. S., Ferreira, J. B., Zampar, A., Ono, E. A., & Affonso, E. G. (2018). Soybean meal in diets for juveniles of pirarucu. *Boletim do Instituto de Pesca, 44*(3), e318. DOI: https://doi.org/10.20950/1678-2305.2018.318

Cipriano, F. S., Lima, K. S., Souza, R. H. B., Tonini, W. C. T., Passinato, É. B., & Braga, L. G. T. (2016). Digestibility of animal and vegetable protein ingredients by pirarucu juveniles, *Arapaima gigas*. *Revista Brasileira de Zootecnia, 45*(10), 581-586. DOI: https://doi.org/10.1590/S1806-92902016001000001

Corrêa, R. O., Lisboa, V., Oliveira, R. S., Aires, L. N. A., Silva, R. S., Queiroz, C. A., & Sousa, T. M. (2022). *Substituição da farinha de peixe por farelo de soja em dietas para pirarucu*. Belém, PA: Embrapa Amazônia Oriental.

Elangovan, A., & Shim, K. F. (2000). The influence of replacing fish meal partially in the diet with soybean meal on growth and body composition of juvenile tin foil barb (*Barbodes altus*). *Aquaculture, 189*(1-2), 133-144. DOI: https://doi.org/10.1016/S0044-8486(00)00365-3

Fuentes-Quesada, J. P., Viana, M. T., Rombenso, A. N., Guerrero-Rentería, Y., Nomura-Solís, M., Gomez-Calle, V., ... Mata-Sotres, J. A. (2018). Enteritis induction by soybean meal in *Totoaba macdonaldi* diets: Effects on growth performance, digestive capacity, immune response and distal intestine integrity. *Aquaculture, 495*, 78-89. DOI: https://doi.org/10.1016/j.aquaculture.2018.05.025

Glencross, B., Fracalossi, D. M., Hua, K., Izquierdo, M., Mai, K., Øverland, M., ... Yakupityage, A. (2023). Harvesting the benefits of nutritional research to address global challenges in the 21st century. *Journal of the World Aquaculture Society, 54*(2), 343-363. DOI: https://doi.org/10.1111/jwas.12948

Gu, M., Bai, N., Zhang, Y., & Krogdahl, Å. (2016). Soybean meal induces enteritis in turbot *Scophthalmus maximus* at high supplementation levels. *Aquaculture, 464*, 286-295. DOI: https://doi.org/10.1016/j.aquaculture.2016.06.035

Hernández, M. D., Martínez, F. J., Jover, M., & García, B. (2007). Effects of partial replacement of fish meal by soybean meal in sharpsnout seabream (*Diplodus puntazzo*) diet. *Aquaculture, 263*(1-4), 159-167. DOI: https://doi.org/10.1016/j.aquaculture.2006.07.040

Hien, T. T. T., Be, T. T., Lee, C. M., & Bengtson, D. A. (2015). Development of formulated diets for snakehead (*Channa striata* and *Channa micropeltes*): can phytase and taurine supplementation increase use of soybean meal to replace fish meal? *Aquaculture, 448*, 334-340. DOI: https://doi.org/10.1016/j.aquaculture.2015.06.020

Instituto Brasileiro de Geografia e Estatística [IBGE]. (2021). *Pesquisa da pecuária municipal*. Recuperado de http://www.sidra.ibge.gov.br/

Krogdahl, Å., Bakke-McKellep, A. M., & Baeverfjord, G. (2000). Feeding atlantic salmon *Salmo salar* L. soybean products: effects on disease resistance (furunculosis), and lysozyme and IgM levels in the intestinal mucosa. *Aquaculture Nutrition, 6*(2), 77-84. DOI: https://doi.org/10.1046/j.1365-2095.2000.00129.x

Krogdahl, Å., Bakke-McKellep, A. M., & Baeverfjord, G. (2003). Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquaculture Nutrition, 9*(6), 361-371. DOI: https://doi.org/10.1046/j.1365-2095.2003.00264.x

Krogdahl, Å., Penn, M., Thorsen, J., Refstie, S., & Bakke, A. M. (2010). Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. *Aquaculture Research, 41*(3), 333-344. DOI: https://doi.org/10.1111/j.1365-2109.2009.02426.x

Kumar, S., Sándor Zs, J., Nagy, Z., Fazekas, G., Havasi, M., Sinha, A. K., De Boeck, G., & Gál, D. (2017). Potential of processed animal protein versus soybean meal to replace fish meal in practical diets for European catfish (*Silurus glanis*): growth response and liver gene expression. *Aquaculture Nutrition, 23*(5), 1179-1189. DOI: https://doi.org/10.1111/anu.12487

Lanari, D., D'Agaro, E., & Turri, C. (1998). Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture, 161*(1-4), 345-356. DOI: https://doi.org/10.1016/S0044-8486(97)00282-2

Lima, A. F., Rodrigues, A. P. O., Lima, L. K. F., Maciel, P. O., Rezende, F. P., Freitas, L. E. L., ... Bezerra, T. A. (2017). *Alevinagem, recria e engorda de pirarucu*. Brasília, DF: Embrapa.

Mattos, B. O., Nascimento Filho, E. C. T., Santos, A. A., Barreto, K. A., Sánchez-Vázquez, F. J., & Fortes-Silva, R. (2017). A new approach to feed frequency studies and protein intake regulation in juvenile pirarucu. *Anais da Academia Brasileira de Ciências, 89*(2), 1243-1250. DOI: https://doi.org/10.1590/0001-3765201720160349

Molina-Poveda, C. (2016). Nutrient requirements. In S. F. Nates (Ed.), *Aquafeed formulation* (p. 75-216). San Diego, CA: Academic Press.

National Research Council [NRC]. (2011). *Nutrient requirements of fish and shrimp*. Washington, DC: NRC.

Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., ... Troell, M. (2021). Publisher correction: a 20-year retrospective review of global aquaculture. *Nature, 595*, E36. DOI: https://doi.org/10.1038/s41586-021-03736-4

Ono, E. A., Halverson, M. R., & Kubitza, F. (2004). Pirarucu - o gigante esquecido. *Panorama da Aqüicultura, 14*(81), 14-25.

Ostaszewska, T., Dabrowski, K., Palacios, M. E., Olejniczak, M., & Wieczorek, M. (2005). Growth and morphological changes in the digestive tract of rainbow trout (*Oncorhynchus mykiss*) and pacu (*Piaractus mesopotamicus*) due to casein replacement with soybean proteins. *Aquaculture, 245*(1-4), 273-286. DOI: https://doi.org/10.1016/j.aquaculture.2004.12.005

Pereira-Filho, M., & Roubach, R. (2018). Pirarucu (*Arapaima gigas*). In B. Baldisserotto, & L. C. Gomes (Orgs.), *Espécies nativas para piscicultura no Brasil* (p. 37-66; 2 ed., rev. e ampl.). Santa Maria, RS: UFSM.

Pino-Hernández, E., Costa, W. A., Araujo, E. A. F., Villa, P. M., Lourenço, L. F. H., & Carvalho Junior, R. (2021). Influence of grilling pretreatment and optimization of *sous vide* processing parameters on the physicochemical and microbiological quality of pirarucu fillet. *Food Science and Technology International, 27*(1), 84-96. DOI: https://doi.org/10.1177/1082013220934257

Ramos, A. M. J., Fracalossi, D. M., Freitas, L. E. L., Santos, V. R. V., & Rodrigues, A. P. O. (2022b). Optimizing methodological aspects of stool collection by sedimentation in digestibility studies with pirarucu (*Arapaima gigas*). *Aquaculture Research, 53*(4), 1456-1467. DOI: https://doi.org/10.1111/are.15680

Ramos, A. M. J., Rodrigues, A. P. O., Freitas, L. E. L., Santos, V. R. V., Mattioni, B., & Fracalossi, D. M. (2022a). Starchy plant ingredients in pirarucu (*Arapaima gigas*) feeds: Utilization potential based on apparent digestibility and starch microstructure. *Aquaculture Research, 53*(11), 4128-4140. DOI: https://doi.org/10.1111/are.15914

Rawles, S. D., Fuller, A., Green, B. W., Abernathy, J. W., Straus, D. L., Deshotel, M. B., ... Webster, C. D. (2022). Growth, body composition, and survival of juvenile white bass (*Morone chrysops*) when dietary fish meal is partially or totally replaced by soybean meal, poultry by-product meal, an all-plant protein blend or a commercial plant-animal protein blend. *Aquaculture Reports, 26*, 101307. DOI: https://doi.org/10.1016/j.aqrep.2022.101307

Refstie, S., Korsøen, Ø. J., Storebakken, T., Baeverfjord, G., Lein, I., & Roem, A. J. (2000). Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Aquaculture, 190*(1-2), 49-63. DOI: https://doi.org/10.1016/S0044-8486(00)00382-3

Rodrigues, A. P. O., Bicudo, Á. J. A., Moro, G. V., Gominho-Rosa, M. C., & Gubiani, É. A. (2022b). Muscle amino acid profile of wild and farmed pirarucu (*Arapaima gigas*) in two size classes and an estimation of their dietary essential amino acid requirements. *Journal of Applied Aquaculture, 34*(2), 441-455. DOI: https://doi.org/10.1080/10454438.2020.1866141

Rodrigues, A. P. O., Moro, G. V., Santos, V. R. V., Freitas, L. E. L., & Fracalossi, D. M. (2019). Apparent digestibility coefficients of selected protein ingredients for pirarucu *Arapaima gigas* (Teleostei: Osteoglossidae). *Latin American Journal of Aquatic Research, 47*(2), 310-317. DOI: https://doi.org/10.3856/vol47-issue2-fulltext-11

Rodrigues, A. P. O., Ramos, A. M. J., Fracalossi, D. M., Moro, G. V., Freitas, L. E. L., & Santos, V. R. V. (2022a). *Ingredientes para a formulação de rações para o pirarucu (Arapaima gigas): tabelas de digestibilidade.* Palmas, TO: Embrapa Pesca e Aquicultura.

Rodrigues, R. A., Saturnino, K. C., & Fernandes, C. E. (2017). Liver histology and histomorphometry in hybrid sorubim (*Pseudoplatystoma reticulatum* × *Pseudoplatystoma corruscans*) reared on intensive fish farming. *Aquaculture Research, 48*(9), 5083-5093. DOI: https://doi.org/10.1111/are.13325

Storebakken, T., Shearer, K. D., & Roem, A. J. (1998). Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, *Salmo salar*. *Aquaculture, 161*(1-4), 365-379. DOI: https://doi.org/10.1016/S0044-8486(97)00284-6

Tomás, A., De La Gándara, F., Garcáa-Gomez, A., Párez, L., & Jover, M. (2005). Utilization of soybean meal as an alternative protein source in the Mediterranean yellowtail, *Seriola dumerili*. *Aquaculture Nutrition, 11*(5), 333-340. DOI: https://doi.org/10.1111/j.1365-2095.2005.00365.x

Wang, Y.-R., Wang, L., Zhang, C.-X., & Song, K. (2017). Effects of substituting fishmeal with soybean meal on growth performance and intestinal morphology in orange-spotted grouper (*Epinephelus coioides*). *Aquaculture Reports, 5*, 52-57. DOI: https://doi.org/10.1016/j.aqrep.2016.12.005

Zhang, C., Rahimnejad, S., Wang, Y.-R., Lu, K., Song, K., Wang, L., & Mai, K. (2018). Substituting fish meal with soybean meal in diets for Japanese seabass (*Lateolabrax japonicus*): effects on growth, digestive enzymes activity, gut histology, and expression of gut inflammatory and transporter genes. *Aquaculture, 483*, 173-182. DOI: https://doi.org/10.1016/j.aquaculture.2017.10.029

Zhao, X., Wang, Y., Wang, X., & Ye, J. (2021). Growth performance, plasma components, and intestinal barrier in grouper (*Epinephelus coioides*) are altered by dietary fish meal replacement with extruded soybean meal. *Aquaculture Reports, 21*, 100863. DOI: https://doi.org/10.1016/j.aqrep.2021.100863

Zhou, Z., Ringø, E., Olsen, R. E., & Song, S. K. (2018). Dietary effects of soybean products on gut microbiota and immunity of aquatic animals: a review. *Aquaculture Nutrition, 24*(1), 644-665. DOI: https://doi.org/10.1111/anu.12532