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Effect of fermented fish silage in diets for juvenile *Piaractus brachypomus* on growth performance, blood parameters and meat quality

Carlos Andre Amaringo CORTEGANO[1]*, Marilyn Jenny PAREDES-URBANO[1], Deykie Max WONG-BARDALES[2], Juan Alexander RONDÓN-ESPINOZA[1], Lluvis Lucero GERMANY-GRÁNDEZ[1], Rudy Leandro NÚÑEZ-MONTÚFAR[1], César Augusto VILLANUEVA-CHÁVEZ[1], Nidia Milagros LLAPAPASCA-GARCÍA[1], Ligia Uribe GONÇALVES[3], Fred William CHU-KOO[4]

[1] Universidad Nacional Mayor de San Marcos, Estación IVITA-Pucallpa, Instituto Veterinario de Investigaciones Tropicales y de Altura, Ucayali, Peru

[2] JM Ucayali SAC, Ucayali, Peru

[3] Instituto Nacional de Pesquisas da Amazônia, Coordenação de Tecnologia e Inovação, Manaus, Amazonas, Brazil

[4] Universidad Nacional Autónoma de Alto Amazonas-UNAAA, Facultad de Ciencias, Yurimaguas 16501, Loreto, Peru

* Corresponding author: camaringoc@unmsm.edu.pe

ABSTRACT

Fermented fish silage (FFS) produced from processing waste of the main fishery species in the Amazon could serve as an alternative with a circular economy approach. We determined the dietary effect of FFS as a replacement of fishmeal on growth performance, blood parameters and meat quality of juvenile *Piaractus brachypomus* (58.6 ± 7.4 g). FFS was prepared by mixing local raw materials (70% fish waste, 7.5% molasses, 3.75% papaya, 3.75% pineapple, 15% yogurt) and fermented for nine days. Five isonitrogenous (278 g kg^{-1} CP) and isocaloric (4,262.21 kcal GE kg^{-1}) diets were formulated with an increasing percentual replacement of fishmeal by FFS (0, 25, 50, 75, 100%). *Piaractus brachypomus* were kept in cages in a pond (three replicates per FFS level) and fed for 90 days at a 4% body weight-feeding rate. No pellet refusal nor mortality was registered. Growth performance was not affected by FFS replacement, except for the hepatosomatic index, which was higher in fish fed 100% FFS (2.84 ± 0.51). Blood parameters and meat pH (6.10 ± 0.78) did not vary significantly and samples of fish meat did not exceed the maximum permissible limits for total aerobic mesophyll, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. The highest total protein and ash content in meat were observed in fish fed 100% FFS, followed by 75% FFS, both groups also presenting the second highest values of total lipid content. We conclude that FFS represents a safe alternative for replacing fishmeal in extruded diets for juvenile *P. brachypomus*.

KEYWORDS: fish waste, lactic-acid-bacteria, Neotropical fish, red-bellied pacu

Efecto del ensilado fermentado de pescado en raciones para juveniles de *Piaractus brachypomus* sobre el crecimiento, parámetros sanguíneos y calidad del filete

RESUMEN

El ensilado fermentado de pescado (FFS) producido con residuos del procesamiento de especies pesqueras amazónicas es una alternativa con enfoque de economía circular. Determinamos el efecto dietético del FFS en sustitución a la harina de pescado sobre el crecimiento, parámetros sanguíneos y calidad del filete de juveniles de *Piaractus brachypomus* (58,6 ± 7,4 g). El FFS se preparó mezclando insumos locales (70% residuos de pescado, 7,5% melaza, 3,75% papaya, 3,75% piña, 15% yogurt) y fermentado por nueve días. Cinco raciones isoprotéicas (278 g kg^{-1} PB) e isocalóricas (4262,21 kcal EB kg^{-1}) se formularon con incremento porcentual de sustitución de harina de pescado por FFS (0, 25, 50, 75, 100%). *Piaractus brachypomus* se mantuvieron en jaulas en un estanque (tres repeticiones por ración) y se alimentaron por 90 días a una tasa de alimentación del 4%. No se reportó rechazo al alimento ni mortalidad. Los parámetros de crecimiento no se afectaron por raciones con FFS, excepto el índice hepatosomático con mayor valor en peces alimentados con 100% FFS (2,84 ± 0,51). Parámetros sanguíneos y el pH del filete (6,10 ± 0,78) no variaron y el filete no superó valores a los límites máximos permisibles para aerobios mesófilos totales, *Escherichia coli*, *Staphylococcus aureus* y *Salmonella* spp. El mayor contenido de proteínas y cenizas en el filete se obtuvo en peces alimentados con 100% FFS, seguido por 75% FFS, y ambos grupos presentaron el segundo mayor contenido de lípidos. Concluimos que FFS representa una alternativa segura para sustituir la harina de pescado en raciones extrusadas para *P. brachypomus*.

PALABRAS CLAVE: residuo de pescado, bacterias ácido lácticas, pez neotropical, pacú panza roja, morocó

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INTRODUCTION

Among the important species in artisanal fishing in the Peruvian Amazon are *Prochilodus nigricans* Spix & Agassiz, 1829 (Prochilodontidae) and *Pseudoplatystoma punctifer* (Castelnau, 1855) (Pimelodidae), and both present meat with high-quality nutritional properties (Cortegano *et al.* 2022; PRODUCE 2022). However, up to 30% of the whole *P. nigricans* carcass, as well as the head and viscera of *P. punctifer*, is unfit for human consumption and treated as waste in the fish processing (PRODUCE 2022). When improperly disposed, this waste can present a serious environmental, economic, and social hazard (Mozumder *et al.* 2022). Nonetheless, fish waste is a rich source of essential nutrients such as proteins, lipids, vitamins and minerals and, when appropriately processed, it can become a potential ingredient for aquafeeds (Mozumder *et al.* 2022; Santana *et al.* 2023).

A viable alternative would be to bioconvert fish waste into fish silage, thereby producing a feedstuff that provides partially hydrolyzed protein, peptides, free amino acids, unsaturated fatty acids, vitamins and minerals (Vidotti *et al.* 2002; Santana *et al.* 2023). Fish silage is produced by using whole fish or parts of fish hydrolyzed with acids, enzymes or lactic-acid-producing bacteria, which results in a product with a pasty consistency (Arruda *et al.* 2009; Santana *et al.* 2023). Fish silage is considered a safe product when its pH is below 4.5, which preserves the fish silage and avoids the proliferation of pathogenic microorganisms (Tatterson 1982; Santana *et al.* 2023). Moreover, fermented silage is considered easy to produce and employs low-cost technology (Santana *et al.* 2023), and its production and use in aquafeed at local scale contributes to the mitigation of the global challenges of the 21st century (Glencross *et al.* 2023).

Some studies have tested the partial or total replacement of fishmeal with dried fish silage in aquafeeds to reduce fish farming costs, and have shown that the replacement does not affect the growth performance, physiological parameters and the nutritional quality of the fish meat (Vidotti *et al.* 2002; Carvalho *et al.* 2006; Davies *et al.* 2020; Costa *et al.* 2022). However, few studies have developed practical ways to include wet fish silage in extruded diets, even though the use of wet fish silage instead of dry silage in aquafeed production may reduce processing costs through the elimination of the drying step and allow the use of the silage closer to the production site, aligning with circular economy principles (Vidotti *et al.* 2002; Arruda *et al.* 2009). Despite these potential benefits, there are significant challenges and risks associated with the incorporation of wet fish silage in extruded diets, such as its potential for microbiological contamination and its typically acidic pH levels (below 4), which is a critical factor for ensuring its stability (Tatterson 1982). Moreover, while many studies have evaluated the pH and microbiological characteristics of the meat from fish farmed with conventional feeding and of fish caught in the wild (Rondón-Espinoza *et al.* 2022), no published studies to date have reported these parameters for fish meat produced with diets that include silage, to evaluate the quality and safety of the fish produced with this diet.

The search for cheaper and more sustainable protein alternatives for producing fish feeds for aquaculture is necessary, especially in the Amazon (Grande-Fernández *et al.* 2023). For example, Peruvian feed mills still use traces of fishmeal as a source of essential amino acids and highly unsaturated fatty acids in diets for omnivorous fish, such as *Piaractus brachypomus* (Cuvier, 1818) (Serrasalmidae) (Grande-Fernández *et al.* 2023), the main native species cultivated in freshwater aquaculture in the Peruvian Amazon (Angeles-Escobar *et al.* 2021; PRODUCE 2022). It grows at temperatures of 23 to 30 °C, reproduces in the wild during the rainy season, but requires hormonal induction in captivity, and has an omnivorous feeding habit (Angeles-Escobar *et al.* 2021).

In this context, this study aimed to determine the effect of increasing replacement of fishmeal by fermented silage produced from *P. nigricans* and *P. punctifer* in the aquafeed for juvenile *P. brachypomus* on growth performance, blood parameters and meat quality of the cultured fish.

MATERIAL AND METHODS

Fish silage

Fermented fish silage (FFS) was produced by a fermentation process using waste from the primary processing of Amazonian fisheries (a mixture composed of 70% of whole *P. nigricans* unfit for human consumption but not yet decomposing, 15% of *P. punctifer* heads and 15% of *P. punctifer* viscera - stomach, pyloric caeca, liver and intestine) collected at a processing plant in the city of Pucallpa, Ucayali, Peru. The ingredients chosen for the preparation of the silage correspond to local experience and followed the methodology proposed by Lupín (1984). The fish waste was ground in a meat grinder and natural yogurt was added (15%) as a source of microorganisms for the production of lactic acid (Ozyurt *et al.*, 2015). Ground whole papaya (3.75%) and ground pineapple (3.75%) were added as the source of the proteolytic enzyme papain (Yang *et al.* 2016), and molasses (7.50%) were added as the main source of carbohydrates (Santana *et al.* 2023). All ingredients were well mixed to ensure adequate acidification and protein hydrolysis of the silage. The silage was produced in a 200-L plastic container and then hermetically sealed to guarantee anaerobic fermentation for nine days. The fermentation period was determined as nine days in order to guarantee the hydrolysis of more than 70% of the silage proteins without affecting the protein quality (Tatterson, 1982). After fermentation, samples (n = 3) of the silage were taken in order to analyze the proximate composition and the data was used for the formulation of the experimental diets. Finally, the temperature and pH of the silage (Portable pHmeter, PH-

013) were registered. After preparation, the silage was removed from the container and exposed to direct sunlight for 12 h in order to reduce moisture slightly.

Experimental diets

Considering the nutritional requirements for *P. brachypomus* (Guimarães and Martins, 2015), five isonitrogenous (278.00 g kg^{-1} of crude protein) and isocaloric (4,262.21 kcal kg^{-1} of gross energy) diets were formulated with an increasing percentual replacement of fishmeal by fermented fish silage (FFS) at the proportions of 0% (0FFS), 25% (25FFS), 50% (50FFS), 75% (75FFS) and 100% (100FFS). The inclusion of fishmeal was defined based on the usual percentage included in commercial feed for *P. brachypomus* in Peru for the initiation phase (Table 1).

The feed ingredients were homogenized in a horizontal mixer for 20 min. First, the dried ingredients were mixed and then the silage was slowly added to allow for easier incorporation into the mixture. The mixtures were extruded as 4-mm-diameter pellets (Light M&E, ft95; 800-1000 kg per hour) and dried at 60 °C for 24 h.

Experimental design and procedures

The trial was performed at the aquaculture station of the company JM Ucayali SAC, Ucayali, Peru (8°35'10.8"S, 74°54'50.6"W). A total of 2,000 fish (3.00 ± 1.02 g; 4.00 ± 2.01 cm) were obtained locally. For sanitary control, we examined twenty fish for the absence of endoparasites and ectoparasites according to procedures described by Rondón *et al*. (2021). The fish were then placed in a 3,600-L tank that was housed in an indoor open system with controlled aeration (4.52 ± 1.42 mg L^{-1} dissolved oxygen), temperature (28.45 ± 1.98 °C), pH (7.14 ± 0.69), and a 12-h photoperiod. The fish were subsequently quarantined for 30 days to guarantee their welfare before the initiation of the feeding trial. During this period, the fish were fed twice a day (at 9:30 and 15:30) with a commercial diet (320.00 g kg^{-1} CP and 3,900.00 kcal GE kg^{-1}) until apparent satiation.

The experiment followed a completely randomized design with five treatments (0FFS, 25FFS, 50FFS, 75FFS and 100FFS) and three replications per treatment. The experimental units were 157.50-m^3 cages (12.50 m × 10.50 m × 1.20 m) installed in a pond. The pond had a 1% daily

Table 1. Formulation and proximate composition of the experimental diets containing increasing fermented fish silage proportions for juvenile *Piaractus brachypomus*.

Component	Fermented fish silage	Treatment[1]				
		0FFS	25FFS	50FFS	75FFS	100FFS
Ingredients (g kg^{-1})						
Fishmeal	-	170.00	127.50	85.00	42.50	-
Fermented fish silage[2]	-	-	42.50	85.00	127.50	170.00
Soybean meal	-	290.00	315.00	340.00	360.00	380.00
Corn grain	-	345.00	345.00	345.00	345.00	345.00
Wheat flour	-	40.00	40.00	40.00	40.00	40.00
Rice powder	-	30.00	30.00	35.00	40.00	40.00
Cellulose	-	100.00	75.00	45.00	20.00	-
Premix[3]	-	20.00	20.00	20.00	20.00	20.00
Fungibam[4]	-	2.50	2.50	2.50	2.50	2.50
BHT[5]	-	2.50	2.50	2.50	2.50	2.50
Proximate composition based on dry matter (g kg^{-1})						
Dry matter	426.00	926.60	921.70	925.60	924.60	926.20
Crude protein	176.49	278.30	278.40	279.30	277.90	275.90
Gross energy (kcal kg^{-1})[6]	5,354.1	4,249. 8	4,211.9	4,268.6	4,299.2	4,281.6
Fiber	67.12	94.50	77.60	57.00	39.90	27.30
Ash	70.30	21.00	57.00	74.00	93.00	119.00
Total lipids	288.72	35.40	43.00	50.60	58.20	65.80
USD per kg of feed[7]	1.07	1.49	1.20	1.11	1.01	0.92

[1] Treatments 0FFS, 25FFS, 50FFS, 75FFS and 100FFS are, respectively, 0, 25, 50, 75 and 100% replacement of fishmeal by fermented fish silage. Means of triplicate analyses per sample are shown. [2] Prepared by mixing local inputs (70.00% fish waste + 7.50% molasses + 3.75% papaya + 3.75% pineapple + 15.00% yogurt) and fermented for 9 days. [3] Premix of vitamins and minerals (DSM AQUACULTURE) – DSM is a premix of ROVIMIX® vitamins, MICROGRAN® minerals, BHT and BHA (antioxidants) for animal use, produced by DSM NUTRITIONAL PRODUCTS PERU. Content (per kg of product): vitamin A = 9'334,000.00 IU; vitamin D3 = 1,866,800.00 IU; vitamin E = 93,333.00 IU; vitamin K3 = 5.33 g; thiamine (B1) = 12.00 g; riboflavin (B2) = 13.32 g; pyridoxine (B6) = 10.00 g; vitamin B12 = 0.02 g; ascorbic acid = 210.00 g; niacin = 100.00 g; pantothenic acid = 33.32 g; folic acid = 2.67 g; biotin = 0.53 g; copper = 1.00 g; iron = 13.33 g; manganese = 26.66 g; cobalt = 0.10 g; iodine = 1.00 g; zinc = 13.33 g; selenium = 0.20 g; antioxidants = 26.60 g; excipients q.s.p. = 2,000.00 g. [4] Antifungal. [5] Butyl-hydroxy-toluene. [6] Gross energy based on values calculated for proteins = 5.64 kcal g^{-1}; lipids = 9.44 kcal g^{-1}; carbohydrates = 4.11 kcal g^{-1} (NRC 2011). [7] Feed cost in USD was estimated using the conversion rate of 1 USD = 3.80 Peruvian Sols as of 29 Feb 2022.

water replenishment to allow for water loss due to evaporation. Juvenile *P. brachypomus* (58.57 ± 7.42 g; 12.96 ± 0.68 cm) were placed in the fifteen cages (96 fish per cage), and all fish were fed twice a day (at 9:30 and 15:30) at a feeding rate of 4% of body weight during 90 days, with feeding adjustments every 18 days after measuring ten fish per cage.

Fish were reared under a natural photoperiod, and water quality parameters were monitored daily at 9:00 at three different points in the pond (HANNA-HI98194). The parameters remained relatively constant throughout the experiment (dissolved oxygen = 5.90 ± 1.70 mg L^{-1}; temperature = 26.90 ± 1.48 °C; pH = 7.10 ± 0.75) and within the comfort range for the species (Rios 2021).

The study was conducted in Peru, where no specific authorizations, permissions, or licenses were required from Peruvian authorities to carry out this research. However, we adhered to international procedures and ethical standards for the use of animals in experimentation (Jenkins *et al.* 2014).

Growth performance and biometric indices

At the end of the experimental period, the fish were measured to evaluate their growth performance in terms of (1) final weight (g); (2) final length (cm); (3) weight gain (g) (final weight - initial weight); (4) length gain (cm) (final length - initial length); (5) feed intake (g) (total feed consumed during the experiment); (6) feed conversion rate (feed intake/weight gain); (7) relative growth rate (% day^{-1}) [(e^{g-1}) × 100; where e = nepper number, g = (ln(final weight) - ln(initial weight))/(feeding time)] and ln = natural logarithm; (8) survival [(final number of fish × 100)/initial number of fish]; and (9) Fulton's allometric condition factor [weight $length^{-3}$].

At the end of the 90-day period, 10 fish from each cage were euthanized via brain puncture. A sample of 250 g of the edible meat portion was obtained from each cage by filleting whole fish muscle and was used for the analysis of the proximate composition. The liver and viscera (stomach, pyloric caeca, liver, and intestine) were removed for analysis of the hepatosomatic index [(liver weight)/(total fish weight) × 100] and the lipo-somatic index [(visceral fat weight)/(total fish weight) × 100], respectively (NRC 2011).

Blood parameters

At the end of the experiment, blood was collected from three fish per cage via caudal vessel puncture using 10% EDTA solution as the anticoagulant (Dos Santos *et al.* 2021). The fish were previously anesthetized with 1.5 ml L^{-1} of eugenol. We evaluated the levels of hematocrit (%) using the scale by Goldenfarb *et al.* (1971). The white blood cells were counted in 1 mm^3 (neutrophils, eosinophils, basophils, lymphocytes, and monocytes, all in %) according to De Oliveira *et al.* (2018). In the blood plasma, we analyzed glucose (mg dL^{-1}) via the enzymatic-colorimetric method (glucose oxidase); triglycerides (mg dL^{-1}) using the colorimetric system; and total proteins (g dL^{-1}) following the modified biuret method (Epifânio *et al.* 2023).

Analysis of proximate composition

The proximate composition was determined for the samples of the fermented fish silage (n = 3), the experimental diets (n = 3 per diet) and the pooled samples of fish meat per cage (obtained from three fish per cage), all in triplicate, according to the procedures described by AOAC (2005).

Microbiological and pH evaluation of meat

Five fish were randomly collected per treatment at the end of the experimental period, according to the number of samples recommended by SANIPES (2016), and were stored in plastic bags in an isothermal box with ice at a 1:1 ratio and then sent to the IVITA-Pucallpa's Animal Health Laboratory (Pucallpa, Peru). The samples were ground and their pH was measured (Hinotek, portable pH meter PH-013). The samples were evaluated for the quantification of CFU per gram of meat in terms of total aerobic mesophyll (30°), *Staphylococcus aureus*, and *Escherichia coli* according to the American Public Health Association (APHA) method (10 repetitions per sample). The estimation of the probable presence or absence of *Salmonella* spp. was determined in 25 g of meat according to ISO 6579 (2002)/Cor.1 (2004)/Amd.1 (2007). The microbiological analysis procedures were described by Da Silva *et al.* (2013). Maximum permissible values for the analyzed parameters in hydrobiological products destined for human consumption in Peru followed SANIPES (2016).

Statistical analysis

The homogeneity of variance of the initial weight of the fish was confirmed using Cochran's Q test. Normality of the data distribution was ascertained with the Shapiro-Wilk test and homoscedasticity by the Breusch–Pagan test. Growth performance, blood parameters, and proximate composition and pH of the meat were compared among treatments using one-way ANOVA and the *post-hoc* Tukey's test. The association between the hepatosomatic and lipo-somatic indices with the increasing levels of fermented fish silage in the diets was evaluated with a Pearson's linear correlation analysis. As results of the microbiological evaluation were below the maximum permissible limit, the application of statistical tests was not necessary. A significance level of 5% was used in all analyses. Data were processed using Statistica Software 10.0 (StatSoft 2011).

RESULTS

At the end of the nine days of storage, the fermented fish silage had pH = 3.83 ± 0.43 and temperature = 29.58 ± 1.98 °C. The average room temperature during the fermentation of the silage was of 31.11 ± 2.18 °C.

Growth performance and biometric indices

No mortality was registered. Fish growth performance parameters did not differ significantly among treatments (final weight = 238.00 ± 20.44 g; final length = 23.21 ± 0.74 cm; weight gain = 179.52 ± 15.55 g; length gain = 10.26 ± 0.66 cm; feed intake = 282.57 ± 33.75 g per fish; feed conversion rate = 1.57 ± 0.14; relative growth rate = 1.02 ± 0.001% day^{-1}; condition factor = 1.90 ± 0.13; lipo-somatic index = 1.98 ± 0.83). However, only the hepatosomatic index increased significantly with increasing levels of dietary silage ($R = 0.9618$; $p < 0.05$), from 1.73 ± 0.51 for 0FFS to 2.84 ± 0.51 for 100FFS (Table 2).

Blood parameters

There were no significant differences among treatments in any haematological parameter (hematocrit = 13.2 ± 3.11%; leukocytes = 1,115.00 ± 91.93 mm^3; neutrophils = 64.90 ± 5.22%; lymphocytes = 35.10 ± 5.22%) or biochemical component (glucose = 93.70 ± 6.16 mg dL^{-1}; triglycerides = 272.00 ± 24.87 mg dL^{-1}; total proteins = 1.10 ± 1.03 g dL^{-1}) (Table 3).

Proximate composition of meat

Moisture (77.02 ± 0.15%) was similar among treatments. The total protein content was significantly higher in 100FFS, while the lipid content was significantly higher in 50FFS relative to all other treatments (Figure 1). There was no clear differentiation in ash content among the treatments, with the highest values in 0FFS and 100FFS (Figure 1).

Microbiological evaluation and pH of meat

The pH did not vary significantly among the treatments (6.10 ± 0.78). No colony-forming units (CFU) were quantified for total aerobic mesophylls, *S. aureus*, and *E. coli*, nor was the presence of *Pseudomonas* spp. detected in any of the analyzed samples.

Table 2. Growth performance of juvenile *Piaractus brachypomus* fed diets containing increasing levels of fermented fish silage. Survival was 100% for all treatments.

Growth parameter	**Treatment**[1]					**Pooled standard error**	**p-value**[2]
	0FFS	25FFS	50FFS	75FFS	100FFS		
Final weight (g)	241.37 ± 21.71	252.15 ± 19.01	227.23 ± 25.59	237.58 ± 14.08	232.08 ± 26.38	5.278	0.69
Final length (cm)	23.27 ± 0.65	23.18 ± 1.20	22.67 ± 0.50	23.47 ± 0.59	23.48 ± 0.84	0.191	0.72
Weight gain (g)	180.47 ± 17.48	186.55 ± 17.49	175.00 ± 19.05	178.68 ± 13.98	176.88 ± 20.16	4.016	0.94
Length gain (cm)	10.02 ± 0.69	9.63 ± 0.78	10.36 ± 0.69	10.53 ± 0.38	10.76 ± 0.37	0.169	0.25
Feed intake (g)	286 ± 26.82	316.24 ± 23.15	251.64 ± 40.78	281.79 ± 21.89	276.50 ± 36.87	8.714	0.23
Feed conversion rate	1.59 ± 0.14	1.71 ± 0.21	1.43 ± 0.10	1.58 ± 0.02	1.56 ± 0.05	0.036	0.20
Relative growth rate (% day^{-1})	1.015 ± 0.001	1.015 ± 0.001	1.017 ± 0.001	1.016 ± 0.001	1.016 ± 0.001	0.002	0.42
Condition factor[3]	1.91 ± 0.02	2.03 ± 0.18	1.95 ± 0.13	1.84 ± 0.05	1.79 ± 0.05	0.048	0.12
Hepatosomatic index	1.73 ± 0.51a	1.88 ± 0.47ab	2.08 ± 0.62ab	2.54 ± 0.83ab	2.84 ± 0.51b	0.064	0.01
Lipo-somatic index	1.75 ± 0.68	2.19 ± 0.65	1.79 ± 0.90	2.29 ± 0.95	1.99 ± 1.07	0.078	0.13

[1] Treatments 0FFS, 25FFS, 50FFS, 75FFS and 100FFS are, respectively, 0, 25, 50, 75 and 100% replacement of fishmeal by fermented fish silage. Values are the mean ± standard deviation (n = 3). [2] p-value of a one-way ANOVA. [3] Fulton's allometric condition factor.

Table 3. Biochemical blood parameters of juvenile *Piaractus brachypomus* fed diets containing increasing levels of fermented fish silage.

Parameter	**Treatment**[1]					**Pooled standard error**	**p-value**[2]
	0FFS	25FFS	50FFS	75FFS	100FFS		
Hematocrit (%)	13.50 ± 0.71	12.50 ± 0.71	13.50 ± 2.12	13.50 ± 7.78	13.00 ± 4.24	1.576	0.22
Leukocytes (mm^3)	1,125 ± 176.78	1,125 ± 35.36	1,075 ± 176.78	1,175 ± 35.36	1,075 ± 35.36	5.698	0.07
Neutrophils (%)	65.50 ± 3.54	68.00 ± 5.56	64.50 ± 4.95	64.50 ± 9.19	62.00 ± 2.83	2.932	0.37
Lymphocytes (%)	34.50 ± 3.54	32.00 ± 5.56	35.50 ± 4.95	35.50 ± 9.19	38.00 ± 2.83	2.830	0.42
Glucose (mg dL^{-1})	90.50 ± 6.06	96.00 ± 6.67	95.00 ± 8.28	90.50 ± 5.46	96.50 ± 4.35	1.577	0.84
Triglycerides (mg dL^{-1})	266.00 ± 38.08	271.50 ± 21.12	267.50 ± 16.26	290.50 ± 14.76	264.50 ± 34.15	3.322	0.36
Total proteins (g dL^{-1})	1.10 ± 0.28	1.15 ± 0.18	1.10 ± 0.11	1.10 ± 0.42	1.05 ± 0.21	0.113	0.86

[1] Treatments 0FFS, 25FFS, 50FFS, 75FFS and 100FFS are, respectively, 0, 25, 50, 75 and 100% replacement of fishmeal by fermented fish silage. Values are the mean ± standard deviation (n = 3). [2] p-value of a one-way ANOVA.

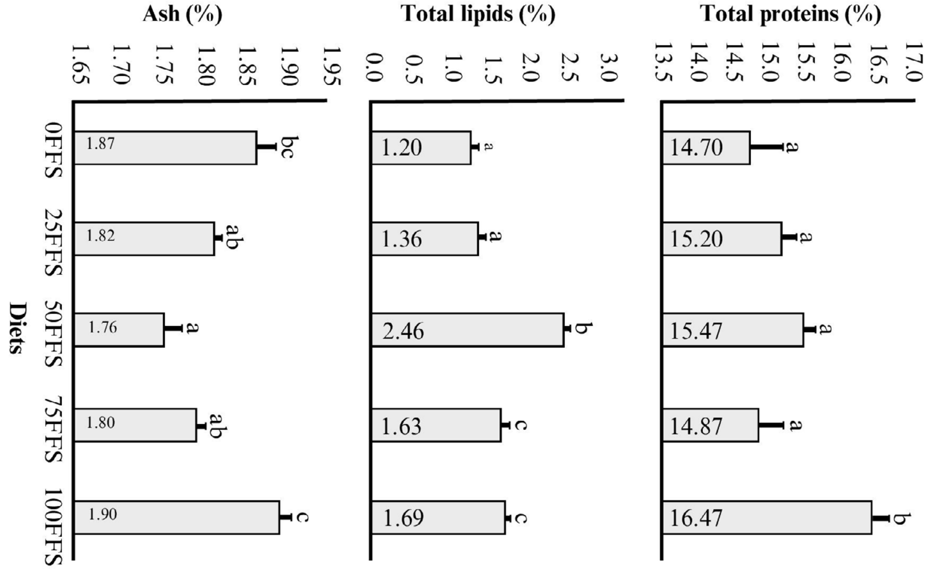

Figure 1. Content of total proteins, total lipids and ash (% in wet basis) in the meat of juvenile *Piaractus brachypomus* fed diets containing increasing levels of fermented fish silage. Treatments 0FFS, 25FFS, 50FFS, 75FFS and 100FFS correspond, respectively, to 0, 25, 50, 75 and 100% replacement of fishmeal by fermented fish silage in the experimental diets. Columns are the mean and bars the standard deviation of three replicates. The pooled standard error is 0.178, 0.117 and 0.016, for the content of total proteins, total lipids and ash, respectively. Different letters above the bars indicate statistically significant pairwise differences between treatments according to a *post-hoc* Tukey test ($p < 0.05$).

DISCUSSION

The consistency of the silage was similar to that usually observed for fermented fish silage using molasses as the main carbohydrate source for lactic acid bacteria (Santana *et al.* 2023). The pH at the end of nine days of storage was within the safe pH range (up to 4.50) that avoids spoilage and the propagation of pathogenic microorganisms (Tatterson 1982; Santana *et al.* 2023) and helps to maintain the physical and nutritional qualities of the silage in order to be used as an ingredient in aquafeeds (Santana *et al.* 2023). The temperature of the silage was in the optimal range, between 25 °C to 35 °C, to guarantee the activity of the lactic acid-producing bacteria (Zahar *et al.* 2002). The high lipid content of fish silage (288.72 g kg^{-1} of lipids in this study) may be a concern for the aquafeed industry, since lipids are related to diet rancidity, which can affect the palatability of the feed (Tatterson 1982; Vidotti *et al.* 2002; Arruda *et al.* 2009). The inclusion of antioxidants in the formulation of the silage and/or its use after 7 to 14 days of storage is recommended when using fish silage in aquafeeds (Tatterson 1982; Vidotti *et al.* 2002; Arruda *et al.* 2009; Santana *et al.* 2023). No additional antioxidants were specifically included in the preparation of the silage in this study, as the experimental diets were prepared immediately after nine days of fermentation. However, as part of good manufacturing practices in diet preparation, antioxidants present in the premix, including BHT, were incorporated into the formulation of the experimental diets. This ensured stability and reduced the risk of diet rancidity.

Growth performance parameters were not affected by the use of silage in the diet, which suggests that the dietary silage was used efficiently by the juvenile *P. brachypomus* as a source of nutrients and energy. Other studies have also shown that the partial or total replacement of fishmeal by fish silage, either acid or fermented, did not hamper the growth performance in black bass, *Micropterus salmoides* (Lacepède, 1802) (Centrarchidae) (Arruda *et al.* 2009), rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) (Salmonidae) (Güllü *et al.* 2014), common carp *Cyprinus carpio* Linnaeus, 1758 (Cyprinidae) (Ramasubburayan *et al.* 2013), Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (Cichlidae) (Carvalho *et al.* 2006), Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852) (Cichlidae) (Goosen *et al.* 2014), European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758) (Moronidae) (Davies *et al.* 2020), and Brazilian pacu (*Piaractus mesopotamicus* (Holmberg, 1887) (Serrasalmidae) (Vidotti *et al.* 2002). The similarity in feed intake of aquacultured fish is due to the isoenergetic levels of experimental diets, as dietary energy plays an important role in feed consumption in fish (NRC 2011). The values of weight gain (179.43 ± 15.55 g), relative growth rate (1.02 ± 0.001% day^{-1}) and feed conversion rate (1.57 ± 0.14) of fish fed with FFS in our study indicate good acceptance of the experimental diets and are consistent with those obtained for *P. brachypomus* fed commercial and experimental diets reared in different aquaculture systems (Angeles-Escobar *et al.* 2021; Favero *et al.* 2021). Our results also were similar to those obtained for *P. mesopotamicus* in experiments including dietary fish silage, with a relative growth rate of 1.38% day^{-1} (Vidotti *et al.* 2002) and a feed conversion rate of 1.34 to 1.52 (Fernandes *et al.* 2000; Vidotti *et al.* 2002). The similarity among treatments in Fulton's allometric condition factor, which estimates the overall condition of fish (NRC 2011), suggests that the FFS diets did not affect the welfare of the fish, as also evidenced by the absence of mortality during the trial. The significant increase of hepatosomatic index with the increase of FFS in the diet was a consequence of the increase in dietary lipid content (NRC 2011), as the FFS contained 288.72 g kg^{-1} of lipids, while the fishmeal contains only 100.00 g kg^{-1}. Nevertheless, the hepatosomatic index values were within the normal range observed in *P. brachypomus* under nutritional management not containing FFS (Favero *et al.* 2021) and did not influence the other growth performance parameters.

The analyzed blood parameters are significant indicators for estimating the health status of farm animals and are associated with nutrition (Dos Santos *et al.* 2021; Grande-Fernández *et al.* 2023). Subnutrition or unbalanced diets are among the main stressing conditions that impair biological homeostasis in fish (NRC 2011). The hematocrit values in our study were lower than those reported for *P. brachypomus* reared in a biofloc technology system (37.64%) (Angeles-Escobar *et al.* 2021). In the latter case, it is possible that the exposure to substances such as nitrites and total suspended solids in intensive systems lead to an increase in hematocrit as a means of compensation by the fish to maintain adequate oxygen transport. The white blood cell count in our trial was close to that reported for *P. brachypomus* reared in floating cages installed in a natural lake in the Peruvian Amazon (Acosta *et al.* 2017), but lower than that for *Colossoma macropomum* (Cuvier, 1816) reared in ponds (Grande-Fernández *et al.* 2023). In aquaculture, blood parameters of *P. brachypomus* may be affected by the rearing system, stressors, nutritional factors, age and species (Angeles-Escobar *et al.* 2021). Several studies have shown that fish silage included in aquafeeds does not affect the blood parameters of fish (Najim *et al.* 2014; Davies *et al.* 2020; Costa *et al.* 2022), which was corroborated by our study, further demonstrating the safe application of fish silage as a feedstuff in diets for *P. brachypomus*.

Even though the experimental diets were balanced to have a similar protein and energy content, the proximal composition of the fish meat samples varied significantly among the treatments. The higher protein and ash contents in fish fed 100FFS suggest a potential interaction between diet composition and nutrient deposition in the meat (Overturf *et al.* 2016). However, as the ash content in 100FFS did not differ significantly from the control (0FFS), these variations may also result from factors beyond the dietary treatments, underscoring the need for cautious interpretation. On the other hand, the lipid content of the experimental diets increased with the inclusion levels of FFS. Given the role of dietary lipids as primary energy sources in fish (Gonçalves *et al.* 2021), it is plausible that the elevated lipid levels in the diets facilitated greater protein deposition in the muscle tissue by sparing protein for growth and maintenance.

Juvenile *P. brachypomus* in our study reached their maximum lipid storage capacity in the meat with 50FFS. The decrease in the lipid content in 75FFS and 100FS was probably due to the mobilization of lipids to other tissues, as a strategy for lipid storage (NRC 2011; Cortegano *et al.* 2019). The variability in ash content in the fish meat among treatments was not related to dietary ash content. In general terms, the protein, ash, and lipid content in the meat was similar to the values obtained for the same species under different management and feeding strategies (Angeles-Escobar *et al.* 2021; Favero *et al.* 2021) and for other characids related to *P. brachypomus*, such as *C. macropomum* and *P. mesopotamicus* (Cortegano *et al.* 2019; Gonçalves *et al.* 2021; Grande-Fernández *et al.* 2023).

The pH of the meat indicated an adequate degree of freshness (Ponce *et al.* 2021), and the values were consistent with the typical range for fresh hydrobiological products (Ponce e*t al.* 2021). No spoilage bacteria or pathogenic bacteria are likely to multiply below a pH of 4.5, but, for safety, a limit of about pH 4.0 must be achieved in the final product (Tatterson 1982). Our silage achieved a safe pH of 3.83 to avoid contamination by spoilage and pathogenic bacteria. Fermented fish silage in aquafeed may protect against pathogenic bacteria, not only Gram-negative bacteria in the gastrointestinal tract, but also bacteria that can alter the quality of the fish meat (Olsen and Toppe 2017). However, proper rearing, slaughtering, and commercial practices are the most important in order to guarantee the safety of hydrobiological products (Rondón-Espinoza *et al.* 2022). In our study, no meat sample exceeded the maximum permissible limits for any of the analyzed microbiological parameters according to Peruvian fishing and aquaculture regulations (SANIPES 2016). Although these values denote freshness and safety, it is important to consider that various factors favor the growth of microorganisms and affect the degree of freshness of fish and safety of fish meat, therefore it is recommended to properly maintain the cold chain in aquaculture (Rondón *et al.* 2020).

CONCLUSIONS

Fermented fish silage has been shown to be a promising ingredient for the total replacement of fishmeal in the dietof farmed juvenile *P. brachypomus*, as it did not adversely affect the growth performance or blood parameters indicative of fish health and welfare, guaranteeing the quality of the meat. The bioconversion of fish waste into aquafeed can decrease the cost of *P. brachypomus* farming, while providing a solution for the elimination of the waste.

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