



Effects of commercial feeds and frozen trash fish on growth and hematological parameters of juvenile silver arowana *Osteoglossum bicirrhosum*

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Abstract

Silver arowana *Osteoglossum bicirrhosum* is a native species of the Amazon basin and presents opportunistic omnivorous habits with ichthyophagous preference. It is subjected to great fishing pressure for consumption and ornamental purposes. Fish farming for this fish is being developed, and one of the main difficulties is food availability for the different stages. This study assessed the effect of feeding with frozen trash fish and commercial feed on the growth and hematological parameters of juvenile silver arowana *O. bicirrhosum*. A total of 72 juveniles (74.2 ± 0.1 g) were randomly distributed into 12 net cages (6 fish per cage) and fed diets with different protein (P) and lipid (L) levels: frozen trash fish (52P:10L), and commercial tilapia (28P:4L), trout (40P:8L), and arapaima feed (50P:10L) with three replicates twice daily at a daily feeding rate of 6% of biomass for 90 days. At the end of the experiment, growth, hematological parameters, and cost per kilogram of weight gain were evaluated. The results revealed improved growth ($P < 0.05$) among fish fed with frozen trash fish (172.6 ± 26.9 g) and commercial arapaima feed (174.6 ± 48.8 g), in comparison with those fed commercial trout (120.5 ± 28.9 g) and tilapia feed (106.0 ± 37.1 g). Fish fed with frozen trash fish had lower daily feed intake (0.9 ± 0.1 g) and feed conversion ratio (0.8 ± 0.0), and greater hematocrit ($36.2 \pm 1.3\%$), erythrocyte sizes (348.7 ± 37.5 fL), total leukocyte ($14.7 \pm 0.3 \times 10^3 \mu\text{L}^{-1}$), and lymphocyte levels ($10.8 \pm 1.1 \times 10^3 \mu\text{L}^{-1}$) than juveniles that consumed the three commercial diets ($P < 0.05$). Feeding with frozen trash fish reduced ($P < 0.05$) the cost per kg of weight (USD 2.5) compared to feeding commercial diets for trout (USD 4.3) and tilapia (USD 4.8). The results show that feeding fresh frozen fish allows for healthy and less costly growth of juvenile silver arowana *O. bicirrhosum*.

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Introduction

In recent decades, aquaculture has become a significant and growing activity in the production of aquatic organisms (FAO 2024). However, balanced diets, which represent 50–70% of production costs, have suffered a continuous price increase (Rana et al. 2009; Gong et al. 2019). Protein is one of the most important macronutrients that affect the production cost and growth of farmed fish (NRC 1993; Younis et al. 2018). Excess protein in the diet leads to increased nitrogen excretion (Shin et al. 2016), reducing water quality, while protein deficiency reduces the growth and health of fish (Craig and Helfrich 2009).

The Peruvian Amazon has poor land connectivity and high transportation costs due to its geographic location, which generates a shortage of commercially balanced diets for fish farming. Fish farmers use commercial diets available at their farms that are not specific for the species farmed, and for fish with piscivorous habits, such as arapaima *gigas* and silver arowana *Osteoglossum bicirrhosum*, additionally use small, less valuable fish (forage or trash fish), generally obtained through fishing, as food (Vásquez et al. 2007; Núñez et al. 2011). Studies that have compared the effect of feeding with balanced diets and trash fish on snakehead *Channa striata*, largemouth bass *Epinephelus coioides*, and grouper *Microp-terus salmoides* found improved results in growth, feed conversion ratio, protein efficiency, and meat quality with balanced diets, and better cost results when feeding with trash fish (Bunlipatanon et al. 2014; Hien et al. 2016; Xu et al. 2022). However, the use of trash fish has disadvantages, such as variable nutritional composition, the risk of introducing diseases, eutrophication of water, and the reduction of natural populations (Tacon and Metian 2009; Kim et al. 2007; Xu et al. 2007). It is also important to evaluate the costs generated by the use of fresh fish versus balanced feed in fish farming.

The silver arowana *O. bicirrhosum* is a species native to the Amazon basin, the Rupununi, and the Oyapock rivers that inhabit shallow bodies of water (Goulding 1980; Reis et al. 2003), in the wild presents opportunistic omnivorous habit with ichthyophagous preference (Torres et al. 2012). It is fished for local human consumption (García et al. 2009) and its larvae are extracted for ornamental purposes, and exported to European and Asian markets (Moreau and Coomes 2007; Maldonado et al. 2017). Natural populations of this species have been subject to strong fishing pressure, resulting in it being classified as threatened and vulnerable in Peru and Colombia, respectively (Moreau and Coomes 2007; Mojica et al. 2012). Therefore, it is important to develop captive breeding to satisfy existing demand and reduce pressure on the natural environment. Larvae are obtained in captivity by opening the oral cavity of males in earthen tanks (Verba et al. 2014). Cultivated larvae, juveniles, and adults are fed small fish, insects, and/or balanced diets for other species (Vásquez et al. 2007; Fernández-Méndez et al. 2019). In early juveniles, it has been established that a diet containing 45% protein and 8% lipids provided good growth and metabolism (Darias et al. 2015). The hematological parameters of silver arowana *O. bicirrhosum* in the wild were recorded (Val et al. 2016; Tostes et al. 2019), and have been found to have been influenced by macro (protein and lipids) and micronutrients (vitamins and minerals) in diets in cultivation systems (Ahmed et al. 2020). This study aimed to evaluate the effect of feeding with frozen trash fish and commercial diets on the productive performance and hematological parameters of juvenile silver arowana *O. bicirrhosum*.

Materials and methods

Experimental diets

Four dietary treatments were used: frozen trash fish, and three commercial diets for tilapia, trout, and arapaima with different protein levels (28, 40, and 50%), with three replicates of each treatment. The fish were hand-fed twice daily (8:00 and 16:00 h) at a daily feeding rate of 6% of biomass for 90 days.

The frozen trash fish were forage fish known as mojarra (*Astyanax* sp.), which were approximately 5 cm in total length, captured in existing excavated tanks at the Institute and frozen whole (-20°C). To be used, the fish were thawed 2 h before feeding. Commercial diets were obtained from a local fish feed supplier, using extruded commercial feeds for tilapia, trout, and arapaima with a pellet of 6 mm (Purina, Cargill Incorporated, Lima, Perú). The proximal composition of the experimental diets is shown in Table 1 and was determined according to the methods described by the Association of Official Analytical Chemists (AOAC 2012). Moisture content was determined by drying at 105°C , and crude fat was determined by Soxhlet extraction (using hexane as a solvent). Crude protein was determined using the Kjeldahl method ($\text{N} \times 6.25\%$) and crude ash was determined by calcination at 600°C in an oven for 6 h.

Fish and experimental conditions

Juveniles of silver arowana *O. bicirrhosum* were obtained by induced reproduction in captivity and were kept in 1000 m^2 excavated tanks. The collected fish were transferred to aquariums ($40 \times 30 \times 30\text{ cm}$) to adapt to the consumption of commercial balanced feed (Purina, Cargill Incorporated, Lima, Perú: 50% protein, 10% lipids, 2% fiber, and 10% ash) for 30 days (Fernández-Méndez et al. 2019), and were then acclimatized in an excavated tank of 10 m^2 for another 30 days. After acclimatization, 72 juvenile silver arowana *O. bicirrhosum* ($74.2 \pm 0.1\text{ g}$ weight and $26.4 \pm 1.3\text{ cm}$ total length) were randomly distributed in 12 floating net cages ($1 \times 1 \times 1.1\text{ m}$) made of PVC pipes and nylon nets with a capacity of 1000 L (6 fish per cage), located in a 5000 m^2 water reservoir at the Instituto de Investigaciones de la Amazonia Peruana (IIAP), Iquitos, Peru. Water conditions were kept constant during the experiment, and temperature ($30.8 \pm 1.5^{\circ}\text{C}$) and dissolved oxygen ($7.0 \pm 0.4\text{ mg}$

Table 1 Proximate composition (% dry matter basis) and gross energy of commercial diets and the frozen trash fish used in the present study

	Commercial tilapia	Commercial trout	Commercial arapaima	Frozen trash fish
Dry matter (%)	91.6	91.6	92.3	36.2
Protein (%)	28.4	40.9	50.2	52.3
Lipid (%)	4.1	8.5	9.9	9.8
Fiber (%)	3.7	3.1	3.1	-
Ash (%)	9.8	11.5	11.5	20.5
Gross energy (MJ/kg)*	17.6	19.2	20.2	19.2

* Calculated based on 23.6, 39.5, and 17.2 kJ/g of protein, lipid, and non-nitrogenous extract (NNE) respectively. $\text{NNE} = 100 - (\text{protein} + \text{lipid} + \text{fiber} + \text{ash})$

L^{-1}) were measured twice a day (8:00 and 16:00 h) with an oximeter (55, YSI, USA). Ammonium ($0.3 \pm 0.2 \text{ mg L}^{-1}$), nitrites ($0.1 \pm 0.0 \text{ mg L}^{-1}$), pH (7.0 ± 0.6), alkalinity ($22.0 \pm 2.2 \text{ mg L}^{-1}$), carbon dioxide ($3.6 \pm 1.3 \text{ mg L}^{-1}$), and hardness ($22.7 \pm 4.8 \text{ mg L}^{-1}$) were evaluated once a week using a colorimetric kit (AQ2, LaMotte, USA).

Sample collection

For all procedures, fish were anesthetized with $20 \mu\text{L L}^{-1}$ of eugenol (Moyco, Lima, Perú). At the end of 90 days of feeding, all fish were weighed and measured to determine their growth parameters. Three fish from each replicate were randomly selected for hematological analysis.

Growth parameters

Growth parameters and somatic indexes were calculated from weight, total length, and feed intake using the following formula:

$$\text{Weight gain (g)} = \text{final weight (g)} - \text{initial weight (g)}$$

$$\text{Daily weight gain (g day}^{-1}\text{)} = \text{weight gain (g)}/\text{days}$$

$$\text{Daily feed intake (g day}^{-1}\text{)} = \text{feed intake in dry matter (g)}/\text{days}$$

$$\text{Specific growth rate (\% day}^{-1}\text{)} = [\text{Ln}(\text{final weight (g)}) - \text{Ln}(\text{initial weight (g)})] \times 100/\text{days}$$

$$\text{Relative growth rate (\%)} = (\text{final weight (g)} - \text{initial weight (g)}) \times 100/\text{initial weight (g)}$$

$$\text{Feed conversion ratio} = \text{feed intake in dry matter (g)}/\text{weight gain (g)}$$

$$\text{Protein efficiency ratio (\%)} = (\text{weight gain (g)}/\text{protein ingested in dry matter (g)}) \times 100$$

$$\text{Condition factor} = [\text{weight (g)}/(\text{total length (cm)})^3] \times 100$$

Blood parameters analyses

Blood sampling was performed by caudal vein puncture with disposable syringes containing ethylenediaminetetraacetic acid (EDTA 10%) (Gonzales-Flores et al. 2022). The collected blood was divided into two aliquots; the first aliquot was used to determine the hematocrit level, using the microhematocrit method (Goldenfarb et al. 1971); hemoglobin concentration by the cyanmethemoglobin method; and the erythrocyte and total leukocyte counts in a Neubauer chamber (Natt and Herrick 1952). With these data, the hematimetric indices were calculated: mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) according to the Wintrobe (1934) method. For the differential leukocyte count, blood smears stained with Wright (Hawkey and Dennett 1989) were taken and at least 200 cells of interest were counted under a light microscope.

The second aliquot was centrifuged at 75G for 5 min to obtain blood plasma for the measurement of glucose by the glucose oxidase method and total protein by the Biuret method in a visible UV spectrophotometer (Agilent Technologies, Cary 60. California, United States).

Cost analysis

The cost of the commercial diets was calculated using the retail price of the local distributor, and the cost of the frozen trash fish was calculated using the selling price of fish from natural water bodies. These data were used to calculate the cost estimations in US dollars per kilogram of weight gain (1 US dollar = 3.56 Peruvian sol; average exchange rate used over the research period), using the following formula:

$$\text{Cost per kg gain(USD)} = \frac{\text{cost per kg feed(USD)} \times \text{feed fed [dry weight(kg)]}}{\text{body weight gain [wet weight (kg)]}}$$

Statistics

The results were expressed as mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) was used, followed by Tukey's test ($P \leq 0.05$) to determine the differences between the means, and orthogonal polynomial contrasts (linear and quadratic) were used to assess the responses of all dependent variables concerning the protein level of the diets. All analyses were performed after checking for normality (Shapiro–Wilk test) and homoscedasticity (Levene test) using the Sigma Plot 11 software package.

Results

Growth parameters

Fish fed frozen trash fish and the commercial arapaima diet showed the most significant positive results ($P < 0.05$) in final total length, final weight, weight gain, daily weight gain, specific growth rate, and relative growth rate compared with the other two commercial diets (tilapia and trout), between which there were no differences in results ($P > 0.05$). The fish consumed a greater amount of daily feed in dry matter with the arapaima diet and less with frozen trash fish, while the best dry matter feed conversion ratio was obtained with the frozen trash fish diet and the worst with the tilapia diet. Condition factor, protein efficiency ratio, and survival did not differ significantly between feeding treatments. It was also observed that all the growth performance parameters evaluated increased linearly and quadratically as crude protein levels were increased in diets ($P < 0.05$), except protein efficiency ratio and daily feed intake ($P > 0.05$) (Table 2).

Hematological parameters

Fish fed frozen trash fish had higher ($P < 0.05$) hematocrit, mean cell volume, and mean cell hemoglobin levels compared to fish fed commercial diets (tilapia, trout, and arapaima), which did not differ between them ($P > 0.05$). The number of white blood cells and lymphocytes in fish fed frozen trash fish increased significantly ($P < 0.05$) compared to fish fed commercial diets, for which the results for these parameters were similar ($P > 0.05$). Red blood cells, mean cell hemoglobin concentration, monocytes, eosinophils, neutrophils, total protein, and glucose did not differ ($P > 0.05$) in any of the feeding treatments. Of all

Table 2 Growth performance of young silver arowana *Osteoglossum bicirrhosum* in net cage fed three commercial diets and frozen trash fish for 90 days. Different letters within a row indicate significant differences among the treatments

Parameters	Commercial tilapia (28.4P)	Commercial trout (40.9P)	Commercial arapaema (50.2P)	Frozen trash fish (52.3P)	Linear		Quadratic	
					P	R ²	P	R ²
Final total length (cm)	28.58 ± 3.23a	29.40 ± 2.16a	32.80 ± 2.70b	32.15 ± 1.43b	<0.001	0.797	<0.001	0.856
Final weight (g)	106.01 ± 37.05a	120.53 ± 28.97a	174.58 ± 48.78b	172.58 ± 26.89b	<0.001	0.779	<0.001	0.826
Weight gain (g)	31.17 ± 9.43a	44.87 ± 12.34a	99.86 ± 15.50b	100.1 ± 11.6b	<0.001	0.767	0.001	0.819
DWG (g day ⁻¹)	0.34 ± 0.10a	0.49 ± 0.13a	1.09 ± 0.17b	1.11 ± 0.13b	<0.001	0.738	0.002	0.812
SGR (% day ⁻¹)	0.39 ± 0.12a	0.52 ± 0.11a	0.91 ± 0.10b	0.98 ± 0.27b	<0.001	0.707	0.004	0.773
RGR (%)	39.69 ± 11.09a	43.01 ± 28.04a	129.80 ± 16.83b	145.31 ± 55.63b	0.003	0.613	0.004	0.761
DFI (g day ⁻¹)	1.35 ± 0.01a	1.33 ± 0.16a	1.88 ± 0.10b	0.92 ± 0.04c	0.974	0.000	0.647	0.048
FCR	4.25 ± 0.83a	2.45 ± 0.09b	1.72 ± 0.21b	0.82 ± 0.01c	<0.001	0.896	<0.001	0.896
PER (%)	0.85 ± 0.18a	0.91 ± 0.04a	1.08 ± 0.12a	0.85 ± 0.01a	0.337	0.093	0.437	0.128
Condition factor	0.45 ± 0.01a	0.47 ± 0.04a	0.49 ± 0.02a	0.52 ± 0.02a	0.007	0.539	0.033	0.584
Survival (%)	100	100	100	100	-	-	-	-

DWG, daily weight gain; SGR, specific growth rate; RGR, relative growth rate; DFI, daily feed intake; FCR, feed conversion ratio; PER, protein efficiency ratio; P, protein diet

the hematological parameters evaluated, only hematocrit and white blood cells increased ($P < 0.05$) linearly and quadratically with increasing crude protein in the diet (Table 3).

Cost per kg

Feeding with frozen trash fish significantly decreased ($P < 0.05$) the cost per kilogram of weight gain compared to fish fed commercial tilapia and trout diets, which exhibited similar results ($P > 0.05$). Fish fed with a commercial arapaima diet, meanwhile, showed no significant differences ($P > 0.05$) with the other feeding treatments (Fig. 1).

Discussion

Nutrients are the basis for the formation of living tissues and a source of energy for the metabolic, physiological, and growth processes of fish. Diets must contain a balanced proportion of protein and energy to meet the nutritional needs of each species (Craig and Helfrich 2009). Silver arowana *O. bicirrhosum* is an opportunistic omnivore with carnivorous tendencies (Torres et al. 2012). Carnivorous fish have high protein nutritional requirements for the production of tissues that allow adequate development (Kaushik 1990). Juveniles silver arowana *O. bicirrhosum* fed diets containing more than 50% protein (frozen trash fish and arapaima commercial diet) exhibited better growth performance than fish fed commercial trout and tilapia diets with protein levels of 40.9% and 28.4%, respectively. The protein needs of silver arowana *O. bicirrhosum* are similar to those reported for arapaima *Arapaima gigas* (Ituassú et al. 2005), which is a phylogenetically close species with a preferentially piscivorous feeding habit (Goulding 1980). Protein levels above 45% are adequate for juvenile silver arowana *O. bicirrhosum* (Cuaical et al. 2013; Darias et al. 2015), which coincides with the short intestine and protein nutritional requirements typical of piscivorous fish (Kubitza and Lovshin 1999; Duque-Correa et al. 2024). There are no reports of growth performance parameters in this species for the sizes evaluated in the present study, as existing reports are of smaller stages, such as early juveniles (Cuaical et al. 2013; Darias et al. 2015; Fernández-Méndez et al. 2019), which present higher growth rates because as fish develop in body size, they have slower growth rates (Craig and Helfrich 2009). Daily feed intake in dry matter was higher in fish fed the arapaima diet, due to greater growth, while those that consumed frozen trash fish, which also exhibited greater growth, had lower daily feed intake, as their diet contained 63% moisture, unlike the commercial diets, which contained 8 to 10% moisture. Frozen trash fish, due to the higher moisture content, maintain a higher source of vitamins and fatty acids and higher ash content as a source of minerals compared to commercial diets (Jeyasanta and Patterson 2014). The feed conversion factor was higher, and above recommended values, in fish fed with the tilapia diet, while fish fed with the arapaima diet presented values within the range reported for the species (Cuaical et al. 2013), and among those fed with the fresh frozen diet the value was lower due to moisture in the diet. The condition factor shows the physiological state of fish in terms of well-being or fat (Nash et al. 2006), and the results obtained revealed that the protein levels of the diets used did not influence the weight-length relationship and that the values obtained are extremely similar to other studies in captivity (0.4–0.5) of the species with smaller individuals (Darias et al. 2015; Fernández-Méndez et al. 2019). The survival rate obtained is similar or superior to other studies with smaller silver arowana *O. bicirrhosum* (Cuaical et al. 2013), considering that the fish in this study was adapted to

Table 3 Hematological parameters of young silver arowana *Osteoglossum bicirrhosum* in net cage fed three commercial diets and frozen trash fish for 90 days. Different letters within a row indicate significant differences among the treatments

Parameters	Commercial tilapia (28.4P)	Commercial trout (40.9P)	Commercial arapaema (50.2P)	Frozen trash fish (52.3P)	Linear		Quadratic	
					P	R ²	P	R ²
Hematocrit (%)	28.1 ± 3.4a	30.5 ± 1.6a	30.5 ± 2.9a	36.2 ± 1.3b	0.014	0.469	0.049	0.536
Hemoglobin (g dL ⁻¹)	8.7 ± 1.5	8.5 ± 0.9	9.7 ± 1.8	11.3 ± 2.9	0.155	0.192	0.326	0.193
RBC (10 ⁶ µL ⁻¹)	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	0.374	0.080	0.520	0.091
MCV (fL)	272.6 ± 35.4a	280.0 ± 15.6a	265.2 ± 25.1a	348.7 ± 37.5b	0.154	0.192	0.181	0.319
MCH (pg)	80.8 ± 10.3a	80.5 ± 10.0a	81.8 ± 11.0a	107.9 ± 24.2b	0.163	0.185	0.196	0.302
MCHC (g dL ⁻¹)	31.1 ± 5.0	28.0 ± 3.4	31.2 ± 6.8	31.1 ± 7.5	0.935	0.001	0.600	0.062
WBC (10 ³ µL ⁻¹)	11.7 ± 0.8a	11.7 ± 0.9a	11.8 ± 0.7a	14.7 ± 0.3b	0.025	0.409	0.012	0.684
Lymphocyte (10 ³ µL ⁻¹)	8.7 ± 0.6a	8.6 ± 1.3a	8.2 ± 1.0a	10.9 ± 1.1b	0.210	0.152	0.163	0.339
Monocyte (10 ³ µL ⁻¹)	1.7 ± 0.6	1.7 ± 0.4	2.1 ± 0.4	1.9 ± 0.4	0.252	0.129	0.375	0.162
Eosinophil (10 ³ µL ⁻¹)	0.7 ± 0.4	0.9 ± 1.1	0.8 ± 0.5	1.5 ± 0.8	0.343	0.090	0.520	0.090
Neutrophil (10 ³ µL ⁻¹)	0.6 ± 0.3	0.5 ± 0.2	0.7 ± 0.5	0.4 ± 0.2	0.667	0.019	0.703	0.033
Total protein (g dL ⁻¹)	3.5 ± 0.5	3.4 ± 0.3	3.7 ± 0.4	3.9 ± 0.2	0.489	0.049	0.204	0.294
Glucose (mg dL ⁻¹)	77.2 ± 13.5	82.7 ± 20.1	70.4 ± 16.6	65.7 ± 12.6	0.512	0.044	0.224	0.275

RBC, red blood cell; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; WBC, white blood cells; P, protein diet

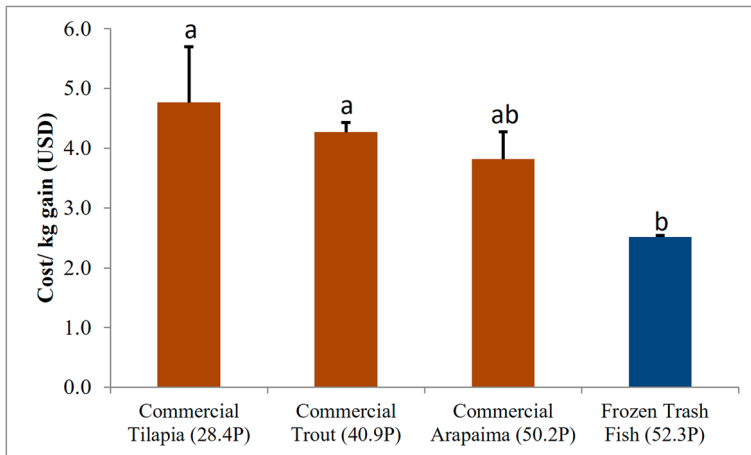


Fig. 1 Estimated cost (USD) per kilogram of gain young silver arowana *Osteoglossum bicirrhosum* in net cage fed three commercial diets and frozen trash fish for 90 days. Results are expressed as mean $\pm$ SD ($n=3$). Different letters indicate significant differences (ANOVA, $P<0.05$). P, protein diet

balanced diets and were larger in size. The increase in dietary protein reflects the linear and quadratic increase of all growth parameters except daily feed intake and protein efficiency ratio, confirming the dependence of the growth of silver arowana *O. bicirrhosum* on dietary protein content. Similar results have been reported in other piscivore fish such as pikeperch *Sander lucioperca* and tiger grouper *Epinephelus lanceolatus*, which exhibited the same relationship (Schulz et al. 2007; Gao et al. 2019).

Hematological parameters are used in aquaculture as indicators of the physiological state of fish (Ahmed et al. 2020) and can be affected by several factors, such as disease, stress, feeding, and cultivation conditions (Yousefi et al. 2012; Fernández-Mendez et al. 2024). It has been demonstrated that variation in nutrients such as protein, lipids, vitamins, and minerals in the diet influences the hematological parameters of fish (Daniels and Gallagher 2000; Lim et al. 2000; Garcia et al. 2007; Babalola et al. 2009). For silver arowana *O. bicirrhosum*, no hematological parameters have been recorded under cultivation conditions, with the only studies among adults in the natural environment (Val et al. 2016; Tostes et al. 2019). The hematological parameters indicated in the present study are within the values indicated by Tostes et al. (2019), except for the number of erythrocytes and the MCV value, which are higher and lower, respectively. These variations within the same species have been reported for other teleosts (Gonzales et al. 2019, 2020). The hematological parameters of the fish that consumed the three commercial diets (tilapia, trout, and arapaima) did not differ between feeds, despite the variation in macronutrients such as protein. Similar results were obtained in other studies on tilapia *Oreochromis niloticus* fed different levels of protein in balanced diets (Kpundeh et al. 2015). However, juveniles fed with frozen trash fish had increased hematocrit, white blood cell, and lymphocyte values, which is considered an immunostimulatory effect, probably due to the higher content of nutrients such as vitamins, minerals, fatty acids, and essential amino acids (Xu et al. 2022), which have been shown to have effects on the immune system of fish (Ming et al. 2012; Lim et al. 2015; El Basuini et al. 2016; Tan et al. 2016). The linear increase in hematocrit and white blood cell levels concerning dietary protein has been recorded in other studies with tilapia *Oreochromis niloticus*

and siberian sturgeon *Acipenser baerii* (Docan et al. 2012; Paul et al. 2022), an increase that may be attributed to the higher amino acid content available in diets with higher protein content (Zhou et al. 2006; Liao et al. 2014).

In aquaculture, balanced feed represents up to 60% of production costs (Tacon 1987). Protein is one of the nutrients that affect cultivation performance and comes mainly from fishmeal, whose market price is constantly increasing (Jannathulla et al. 2019), with the price of balanced diets directly related to protein content. The cost per kg of weight gain results obtained were similar (USD 4.5) to those obtained in *Arapaima gigas* grown in cages with a balanced diet (De Oliveira et al. 2012). Balanced diets for tilapia and trout generated the highest cost per kg of weight gain due to the lower protein content of such feed, below that recommended by studies (45%) for juveniles silver arowana *O. bicirrhosum* (Cuaical et al. 2013; Darias et al. 2015), and produced lower growth and a high conversion factor. In arapaima *A. gigas*, higher conversion factor values have been reported for diets with protein levels below the nutritional requirements of the species (Epifânio et al. 2023). The frozen trash fish diet had the lowest cost per kg of weight as it generated optimal growth of silver arowana *O. bicirrhosum* juveniles, the best feed conversion ratio, and the lowest cost of frozen trash fish compared to balanced diets. It is important to establish diets that are not only economically viable but also environmentally sustainable, as the use of fish as food will generate environmental impacts such as eutrophication of the aquatic culture environment and overfishing of forage fish in the natural environment (Xu et al. 2007; Tacon and Metian 2009). Likewise, feeding with this type of diet is also considered a risk to the health of fish, as they can carry pathogens resistant to freezing and be transmitted to the fish that consume them (Kim et al. 2007). However, despite the knowledge generated herein, more studies are still needed on the protein, lipid, amino acid, and nutrient digestibility needs for the development of diets for this species (Booth et al. 2013).

In conclusion, juvenile silver arowana *O. bicirrhosum* has a high dietary protein requirement and feeding frozen trash fish allows for healthy fish growth at a lower cost.

Author contribution C.F.M. Conceptualization; Methodology; Writing - original draft; Formal analysis. G.C.U. and R.R.V. Investigation; Visualization; Writing - review & editing. A.P.F.G. Conceptualization; Methodology; Writing - review & editing. All authors read the manuscript and approved it in its final version.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Ethics approval Experimental procedures with animals were performed following the European Union Council Guidelines (2010/63/EU) for the use of laboratory animals and with the Peruvian legislation on animal protection and welfare (30407/2016).

Conflict of interest The authors declare no competing interests.

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