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Growth and blood chemistry of juvenile Neotropical catfish (*Lophiosilurus alexandri*) self-feeding on diets that differ in protein-to-energy (P:E) ratio

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Abstract

This study evaluated the growth and blood biochemistry were examined in juvenile *Lophiosilurus alexandri* that were self-feeding on feeds with different protein-to-energy (P:E) ratios. Juveniles (21.10 ± 0.39 g) were stocked at a density of six fish/tank (40 L) photoperiod 12L:12D, equipped with an on-demand feeder connected to a photoelectric cell. The 50-day experiment evaluated isoenergetic diets (17.65 MJ/kg) with crude protein levels from 25 to 42% and P:E of 14.56, 17.43, 20.44, and 23.91 g protein/MJ, in four replications, in a completely randomized design. The 23.91 g protein/MJ diet had the lowest leftover food and daily intake, while the 14.56 g protein/MJ diet had the highest leftovers. Polynomial regression analysis showed that the P:E ratios affected weight, average daily consumption per fish, protein efficiency, and weight gain had their lowest estimated values at 17.80, 21.23, 19.24, and 17.77 g protein/MJ, respectively. Feed conversion ratio peaked at 15.48 g protein/MJ, while the viscerosomatic index and carcass lipid had the lowest values at 22.74 and 20.03 g protein/MJ, respectively. Glucose, cholesterol, and low-density lipoprotein (LDL) were lower for animals fed a diet containing 24.17, 22.38, and 17.25 g protein/MJ, respectively. The total protein showed a increasing linear effect as the P:E ratio increased. High-density lipoprotein (HDL) had its highest value at 22.28 g protein/MJ. Thus, diets with an P:E ratio close to 23.91 g protein/MJ provide better adaptation of *L. alexandri* juveniles to the self-feeding system, along with better growth rates and blood biochemistry.

Keywords Growth metrics · On-demand feeding · Blood analysis · Nutrition · Carnivorous fish

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Introduction

To bridge the gap between demand and supply, efforts must be made to increase aquaculture; nutrition is a keyword for this development. An animal's diet must meet essential nutrients requirements, and food must also be consumed in adequate amounts to sustain growth (Houlihan et al. 2008). In commercial production of fish, feeding represents most of the total cost during the production period (Baki and Yücel 2017). Its composition and provisioning are crucial to sustaining intensive farming's economic viability (Stejskal et al. 2020). Thus, feeding management is critical for the financial performance of a fish farm (Benhaïm et al. 2017), where problems such as underfeeding, which inhibits growth and promotes competition (Mccarthy et al. 1992), or overfeeding, which increases food waste (Thorpe and Cho 1995) and feed conversion ratios (Talbot 1993), need to be avoided. Among the nutrients needed to formulate a balanced diet for fish, protein is the main cost factor, as it plays a critical role in the maintenance of fast growth (NRC 2011) and animal health (Wang et al. 2017).

Three main strategies are currently available for diet distribution: manual feeding, automatic feeders, and self-feeders (Stejskal et al. 2020). Self-feeding has received particular attention in dietary management. Such systems were developed to avoid the abovementioned problems by allowing fish to seek food according to their nutritional needs (Covès et al. 2006). The main advantage of these systems is the dispensation of a precise amount of feed according to the demand of the animals (Stewart et al. 2012; Cho 1992; Fortes-Silva et al. 2011), allowing fish to choose their feeding time and frequency which are not guaranteed by other systems—and to feed according to their biological rhythms (Benhaïm et al. 2017; López-Olmeda et al. 2012). This type of system can also be beneficial in avoiding food competition, as demonstrated in a study with Atlantic salmon *Salmo salar* L., sea bream *Sparus aurata* L. and European sea bass *Dicentrarchus labrax* L. (Andrew et al. 2002). Self-feeders have also been shown to improve the growth and feed conversion rate of rainbow trout, *Oncorhynchus mykiss* L. (Noble et al. 2007), as well as decrease stress in Nile tilapia, *Oreochromis niloticus* L. (Endo et al. 2002), and damage to fins (Stewart et al. 2012), caused mainly by conspecific attacks on Atlantic salmon, *Salmo salar* L. (Turnbull et al. 1998; MacLean et al. 2000), in addition to handling procedures, bacterial infection, and fin abrasion by contact with rough surfaces in enclosures (Hoyle et al. 2007; Latremouille 2003).

The neotropical catfish *Lophiosilurus alexandri*, a carnivorous species with tasty white meat (Salaro et al. 2015) and no intramuscular spines, and native to the São Francisco River basin in Brazil (Tenório et al. 2006), can be conditioned to formulated diets (Luz et al. 2011; Silva et al. 2014; Salaro et al. 2015) allowing studies of different management and cultivation conditions with the exclusive supply of formulated foods (Melillo Filho et al. 2014; Kitagawa et al. 2015; Cordeiro et al. 2016; Costa et al. 2016 2017). This species is threatened with extinction, especially by fishing pressure in its natural environment (Lins et al. 1997). Thus, its production in captivity would help reduce the capture of wild individuals (Ananias et al. 2022), making it a promising species for aquaculture (Becker et al. 2017; Costa et al. 2015; Kitagawa et al. 2015). Knowledge about the nutritional requirements of this species is still scarce. Still, its digestibility of fish, meat, bone, soybean, roasted whole soybean, wheat bran, and corn protease (Melo et al. 2016), effects of corn in diets on metabolic and performance parameters (Oliveira et al. 2020), regulation of voluntary intake of different protein/

energy ratios (Santos et al. 2019), and effects of protein:carbohydrate ratio on performance and metabolism (Figueiredo et al. 2014) have already been evaluated.

This study aimed to evaluate growth performance, blood biochemical profile, and efficiency of the self-feeding system using different protein:energy ratios in the diet of *Lophiosilurus alexandri* juveniles.

Material and methods

Ethical approval.

The experiment was carried out at the Laboratório de Aquacultura of the Universidade Federal de Minas Gerais, Brazil, using juvenile *L. alexandri* from the laboratory itself. All procedures described here were approved by the Comitê de Ética no Uso de Animais (CEUA / UFMG—nº 208/2018).

Animal housing

Ninety-six juvenile *L. alexandri* were used and stored at a density of six fish per tank in 16 40-L tanks kept in a water recirculation system. The temperature was maintained at 28.0 ± 0.01 °C with a photoperiod of 12L:12D (L-light period and D-dark period) controlled by a digital timer (Key West group DNI). Lighting was provided by LED (4.5 W), with an average intensity of 230 lx (Digital lux meter Instrutemp ITL 260) on the surface of the tanks. During the entire experimental period, dissolved oxygen remained above 5.2 ± 0.12 mg/L (Probe model HI9146, Hanna instruments), total ammonia below 0.5 ± 0.02 mg/L (Toxic ammonia, Labcon Test), and pH at 7.2 ± 0.36 (Tropical pH, Labcon Test), all measured daily.

The fish were submitted to a 14-day adaptation phase. During the first 7 days of adaptation, the fish were manually fed with extruded commercial feed (SUPRA® Acqua line, 36% crude protein-CP, 12% moisture, 13% mineral matter, and 4-mm pellet diameter). The animals were conditioned to use the self-feeding system on demand for the next 7 days of adaptation (until the beginning of the experimental period). An automatic demand feeder (EHEIM 3581, Deizisau, Stuttgart, Germany) connected to a photoelectric cell (Omron model, E3SAD62, Japan) was installed in each tank at 3 cm below the water surface and 33 cm from the bottom (Fig. 1). The automatic feeder was activated by fish crossing the infrared light beam, causing it to release 0.2 g of feed (pellets). Each feeder had a specific food hall composed of a semicircular PVC structure (length: 10 cm, radius: 10 cm, width: 14 cm) to keep feed close to the aquarium wall and prevent the dispersion of pellets. This was done because of the feeding behavior of juvenile *L. alexandri*, which swim, leaning against the wall of the tank, to the surface for feeding.

After adaptation, the animals were weighed and kept in their respective tanks. Juveniles weighing 21.10 ± 0.39 g were subjected to treatments consisting of different protein:energy (P:E) ratios in the diet (Isoenergetic diets, with variation in crude protein content: 14.56 (Crude Protein-CP 25.14% and Gross Energy-GE 17.27 MJ/kg diet), 17.43 (CP 31.12% and GE 17.85 MJ/kg diet), 20.44 (CP 36.60% and GE 17.91 MJ/kg diet) and 23.91 g protein/MJ (CP 42.05% GE 17.59 MJ/kg diet) g protein / MJ. Thus, totaling four treatments, with each treatment containing four replications, distributed in a completely randomized design. The composition of the experimental diets is shown in Table 1.

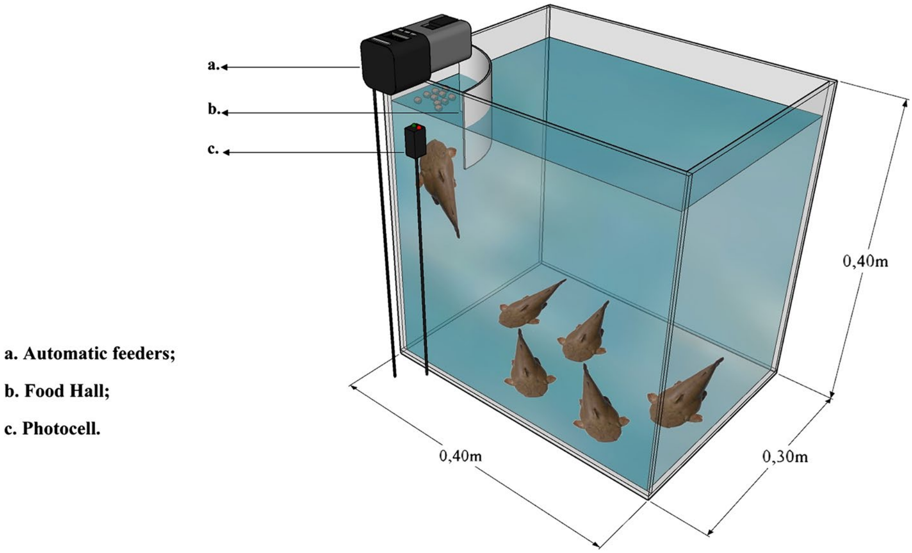

Fig. 1 Self-supply with the infrared sensor. The feeder (a) is activated when a fish crosses the infrared light beam of the photocell (c). The auto-feeder is only activated by the presence of fish inside the feeder (b), avoiding possible unexpected activations

The diets were prepared, first, grinding and homogenizing the ingredients. Floating extruded pellets of 6–8 mm in diameter were then produced (Imbramx40, Imbramaq Ltda., Ribeirão Preto, São Paulo, Brazil). Oil was sprayed after extrusion, and the diets were then dried and stored in a cold chamber (− 20 °C) until used. The chemical composition of the ingredients and diets were analyzed at the Nutrition Laboratory at the Veterinary School of the Federal University of Minas Gerais according to AOAC methods (AOAC 2012). Briefly, moisture was determined after drying in an oven at 105 °C for 24 h; ash by incineration in a muffle furnace at 550 °C for 24 h; crude protein (N6.25) by the Kjeldahl method after acid digestion using a Kjeldahl system. Gross energy was determined by direct combustion in an adiabatic bomb calorimeter (PARR 6200, Parr Instrument Company, Illinois, USA); ANKON for crude fat extraction. The amino acid analysis was analyzed in laboratory CBO, Campinas, São Paulo, Brazil (values determined by means of high performance liquid chromatography (HPLC) (White et al. 1986) and enzymatic method (Lucas and Sotelo 1980).

Productive performance

During the experiment, the remaining feed (Floating extruded pellets) from the feeders was weighed in the morning and supplemented with the respective diet to reach 30 g per feeder. Unconsumed pellets were collected daily (Food hall), early in the morning and late in the afternoon, dried in an oven (Nova Ethics/Ethink) at 55 °C, and weighed to estimate daily consumption. All tanks were cleaned to remove feces at that same time, with the volume of water removed being replenished to maintain the level of the recirculation system.

Table 1 Composition of experimental diets containing different protein:energy (P:E) ratios

Ingredients	Protein:Energy ratio (g protein/MJ)			
	14.56	17.43	20.44	23.91
Fish meal	15.50	21.00	24.00	29.00
Soybean meal	24.00	31.00	33.00	37.00
Corn	40.00	31.50	22.90	1.80
Sour rice	5.00	5.00	5.00	5.00
Albumin	1.00	1.60	6.50	9.10
Soy oil	8.80	6.60	5.30	3.70
Cellulose	3.00	1.00	1.00	0.00
Dicalcium phosphate	0.50	0.50	0.50	0.50
[1]Vitamin/Mineral supplement	0.50	0.50	0.50	0.50
[2]BHT	0.02	0.02	0.02	0.02
Salt	0.50	0.50	0.50	0.50
Inert	1.18	0.78	0.78	0.88
[3]Composition analyzed				
Crude protein (%)	25.14	31.12	36.60	42.05
Gross energy (MJ/kg diet)	17.27	17.85	17.91	17.59
Ash (%)	6.66	7.02	7.49	10.08
Ethereal extract (%)	9.98	7.77	7.86	6.35
Energy:Protein (E:P) Ratio	0.068	0.057	0.048	0.041
[4]Amino acid profile (g/100 g)				
Tryptophan	10.20	13.80	15.30	18.00
Valine	12.60	16.20	19.30	21.70
Methionine	4.90	6.50	7.30	9.50
Isoleucine	11.50	14.90	17.60	20.30
Leucine	21.30	26.30	29.80	32.90
Phenylalanine	11.30	14.50	17.50	19.80
Lysine	16.50	22.80	26.10	30.00
Histidine	6.30	8.40	9.90	11.10
Argine	17.00	24.10	27.70	31.30
$\sum$ EAA	111.60	147.50	170.50	194.60
Aspartic Acid	26.20	36.90	41.20	46.50
Glutamic Acid	39.50	50.30	56.10	64.50
Glycine	15.20	22.30	24.40	29.70
Alanine	15.50	19.90	22.40	26.00
Proline	14.50	18.00	19.90	22.60
Tyrosine	8.80	12.30	13.30	15.60
Cystidine	2.80	3.90	5.10	6.60
Serine	12.70	17.10	19.80	23.70
$\sum$ NEAA	135.20	180.70	202.20	235.20
$\sum$ EAA/$\sum$ NEAA	0.83	0.82	0.84	0.83

[1] Vitamin and mineral supplement composition: Vit. A. 1,200,000 UI; Vit. D3. 200,000 UI; Vit. E. 12,000 mg; Vit. K. 2400 mg; Vit. B1. 4800 mg; Vit. B2. 4800 mg; Vit. B_6. 4000 mg; Vit. B_{12}. 4800 mg; Ac. folic. 1200 mg; Ac. Pantothenic. 3750 mg; Vit. C. 48,000 mg; Biotin. 48 mg; Hill. 65,000 mg; Niacin. 24,000 mg; Fe. 10,000 mg; Cu. 6000 mg; Mn. 4000 mg; Zn. 6000 mg; I. 20 mg; Co. 2 mg; Se. 20 mg

Table 1 (continued)

[2] Butil-Hidroxi-tolueno (antioxidant)

[3] The chemical composition of the ingredients and diets were analyzed according to AOAC methods (AOAC 2012). Briefly, moisture was determined after drying in an oven at 105 °C for 24 h; ash by incineration in a muffle furnace at 550 °C for 24 h; crude protein (N6.25) by the Kjeldahl method after acid digestion using a Kjeldahl system. Gross energy was determined by direct combustion in an adiabatic bomb calorimeter (PARR 6200, Parr Instrument Company, Illinois, USA); ANKON for crude fat extraction

[4] Values analyzed in laboratory CBO, Campinas, São Paulo, Brazil; (values determined by means of high performance liquid chromatography (HPLC) (White, 1986) and enzymatic method (Lucas and Sotelo 1980)

Biometric index

After the 50 days of the experiment, the fish were fasted for 8 h and weighed (Shimadzu model BL3200S scale, 0.01 g precision). The data obtained were used to calculate the following:

Final weight (g) = final biomass (g) / number of animals per aquarium;

Daily weight gain (g) = weight gain (g) / experiment time (days);

Daily feed consumption per fish (g) = total feed consumption / experiment time (days) / number of animals per tank;

Feed conversion ratio (FCR) = apparent total feed consumption (g) / weight gain (g).

Protein efficiency ratio (PER) (%) = weight gain / protein consumption.

Survival (%) = (final number of fish/initial number of fish) × 100.

Blood samples

After biometrics, 12 animals from each treatment (Three fish from each tank) were removed for blood collection. The animals were anesthetized with benzocaine at 120 mg/L for blood collection by puncture of the tail vein (Ribeiro et al. 2019). A total of 1.0 mL of blood was collected from each fish through caudal vein puncture with 3.0 mL syringes containing heparin (0.1 to 0.2% mg mL^{-1} of blood). Aliquots of blood were centrifuged at 1000 rpm for 5 min and then at 3000 rpm for 4 min to separate the supernatant fraction (Mattioli et al. 2017). The biochemical profile was obtained from plasma samples using an automated device (Mindray BS-200E; Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). Bioclin/Quibasa kits (Minas Gerais, Brazil) were used to analyze plasma concentration of glucose (kit # K082-3), total protein (kit # K031-1), albumin (Kit # k040-1), triglycerides (kit # K117-3), cholesterol (kit # K083-3), high-density lipoprotein (HDL; kit # K071-23), low-density lipoprotein (LDL; kit # K088-27), aspartate aminotransferase (AST; kit # K048 -6), and alanine aminotransferase (ALT; kit # K049-6).

Viscerosomatic Index and crude lipid composition of the carcass

After the blood collection, the animals were euthanized with a solution containing 300 mg Benzocaine (Ross and Ross 2008) and had their viscera collected to determine the Visceosomatic Index as:

Viscerosomatic Index (VSI) = 100 × (viscera weight (g) / body weight (g)).

The eviscerated carcasses ($n = 12$) were stored at – 20 °C, for later lyophilization. After the samples were ground to determine dry lipid amount of the eviscerated carcasses.

The crude lipid amount of the eviscerated carcass was determined using the semi-automatic equipment ANKOM XT15 (ANKON Technology Corporation, Fairport, NY, USA). One gram of sample was weighed and placed in an ANKON XT4 filter bag, and the analysis was performed as indicated by the manufacturer using petroleum ether.

Data analysis

All data were submitted to the Shapiro–Wilk homoscedasticity and normality test. The daily consumption of diets and daily amount of leftover food were submitted to ANOVA, followed by Tukey's test ($P < 0.05$). Regression analysis ($P < 0.05$) was performed for data of final weight, average daily consumption, feed conversion ratio, viscerosomatic index, glucose, total protein, cholesterol, HDL, LDL, and the carcass crude lipid content that showed differences by ANOVA ($P < 0.05$).

Results

Growth *performance*

There was no significant effect of different P:E ratios on survival rate (100%). Average daily consumption and average daily leftover feed were affected by diet (Fig. 2) ($P < 0.05$), with lower leftover feed for the 23.91 g protein/MJ diet and lower daily consumption for the 17.43, 20.44, and 23.91 g protein/MJ diets. The 14.56 g protein/MJ diet had the highest daily consumption and the highest number of leftover pellets from diets.

The different P:E ratios of the diet significantly ($P < 0.05$) influenced the final weight, demonstrating a positive quadratic effect ($Y = 0.06718x^2 - 23.921x + 269.84$; $R^2 = 80.04\%$). A value of 17.80 g protein/MJ of P:E was estimated for the minimum final weight of 56.90 g (Fig. 3A). Different P:E ratios of the diet significantly influenced ($P < 0.05$) the average daily consumption per fish, demonstrating a positive quadratic ($Y = 0.0048x^2 - 0.2038x + 3.0351$; $R^2 = 97.05\%$). A value of 21.23 g protein/MJ of P:E was estimated for the minimum average daily consumption per fish of 0.87 g (Fig. 3B). A significant effect ($P < 0.05$) of the different P:E ratios of the diet was also observed on FCR, demonstrating

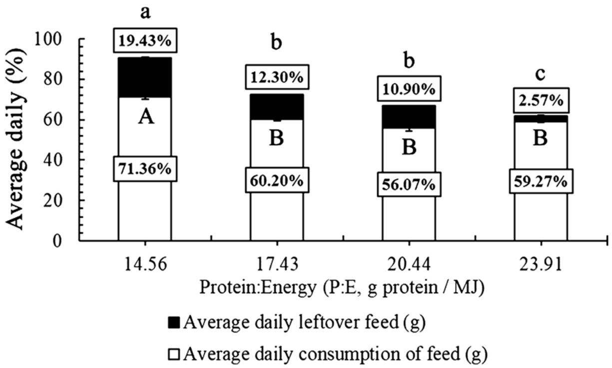

Fig. 2 Mean values (± standard error) for daily consumption of diets and daily leftovers for juvenile *L. alexandri* submitted to self-feeding with diets containing different energy:protein ratios (Tukey, $P < 0.05$)

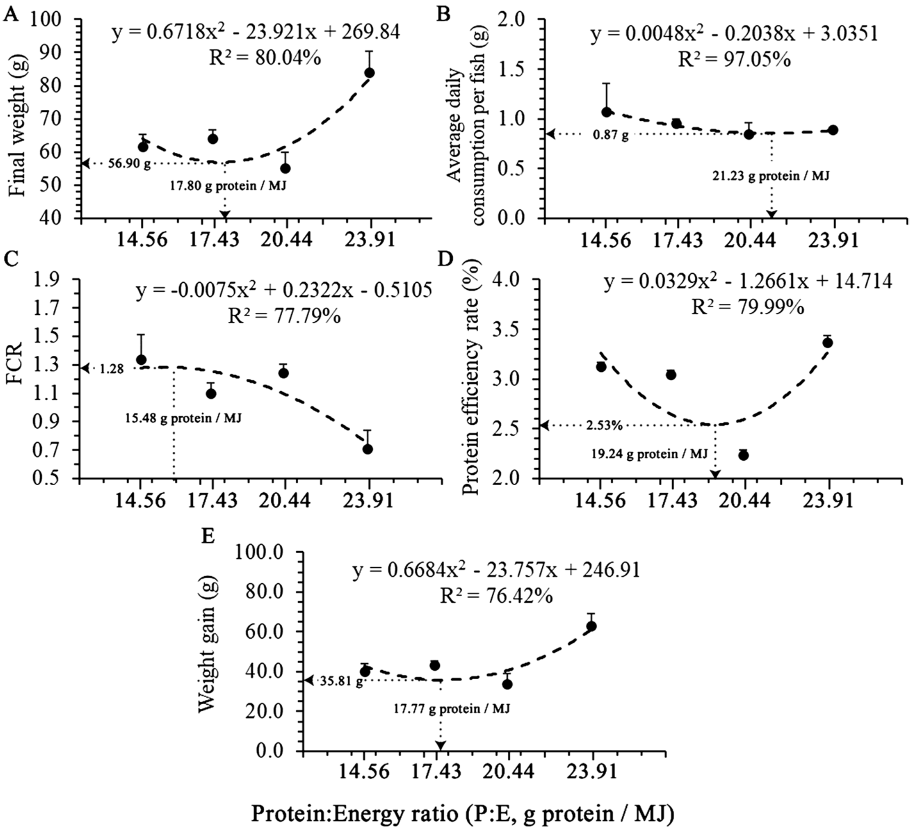

Fig. 3 Mean values (± standard error) for the final weight (**A**), average daily consumption per fish (**B**), FCR-feed conversion ratio (**C**), protein efficiency rate (**D**), and weight gain (**E**) for juvenile *L. alexandri* submitted to self-feeding with diets with different energy:protein ratios. The arrow represents the value estimated by the derivative of the equation

a negative quadratic effect ($Y = -0.0075x^2 + 0.2322x - 0.5105$; $R^2 = 77.79\%$). A value of 15.48 g protein/MJ of P:E was estimated for a maximum FCR of 1.28 (Fig. C). Different diet P:E ratios significantly influenced ($P < 0.05$) the protein efficiency rate, demonstrating a positive quadratic effect ($Y = 0.0329x^2 - 1.2661x + 14.714$; $R^2 = 79.99\%$). A value of 19.24 g protein/MJ of P:E was estimated for the minimum average daily consumption per fish of 2.53% (Fig. 3D). Different diet P:E ratios significantly influenced ($P < 0.05$) weight gain, demonstrating a positive quadratic effect ($Y = 0.6684x^2 - 23.757x + 246.91$; $R^2 = 76.42\%$). A value of 17.77 g protein/MJ of P:E was estimated for a minimum weight gain of 35.81 g (Fig. 3E).

Viscerosomatic index and crude lipid composition of the carcass

Different diet P:E ratios significantly ($P < 0.05$) influenced the VSI, demonstrating a positive quadratic effect ($Y = 0.0454x^2 - 2.0649x + 31.302$; $R^2 = 99.18\%$). A value of 22.74 g protein/MJ of P:E was estimated for the minimum VSI of 7.82% (Fig. 4A).

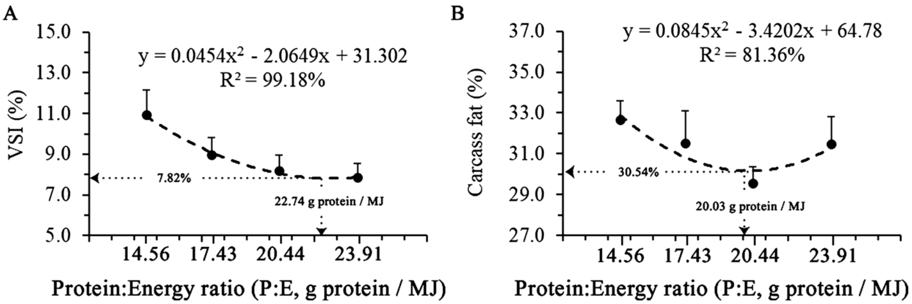

Fig. 4 Mean values (± standard error) for the **A** viscerosomatic index (VSI) and **B** carcass crude lipid for juvenile *L. alexandri* submitted to self-feeding with diets with different energy:protein ratios. The arrow represents the value estimated by the derivative of the equation

Different diet P:E ratios significantly influenced ($P < 0.05$) the composition of the crude lipid in the carcass, demonstrating a positive quadratic effect ($Y = 0.0845x^2 - 3.4202x + 64.78$; $R^2 = 81.36\%$). A value of 20.03 g protein/MJ of P:E was estimated for the minimum carcass lipid composition of 30.54% (Fig. 4B).

Blood biochemistry parameters

Different diet P:E ratios significantly influenced ($P < 0.05$) glucose, demonstrating a positive quadratic effect ($Y = 0.6916x^2 - 33.431x + 459.38$; $R^2 = 91.53\%$). A value of 24.17 g protein/MJ of P:E was estimated for minimum glucose of 55.38 mg/dL (Fig. 4A). The total protein showed a increasing linear effect as the P:E ratio increased (Fig. 5B) ($P < 0.05$). Different diet P:E ratios significantly influenced ($P < 0.05$) cholesterol, demonstrating a positive quadratic effect ($Y = 0.9302x^2 - 41.637x + 597.11$; $R^2 = 71.76\%$). A value of 22.38 g protein/MJ of P:E was estimated for minimum cholesterol of 131.18 mg/dL (Fig. 5C). A significant effect ($P < 0.05$) of different diet P:E ratios was also observed on HDL, demonstrating a negative quadratic effect ($Y = -7177.1x^2 + 540.39x + 2.5663$; $R^2 = 88.87\%$). A value of 22.28 g protein/MJ of P:E was estimated for a maximum HDL of 12.70 mg/dL (Fig. 5D). Different diet P:E ratios significantly influenced ($P < 0.05$) the LDL, demonstrating a positive quadratic effect ($Y = 0.0968x^2 - 3.3394x + 43.263$; $R^2 = 99.05\%$). A value of 17.25 g protein/MJ of P:E was estimated for a minimum LDL of 14.46 mg/dL (Fig. 3D).

Triglycerides (525.51 ± 231.20 mg/dL), ALT (11.84 ± 4.69 U/L), AST (38.97 ± 21.65 U/L), and albumin (1.39 ± 0.65 g/dL) were similar among diets ($P > 0.05$).

Discussion

Juveniles of *L. alexandri* adapted well to the self-feeding system and revealed differences in performance and consumption when fed with diets containing different protein:energy ratios. This adaptation confirms what was verified by Kitagawa et al. (2015), and Santos et al. (2019), for this species. Furthermore, Santos et al. (2019) emphasized the ability of *L. alexandri* to adjust its consumption of protein and energy in the diet in a self-feeding system, an ability that, according to Noble et al. (2007), can improve fish growth and feed conversion. The present study demonstrated the self-feeding system's efficiency for juvenile

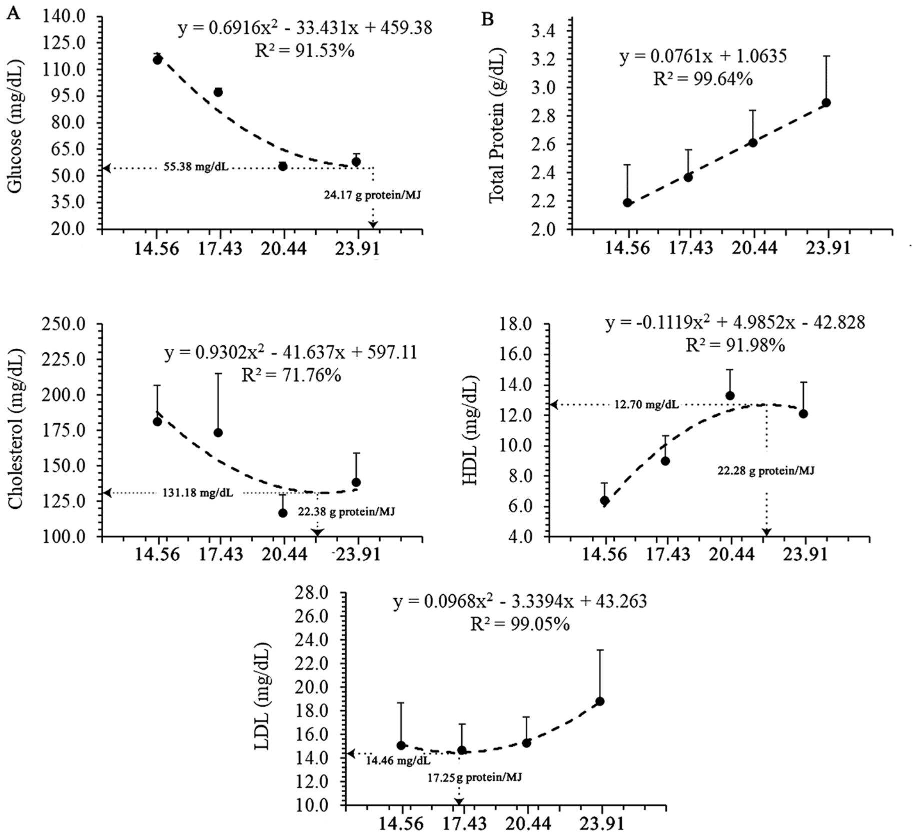

Fig. 5 Mean values (± standard error) for glucose (**A**), total protein (**B**), cholesterol (**C**), HDL (**D**), and LDL (**E**) for juvenile *L. alexandri* submitted to self-feeding with diets containing different energy:protein ratios. The arrow represents the estimated value by the derivative of the equation

L. alexandri (Fig. 2), corroborating Boujard and Médale (1994), Sánchez-Vázquez et al. (1999), and Yamamoto et al. (2000). The self-feeding system's efficiency depended on the diet used, since diets with 14.56, and 23.91 g protein/MJ resulted in higher (19.46%) and lower (2.56%) food waste, respectively. The higher consumption and waste in animals that received diets with 14.56 g protein/MJ can be explained by the fact that *L. alexandri* is a carnivorous species and could not meet its metabolic needs because this diet contains a lower level of protein (25.14% CP). When the food intake target is suboptimal (in this case, due to low protein), fish are forced to make behavioral compromises resulting in excessive energy consumption (Simpson and Raubenheimer 2001). Santos et al. (2019) demonstrated that juveniles of *L. alexandri* chose to make up for their energy needs when fed with two diets considered low in protein for a carnivorous species. When fed a protein-deficient diet, these animals would increase their daily feed intake and, consequently, their energy intake rate, as already demonstrated for other animal species (Emmans 1981).

The lowest consumption rate when using self-feeders was for animals fed diets with an E:P ratio of around 21.23 g protein/MJ. Figueiredo et al. (2014) found the E:P ratio to affect total apparent feed consumption, with a longitudinal increase in consumption for diets containing higher amounts of carbohydrates and the lowest feed consumption for animals fed with E:P ratios of 15.47 g protein/MJ (34.06% CP and 22.02 MJ of GE/kg of diet) and 17.44 g protein/MJ (38.52% CP and 22.09 MJ of GE/kg of diet). Consumption is regulated by the concentration of energy in the diet (Lovell 1989). Two main nutrients proteins and lipids are the primary energy sources for fish (Lu et al. 2020). This means that dietary protein can be used as energy, while it can be spared for anabolic functions when other energy source nutrients are adequately balanced (Lu et al. 2020). Thus, diet energy content often affects protein requirements (Meyer and Fracalossi 2004; Zhang et al. 2017).

Protein and energy content are the main factors in formulating fish diets. The right E:P or P:E balance provides adequate nutrient intake to optimize fish growth (Lee et al. 2000) at all stages of life (Twibell et al. 2016). The present study found that the diet P:E ratio influences fish performance. Final weight, weight gain, and protein efficiency rate were higher for animals fed diets containing 23.91 g protein/MJ (42.05% CP and 17.59 MJ of GE/kg of diet). Souza et al. (2013) reported that juvenile *L. alexandri* (5.19 ± 0.01 g) fed diets containing P:E ratios between 34.00 g protein/MJ (48.8% CP and 14.35 MJ of GE/kg of diet) and 26.27 g protein/MJ (36.2% CP and 13.78 MJ of GE/kg of diet) had higher mean final weight and weight gain with the 26.27 g protein/MJ diet.

Adjustments to a diet's protein content and amino acid profile are essential to achieve a good performance, which is reflected in better growth and body protein retention (Mora Sanchez et al. 2009; Luo et al. 2006; Peres and Oliva-Teles 2009; Furuya et al. 2004). The FCR values of the present study agree with those recorded for this species when manually fed (Figueiredo et al. 2014; Oliveira et al. 2020; Silva et al. 2019; Costa et al. 2016), confirming the possibility of using self-feeding for this species. Noble et al. (2007) found that, for rainbow trout (*Oncorhynchus mykiss*), self-feeding regimes have similar results—such as growth rate, condition factor, size heterogeneity, and FCR (1.27–1.45)—to those of imposed self-feeding regimens while minimizing feed waste, which is essential to reach a low production cost. Animals that received diets containing an P:E ratio of 23.91 g protein/MJ had a better feed conversion ratio. Such diets probably had an adequate dietary balance mainly of essential amino acids making the protein metabolism more efficient and leading to more significant growth, better consumption efficiency, and lower feed conversion. In addition, the fish feed intake and its conversion ratio generally decrease with increasing dietary lipid levels (Liu et al. 2011; Chai et al. 2013; Wang et al. 2013). This behavior agrees with that found for juveniles of Snakehead *Channa striatus* (Aliyu-Paiko et al. 2010), hybrid grouper *Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀ (Jiang et al. 2015), and Acará Severo *Heros severus* (Sousa et al. 2021).

Carcass lipid content and VSI are essential to detect possible problems affecting fish production as they reflect body lipid deposition, especially from nutritional imbalance in the diet (Sagada et al. 2017). Among the P:E ratios evaluated in the present study, animals fed diets with lower P:E ratios had higher visceral fat deposition than animals fed diets with higher P:E ratios, with the lowest VSI being estimated for diets with an P:E ratio of 22.74 g protein/MJ. Increased VSI is related to excess energy in the diet, leading to more significant visceral fat deposition. Any imbalance of non-protein energy sources and/or their levels in the diet can directly affect fish body composition (Erfanullah and Jafri 1998). The excess of energy relative to the protein content in a diet can result in high lipid deposition since fish feed to meet their energy needs (NRC 1993).

The lipid content of the substrate depends on the diet's composition and the species to which it is provided. In the present study, animals fed with an P:E ratio estimated at 20.03 g protein/MJ had lower body lipid deposition. A decrease or increase in dietary protein levels changing the P:E ratio may increase carcass lipid content. Carcass lipid increases in

Senegalese sole *Solea senegalensis* juveniles were higher when fed a high-fat, low-protein diet, suggesting metabolic disturbances when high levels of fat are combined with high levels of dietary protein (Borges et al. 2013). The increased deposition of body lipids in juveniles fed diets containing lower levels of CP is due to the high levels of carbohydrates in these diets (Sousa et al. 2021). Lower levels of protein and higher concentrations of carbohydrates in their composition lead to an excess of glucose that is not used by the tissues but instead deposited for the formation of liver and muscle glycogen or converted into fat. Body fat deposition also increased with increasing dietary protein for parrot fish *Oplegnathus fasciatus* (Kim et al. 2017), grouper *Epinephelus malabaricus* (Shiau and Lan 1996), and yellow puffer *Takifugu obscyrus* (Bai et al. 1999). Excess glucogenic amino acids can be directed to glucose synthesis and stored as glycogen (gluconeogenesis) (Sousa et al. 2021). Ketogenic amino acids, in turn, can be deposited as fat (lipogenesis) in the liver (Peres and Oliva-Teles 2009), intramuscular, subcutaneous tissue, and viscera (Mohanta et al. 2009; Signor et al. 2010). Body lipid deposition can be influenced by the diet's carbohydrate content (Papaparaskeva-Papoutsoglou and Alexis 1986). Storage of lipids from carbohydrates and/or dietary lipids in the perivisceral adipose tissue and carcass was observed for *L. alexandri*.

The estimated lowest levels of blood glucose are for animals that receive a diet with an P:E ratio of 24.17 g protein/MJ. Lundstedt et al. (2004) found similar results for juveniles of the siluriform *Pseudoplatystoma corruscans*, a carnivorous species like *L. alexandri*, for which the highest crude protein level in the diet (50%) provided the lowest plasma glucose value. Carnivorous fish can regulate blood glucose through gluconeogenesis (Hemre et al. 2002) and demonstrate a lower ability to control blood glucose concentrations than do omnivorous or herbivorous fish (Cowey et al. 1977; Hemre et al. 2002), generating prolonged postprandial hyperglycemia (Enes et al. 2009; Moon 2001). Higher blood glucose levels occur with animals fed diets with higher E:P ratios, for which carbohydrate levels are higher. Oliveira et al. (2020) observed higher blood glycemic levels in juvenile *L. alexandri* fed diets containing more elevated amounts of corn.

Blood cholesterol was higher for animals that received a diet with an P:E ratio above 22.38 g protein/MJ (estimated value). Cholesterol homeostasis is achieved by balancing cholesterol uptake, biosynthesis, and transport (Yun et al. 2011). The present study's data are similar to those recorded for juvenile black sea bream *Sparus macrocephalus* (Zhang et al. 2010), grass carp *Ctenopharyngodon idella* (Jin et al. 2015), *T. blochii* (Prabu et al. 2020), *O. niloticus* (Chen et al. 2009), and *Spinibarbus hollandi* (Yang et al. 2003). The decreased cholesterol may have been because, with increasing crude protein levels in the diet, fatty acids block the expression of acetyl-CoA carboxylase in the liver (Jin et al. 2015). Acetyl-CoA carboxylase is sensitive to nutritional status and increases its expression in carbohydrate-rich diets (Ishii et al. 2004). LDL transports cholesterol from the liver to peripheral tissues, while HDL transports cholesterol from peripheral tissues to the liver (Nelson and Cox 2002). Animals fed diets containing an P:E ratio of 17.25 g protein/MJ (estimated value) had lower LDL levels than the others. The highest indices of HDL were observed for *L. alexandri* fed diets with an P:E ratio of 22.38 g protein/MJ (estimated value). Animals fed diets containing a lower P:E ratio have lower circulating cholesterol levels and higher blood HDL content, which can be harmful in the long term (Ye et al. 2019; Wang et al. 2016).

Total circulating protein increased linearly for animals fed higher P:E ratio diets. The same was also recorded for common carp *Cyprinus carpio* (Al-Saraji and Nasir 2013), jundiá *Rhamdia quelen* (Coldebella et al. 2011), pacu *Piaractus mesopotamicus* (Almeida Bicudo et al. 2009), and hybrid surubim *Pseudoplatystoma fasciatum* x *Leiarius* *marmoratus* (Campeche et al. 2018). Albumin represents 52 to 60% of the total content of plasma protein, playing an essential role in the transport of endogenous and xenobiotic ligands through the formation of non-covalent complexes at specific binding sites (Kragh-Hansen 1990; Curry et al. 1998; Sugio et al. 1999; Bertucci and Domenici 2012). In the present study, albumin remained the same for all treatments. Triglycerides, ALT, and AST were not influenced by the P:E ratios studied here. Triglycerides are made of glycerol and long-chain fatty acids and appear in the bloodstream linked to lipoproteins (Oliveira et al. 2014). Blood triglyceride concentration is influenced mainly by the lipid content of the diet and by the transport of triglycerides among different tissues (Van Der Boon et al. 1991; Thrall 2004), which was not observed in the present work. The enzymes ALT and AST are involved in protein metabolism and may help assess animal dietary nutrient utilization (Abdel-Tawwab et al. 2010). They can signal the use of proteins for energy formation (Melo et al. 2006) and indicate liver damage when released into the bloodstream in large amounts (Sparling et al. 1998). ALT and AST observed in the present study agree with those of Seong et al. 2018, who found that different amounts of crude protein did not affect the levels of these enzymes for *P. olivaceus*.

Conclusion

Diets with protein:energy ratio close to 23.91 g protein/MJ (42.05% CP and 17.59 MJ of GE/kg of diet) provide better adaptation of juvenile *L. alexandri* to a self-feeding system, as well as better performance and blood biochemistry. Furthermore, the use of the self-feeding system is efficient in the cultivation of juveniles *L. alexandri*.

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Data availability The data supporting this study's findings are available from the author, Fabio Aremil Costa dos Santos, upon reasonable request.

Code availability Not applicable.

Declarations

Ethics approval The authors followed all applicable international, national, and/or institutional guidelines for animal welfare. The procedures of this work abide by the protocols approved by the Animal Use Ethics Committee (Comissão de Ética no Uso de Animais CEUA-UMG) (nº 208/2018).

Consent to participle All names in the author list have been involved in various stages of experimentation or writing.

Consent for publication All names on the list of authors agree with this study's publication.

Conflict of interest The authors declare no conflict of interest.

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