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Digestible protein levels and metabolic responses in juvenile piapara (*Megaleporinus obtusidens*)

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ABSTRACT

This study aimed to determine the effects of dietary digestible protein (DP) on growth and physiological indicators in piapara. Three hundred piapara juveniles (24.0 ± 1.3 g) were distributed in 20 tanks of 130 L (15 fish/tank) with continuous aeration and water. Five isoenergetic diets (14.0 MJ·kg^{-1}) were formulated to contain increasing levels of DP (21, 24, 27, 30 and 33%). After 77 days of feeding, increasing levels of digestible protein influenced final weight, specific growth rate and weight gain linearly ($p < 0.05$). Water ammonia concentration and liver alanine aminotransferase activity also showed a linear effect with an increasing DP level ($p < 0.05$), but no relationship was found between DP level and serum ammonia ($p > 0.05$). There was no linear and quadratic effect for hepatosomatic index, liver glycogen and liver lipid content ($p > 0.05$). On the other hand, the muscle lipid content decreased linearly with the increase in the DP level ($p < 0.05$), while the mesenteric fat index showed a linear and quadratic effect ($p < 0.05$) with an increasing curve until the peak of 25.77% DP. The activity of the hepatic malic enzyme also followed a quadratic pattern ($p < 0.05$) with a maximum point of 27.08% of DP. This contrasts with the hepatic enzyme glucose-6-phosphate dehydrogenase, which increased linearly with the increase in the DP level ($p < 0.05$). The results showed better productive performance for fish fed with the highest levels of DP, though greater excretion of ammonia in the water was also shown. Diets below 27% DP resulted in greater energy reserve, amino acid catabolism and lipogenesis. Therefore, the inclusion of 28 to 30% DP in the diet will be ideal for growth and physiological responses in piapara.

Keywords: Performance; Physiological indicators; Omnivorous; Energy retention.

Níveis de proteínas digestíveis e respostas metabólicas em juvenis de piapara (*Megaleporinus obtusidens*)

RESUMO

Este estudo teve como objetivo determinar os efeitos da proteína digestível (PD) da dieta sobre o crescimento e indicadores fisiológicos em piapara. Trezentos juvenis de piapara (24 ± 1,3 g) foram distribuídos em 20 tanques de 130 L (15 peixes/tanque) com aeração contínua e água. Cinco dietas isoenergéticas (14,0 MJ·kg^{-1}) foram formuladas para conter níveis crescentes de PD (21, 24, 27, 30 e 33%). Após 77 dias de alimentação, níveis crescentes de proteína digestível influenciaram o peso final, taxa de crescimento específico e ganho de peso de forma linear ($p<0,05$). A concentração de amônia na água e a atividade hepática da alanina aminotransferase também apresentaram efeito linear com o aumento do nível de PD ($p < 0,05$), mas não foi encontrada relação entre o nível de PD e amônia sérica ($p > 0,05$). Não houve efeito linear nem quadrático no índice hepatossomático, no glicogênio hepático e na concentração de lipídios hepáticos ($p > 0,05$). Por outro lado, o conteúdo de lipídio muscular diminuiu de forma linear com o aumento do nível de PD ($p < 0,05$), enquanto o índice de gordura mesentérica apresentou efeito linear e quadrático ($p < 0,05$) com curva crescente até o ponto máximo em 25,77% de PD. A atividade hepática da enzima málica também seguiu um padrão quadrático ($p < 0,05$) com ponto máximo em 27,08% de PD, diferentemente da enzima glicose-6-fosfato

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desidrogenase hepática, que aumentou linearmente com o aumento do nível de PD ($p < 0,05$). Os resultados mostraram melhor desempenho produtivo para os peixes alimentados com os maiores níveis de PD. Por outro lado, também proporcionaram maior excreção de amônia na água. Dietas abaixo de 27% PD resultaram em maior reserva energética, catabolismo de aminoácidos e lipogênese. Portanto, a inclusão de 28 a 30% PD na dieta será ideal para o crescimento e respostas fisiológicas em piaparas.

Palavras-chave: Desempenho; Indicadores fisiológicos; Onívoro; Retenção de energia.

INTRODUCTION

Protein is a fundamental macronutrient for the fish metabolism, as it performs important functions in skeletal structures, as well as enzymatic, hormonal, and immune defense functions (Tu et al., 2015) in contributing to growth, maintenance, tissue repair and animal health (Oliva-Teles et al., 2020). While dietary protein is important for fish growth and survival, it also accounts for a large proportion of total feed costs (Kabir et al., 2019). Up to the optimal level, dietary protein intake can improve fish growth (Zhang et al., 2017; Sivaramakrishnan et al., 2022); however, excess dietary protein is catabolized for energy production, increasing feed costs, and affecting water quality via nitrogen (ammonia) excretion (Ali and Rawal, 2022; Peres et al., 2022).

Protein requirements can be determined experimentally in dose-response studies using growth performance as a measure of the response to increasing nutrient levels (Radhakrishnan et al., 2020). However, nutrients play a key role in fish metabolism and physiology and as such should also be investigated (Azaza et al., 2015; Arenas et al., 2021). For example, intermediary metabolism and enzyme activity are modulated by the nutritional status of fish, while nitrogen metabolism is influenced by the protein to energy (P/E) ratio of the diet (Fang et al., 2017; Alam et al., 2020; Singha et al., 2021).

Increased dietary protein has often been associated with higher growth rates because protein provides essential amino acids for protein synthesis (Hassan et al., 2022). Thus, in fish fed lower levels of protein, the mobilization of body protein must be higher to meet nutrient demands for protein synthesis, maintenance, and metabolism, while excessive protein in the diet is catabolized or deaminated and excreted (Yan et al., 2019; Sivaramakrishnan et al., 2022). Many studies have shown that increasing the dietary protein content to a certain level results in optimal growth, and that upon reaching a plateau any further increase in protein level leads to reduced growth (Ye et al., 2017; Jayant et al., 2018; Ahmed and Ahmad, 2020; Cai et al., 2020; Prabu et al., 2020; Karapanagiotidis et al., 2022), but this did not occur in the present study.

The piapara (*Megaleporinus obtusidens*) formerly *Leporinus obtusidens*, (Characiformes: Anostomidae) (Ramirez et al., 2017), is a freshwater fish with omnivorous habits and characterized by a subterminal mouth associated with preferential feeding on the bottom (Balassa et al., 2004; Ramirez et al., 2017), as well as other Anostomidae genera characterized by subterminal or lower mouths that exhibit downward-facing behavior related to bottom feeding, for example, chimboré (*Schizodon nasutus*), piava (*Leporellus vittatus*), piau (*Leporinus friderici*) and piauçu (*Leporinus macrocephalus*) (Teresa et al., 2014; Machado-Evangelista et al., 2015).

The target species of the study is one of the largest of the family, reaching up to 60 cm in length and more than 1 kg of live weight in the first year (Reynalte-Tataje and Zaniboni-Filho, 2010). Fish of the Anostomidae family of economic importance (piau, piauçu, piava, and piapara) produced the total of 2,806 kg (IBGE, 2021). In addition, piapara presents aggressive behavior when caught on a hook and is highly appreciated for sport fishing (Moro et al., 2013).

Despite its high production potential and commercialization, there is a lack of development of nutritionally balanced feeds that meet piapara's nutritional requirements for ideal growth, which is essential for its large-scale cultivation. In species similar to piapara that show the same eating habits and environmental characteristics, some studies have already evaluated the ideal dietary protein levels for different stages of development. For example, Pinto et al. (2017) report that, for piauçu juveniles with the initial weight of 22.23 g, the ideal crude protein (CP) for growth should be 380 $g \cdot kg^{-1}$ with a digestible energy ratio of 2,300 $kcal \cdot kg^{-1}$. On the other hand, for fingerlings of 0.40 g, Bittencourt et al. (2010) found better zootechnical results with 35% CP. Meanwhile, Feiden et al. (2009) found better results with diets containing 34 and 38% CP for fingerlings of 0.62 g.

Therefore, this study aimed to determine the effects of digestible protein on growth and physiological indicators of piapara.

MATERIALS AND METHODS

This study was performed in accordance with the National Council for the Control of Animal Experimentation, and it was approved by the Ethics Commission on the Use of Animals, from the Universidade Estadual Paulista "Júlio de Mesquita Filho" (Unesp), College of Agricultural and Technological Sciences, Dracena (SP), Brazil (Protocol No. 23/2018.R2).

Experimental diets

Isoenergetic diets with 14 MJ·kg^{-1} of digestible energy were formulated to contain five levels of digestible protein (DP) (21, 24, 27, 30 and 33% DP), with fish meal and soybean meal as the main sources of protein. The diet formulation was based on the apparent digestibility coefficients (ADC) of the ingredients (Tanaka et al., 2021), which were used to estimate the DP and energy of each ingredient used in the experimental diets (Table 1).

All ingredients were obtained from local commercial suppliers. To prepare the diets, the ingredients were ground to pass a 0.8-mm sieve, mixed, and extruded. The pellets were dried in an air circulation oven at 40°C, cooled at room temperature, put into separate plastic bags, and kept in a cold chamber at -12°C until use.

The chemical composition of the ingredients and experimental diets were determined according to the Association of Official Analytical Chemists' methodologies (AOAC, 2005). Dry matter content was determined in an oven at 105°C for 12 h (method 930.15), mineral matter by incineration in a muffle furnace at 550°C (method 942.05) and ether extract through extraction with petroleum ether in a Soxhlet extractor (method 920.39). The crude energy analyses were performed by combustion of the samples in a calorimetric pump (Compact combustion calorimeter – C-200, IKA, Staufen, Germany).

Fish and experimental conditions

Three hundred piapara juveniles (initial weight: 24 ± 1.3 g) were randomly distributed in 20 130-L tanks with the capacity of 15 fish/tank in a continuous water flow system. The fishponds were provided with continuous aeration and artesian well water at a renewal rate of approximately 3.5 times per day. The photoperiod was maintained at 12 hours of light and 12 hours of dark with fluorescent lamps.

The fish were fed twice daily (10 a.m. and 4 p.m.) until apparent satiety for 77 days. Every seven days, the water parameters (temperature, dissolved oxygen concentration, electrical conductivity, and pH) in the tanks were monitored and maintained within the acceptable range for the species: temperature, 26.4 ± 0.1°C; pH, 8 ± 0; dissolved oxygen, 7.4 ± 0.2 mg·L^{-1}; and electrical conductivity, 216 ± 1.4 µS.

At the end of the experimental period and 12 h after the last meal, the fish were anesthetized (eugenol, 0.2 g·L^{-1}) and weighed individually. Biometric data were recorded to determine the following parameters of productive performance: final weight, specific growth rate, feed intake, weight gain and apparent feed conversion ratio, as follows (Eqs. 1 to 5):

Table 1. Composition and proximate analysis of the experimental diets.

Ingredient (%)	Digestible protein %				
	21	24	27	30	33
Soybean meal[1]	34.00	36.00	38.00	40.00	42.00
Fish meal[2]	11.50	15.70	19.90	24.10	28.30
Corn[1]	17.00	16.50	16.00	15.50	15.00
Maize starch[3]	30.00	25.50	21.00	16.50	12.00
Soybean oil[4]	0.80	0.70	0.60	0.50	0.40
Dicalcium phosphate	2.30	1.90	1.40	1.00	0.60
Vitamin and mineral supplement[5]	0.40	0.40	0.40	0.40	0.40
Microfine cellulose[6]	1.20	1.10	0.90	0.80	0.60
Vitamin C[7]	0.08	0.08	0.08	0.08	0.08
Calcitic limestone	1.70	1.30	1.00	0.60	0.30
Kaolin	1.00	0.80	0.70	0.50	0.30
Antioxidant[8]	0.02	0.02	0.02	0.02	0.02
Analyzed composition (%)					
Dry matter	92.46	92.03	94.08	92.66	92.63
Digestible protein[9]	21.06	24.01	27.02	30.02	33.03
Crude protein	24.79	28.57	30.69	34.13	37.48
Crude lipid	6.52	3.34	3.04	3.30	7.55
Crude fiber	1.60	1.58	1.99	2.55	2.52
Mineral material	10.62	12.37	12.9	13.12	14.20
NFE[10]	52.72	50.73	49.13	43.69	35.33
Gross energy, MJ·kg^{-1}	16.26	16.44	16.71	17.13	17.29
Digestible energy, MJ·kg^{-1}	13.97	13.98	13.99	14.00	14.40
Energy/Protein ratio, MJ·g^{-1}	15.90	13.89	12.36	11.13	10.12

[1]Granol Indústria, Comércio e Exportação S.A., Bebedouro (SP), Brazil; [2]Indústria Brasileira do Peixe LTDA, Buritama (SP), Brazil; [3]Raguife Indústria e Comércio de Rações LTDA, Santa Fé do Sul (SP), Brazil; [4]Moinho Globo Alimentos S.A., Sertanópolis (PR), Brazil; [5]Premix AcquaMeal Fishes – Mcassab Animal Nutrition, Valinhos (SP), Brazil, composition/kg of product: choline, 100 g; vitamin A, 1,750,000 IU; vitamin D3, 375,000 IU; vitamin E, 20,000 IU; vitamin K3, 500 mg; vitamin B1, 2,000 mg; vitamin B2, 2,500 mg; vitamin B6, 2,500 mg; vitamin B12, 5.0 mg; niacin, 8,750 mg; pantothenic acid, 7,500 mg; folic acid, 625 mg; biotin, 50 mg; vitamin C, 37.5 g; inositol, 12.5 g; iron, 15.0 g; copper, 1,250 mg; manganese, 3,750 mg; zinc, 17.5 g; cobalt, 50 mg; iodine, 100 mg; selenium, 75 mg; [6]Microfine cellulose. Rhoster Indústria and Comércio LTDA, Vargem Grande Paulista (SP), Brazil; [7]Vitamin C Rovimix Stay-35, DSM Nutritional Product, Switzerland; [8]Etoxiquim. 66,6, MCassab Animal Nutrition, São Paulo (SP), Brazil; [9]calculated from the digestibility coefficients for the piapara (Tanaka et al., 2021) and the crude protein values determined for the ingredients; [10]nitrogen-free extract (NFE) = dry matter − (crude protein + lipid + mineral matter + crude fibre).

$$\text{Final weight} = \text{final body weight (g)} \quad (1)$$

$$\text{Specific growth rate} = (\textit{ln}\ \text{final weight} - \textit{ln}\ \text{initial weight}) \times 100/\text{days} \quad (2)$$

$$\text{Weight gain} = \text{final body weight (g)} - \text{initial body weight (g)} \quad (3)$$

$$\text{Feed intake} = \text{feed intake per fish (g)/days} \quad (4)$$

$$\text{Feed conversion ratio} = \text{feed intake (g)/weight gain (g)} \quad (5)$$

Ammonia in water, serum ammonia, and amino acid metabolism

Three fish from each tank were anesthetized (eugenol, 0.2 $g \cdot L^{-1}$), and blood samples were collected by caudal puncture with heparinized syringes and centrifuged at 827 x *g* for 10 min at 10°C (Allegra x- 30R Centrifuge, Beckman Coulter, Brea, California, United States of America). Serum ammonia levels were determined by the sodium salicylate indophenol formation method as described by Verdouw et al. (1978). Briefly, 20 µL of trichloroacetic acid (15%) was added to a 60-µL aliquot of serum. Next, sodium nitroprusside (0.01 mM), sodium hypochlorite (0.32%), sodium hydroxide (64.5 mM), sodium citrate (87.1 mM), and sodium salicylate (161.2 mM) were added to the supernatant. After homogenization, the sample was incubated for two hours in the dark followed by reading on a spectrophotometer at 540 nm (Evolution 60S UV-Visible Spectrophotometer, Thermo Fisher Scientific Inc., Madison, Wisconsin, United States of America). Ammonium chloride was used as the standard.

The concentration of total ammonia nitrogen (NH_3–N) in water was determined weekly throughout the study period. To adjust for the effect of water renewal, 15 minutes after the first meal of the day, water flow was interrupted two hours before sample collection. NH_3–N was determined by the indophenol blue reaction method (Koroleff, 1976), which is based on the quantification of total ammonia nitrogen with reading on a spectrophotometer at 630 nm (Evolution 60S UV-Visible Spectrophotometer, Thermo Fisher Scientific Inc., Madison, Wisconsin, USA).

Tissue energy reserves

The fish used for blood sampling (three fish/tank) were euthanized, and laparotomy was performed to remove and weigh the visceral fat and liver. The mesenteric fat index (MFI) and hepatosomatic index (HSI) were calculated using the Eq. 6:

$$\text{MFI or HSI} = 100 \times (\text{tissue weight/live weight}) \quad (6)$$

The total liver and muscle lipid content was determined gravimetrically by solvent extraction (Bligh and Dyer, 1959), and the liver glycogen content was determined by the colorimetric enzymatic method (Perry et al., 1988).

Liver enzymes activity

Liver samples (three fish/tank) were homogenized (1:4 dilution) in Tris buffer [100 mM Tris-HCl, 0.1 mM EDTA, and 0.1% (v/v) Triton X-100 at pH 7.8] and centrifuged (30 min, 28,000 x *g* at 4°C) in a refrigerated centrifuge (Allegra x- 30R Centrifuge, Beckman Coulter, Brea, California, United States of America). From the supernatant, the activity of alanine aminotransferase (ALT; EC 2.6.1.2) was determined by the enzymatic-colorimetric method using a commercial kit (ALT - PP, Gold Analisa Diagnóstica LTDA, Belo Horizonte, MG, Brasil), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) activity was determined according to Bautista et al. (1988), and malic enzyme (ME) activity was determined by the method of Chakrabarty and Leveille (1969). All enzyme activities were measured in a spectrophotometer (Evolution 60S UV-Visible Spectrophotometer, Thermo Fisher Scientific Inc., Madison, Wisconsin, United States of America).

Enzyme activities are expressed in milliunits per milligram of protein (specific activity). An enzyme unit was defined as the amount of enzyme required to transform 1 µmol of substrate per minute at assay temperature (25°C). The protein concentration was determined by the method described by Cain and Skilleter (1987) using a biuret reagent and by performing a spectrophotometer reading with bovine serum albumin as the standard.

Statistical analysis

The data were expressed as the mean and standard error of the pooled mean. Initially, data normality and variance homogeneity were tested using the Shapiro-wilk test and the Levene test, respectively. Subsequently, regression models commonly used in dose-response fish nutrition studies were applied, such as a broken straight-line model and a first- and second-order polynomial model (quadratic model) to estimate the optimal level of protein according to the methodology of Lee et al. (2021). All analyses were performed using the R.1 statistical software package (RStudio 4.2.1; R Development Core Team, 2013).

RESULTS

Growth performance

There was no mortality of piapara during the feeding experiment. There were differences in the linear regression in

the final weight, specific growth rate, weight gain, and feed conversion ($p < 0.05$) of piapara fed with increasing levels of DP. There was no difference in feed intake ($p > 0.05$) (Table 2).

The regression graphs show increasing linear patterns, and it is possible to observe a decreasing linear pattern for feed conversion as the levels of DP in the diets increase (Fig. 1).

Ammonia in water, serum ammonia, and amino acid metabolism

The total ammonia concentration in water and the hepatic alanine aminotransferase enzyme showed differences for linear regression ($p < 0.05$) (Table 3). However, no effect was found between PD level and serum ammonia concentration ($p > 0.05$). The regression graphs show increasing linear patterns (Fig. 2).

Tissue energy reserves and liver activity of lipogenic enzymes

Liver glucose-6-phosfate-dehydrogenase activity increased linearly with the increase in DP level, whereas the mesenteric fat index and liver malic enzyme activity followed a quadratic pattern. Conversely, there was no relationship between DP level and the hepatosomatic index, liver glycogen or liver lipid content (all, $p > 0.05$) (Table 4).

Table 2. Growth performance of piapara fed increasing levels of digestible protein for 77 days[#].

Variable	Digestible protein (%)					SEM	p-value*	
	21	24	27	30	33		Linear	Quadratic
Final weight (g)	43.4	46.2	47.3	48.0	47.4	0.61	0.02	0.08
Specific growth rate (%)	0.8	0.8	0.8	0.8	0.9	0.02	0.01	0.72
Feed intake (g/day)	30.3	31.4	31.7	33.3	31.0	0.62	0.50	0.32
Weight gain (g)	19.9	21.9	22.5	23.5	23.9	0.53	0.001	0.43
Feed conversion	1.5	1.4	1.4	1.4	1.2	0.03	0.001	0.89

[#]Values are presented as mean (n = 4) and SEM, pooled standard error of means; SEM: standard error of means; *the polynomial regression generated the following equations: Final weight = 43.581 + 0.979PD, $R^2 = 0.717$; Final weight = 39.981 + 4.064PD – 0.514PD^2, $R^2 = 0.995$; Specific growth rate = 0.771 + 0.023PD, $R^2 = 0.615$; Weight gain = 13.78 + 0.318PD, $R^2 = 0.929$; Feed conversion = 1.55 – 0.046PD, $R^2 = 0.774$.

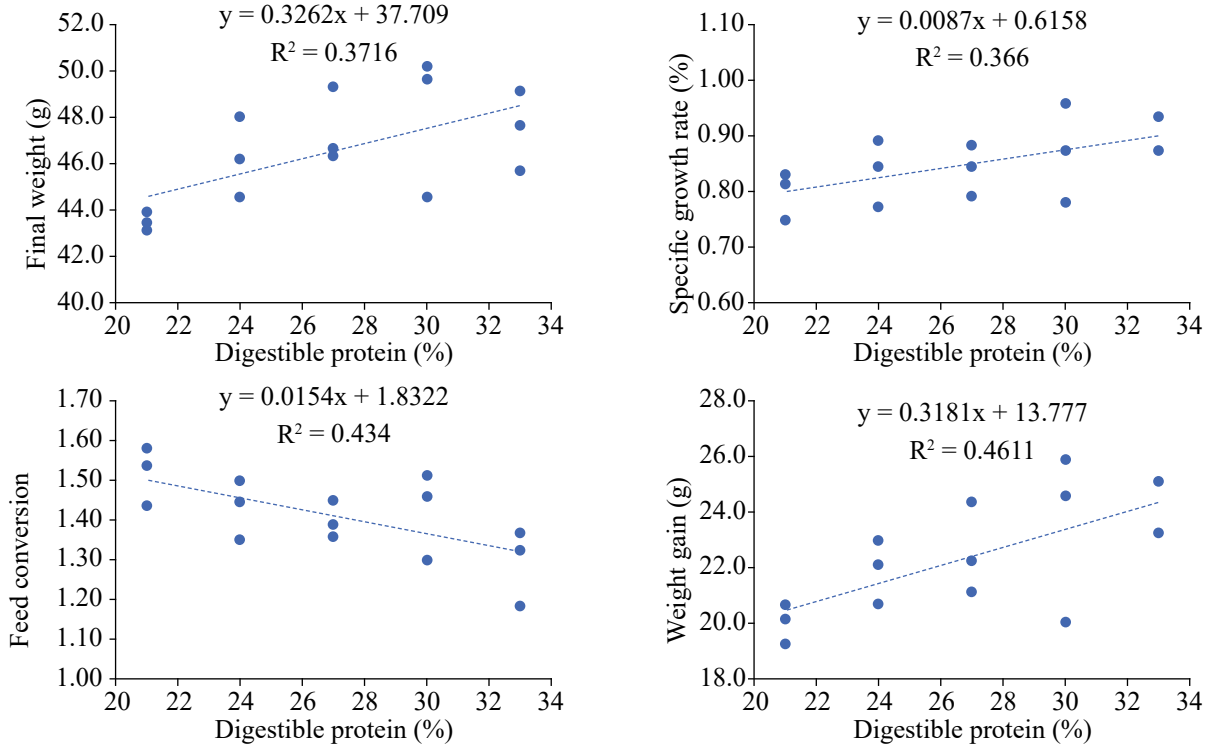

Figure 1. Linear regression analysis for the productive performance of piapara as a function of digestible protein levels (21, 24, 27, 30 and 33%). Each point represents the average of three replicates per treatment.

Table 3. Ammonia in water, serum ammonia, and liver activity of amino acid metabolism enzymes in piapara fed increasing levels of digestible protein for 77 days[#].

Variable	Digestible protein (%)					SEM	p-value*	
	21	24	27	30	33		Linear	Quadratic
Water ammonia (mg·L^{-1})	8.6	11.2	10.9	12.3	13.0	0.47	0.00	0.41
Serum ammonia (µg·µL^{-1})	0.7	0.6	0.7	0.6	0.7	0.02	0.70	0.36
Alanine aminotransferase (mU·mg protein^{-1})	379.3	381.3	392.7	388.1	546.5	0.25	0.01	0.05

[#]Values are presented as mean (n = 4) and SEM, pooled standard error of means; SEM: standard error of means; *the polynomial regression generated the following equations: Water ammonia = 8.241 + 0.997PD, R^2 = 0.871; Alanine aminotransferase = 315.22 + 34.12PD, R^2 = 0.557; Alanine aminotransferase = 463.62 – 93.08PD + 21.20PD^2, R^2 = 0.858.

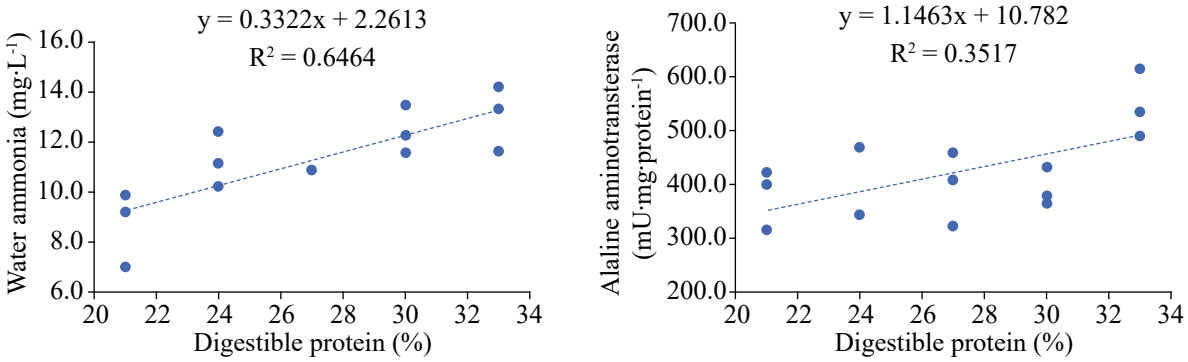

Figure 2. Linear regression analysis for water ammonia and liver alanine aminotransferase of piapara as a function of digestible protein levels (21, 24, 27, 30 and 33%). Each point represents the average of three replicates per treatment.

Table 4. Tissue energy reserves and liver activity of lipogenic enzymes in piapara fed increasing levels of digestible protein for 77 days[#].

Variable	Digestible protein (%)					SEM	p-value*	
	21	24	27	30	33		Linear	Quadratic
Hepatosomatic index (%)	0.8	0.8	0.8	0.8	0.9	0.01	0.17	0.48
Mesenteric fat index (%)	0.9	1.0	1.3	0.8	0.6	0.04	0.01	0.001
Liver glycogen (g·100 g^{-1})	5.9	5.3	5.5	5.8	5.3	0.16	0.53	0.80
Liver lipid (g·100 g^{-1})	4.6	3.6	3.6	3.7	3.6	0.17	0.14	0.28
Muscle lipid (g·100 g^{-1})	1.1	1.5	0.8	0.7	0.7	0.06	0.001	0.79
Glucose-6-phosphate dehydrogenase (mU·mg of protein^{-1})	121.6	142.8	177.4	183.4	206.2	0.02	0.001	0.72
Malic enzyme (mU·mg of protein^{-1})	168.66	71.33	107.8	132.9	141.7	6.02	0.91	0.01

[#]Values are presented as mean (n = 4) and SEM, pooled standard error of means; SEM: standard error of means; *the polynomial regression generated the following equations: Mesenteric fat index = 1.160 – 0.080PD, R^2 = 0.238; Mesenteric fat index = 0.460 + 0.520PD – 0.100PD^2, R^2 = 0.761; Muscle lipid = 1.440 – 0.160PD, R^2 = 0.542; Glucose 6-phosphate dehydrogenase = 103.260 + 21.020PD, R^2 = 0.966; Malic enzyme = 231.03 – 101.02PD + 19.96PD^2, R^2 = 0.722.

In addition, the muscle lipid content decreased linearly with the increase in dietary protein level.

The regression graph for muscle lipid showed a decreasing linear pattern as the levels of DP increased, which was similar to the mesenteric fat index, which also showed the same linear pattern. However, the second-order polynomial regression for mesenteric fat showed an upward trend with a maximum point of 25.77% and subsequent decline. The malic enzyme showed a downward trend with a maximum point of 27.08% and subsequently increased. As for glucose-6-phosphate dehydrogenase, there was an increasing linear pattern (see Fig. 3).

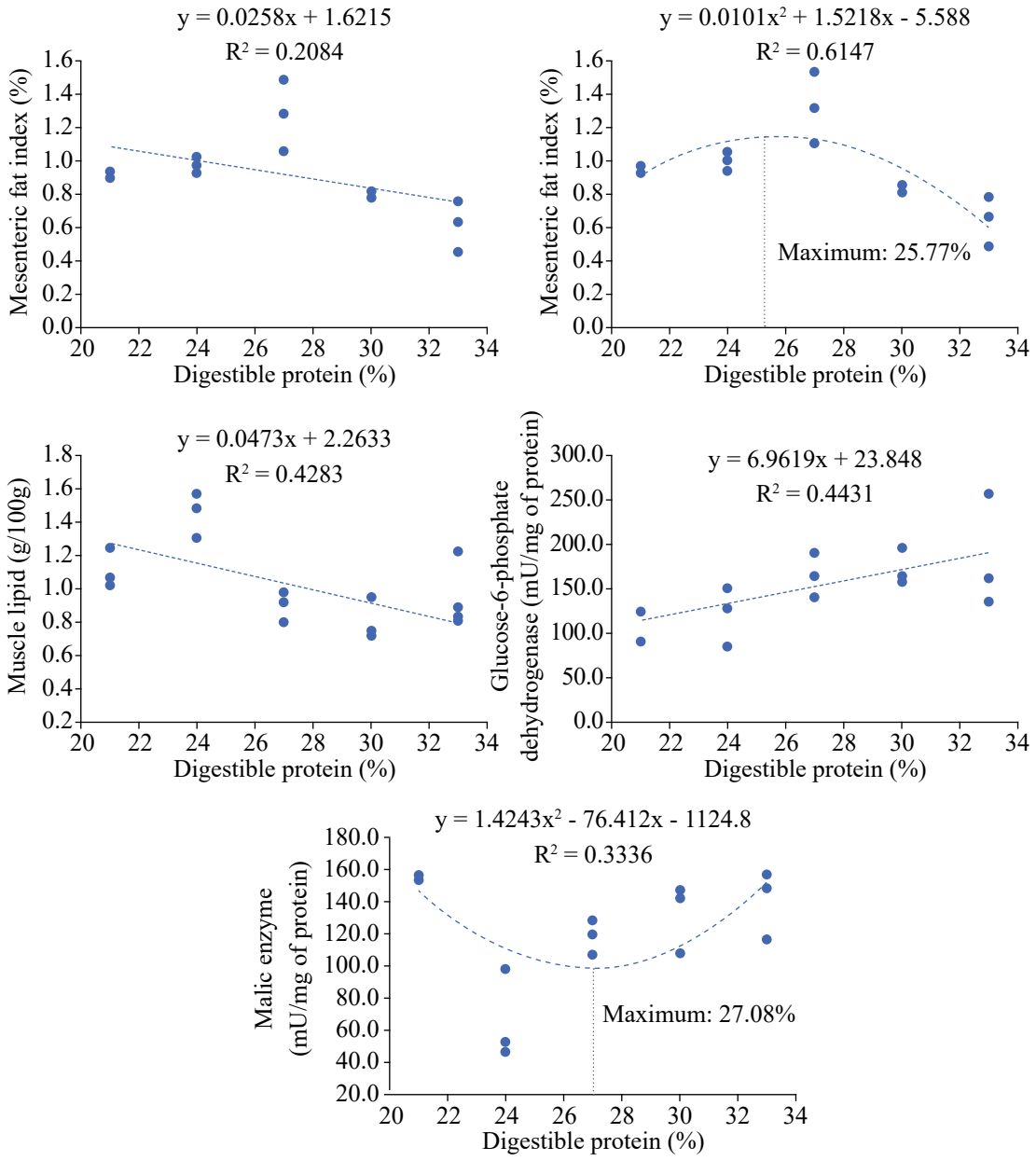

Figure 3. Regression analysis linear and second-order polynomial for tissue energy reserves and liver activity of lipogenic enzymes of piapara as a function of digestible protein levels (21, 24, 27, 30 and 33%). Each point represents the average of three replicates per treatment, and the blue line represents the maximum point.

DISCUSSION

Generally, protein is the first nutrient considered when determining the nutritional requirements of fish species (Oliva-Teles et al., 2020; Cho et al., 2021; Qian et al., 2022). There are several species exploited in aquaculture that have different eating habits, live in different habitats and temperatures, and may grow slower or faster (NRC, 2011), directly reflecting different nutritional requirements. In this study, for the first time, we investigated the effect of DP levels on the productive performance and physiological indicators of piapara. The data showed that protein levels positively influenced the productive performance of fish; however, we noticed the slow growth of piapara in 77 days.

Ammonia is the main nitrogenous residue of amino acid deamination in fish (Carvalho et al., 2017), i.e., the higher the dietary protein level, the greater the excretion of ammonia and phosphate, which is excreted by the gills through passive

diffusion along a partial gas gradient between fish blood and water (Wright, 2021). However, several studies report that, when dietary protein is provided up to the ideal level, the excretion of nitrogenous waste is low (Li et al., 2016; Yigit et al., 2018; Bhatnagar and Raparia, 2020; Ali and Rawal, 2022), resulting in increased protein synthesis. Thus, the increase in ammonia in the water observed in the present study could indicate protein catabolism, which would be consistent with the increase in the enzyme alanine aminotransferase. However, the linear performance suggests that the growing fish had more protein metabolism when they ingested more protein in the diet (Gao et al., 2019).

In the present study, fish fed diets containing low protein content consumed large amounts of carbohydrates (see Table 1), which could have increased fatty acid synthase activity, promoting the conversion of sugar into lipids that are transported and stored in tissues (Yang et al., 2017; Chen et al., 2021). Most of this fat was deposited in the abdominal cavity, resulting in lower carcass yield and higher amounts of processing waste (Ribeiro et al., 2016), which is not desirable in the industry. In fact, tissue energy reserves (linear muscle lipid and mesenteric fat index with a maximum of 25.77%) were increased for fish that received diets with lower levels of DP and higher levels of carbohydrates, but lipid stores were reduced with the increase in protein, confirming that carbohydrates were used to meet energy demands. On the other hand, an increase in the activity of glucose-6-phosphate dehydrogenase and malic enzyme was observed with the increasing levels of DP; these are responsible for supplying reducing energy in the form of NADPH, which is necessary for lipogenesis (Coutinho et al., 2016). These results may be related to the lipid in the diets, despite the formulations being isoenergetic, as when protein levels increased, the non-protein energy source used to meet isoenergetic status was lipid, which suggests that piapara efficiently uses carbohydrates as a non-protein energy source.

CONCLUSION

Diets containing increasing levels of DP resulted in greater productive performance for piapara, but also led to the increased excretion of ammonia in the water. Diets below 27% DP resulted in higher energy reserves, amino acid catabolism and lipogenesis. Therefore, the inclusion of 28 to 30% DP in the diet will be ideal for growth and physiological responses in piapara.

CONFLICT OF INTERESTS

The authors certify that they do not have any potential sources of conflict of interest, such as any interest or relationship, financial or non-financial interest that might influence the author's objectivity.

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