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Antinutritional effect of lectin from faveira (*Parkia platycephala*) seeds in tambatinga (*Colossoma macropomum x Piaractus brachypomus*)

Rafael Carvalho da Silva[1]
Claudener Souza Teixeira[2]
Alexandra Pretto[3]
Thaisa Sales Costa[4]
Jefferson Costa de Siqueira[4]
Bruna Tássia Santos Pantoja[4]
Bernardo Baldisserotto[5]
Jane Mello Lopes[4]*

[1]Universidade Federal Rural do Rio de Janeiro, Programa de Pós-Graduação em Zootecnia – Seropédica (RJ), Brazil.

[2]Universidade Federal do Cariri, Centro de Ciências Agrárias e da Biodiversidade – Crato (CE), Brazil.

[3]Universidade Federal do Pampa, Curso Superior de Tecnologia em Aquicultura – Uruguaiana (RS), Brazil.

[4]Universidade Federal do Maranhão, Centro de Ciências de Chapadinha – Chapadinha (MA), Brazil.

[5]Universidade Federal de Santa Maria, Departamento de Fisiologia e Farmacologia – Santa Maria (RS), Brazil.

*Corresponding author: Jane Mello Lopes, BR 222, km 4, s/n, CEP: 65500-000 – Chapadinha (MA), Brazil. E-mail: janemellolopes@hotmail.com

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ABSTRACT

This study investigated the possible antinutritional effects of *Parkia platycephala* lectin (0, 20, 40, or 60 mg kg^{-1} of diet) on tambatinga feeding for 60 days as well as methods of inactivating this protein. Weight gain, specific growth rate, and relative weight gain decreased, and the feed conversion rate increased with the increase in dietary lectin. The hepatic glycogen levels of fish fed 60 mg kg^{-1} were higher than those of fish fed 20 and 40 mg kg^{-1}. Diets containing 40 and 60 mg kg^{-1} increased muscle glucose levels compared to the control group. Fish-fed diets containing lectin showed reduced muscle glycogen compared to those receiving the control diet. Fish fed 60 mg kg^{-1} presented lower muscle protein levels than those fed 20 mg kg^{-1}. *In vitro* tests showed that the hemagglutination activity of lectin was inhibited by D-mannose, D-glucose, and α-methyl-D-mannopyranoside. Thermal treatment at 50–60°C was sufficient to reduce the action of lectin, as well as a pH below and above the 6–7 range. Therefore, the use of *P. platycephala* meals as a dietary ingredient for tambatinga with no lectin inactivation is not recommended as it can negatively affect the fish's biochemical parameters and growth. Acid or alkaline solutions can be an alternative for inactivating the protein and improving its use by fish and other animals.

Keywords: alternative feeds; amazon fish; fava-de-bolota; growth performance; lectins.

Efeito antinutricional da lectina de sementes de faveira (*Parkia platycephala*) em tambatinga (*Colossoma macropomum x Piaractus brachypomus*)

RESUMO

Este estudo investigou possíveis efeitos antinutricionais da lectina de *Parkia platycephala* (0, 20, 40 ou 60 mg kg^{-1} de dieta) na alimentação de tambatinga por 60 dias, bem como métodos de inativação dessa proteína. O ganho de peso, a taxa de crescimento específico e o ganho de peso relativo diminuíram, enquanto o índice de conversão alimentar aumentou com o incremento de lectina na dieta. Os níveis de glicogênio hepático dos peixes alimentados com 60 mg kg^{-1} foram superiores aos dos que ingeriram 20 e 40 mg kg^{-1}. Dietas contendo 40 e 60 mg kg^{-1} aumentaram os níveis de glicose muscular em comparação com os que receberam a dieta controle. Nos animais cuja dieta continha lectina, reduziu-se o glicogênio muscular em comparação com os da dieta controle. Os espécimes alimentados com 60 mg kg^{-1} apresentaram menor nível de proteína muscular do que aqueles que consumiram 20 mg kg^{-1}. Testes *in vitro* mostraram que a atividade de hemaglutinação da lectina foi inibida por D-manose, D-glicose e α-metil-D-manopiranosídeo. O tratamento térmico entre 50 e 60°C foi suficiente para reduzir a ação da lectina, assim como o pH abaixo de 6 e acima de 7. Portanto, usar farelo de *P. platycephala* como ingrediente em dieta para tambatinga sem inativação da lectina não é recomendado, pois pode afetar negativamente os parâmetros bioquímicos e o crescimento dos exemplares. Soluções ácidas ou alcalinas podem ser uma alternativa para inativar a proteína e melhorar sua utilização em peixes e outros animais.

Palavras-chave: alimentos alternativos; peixe amazônico; fava-de-bolota; desempenho de crescimento; lectinas.

INTRODUCTION

Tambatinga (*Colossoma macropomum* × *Piaractus brachypomus*) is a hybrid fish of economic importance in the northern and northeastern regions of Brazil (Ribeiro et al., 2019). The demand for cultured finfish has increased tremendously during the past decades, but the success of intensive farming is dependent on several factors, such as management, dietary supplementation, and animal welfare. Tambatinga is mainly

raised in intensive systems (Hashimoto et al., 2011), where feed represents the main production cost (Welengane et al., 2019). Therefore, feed costs can be characterized as major obstacles to the production of this species.

Alternative feeds from North Brazil have been investigated and used instead of conventional feeds for developing nutritional or economic strategies (Araújo et al., 2019). In this context, *Parkia platycephala* Benth (fava-de-bolota) has attracted attention for its nutritional potential and ease of acquisition (Alves et al., 2007). This tree is commonly found in the Caatinga biome, and its fruit is valuable fodder for livestock in the semi-arid region (Araújo et al., 2019). In addition, research has demonstrated that *P. platycephala* can be used in feeding nonruminants as it has a high content of nonfibrous carbohydrates, which makes it a viable alternative to the energy ingredients commonly used in aquaculture, such as corn, consequently reducing feed costs (Araújo et al., 2019). More recently, *Parkia biglobosa* seeds were also used in the diet of *Clarias gariepinus* (Abafi et al., 2019; Abdul-Qadir et al., 2020; Michael and Mathias, 2020). However, an important issue in their use is the presence of antinutritional factors, which, when consumed in excess or inappropriately, can affect fish performance and health (Von Danwitz and Schulz, 2020).

P. platycephala seeds contain some substances with antinutritional properties, of which we highlight a group of proteins called lectins (Alves et al., 2007; Silva et al., 2019). Lectins are proteins found in greater proportion in legumes. They can present at least one noncatalytic domain that selectively recognizes and reversibly binds specific sugars or glycans present in glycoproteins (Santos et al., 2019). The ability to recognize carbohydrates allows these proteins to agglutinate erythrocytes and interact with the carbohydrate residues present on the surface of the intestinal mucosa, inflicting damage on the surface of the small intestine (Martins et al., 2017; Popova and Mihaylova, 2019). However, the use of certain chemical and physical procedures can minimize the action of these proteins, such as exposure to high temperatures, a wide pH range, and the use of carbohydrates (Santos et al., 2019).

Recently, *P. platycephala* lectin (PPL) has attracted attention by combating multidrug-resistant bacterial strains and preventing the development of the larvae of *Haemonchus contortus*, a gastrointestinal parasite in ruminants (Silva et al., 2019), showing that this protein is possibly capable of interacting with cells of living organisms and altering them. The PPL is a glucose/mannose-binding protein formed by three domains of the β prism organized in tandem, each having a different carbohydrate recognition domain (CRD) (Del Sol et al., 2005). This lectin is classified as a jacalin-related lectin (JRL), a specific subgroup of proteins commonly associated with plant defense mechanisms against phytopathogens (Xiang et al., 2011).

It is believed that the three domains of carbohydrate recognition possibly enable PPL to bind to remnants of monosaccharides present in the intestinal mucosa and consequently interfere in the absorption of nutrients (Popova and Mihaylova, 2019). In this study, we report, for the first time, the possible antinutritional effect of lectin from *P. platycephala* seeds and point out different strategies to minimize its deleterious effects. Thus, it was hypothesized that the addition of PPL in tambatinga diets can affect their productive performance. The aim of this study was to investigate the antinutritional effects of the dietary addition of PPL on growth and biochemical parameters in tambatinga and to evaluate different methods of inactivating them, indicating possible strategies to decrease the antinutritional effect and favor greater use of this food source by animals.

MATERIAL AND METHODS

Test animals and culture conditions

The experiment was conducted at the Universidade Federal do Maranhão (UFMA), Maranhão State (MA), Brazil. All applicable institutional guidelines for the care and use of animals were followed. A total of 160 fish (2.62 ± 0.44 g and 5.44 ± 0.37 cm) were divided into twenty 150-L tanks (8 fish per tank) in a recirculation aquaculture system, fed a control diet (0 mg PPL kg^{-1}; Table 1), and acclimated to laboratory conditions for 10 days.

Protein purification

Seeds from *P. platycephala* were collected from plants located at Chapadinha, MS, Brazil. The seeds of *P. platycephala* were ground to a fine powder in a coffee mill, and the soluble proteins were extracted at 25°C by continuous stirring with 150 mM NaCl [1:10 (w:v)] for 4 h, followed by centrifugation at 10,000 × *g* at 4°C for 20 min. Protein purification was carried out by the affinity chromatography protocol, as previously described by Cavada et al. (1997), using a Sephadex®-G75 column (Sigma, St. Louis, MO, USA) (2 × 10 cm).

Diets and experimental design

Four diets were formulated based on the protocols previously described by Oishi et al. (2010; Table 1). All ingredients were finely ground, weighed, and mixed until a homogeneous mixture was obtained. Different levels of PPL (0 — control, 20, 40, or 60 mg kg^{-1} of diet) were then added, along with powdered ingredients and water. The mixtures were pelleted in a meat grinder and dried in a forced air circulation oven for 24 h at 40°C. Finally, the pellets were crumbled, sieved, and stored in a freezer at -4°C until use. The fish were fed the experimental diets until apparent satiation three times a day (9 a.m., 1 p.m., and 5 p.m.) for 60 days. Tanks were cleaned 30 min after feeding via siphoning to remove any remaining waste (feed and feces). The experimental design was completely randomized, with four groups and five repetitions each.

Water sampling and analyses

The water parameters were checked daily (dissolved oxygen, temperature, and pH) or weekly (alkalinity, hardness, nitrite, and ammonia). Dissolved oxygen and temperature were measured with a Y5512 oxygen meter (YSI Inc., Yellow Springs, OH, USA), and pH was verified with a DMPH-2 pH meter (Digimed, São Paulo, SP, Brazil). Total ammonia nitrogen (TAN) and nonionized ammonia (NH_3) were measured with a colorimetric kit (Alfakit, Florianópolis, Brazil). Throughout the experiment, the temperature was maintained at 26.3 ± 0.37°C, pH at 7.10 ± 0.06, and dissolved oxygen at 7.2 ± 0.03 mg L^{-1}. Total ammonia (0.3 ± 0.04 mg L^{-1}) and nonionized ammonia levels (0.08 ± 0.02 mg L^{-1}) were kept in the desired ranges.

Table 1. Composition and proximate analysis of the experimental diets containing *Parkia platycephala* lectin (PPL).

Ingredient (g kg^{-1})	Diet (PPL mg kg^{-1})			
	0	20	40	60
Soybean meal	300	300	300	300
Meat and bone meal	350	350	350	350
Rice bran	120	120	120	120
Ground corn	150	150	150	150
Canola oil	30	30	30	30
Common salt	10	10	10	10
Vitamin and mineral premix[a]	30	30	30	30
Dicalcium phosphate	10	10	10	10
PPL[b]	0.00	0.02	0.04	0.06
Proximate composition (%)				
Dry matter[c]	95.14	94.61	94.75	94.84
Crude protein[c]	35.60	33.83	34.26	35.54
Fat[c]	8.17	7.99	7.77	7.96
Mineral matter[c]	22.62	23.07	23.97	22.71
Neutral detergent fiber[c]	33.34	35.70	33.83	33.62
Digestible energy[d] (kcal kg^{-1})	3,278.25	3,278.25	3,278.25	3,278.25

[a]Vitamin and mineral mixture (minimum levels per kilogram of diet) — folic acid: 250 mg, pantothenic acid: 5,000 mg, antioxidant: 0.60 g, biotin: 125 mg, cobalt: 25 mg, copper: 2,000 mg, iron: 820 mg, iodine 100 mg, manganese: 3,750 mg, niacin: 5,000 mg, selenium: 75 mg, vitamin A: 1,000,000 UI, vitamin B1: 1,250 mg, vitamin B12: 3,750 mg, vitamin B2: 2,500 mg, vitamin B6: 2,485 mg, vitamin C: 28,000 mg, vitamin D3: 500,000 UI, vitamin E: 20,000 UI, vitamin K: 500 mg, zinc: 17,500 mg; [b]*Parkia platycephala* lectin (PPL) added levels in the diet; [c]analyzed proximate composition; [d]based on the digestible energy values of the ingredients (Soares, 2017).

Growth performance

The fish were weighed and measured at 0, 30, and 60 days of the experiment. Before each biometry, fish were fasted for 24 h and then anesthetized with eugenol at (40 mg L^{-1}). At the end of the experimental period and after performing the biometrics, the fish were euthanized by sectioning the spinal cord. Liver and muscle were removed and immediately stored at -20°C until biochemical analyses, with 8 fish per treatment.

Feed efficiency and growth parameters were calculated by applying the following formulas:

- Weight gain (WG) = [(final body weight − initial body weight)];
- Specific growth rate (SGR) = 100 × [(ln final weight − ln initial weight)/days of experiment];
- Relative weight gain (RW) = 100 × [(final body weight − initial body weight)/initial body weight];
- Condition factor (CF) = 100 × [(body weight)/(body length)^3];
- Feed conversion rate (FCR) = 100 × [(feed intake/weight gain)];
- Hepatosomatic index (HSI) = 100 × [(liver weight)/(whole-body weight)].

Biochemical parameters

Hepatic and muscular glycogen was evaluated using the methodology proposed by Bidinotto et al. (1997). For this, 50 mg of each tissue was weighed, and 1 mL of potassium hydroxide (KOH 6N) was added under heating and 3 mL of ethanol at room temperature to hydrolyze and precipitate glycogen, respectively. An aliquot (250 µL) of each tissue hydrolyzed in KOH at 100°C was diluted (1:4 in distilled water) and used to estimate the total protein level (Lowry et al., 1951). The standard was bovine serum albumin. For lactate and glucose (soluble sugar) analysis, 50 mg samples of liver or muscle were homogenized in 10% trichloroacetic acid (1:20 dilution) using a motor-driven Teflon pestle. Homogenates were centrifuged at 1,000 × *g* for 10 min. In the supernatant, the lactate (Harrower and Brown, 1972) and glucose (Dubois et al., 1956) contents were determined.

Histological and histometric analysis

Fish were fasted for 24 h at the end of the feeding experiment, anesthetized with eugenol (40 mg L^{-1} for 3 min), and euthanized by sectioning the spinal cord. The intestines of five fish per treatment were collected, fixed in 10% formalin for 24 h, and preserved in 70% alcohol. The histological slides were made according to the protocols for Harris hematoxylin–eosin, following the methodology described by Prophet et al. (1992), and later observed under an Olympus-Japan trinocular light microscope. The images were visualized with 4×, 10×, 20×, or 40× magnification lenses and captured using a Nikon DXM 1200 system attached to a computer, using the Image-Pro Express program.

For the evaluation of the histometric characteristics of the intestinal mucosa (height and width), six villi per sample were analyzed. Height and width were measured by scanning the samples using the Infostat (version 2017) image analyzer software.

Hemagglutination activity and inhibition assays

Hemagglutination activity tests were performed by the serial dilution of rabbit erythrocytes, either untreated or treated with the proteolytic enzymes (trypsin or papain; Sigma), according to Moreira and Perrone (1977). Results were expressed in hemagglutinating units (HU), with 1 HU being defined as the smallest amount (mg) of protein per mL capable of inducing visible agglutination. Lectin carbohydrate-binding specificity was defined as the smallest sugar concentration capable of totally inhibiting agglutination. Two-fold serial dilutions (initial concentration: 100 mM) of D-glucose, D-galactose, D-mannose, D-fucose, β-lactose, *N*-acetyl-D-glucosamine, α-methyl-D-mannopyranoside, L-rhamnose, α-lactose, adenosine, and mucin were prepared in distilled water. Lectin (4 HU) was added to each dilution. All carbohydrates and glycoproteins were obtained from Sigma.

Inactivation of PPL by physical and chemical agents

The influence of pH and temperature on the hemagglutination of PPL was studied according to the methodology described by Santos et al. (2019). To measure the influence of pH, PPL was incubated for 30 min in different buffers (at 100 mM) containing 150 mM NaCl: sodium citrate (pH 4 and 6), sodium acetate (pH 5), Tris-HCl (pH 7 and 8), and glycine-NaOH (pH 9 and 10). The PPL was subjected to different temperatures (20, 30, 40, 50, 60, 70, 80, 90, and 100°C) for 30 min to assess the influence of temperature on PPL activity. Subsequently, the samples were used in the hemagglutination assays.

Statistical analysis

All results are expressed as the mean ± standard error of the mean (SEM). Levene's test was performed to evaluate the homogeneity of variances, and the Cramér-von Mises test was used to evaluate data normality. The zootechnical parameters were evaluated by linear regression analysis. Evaluation of the effects of dietary PPL concentrations on the biochemical parameters in tambatinga juveniles and morphometric intestinal mucosa analysis was carried out through a one-way ANOVA, followed by the Duncan test. Analyses were performed using the "R" version 3.6.2 software and the SPSS® program (version 20.0; IBM®, Armonk, NY, USA), and differences were considered significant at $p < 0.05$.

RESULTS

Purification of *Parkia platycephala* lectin

To obtain high-purity PPL for use in assays, PPL purification was achieved by applying the crude extract of *P. platycephala* seeds in a Sephadex-G75 column, where it was possible to obtain an unretained peak (PI) and another peak that was obtained after elution with 0.2 M glycine and pH 2.6, called the "active retained peak" (PII) (Figure 1A).

The SDS-PAGE from the Sephadex-G75 fraction PII revealed that PPL is composed of a single chain with an apparent molecular mass of 50 kDa (Figure 1B), indicating the high degree of purity of the PPL.

Growth performance

Fish survival was not affected by any of the experimental diets. There was no significant difference ($p > 0.05$) in the growth parameters evaluated at 30 days of the experiment. However, after 60 days of weight gain, the specific growth rate and relative weight gain decreased linearly, according to the equations (Table 2; Figure 2), while the feed conversion rate value increased as the PPL concentrations in the diet of tambatinga juveniles increased. The condition factor and hepatosomatic index were not affected by dietary PPL.

Biochemical parameters

No significant difference ($p > 0.05$) was observed among the groups regarding hepatic glucose, lactate, and protein levels in tambatinga. The hepatic glycogen levels of fish fed PPL 60 mg kg^{-1} were significantly higher than those of fish fed 20 and 40 mg kg^{-1} (Table 3).

The addition of PPL at concentrations of 40 and 60 mg kg^{-1} increased the glucose levels in fish muscle compared to those in the control group. Similarly, fish-fed diets with all PPL levels (20, 40, and 60 mg kg^{-1} diet) had a lower concentration of muscle glycogen compared to fish fed the control diet. Fish fed a 60 mg kg^{-1} diet presented lower muscle protein levels than those on a 20 mg kg^{-1} diet (Table 3).

Histological and histometric analysis

Evaluation of the morphological and histological characteristics did not show any other cellular differences among the groups. The mucosa presented villi, and the epithelium was simple and cylindrical, with abundant mucous cells. Histometric analysis of the intestine revealed that the height and length of the villi of the intestinal mucosa did not differ among the different treatment groups ($p > 0.05$) (Table 4).

Hemagglutination activity and inhibition assays

Inhibition of the hemagglutinating activity showed that PPL has an affinity for mannose, glucose, and α-methyl-D-

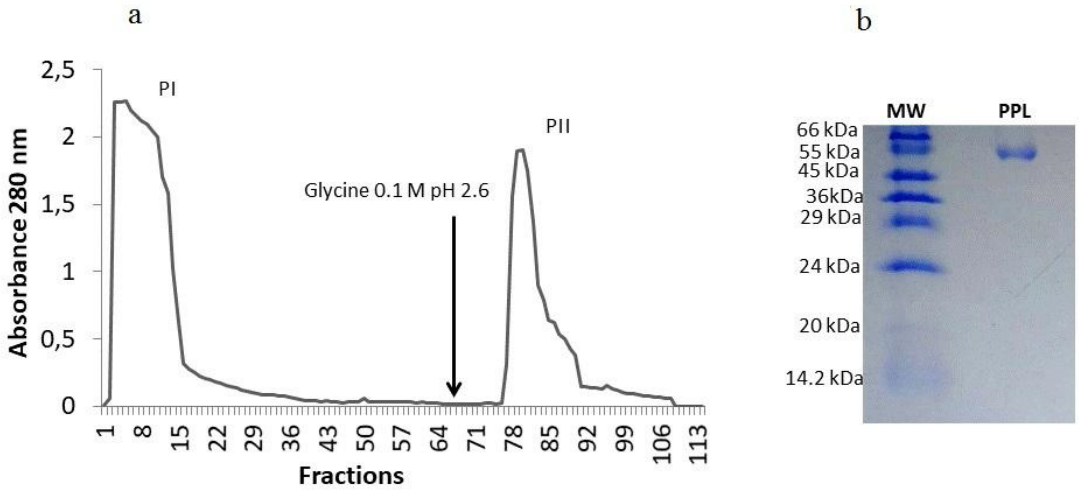


Figure 1. *Parkia platycephala* lectin (PPL) purification. (A) Elution profile of PPL in affinity chromatography. (B) SDS-PAGE profile, (MW) molecular mass markers: phosphorylase B, 97 kDa; bovine serum albumin, 66 kDa; glutamic dehydrogenase, 55 kDa; ovalbumin, 45 kDa; glyceraldehyde-3-phosphate dehydrogenase, 36 kDa; carbonic anhydrase, 29 kDa; trypsinogen, 24 kDa; trypsin inhibitor, 20 kDa; α-lactalbumin, 14.2 kDa; (L2) crude extract; and (PPL) purified lectin.

Table 2. Growth performance of tambatinga juveniles fed diets containing different levels of *Parkia platycephala* lectin (PPL)[#].

Parameters	Diets (PPL mg kg^{-1})			
	0	20	40	60
Initial				
W (g)	2.55 ± 0.13	2.58 ± 0.14	2.60 ± 0.16	2.58 ± 0.09
SL (cm)	5.42 ± 0.07	5.43 ± 0.09	5.53 ± 0.18	5.39 ± 0.05
30 days				
WG (g)	8.30 ± 0.94	8.32 ± 0.99	8.10 ± 0.40	8.21 ± 0.48
SGR (%)	4.77 ± 0.43	4.70 ± 0.10	4.76 ± 0.21	4.60 ± 0.12
RW (%)	322.80 ± 0.11	325.51 ± 0.30	319.10 ± 0.11	320.01 ± 0.12
FCR	0.97 ± 0.13	0.98 ± 0.10	0.83 ± 0.14	0.85 ± 0.13
CF	0.94 ± 0.02	0.92 ± 0.03	0.92 ± 0.02	0.90 ± 0.02
60 days				
WG* (g)	20.20 ± 0.5	18.24 ± 0.5	18.40 ± 0.5	16.63 ± 3.8
SGR* (%)	3.63 ± 0.19	3.57 ± 0.08	3.40 ± 0.20	3.32 ± 0.28
RW* (%)	789.29 ± 102.56	716.53 ± 62.63	675.76 ± 92.37	643.50 ± 138.11
FCR*	1.22 ± 0.01	1.23 ± 0.01	1.40 ± 0.11	1.49 ± 0.15
CF	1.47 ± 0.04	1.60 ± 0.14	1.58 ± 0.08	1.56 ± 0.08
HSI (%)	1.29 ± 0.12	1.25 ± 0.25	1.37 ± 0.06	1.37 ± 0.24

[#]Values are reported as mean ± SEM (n = 5);. R^2: determination coefficient; ns: nonsignificant ANOVA and regression* ($p > 0.05$); W: weight; SL: standard length; WG: weight gain; SGR: specific growth rate; RW: relative weight gain; FCR: feed conversion rate; CF: condition factor; HIS: hepatosomatic index.

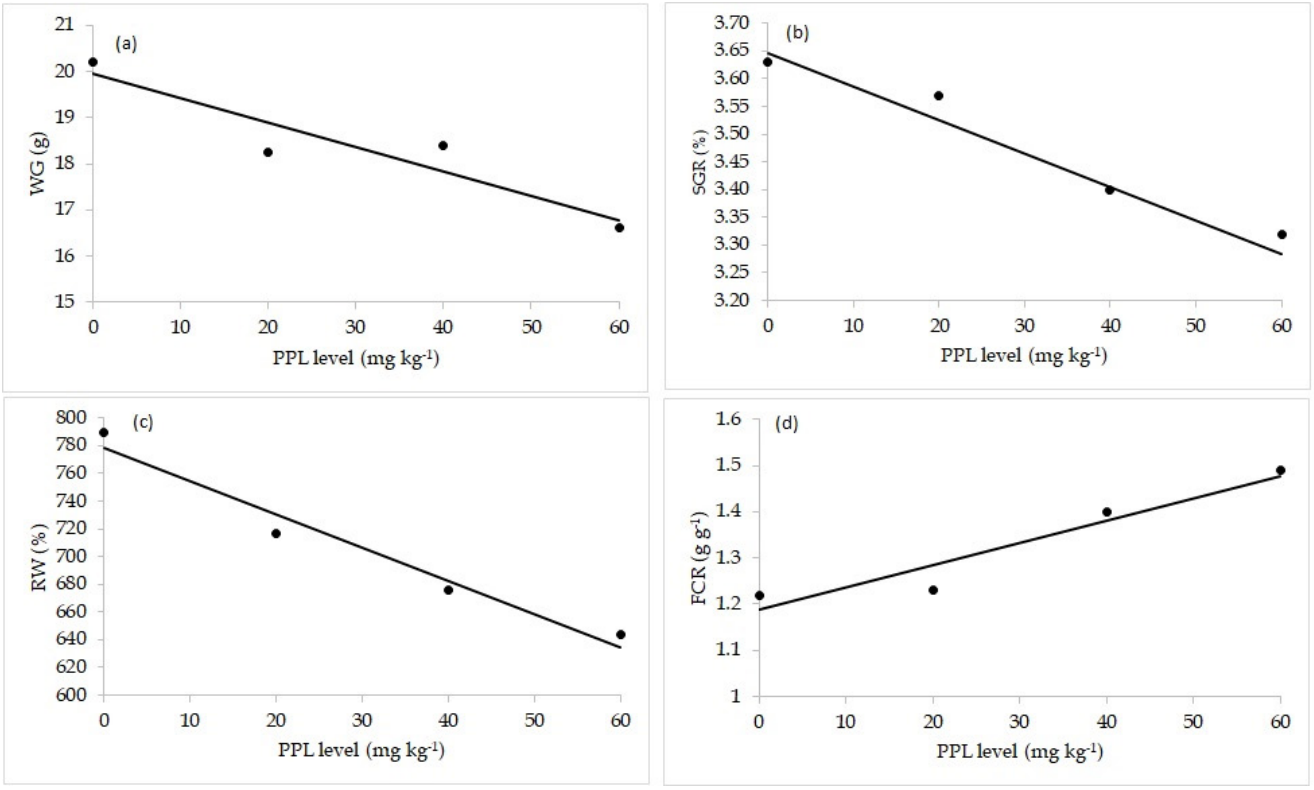


Figure 2. Growth performance of tambatinga juveniles fed diets containing different levels of *Parkia platycephala* lectin (PPL)[#].
[#]Values are reported as mean ± SEM (n = 5); R^2: determination coefficient; (A) WG: (^{a}Y = -0.0528x + 19.95; R^2 = 0.87; p = 0.037); (B) SGR: (^{b}Y = -0.006x + 3.645; R^2 = 0.97; p = 0.017); (C) RW: (^{c}Y = -2.391x + 777.99; R^2 = 0.96; p = 0.024); (D) FCR: (^{d}Y = 0.004x + 1.188; R^2 = 0.92; p < 0.001); WG: weight gain; SGR: specific growth rate; RW: relative weight gain; FCR: feed conversion rate.

Table 3. Biochemical parameters in the liver and muscle of tambatinga juveniles fed with diets containing different concentrations of *Parkia platycephala* lectin (PPL)[#].

Parameters	Diet (PPL mg kg^{-1})			
	0	20	40	60
Liver				
Glucose	489.43 ± 30.37	485.48 ± 24.53	446.14 ± 26.14	480.63 ± 15.65
Glycogen	49.83 ± 2.62^{ab}	46.46 ± 1.61^{b}	45.96 ± 2.68^{b}	55.79 ± 3.53^{a}
Lactate	4.17 ± 0.23	4.15 ± 0.13	3.57 ± 0.28	4.10 ± 0.22
Protein	136.53 ± 5.26	139.78 ± 4.22	144.57 ± 6.13	142.10 ± 5.47
Muscle				
Glucose	22.12 ± 1.34^{c}	24.08 ± 2.01^{bc}	28.01 ± 0.83^{b}	33.07 ± 1.09^{a}
Glycogen	11.22 ± 0.93^{a}	8.90 ± 0.45^{b}	4.85 ± 0.61^{c}	7.81 ± 0.52^{b}
Lactate	16.95 ± 1.18	18.52 ± 1.43	18.10 ± 0.72	19.79 ± 0.83
Protein	143.92 ± 4.07^{ab}	147.57 ± 4.15^{a}	135.65 ± 4.99^{ab}	131.76 ± 5.26^{b}

[#]All values are reported as mean ± SEM (n = 8). Different letters within rows indicate significant differences using the Duncan test (p < 0.05); glucose and glycogen: µmol glucose g tissue^{-1}; lactate: µmol lactate g tissue^{-1}; protein: mg g tissue^{-1}.

mannopyranoside, with minimum inhibitory concentrations of 50, 25, and 6.26 mM, respectively, and that no affinity was displayed for the other carbohydrates tested or glycoproteins (Table 5).

Inactivation of PPL by physical and chemical agents

The PPL maintained its total hemagglutinating activity up to 40°C, as shown in Figure 3A. However, when incubated at

50°C, the activity was considerably reduced. The PPL showed hemagglutinating activity over a wide pH range, but its stability was more evident between pH 6 and 7 and was reduced when exposed to lower or higher pH values, respectively (Figure 3B).

Table 4. Histometric characteristics of the intestinal mucosa of tambatinga juveniles fed with diets containing different concentrations of *Parkia platycephala* lectin (PPL)[#].

Parameters	Diet (PPL mg kg^{-1})				CV (%)
	0	20	40	60	
FH (µm)*	98.32	94.08	101.24	105.47	30.72
FW (µm)**	56.42	57.97	55.81	69.96	25.12

[#]All values are reported as mean ± SEM (n = 5); FH: fold height; FW: fold width; CV: coefficient of variation; *p = 0.9303; **p = 0.3437.

Table 5. Inhibitory effects of carbohydrates and glycoproteins on *Parkia platycephala* lectin (PPL) hemagglutinating activity.

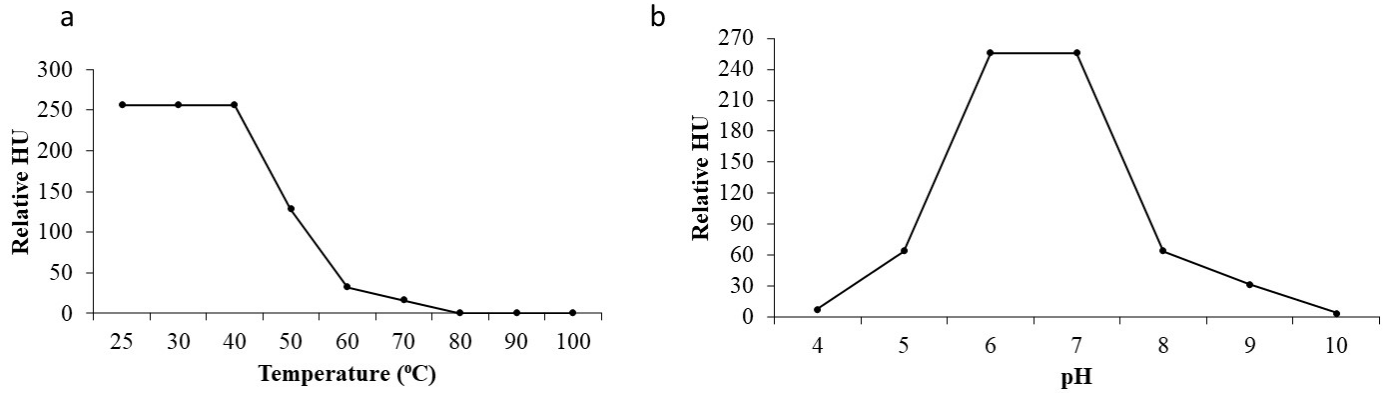
Sugar	MIC (mM)
D-Mannose	50
D-Galactose	NI[a]
D-Glucose	25
D-Fucose	NI
L-Rhamnose	NI
N-Acetyl-D-glucosamine	NI
α-Methyl-D-mannopyranoside	6.25
α-Lactose	NI
β-Lactose	NI
Glycoproteins	
Adenosine	NI
Mucin	NI

MIC: minimum inhibitory concentration; NI[a]: carbohydrate or glycoprotein not inhibitory up to 100 mM.

DISCUSSION

The water parameters evaluated remained stable throughout the experimental period and within the limits considered suitable for the species (Ribeiro et al., 2019). Besides assessing the impact of a diet on growth and feed use efficiency in fish, it is also vital to know the mechanisms responsible for the observed performance. The nutritive value of food depends not only on its nutrient content or antinutrients but also on the capacity of the animal to digest and absorb these substances (Cook et al., 2000). These include, among others, digestibility, absorption in the digestive system, and retention in the body (Aanyu et al., 2014).

The use of plant-derived materials, such as legume seeds, leaf meals, leaf protein concentrates, and root tuber meals, as fish feed ingredients, is limited by the presence of a wide variety of antinutritional substances. The antinutritional factors present in food intended for animal feed can compromise not only growth but also health. Therefore, it is of fundamental importance to know the antinutrients and their main effects on the animal organism (Mohan et al., 2016). In this sense, PPL inclusion is accompanied by negative results for several growth indices in tambatinga. Many of these responses could be associated with poor use of the feed (digestion and absorption), as could be observed in our results for WG, SGR, RW, and apparent FCR. Similarly, Nile tilapia (*Oreochromis niloticus*) fed with raw soybean (6.5 mg g^{-1} lectin, with inclusions of this ingredient ranging from 106 to 247 g kg^{-1}) had a lower final weight, feed intake, and protein retention than those fed with a control diet (Martins et al., 2017). In another study, rainbow trout (*Oncorhynchus mykiss*) showed a reduction in weight gain and specific growth rate when fed diets containing soy lectin (80 mg kg^{-1}) or soy trypsin inhibitor (2.6 g kg^{-1}) added separately to each diet (Hart et al., 2010). In the latter study, rainbow trout showed a moderate increase in concentrations of alanine aminotransferase (ALT) and serum amylase, which, according to the authors, can lead to effects at the level of the gastrointestinal tract and in the secretion of pancreatic enzymes in fish. Still, fingerlings of *C. gariepinus* fed with the lowest dietary



HU: hemagglutinating units.

Figure 3. Inactivation of the lectin from *Parkia platycephala* seeds. Effects of (A) temperature and (B) pH on hemagglutinating activity of PPL.

level (25%) of *P. biglobosa* showed higher weight gain than fingerlings fed with the other tested levels (0, 50, 75, and 100%) (Michael and Mathias, 2020). All these effects can help explain the action of PPL on the growth of tambatinga juveniles fed with lower levels of this antinutrient than reported in other studies.

In relation to the biochemical responses evaluated, there was a breakdown of muscle glycogen and, consequently, an increase in glucose in this tissue in tambatinga-fed diets containing PPL compared to the fish that received the diet without PPL. This response could also be verified in relation to muscle protein, mainly in diets containing the highest levels of PPL. These results are also related to the effects observed in the fish growth response. The inclusion of lectin and soybean trypsin inhibitor in a rainbow trout diet increased the levels of ALT and amylase in fish serum but did not cause changes, for example, in glucose, albumin, globulin, alkaline phosphatase, and serum minerals (Hart et al., 2010). The inclusion of nondetoxified *Jatropha curcas* meal caused a reduction in hematocrit, hemoglobin, and red blood cell count in *C. gariepinus* fingerlings compared to fish-fed diets containing detoxified bran, that is, with acid reduction phytic acid, oxalates, cyanogenic glycosides, and trypsin inhibitors (Musa et al., 2018). The presence of lectins in *J. curcas* meal has also been reported (Makkar, 2016), and probably, lectin effects add to those of the other antinutrients, causing undesirable effects on fish. Another example of metabolic changes in fish fed with vegetable bran containing antinutrients occurred with juveniles of *Rhamdia quelen* fed with a diet containing tung meal (*Aleurites fordii*) in its crude form, after oil extraction, and with the presence of tannins, phytic acid, and possibly esters of phorbol and lectins. The fish showed a reduction in plasma glucose, triglycerides, and cholesterol, as well as a reduction in hepatic glycogen and an increase in aspartate aminotransferase in the liver, and there was a drastic reduction in growth compared to fish that were fed with bran containing a reduced concentration of antinutrients (Pretto et al., 2020).

The physiological effects of diets, such as increased absorption surface area in the intestinal mucosa, nutrient digestibility, and retention, are some of the vital aspects for guiding feed manufacturers to manipulate feed formulae to improve the quality of feed (Aanyu et al., 2014; Lopes et al., 2020). According to the histological analysis performed in this study, the addition of lectin from faveira seeds (*P. platycephala*) to the diet did not result in alterations in the intestinal morphology of tambatinga juveniles.

Thus, some molecules present in vegetable meal and/or extracted and purified from plants can have adverse effects when inserted into animal feed, even at low concentrations. Therefore, understanding their effects and possible inactivation pathways is important both for the inclusion of alternative plant sources in animal nutrition and for possible applications of these molecules in different areas.

In this sense, the hemagglutinating activity of PPL in response to chemical and physical agents, which can act as potential inactivators, was studied. The hemagglutinating activity of PPL was inhibited by D-mannose, D-glucose, and α-methyl-D-mannopyranoside. Silva et al. (2019) also demonstrated that PPL showed an interaction with mannose and glucose during the hemagglutination inhibition assay, providing evidence that these carbohydrates can inhibit the action of PPL. The hemagglutination assay is the most common method used to obtain semi-quantitative data on the carbohydrate binding and specificity of a lectin (Batista et al., 2018).

In this study, PPL was shown to be a thermostable protein up to 40°C and showed thermostability similar to *Machaerium acutifolium* lectin (Santos et al., 2019). However, when subjected to a temperature of 50°C, its activity was reduced. Therefore, thermal treatment of PPL between 50 and 60°C is recommended because this interval is sufficient to reduce the action of this protein. In relation to the PPL activity at different pH values (pH 4–10), lectin exhibited its maximum activity at a pH range of 6–7, and lectin activity declined below and above this pH range. Cavada et al. (1997) also reported that PPL showed maximum activity at a pH of around 7, corroborating our results. Thus, it is suggested that the conditioning of PPL for up to 30 min in an acidic or alkaline medium may be effective to decrease the activity of this protein and, consequently, its antinutritional effect.

CONCLUSION

The addition of PPL in its natural form or without inactivation to the diet of tambatinga is not recommended as it can negatively affect the biochemical parameters and, consequently, the growth of the animals. Additionally, it was possible to identify different methods of inactivating this antinutrient, such as the use of acid or alkaline solutions, thus creating new perspectives for the use of ingredients based on *P. platycephala* in feed for fish and other animals.

CONFLICT OF INTERESTS

Nothing to declare.

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AUTHORS' CONTRIBUTIONS

Lopes, J.M.: Conceptualization, Writing — original draft, Data curation, Writing — review & edition. Silva, R.C.: Conceptualization, Resources, Investigation. Siqueira, J.C.: Sofware, Data curation. Pretto, A.: Formal Analysis, Writing — review & editing. Costa, T.S.: Resources, Investigation. Baldisserotto, B.: Supervision, Validation, Supervision. Pantoja, B.T.S.: Resources. Teixeira, C.S.: Conceptualization, Data curation.

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