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Scientific Article

Fungi influence in the chemical composition, textural quality, buoyancy, and floatation time of poorly stored fish feeds

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ABSTRACT

This study assessed the effects of fungal contamination on the chemical composition, texture, buoyancy, and floating time of poorly stored fish feed. Among 21 commercial and six artisanal feed samples analyzed, 81% showed fungal growth. Nutritional losses were significant: commercial feeds lost 41.97–50.61% of minerals, 17.11–21.69% of crude protein, 38.28% of lipids, 35.16–50.66% of carbohydrates, and 37.77% of raw energy. Artisanal feeds experienced even greater losses, with reductions of 66.12–84.06% in minerals, 43.29–47% in crude protein, and 25.60–42.40% in raw energy. Contamination altered pellet texture, causing hardening, crumbling, and increased fracturability in water, reducing buoyancy (~50%) and causing nutrient leaching. This led to feed accumulation at pond bottoms, accelerating eutrophication. These findings emphasize the economic and environmental risks of fungal contamination in fish farming. Proper storage with humidity and temperature control is crucial to prevent fungal growth, nutrient loss, and mycotoxin contamination.

Keywords: *Colossoma macropomum*; Fish farming; Feed storage; Fungal contamination.

Fungos influenciam na composição química, na qualidade textural, na flutuabilidade e no tempo de flutuação de rações mal armazenadas

RESUMO

Este estudo avaliou a influência de fungos na qualidade de rações para peixes mal armazenadas, analisando flutuabilidade, tempo de flutuação, composição química e textura. Foram examinadas 21 amostras de ração comercial e seis artesanais, com 81% apresentando contaminação fúngica. Perdas nutricionais significativas foram observadas: nas rações comerciais, houve reduções de 41,97–50,61% em minerais, 17,11–21,69% em proteína bruta, 38,28% em lipídios, 35,16–50,66% em carboidratos e 37,77% em energia bruta. Nas rações artesanais, as perdas foram ainda maiores, com redução de 66,12–84,06% em minerais e até 47% em proteína bruta. A textura dos pellets apresentou endurecimento, esfarelamento e aumento da fraturabilidade, reduzindo a flutuabilidade em ~50% e promovendo lixiviação de nutrientes, acumulando-se no fundo dos viveiros e acelerando a eutrofização. O estudo ressalta os impactos econômicos e ambientais da contaminação fúngica e reforça a importância de armazenamento adequado para evitar micotoxinas e preservar a qualidade nutricional.

Palavras-chave: *Colossoma macropomum*; Contaminação fúngica; Armazenamento das rações; Piscicultura.

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INTRODUCTION

Regarding fish feeds farming, the inclusion of vegetable raw material, such as cereal grains and their by-products, is commonly performed, which can increase the risk of fungal contamination, as contamination of this raw material can occur during production, storage and transportation, being sensitive at all stages of production (Tolosa et al., 2019). Tolosa et al. (2019) point out that grains (corn and soybeans, for example) can sometimes already be contaminated by mycotoxins before the feed is manufactured. Although, contamination can occur naturally during the processing, storage, and handling of raw materials, especially in agricultural commodities, that are rich in bran or fiber and have moisture content (Oliveira & Vasconcelos, 2020). In addition, increased fungal contamination in food can affect both the chemical composition and its nutritional value, as well as generate negative effects on palatability, aroma, toxin formation, discoloration, and rotting (Pietsch et al., 2020). Terada-Nascimento et al. (2023) analyzed feed for omnivorous fish in 40 fish farms in the Rondônia state, Brazil, and found Fumonisins B_1 + B_2 (375 to 1418 $\mu g \cdot kg^{-1}$), one of the most common mycotoxins to contaminate corn, which is one of the main ingredients in feed for omnivorous fish.

For the manufacture of diets intended for aquaculture, procedures such as grinding ingredients, mixing, application of moisture, heat, and pressure are used to produce the physical form of the product (Guimarães & Martins, 2015). The main physical forms of feed used in the aquaculture industry are mash, pellets, extrusion, and flakes (Boscolo et al., 2011). Feeding in fish farming has particularities such as the lower of nutrients by leaching and the difficulty of visualizing consumption. Therefore, buoyancy and floatation are important characteristics. The physical stability is necessary to minimize the losses of nutrients until identification, seizure, and consumption of feed by the fish (Pereira Junior et al., 2013). So, the processes that make nutrient losses by leaching extremely difficult are pelletizing, extrusion, and flocculation. They vary because fish need smaller or larger pellets. They determine pellets have to be smaller or larger, so that the fish can ingest the feed, otherwise the food will become a surplus (Campeche et al., 2014).

The physical assessment aspects of the feed are relevant as they can act as an indicator of the feed quality and possible issues in the feed manufacturing process. Therefore, the aim of this study was to evaluate the influence of fungi on the chemical composition, textural quality, buoyancy, and floating time of poorly stored fish feed.

MATERIAL AND METHODS

Commercial feeds for farmed tambaqui (*Colossoma macropomum*)

Fish feed samples were obtained from 27 fish farms in the Vale do Jamari and Centro-Leste microregions in the Rondônia state, Brazil. Fish feed from different brands were used, and those that had the same guaranteed levels were selected. For tambaqui, commercial fish feed was supplied, composed of 36% crude protein from a feed rate of 1% in relation to the fish weight (Table 1). Regarding artisanal feeds, they were composed of 28% crude protein, prepared at a feed rate of 1% in relation to the weight of the fish. This artisanal fish feed was made basically from fish silage and fermented corn.

The frequency of supplying fish feed was provided twice a day, from 8 a.m. to 6 p.m. It is interesting to highlight the information on the guaranteed levels of food provided by fish farmers to attest that fish farmers in the sampled regions adopt a standardized diet.

Sample collection and microbiological analyses in fish feeds

We collected feeds samples from November 2021 to May 2022. Epidemiological data were collected on the use and conservation of foods used in farmed tambaqui, as well as commercial and artisanal feed samples.

A total of 21 samples of commercial feed and six samples of artisanal feed were collected. These samples were 500 grams and stored under refrigeration while being transported to the laboratory. Sample collection was conducted in accordance with European Commission's Regulation No. 401/2006, which

Table 1. Guaranteed composition of the commercial feed for raised tambaqui (*Colossoma macropomum*).

Composition	Content ($g \cdot kg^{-1}$)
Dry matter (g)	910
Crude protein (min., g)	360
Fibrous matter (max., g)	95
Mineral matter (max., g)*	15
Ethereal extract (min, g)	80
Calcium (max., g)	35
Calcium (min., g)	20
Phosphorus (min., g)	15

*Amount of nutrients per kg for crude protein ration (36%): pantothenic acid (min): 3 mg, biotin (min): 50 mg, choline (min): 290 mg, vitamin A (min): 28,000 IU, vitamin B_1 (min): 2 mg, vitamin B_{12} (min): 4 mg, vitamin B_2 (min): 3 mg, vitamin B_6 (min): 2 mg, vitamin D_3 (min): 5,000 IU, vitamin E (min): 45 IU, vitamin K_3 (min): 2 mg, vitamin C (min): 500 mg, copper (min): 10 mg, total iron (min): 90 mg, iodine (min): 0.40 mg, niacin (min): 50 mg, manganese (min): 10 mg, zinc (min): 180 mg, and selenium (min): 0.60 mg.

establishes sampling and analysis methods for the official control of mycotoxin levels in foodstuffs. Therefore, the samples were collected directly from the storage location, placed in sterile bottles with lids, transported in isothermal boxes to the Microbiology Laboratory, at the Universidade Federal de Rondônia, in Rolim de Moura city, and refrigerated between 2 to 8°C until the time of carrying out the microbiological analyses.

Regarding the identification of filamentous fungi in feeds for farmed tambaqui, the samples were diluted according to ISO 6887-4 (ISO, 2003) at a concentration of 10^{-1}, prepared by adding 225 mL of 0.1% peptone water mixed with 25 grams of previously homogenized and ground feed. From this dilution, successive dilutions were carried out up to 10^{-3}. Subsequently, sowing was carried out in a sterilized Petri dish containing 20 mL of DG18 culture medium, incubated at 25°C for five days to isolate molds and yeasts.

Colonies of different morphological types were isolated on potato dextrose agar and identified using the microculture technique for morphological analysis and identification of filamentous fungi, as described by Pinto et al. (2012).

Chemical composition analysis

Regarding proximate composition, moisture, crude protein, lipids, mineral matter, and carbohydrates were determined. Moisture in fish feed is commonly determined by drying a sample at some elevated temperature and reporting the loss in weight in terms of moisture (Draganovic et al., 2011). The mineral matter in fish feed is minutely determined by incineration from either raw or dried samples at about 600-700EC for 5 to 8 hours. The residue is weighed as mineral matter (Pires et al., 2021). The crude protein content was carried out using the micro-Kjeldhal method (Lynch & Barbano, 1999). Finally, the lipid content was quantified through extraction with a chloroform-methanol mixture (2:1). The mixture was allowed to stand overnight, and lower lipid protein was transferred to a pretreatment, and weighed flask was heated to dryness. The difference in the two weights of the round joint flask gave the weight of the fat (Santos et al., 2009).

loric value, it was calculated according to Souci et al. (2000), obtained by the sum of the multiplication of the crude protein, total lipids, and carbohydrates content multiplied by indicators 4, 9, and 4, respectively. The result in kcal·100 g^{-1} was transformed into KJ·100 g^{-1}, registered for energy raw, as shown in Eq. 1 (Souci et al., 2000).

$$EV = (kcal \cdot 100\ g^{-1}) \times 4.184 = CP \times 4 + TL \times 9 + CB \times 4 \quad (1)$$

Where: EV= energy raw; CP= crude protein; TL= total lipids; CB= carbohydrates.

Texture analysis in fish feeds

The methodology to determine the instrumental texture profile was performed according to Karim et al. (2024). The attributes tested were hardness (N), compression strength ($kgF \cdot mm^{-2}$), fracturability (N), chewability ($N \cdot mm^{-1}$), and spreadability ($N \cdot mm^{-2}$), considering the shear stress and viscosity of the water at 26°C (Tonhi and Plepis, 2002). These attributes were analyzed in a TAXT.plus texturometer, using the Exponent Stable Micro Systems software (Stable Micro System Ltd, Vienna Court, UK) (Hwang et al., 2012).

A total of six was randomly selected from each feed sample. In the texturometer analyses, they were positioned vertically and horizontally on a platform (Ashtiani et al., 2016). A cylindrical probe with a flat end, ½ inch in diameter, was used. Concerning the conditions of the instrumental texture tests, the pre-test speed was 2 $mm \cdot s^{-1}$, the post-test 10 $mm \cdot s^{-1}$, and the distance was 4 mm (Cavali et al., 2024).

Buoyancy and floatation tests of fish feed

Initial buoyancy was conducted in a 10-L bucket of water, with 200 g of fish feed, and instantly it was seen how long it took for 0.5% of the fish feed to sink. Then, for the final buoyancy (10 minutes after the 0.5% sink), we waited for at least another 3% of the fish feed to sink. The producer can perform the same test mentioned above, however, for 10 minutes. In that case, less than 3% of the fish feed will be able to sink. Out of 200, if 7% or more of the fish feed sink, the feed does not have adequate buoyancy. Based on these results, Eq. 2 was applied (Abubakar et al., 2016).

$$B = Cw.Sp/(V_{water}) \times 100 \quad (2)$$

Where: B= buoyancy; Cw= chewiness of the fish feed pellets; Sp= spreadability of the fish feed pellets; Vwater= the dynamic viscosity of water of 0.7978 $mPa \cdot s^{-1}$ (at the temperature of 26ºC).

A second step was performed to verify the total percentage of that sank. Regarding the total floatation of the test, fish feed was determined by placing samples containing 10 L from both machines, replicated three times into 2 L of bowls filled to about 75% with water. The number of each sample remaining afloat after every 60-second interval was recorded for 30 minutes. Percentage buoyancy at any time interval was determined as Eq. 3 (Momoh et al., 2016). For more methodological details on the expansion of fish feed pellets and water density considered, see Abubakar et al. (2016).

$$Floatation\ (\%) = (Number\ of\ afloat) / (Number\ of\ in\ sample) \times 100 \quad (3)$$

Database and statistical analysis

Averages of chemical composition, textural quality, buoyancy, and floatation were analyzed using the described statistics shown as average and standard deviation. A previous verification of normality and homoscedasticity by the Shapiro-Wilk's test was performed. Also, after the data were submitted to analysis of variance, the averages were compared by Tukey's test, with a significance level considered 5%.

All statistical analyses were performed using R Development Core Team, version 3.5.3.

RESULTS

Fish feed storage conditions

The feed storage conditions were checked in 40 fish farms. A total of 21 fish farms supplied factory feed (commercial), six produced their own feed (artisanal), and 13 fish farms did not feed the fish. Only one fish farm stored feed properly in a closed environment, free from humidity/rain, high temperature and insects, and the other fish farms stored it in polyethylene water ponds very close to the water, in the fishponds.

A total of 70% of fish farms stored feed under the floor of the sheds, without any protection against humidity and high temperatures (the place was poorly ventilated). The feed bags were stored in small, improvised sheds, without windows and covered without lining, so they were subject to contact with rats, cockroaches, and woodworms (Fig. 1).

Fungi in fish feeds

As previously reported, 13 fish farms did not provide food for the fish. Some of them only provided fermented corn, others provided nothing, forcing the fish to feed only on natural plankton food.

A total of 27 feed was sampled and stored under refrigeration, with 500 g obtained from each bag of fish feed. Among all feed collected, 17/21 commercial feeds and 5/6 artisanal feeds showed growth of potentially mycotoxigenic filamentous fungi (Fig. 2). Therefore, 81.25% of feed for farmed tambaqui in Rondônia were contaminated.

In the presumptive test, colonies with different appearances were submitted to microculture with potato dextrose agar for morphological identification in an optical microscope, and the growth of filamentous fungi of the genera *Fusarium* sp., *Penicillium* sp., and *Aspergillus* sp. was verified (Fig. 3). Coinciding with the fungi found in the fishponds water, a linear model correlation analysis was necessary, which was carried out later.

These genera are considered mycotoxigenic. Therefore, they demonstrate the viability of mycotoxin occurrence in fish feed. After isolation, three colonies of the genus *Penicillium* sp.,

Figure 1. Feed storage conditions. Feed stored in small rooms in bags placed directly on the floor, close to the tank, being exposed to (a) humidity and high temperatures, (b) feeds subject to contact with (c) rat feces and (d) insects.

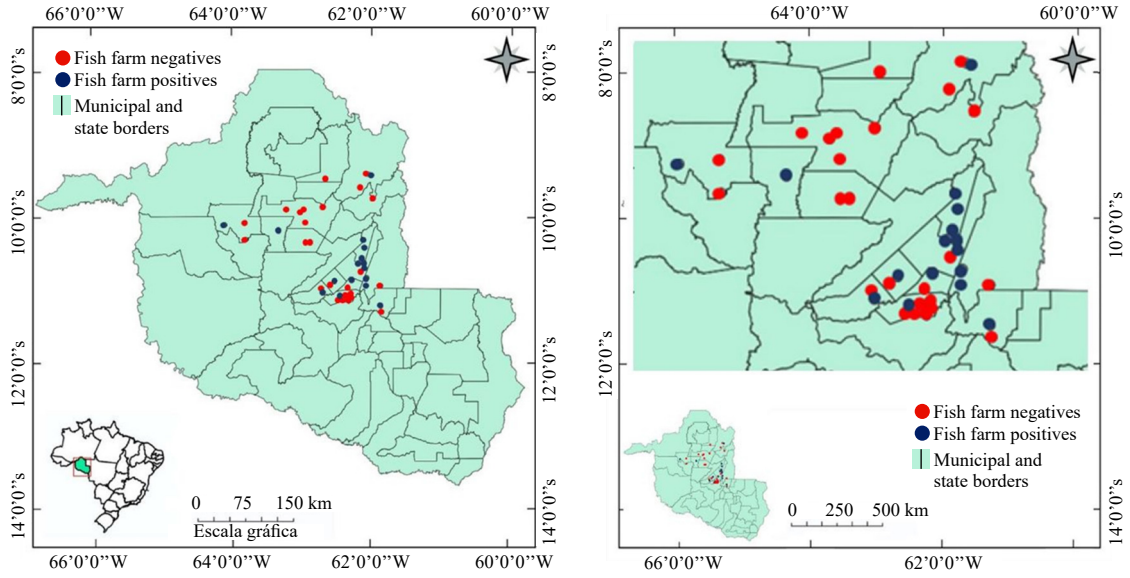

Figure 2. The geographic location of fish farms (negative and positive) for the presence of mycotoxigenic fungi in feeds for raised tambaqui (*Colossoma macropomum*) in Rondônia state, Brazil.

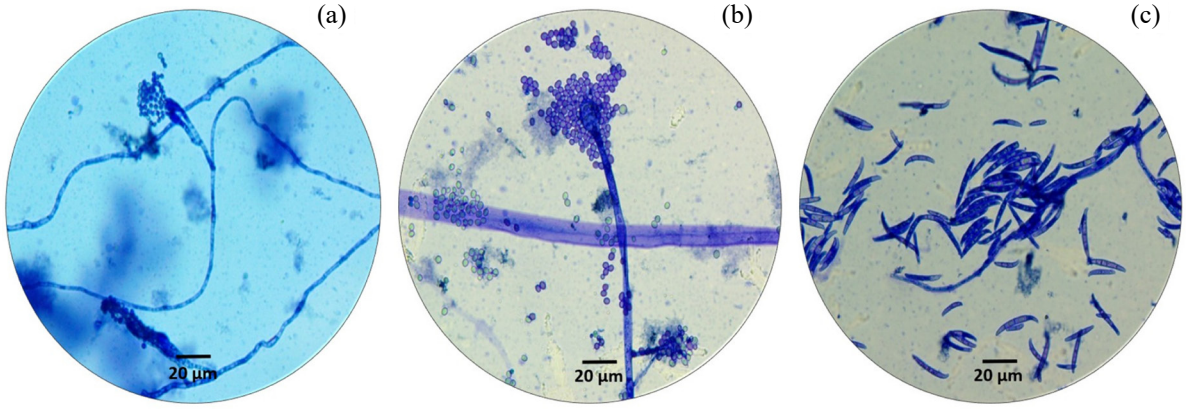

Figure 3. Photomicrographs of mycotoxigenic fungi found in feeds for raised tambaqui (*Colossoma macropomum*): (a) *Aspergillus sp.,* (b) *Penicillium sp.*, (c) *Fusarium sp.*

eight of the genus *Aspergillus* sp., and four of the genus *Fusarium* sp.were identified under an optical microscope (Table 2). From the confirmations of the presumptive test, the samples were isolated to be sent to a specialized and internationally certified laboratory for mycotoxicological analysis.

Chemical composition

As fish farms adopted different ways of storing feed, the chemical composition was compared in different fish farms. The chemical compositions of commercial pelleted feed and artisanal feed were evaluated.

Firstly, regarding the influence of fungi on the moisture content of commercial fish feeds, feeds with fungus D, A, and B showed higher moisture contents in relation to other feeds with fungi (9.62, 5.97 and 5.68%, respectively) and higher than feed without fungi (4.55%) (Table 3).

Regarding mineral material, feeds with fungi showed substantial loss of minerals, the one without fungi was composed of 8.22 g·100g^{-1} of minerals, while the feeds with fungus D and B were composed of 4.06 and 4.77 g·100g^{-1} of minerals, representing losses of 50.61 and 41.97% of minerals. There was a substantial loss of crude protein content with

Table 2. Fungi isolated and identified in feeds for raised tambaqui (*Colossoma macropomum*).

Fish farms	Dilutions		
	10^{-1}	10^{-2}	10^{-3}
P1	*Fusarium* sp.	-	-
P3	*Fusarium* sp.	-	-
P4	*Fusarium* sp.	-	-
P5	-	-	-
P6	-	*Fusarium* sp.	-
P7	-	-	-
P8	-	-	-
P11	*Fusarium* sp.	-	-
P13	*Penicilium* sp. *Fusarium* sp.	*Penicilium* sp. *Fusarium* sp.	*Penicilium* sp. *Fusarium* sp.
P14	*Fusarium* sp.	-	-
P15	-	*Fusarium* sp.	*Fusarium* sp.
P16	*Aspergillus* sp. *Fusarium* sp.	*Aspergillus* sp.	*Aspergillus* sp.
P17	*Aspergillus* sp. *Fusarium* sp.	*Fusarium* sp.	-
P20	-	-	*Fusarium* sp.
P22	*Fusarium* sp.	-	-
P24	*Fusarium* sp.	-	*Fusarium* sp.
P27	*Fusarium* sp.	-	*Fusarium* sp.
P28	-	-	-
P31	-	*Aspergillus* sp. *Fusarium* sp.	-
P32	*Aspergillus* sp.	*Fusarium* sp.	-
P38	*Aspergillus* sp.	*Fusarium* sp.	-

fungal contamination; the feed without fungi was composed of 36.23 g·100 g^{-1}, while the feeds with fungus D and B were composed of 28.37 and 30.03 g·100g^{-1}, representing losses of 21.69 and 17.11% crude protein (Table 3; Fig. 4).

There was also a substantial loss of lipid content with fungal contamination. The feed without fungi was composed of 3.37 g·100g^{-1}, while the feed with fungi D was composed of 2.08 g·100g^{-1}, representing a loss of 38.28% of lipids. Regarding the carbohydrates content, there was also a significant loss. The feed without fungi was composed of 44.88 g·100 g^{-1} of carbohydrates, while the feeds with fungus A, B, C, and D were composed of only 27.38, 28.67, 29.10 and 22.14 g·100g^{-1} of carbohydrates, representing a loss of 50.66 and 35.16% of carbohydrates (Table 3; Fig. 4).

Concerning the raw energy, there was a significant loss. The feed without fungi expressed 1,484.36 kJ·100 g^{-1}, while feed with fungi D expressed only 923.66 kJ·100 g^{-1}, representing losses of 37.77% of raw energy (Table 3; Fig. 4).

Regarding the influence of fungi on the moisture of artisanal fish feed, the feeds with fungi W showed higher moisture content in relation to the other feeds with fungi (17.28%) and higher than the one without fungi feed (5.98%) (Table 4).

Concerning mineral material, the feeds with fungi showed substantial loss of minerals. The one without fungi feed was composed of 5.52 g·100 g^{-1} of minerals, while the feeds with fungi W and S were composed of 0.88 and 1.87 g·100g^{-1} of minerals, representing losses of 84.06 and 66.12% of minerals. There was a substantial loss of crude protein content with fungal contamination. The feed without fungi was composed of 31.88 g·100g^{-1}, while the feeds with fungi W and S were composed of 16.89 and 18.08 g·100 g^{-1}, representing losses of 47 and 43.29% crude protein (Table 4; Fig. 5).

Table 3. Chemical composition in commercial fish feeds without fungus and with fungus*.

Content (g·100 g^{-1})	Without fungus**	With fungus				*p*-value
		A	B	C	D	
Moisture	4.55 ± 0.36^{c}	5.68 ± 0.45^{b}	5.97 ± 0.48^{b}	5.10 ± 0.41^{c}	9.62 ± 0.77^{a}	< 0.01
Mineral matter	8.22 ± 0.49^{a}	5.34 ± 0.32^{b}	4.77 ± 0.28^{bc}	5.42 ± 0.32^{b}	4.06 ± 0.28^{c}	< 0.01
Crude protein	36.23 ± 1.45^{a}	31.52 ± 1.26^{b}	30.03 ± 1.22^{c}	31.05 ± 1.24^{b}	28.37 ± 1.13^{c}	< 0.01
Lipids	3.37 ± 0.20^{a}	2.86 ± 0.17^{b}	2.83 ± 0.17^{b}	2.90 ± 0.17^{b}	2.08 ± 0.12^{c}	<0 .01
Carbohydrates	44.88 ± 6.28^{a}	27.38 ± 3.83^{b}	28.67 ± 4.01^{b}	29.10 ± 4.07^{b}	22.14 ± 3,10^{b}	0.037
Raw energy (kJ·100 g^{-1})	1,484.36 ± 163.28^{a}	1,093.44 ± 120.28^{b}	1,092.74 ± 120.20^{b}	1,115.87 ± 122.75^{b}	923.66 ± 101.57^{c}	< 0.014

*If there are different letters between the means, they are different according to Tukey's test ($p < 0.05$); **confirmed without fungus in fish feed guarantee levels.

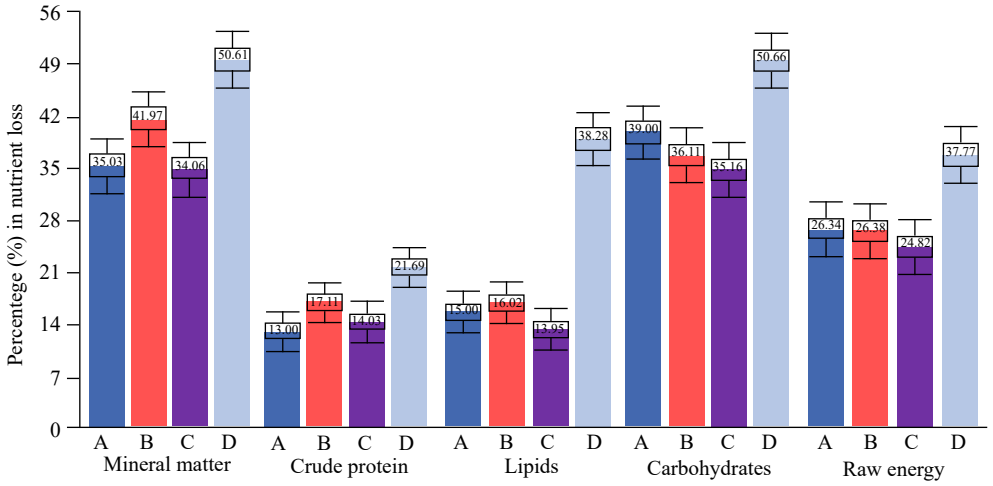

Figure 4. Percentages of nutritional losses caused by fungal contamination in commercial fish feed.

Table 4. Chemical composition in artisanal fish feeds without and with fungus*.

Content (g·100 g^{-1})	Without fungus**	With fungus					*p*-value
		X	Y	Z	S	W	
Moisture	5.98 ± 0.47^{c}	8.94 ± 0.71^{bc}	8.61 ± 0.69^{bc}	9.99 ± 0.80^{b}	11.66 ± 0.93^{b}	17.28 ± 1.38^{a}	< 0.010
Mineral matter	5.52 ± 0.33^{a}	4.00 ± 0.24^{b}	3.86 ± 0.23^{b}	3.59 ± 0.21^{b}	1.87 ± 0.11^{bc}	0.88 ± 0.05^{c}	< 0.010
Crude protein	31.88 ± 1.27^{a}	27.85 ± 1.11^{b}	26.50 ± 1.06^{b}	25.91 ± 1.04^{b}	18.08 ± 0.72^{c}	16.89 ± 0.67^{c}	< 0.010
Lipids	2.90 ± 0.17^{a}	2.60 ± 0.16^{a}	2.42 ± 0.14^{ab}	2.30 ± 0.14^{ab}	2.24 ± 0.13^{ab}	1.90 ± 0.11^{b}	0.029
Carbohydrates	40.94 ± 5.73^{a}	33.75 ± 4.73^{a}	30.30 ± 4.24^{b}	28.25 ± 3.96^{b}	26.20 ± 3.67^{bc}	22.04 ± 3.44^{c}	0.017
Raw energy (kJ·100 g^{-1})	1,327.92 ± 100.90^{a}	1,061.90 ± 80.70^{b}	1,041.73 ± 79.17^{b}	988.00 ± 75.09^{bc}	825.42 ± 62.73^{c}	764.92 ± 58.13^{c}	< 0.010

*If there are different letters between the means, they are different according to Tukey's test ($p < 0.05$); **confirmed without fungus in fish feed guarantee levels.

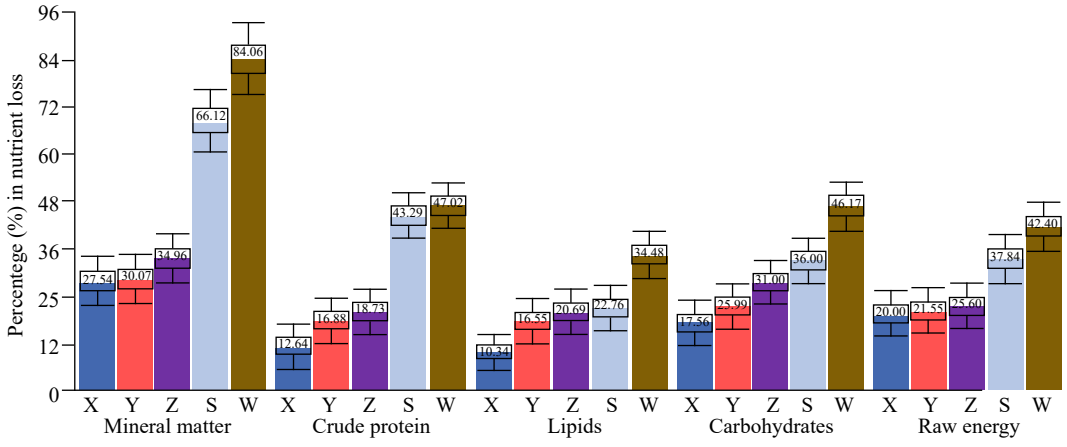

Figure 5. Percentages of nutritional losses caused by fungal contamination in artisanal fish feed.

There was also a substantial loss of lipid content with fungal contamination. The feed without fungi was composed of 2.90 g·100 g^{-1}, while the feeds with fungi W was composed of 1.90 g·100 g^{-1}, representing a loss of 34.48% of lipids. Regarding carbohydrates content, there was also a significant loss. The feed without fungi was composed of 40.49 g·100g^{-1} of carbohydrates, while feeds with W and S fungi were composed of only 22.04 and 26.20 g·100g^{-1} of carbohydrates, representing losses of 46.27 and 36% of carbohydrates (Table 3; Fig. 5).

Concerning the raw energy, there was a significant loss. The feed without fungi expressed 1,327.92 kJ·100 g^{-1}, while the feeds with fungi W, S, and Z expressed only 764.92, 825.42, and 988.00 kJ·100 g^{-1}, respectively, representing losses of 42.40, 37.84 and 25.60% of raw energy, respectively (Table 4; Fig. 5).

Feed texture attributes, buoyancy, and floatation

As fish farms adopted different ways of storing feed, the textural quality was compared in different fish farms. Concerning the texture of commercial fish feed, there were higher averages ($p < 0.05$) of hardness in fish feeds of P1 (10,424.16 N), P15 (10,810.62 N), and P32 (10,471.76 N), while the control (non-fungus feeds) (2,375.22 N), P11 (3,115.16 N), P13 (3,306.10 N), P14 (2,514.31 N), and P24 (3,166.50 N) had the lowest averages ($p < 0.05$) of hardness. The highest averages ($p < 0.05$) of compression strength were required in fish feeds of P4 (22,366.22 kgF·mm^{-2}) and P20 (18,701.16 kgF·mm^{-2}), while the control (5,345.10 kgF·mm^{-2}) showed the lowest ($p < 0.05$) required compression strength. Also, the fish feeds of P1 (13,294.11 N), P4 (13,562.23 N), P15 (12,962.65 N), and P32 (12,097.47 N) showed the highest averages ($p < 0.05$) of fracturability, while the control (2,375.01 N) showed the lowest average ($p < 0.05$) of fracturability (Table 5).

The fish feeds of P3 (4.38 N·mm^{-1}), P16 (4.54 N·mm^{-1}), and P38 (4.40 N·mm^{-1}) showed the highest averages ($p < 0.05$) of chewability, while the control (0.99 N·mm^{-1}), P13 (1.31 N·mm^{-1}), P14 (1.39 N·mm^{-1}), P15 (1.06 N·mm^{-1}), and P27 (1.33 N·mm^{-1}) had the lowest averages ($p < 0.05$) of chewability. Finally, the fish feeds of P1 (1.05 N·mm^{-2}), P3 (0.96 N·mm^{-2}), P11 (1.22 N·mm^{-2}),

Table 5. Averages of texture, hardness, compression strength, fracturability, chewiness, and spreadability attributes of fungus and non-fungus commercial feeds for raised tambaqui (*Colossoma macropomum*).

Fish farms	Texture atributes				
	Hardness (N)	Compression strength (kgF·mm^{-2})	Fracturability (N)	Chewiness (N·mm^{-1})	Spreadability (N·mm^{-2})
Control**	2,375.22 ± 498.80^{c}	5,345.10 ± 1,149.20^{c}	2,375.01 ± 296.88^{c}	0.99 ± 0.11^{c}	0.09 ± 0.01^{c}
P1	10,424.16 ± 2,189.07^{a}	16,164.48 ± 3,475.36^{ab}	13,294.11 ± 1,661.73^{a}	1.12 ± 0.12^{bc}	1.05 ± 0.10^{a}
P3	7,271.82 ± 1,527.08^{ab}	13,990.40 ± 3,007.94^{b}	7,271.61 ± 908.95^{bc}	4.38 ± 0.48^{a}	0.96 ± 0.10^{a}
P4	4,758.65 ± 999.32^{bc}	22,366.22 ± 4,808.74^{a}	13,562.23 ± 1,695.28^{a}	3.05 ± 0.33^{b}	0.67 ± 0.07^{b}
P6	5,323.73 ± 1,117.98^{bc}	10,009.67 ± 2,171.42^{bc}	7,666.49 ± 958.31^{bc}	1.99 ± 0.22^{bc}	0.45 ± 0.05^{bc}
P11	3,115.16 ± 654.18^{c}	14,174.90 ± 3,047.75^{b}	8,644.82 ± 1,080.60^{bc}	2.23 ± 0.24^{bc}	1.22 ± 0.12^{a}
P13	3,306.10 ± 694.28^{c}	16,942.00 ± 3,642.53^{ab}	10,123.84 ± 1,265.48^{b}	1.31 ± 0.14^{c}	0.50 ± 0.05^{bc}
P14	2,514.31 ± 528.00^{c}	8,469.00 ± 1,820.84^{bc}	5,491.45 ± 686.38^{bc}	1.39 ± 0.15^{c}	0.88 ± 0.09^{ab}
P15	10,810.62 ± 2,270.23^{a}	15,115.10 ± 3,249.75^{ab}	12,962.65 ±1,620.33^{a}	1.06 ± 0.12^{c}	0.84 ± 0.08^{ab}
P16	6,178.86 ± 1,297.5^{b}	11,948.40 ± 2,568.91^{bc}	9,063.42 ± 1,132.93^{b}	4.54 ± 0.50^{a}	1.15 ± 0.12^{a}
P17	7,615.33 ± 1,599.22^{ab}	12,594.67 ± 2,707.85^{bc}	10,104.79 ± 1,264.00^{ab}	2.60 ± 0.29^{bc}	1.09 ± 0.11^{a}
P20	5,745.45 ± 1,206.54^{bc}	18,701.16 ± 4,020.75^{a}	12,223.10 ± 1,527.89^{ab}	3.20 ± 0.35^{b}	0.42 ± 0.04^{bc}
P24	3,166.50 ± 664.97^{c}	12,222.26 ± 2,627.79^{b}	7,694.17 ± 961.77^{bc}	2.41 ± 0.26^{bc}	0.67 ± 0.07^{b}
P27	4,379.17 ± 919.63^{bc}	10,740.10 ± 2,309.12^{bc}	7,559.43 ± 922.25^{bc}	1.33 ± 0.15^{c}	1.10 ± 0.11^{a}
P31	4,688.60 ± 984.61^{bc}	16,811.00 ± 3,614.37^{ab}	10,749.59 ± 1,311.50^{ab}	1.84 ± 0.20^{bc}	0.54 ± 0.05^{bc}
P32	10,471.76 ± 2,199.07^{a}	13,723.60 ± 2,950.57^{b}	12,097.47 ± 1,475.89^{a}	1.97 ± 0.22^{bc}	1.00 ± 0.10^{a}
P38	5,939.11 ± 1,247.21^{bc}	14,965.69 ± 3,217.62^{b}	10,452.19 ± 1,275.16^{ab}	4.40 ± 0.48^{a}	0.41 ± 0.04^{bc}
p-value	< 0.010	< 0.010	0.028	< 0.010	0.025

*If there are means ± standard deviation followed by different letters in the columns, they are different according to Tukey's test ($p < 0.05$); *non-fungus commercial feeds for farmed tambaqui.

P16 (1.15 N·mm^{-2}), P17 (1.09 N·mm^{-2}), P27 (1.10 N·mm^{-2}) and P32 (1.00 N·mm^{-2}) showed the highest averages ($p < 0.05$) of spreadability, while the control (0.09 N·mm^{-2}) showed the lowest average ($p < 0.05$) of spreadability (Table 5).

Regarding the texture of artisanal fish feed, there was a higher average ($p < 0.05$) of hardness in fish feeds of PD (8,795.98 N), while the control (non-fungus feeds) (2,660.05 N) and PB (2,186.80 N) had the lowest averages ($p < 0.05$) of hardness. The highest average ($p < 0.05$) of compression strength was required in fish feeds of PE (22,221.47 kgF·mm^{-2}), while the control (5,922.70 kgF·mm^{-2}) had the lowest average ($p < 0.05$) of required compression strength. Also, the control (4,270.38 N) and PE (4,646.51 N) showed the highest averages ($p < 0.05$) of fracturability, while fish feeds of PB (1,885.48 N) showed the lowest average ($p < 0.05$) of fracturability (Table 6).

The fish feeds of CP (3.69 N·mm^{-1}) and PD (3.47 N·mm^{-1}) showed the highest averages ($p < 0.05$) of chewability, while the fish feeds of PA (0.92 N·mm^{-1}) and PB (1.55 N·mm^{-1}) had the lowest averages ($p < 0.05$) of chewability. Finally, the fish feeds of PE (2.71 N·mm^{-2}) showed the highest average ($p < 0.05$) of spreadability, while the control (0.09 N·mm^{-1}) and PB (0.09 N mm^{-1}) showed the lowest averages ($p < 0.05$) of spreadability (Table 6).

Regarding the buoyancy and floatation, the five different brands of feed found were compared. Peletts from control and PA fish feeds showed the highest average ($p < 0.05$) floating time (22 min + 33 sec and 19 min + 28 sec, respectively) and the highest buoyancy percentages (99.70 and 86.14%), while pellets from PD and PE fish feeds showed the lowest average ($p < 0.05$) floating time (13 min + 20 sec and 10 min + 33 sec, respectively) and the lowest percentages of buoyancy (49.54 and 33.19%) (Fig. 6).

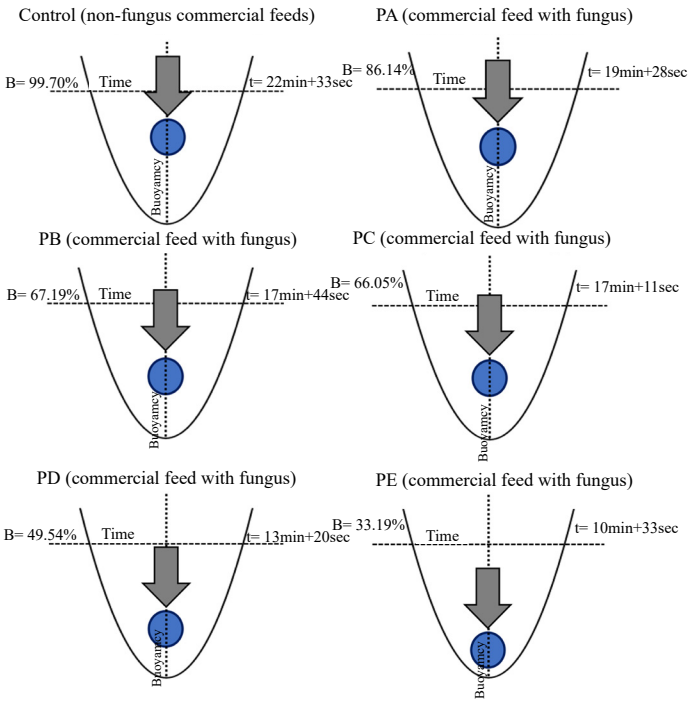

Figure 6. Buoyancy and estimated buoyancy time for non-fungus and with fungus fish feeds.

Pellets from control, PA, and PB fish feeds showed 100% fluctuation up to 15 minutes and more than 90% after 20 minutes of experimentation, while pellets from PD and PE fish feeds showed less than 10% fluctuation after 20 minutes of experimentation. The PC, PD, and PE fish feeds showed the highest means for expansion ratio, bulk density, and relative absorption rate, while the control, PA, and PB fish feeds showed the lowest means for these variables (Table 7).

Table 6. Averages of texture, hardness, compression strength, fracturability, chewiness, and spreadability attributes of fungus and non-fungus artisanal feeds for raised tambaqui (*Colossoma macropomum*)*.

Fish farms	Texture attributes				
	Hardness (N)	Compression strength (kgF·mm^{-2})	Fracturability (N)	Chewiness (N·mm^{-1})	Spreadability (N·mm^{-2})
Control*	2,660.05 ± 558.61^{c}	5,922.70 ± 1,302.99^{c}	4,270.38 ± 854.01^{a}	2.42 ± 0.24^{b}	0.09 ± 0.01^{c}
PA	5,337.45 ± 1,120,.86^{b}	12,165.72 ± 2,676.46^{b}	2,543.85 ± 508.77^{bc}	0.92 ± 0.09^{c}	0.77 ± 0.08^{b}
PB	2,186.80 ± 459.23^{c}	2,586.57 ± 509.05^{bc}	1,885.48 ± 377.10^{c}	1.55 ± 0.16^{c}	0.09 ± 0.01^{c}
PC	3,694.46 ± 775,84^{bc}	16,264.69 ± 3,578.23^{ab}	3,400.94 ± 680.19^{b}	3.69 ± 0.37^{a}	0.80 ± 0.08^{b}
PD	8,795.98 ± 1,847.56^{a}	11,089.11 ± 2,439.60^{b}	2,318.73 ± 463.75^{bc}	3.47 ± 0.35^{a}	2.05 ± 0.21^{ab}
PE	8,271.87 ± 1,737,09^{a}	22,221.47 ± 4,888.72^{a}	4,646.51 ± 929.24^{a}	2.24 ± 0.22^{b}	2.71 ± 0.27^{a}
p-value	0.012	< 0.010	< 0.010	0.024	0.020

*If there are means ±SD followed by different letters in the columns, they are different according to Tukey's test ($p < 0.05$); **non-fungusartisanaleeds for farmed tambaqui.

Table 7. Floatation and stability of non-fungus and fungus fish feed pellets.

Floatation	Control (Non-fungus)	With fungus				
		PA	PB	PC	PD	PE
1 to 5 min	100.00	100.00	100.00	100.00	100.00	75.60
6 to 10 min	100.00	100.00	100.00	100.00	94.00	60.50
11 to 15 min	100.00	100.00	100.00	97.00	77.10	48.40
16 to 20 min	100.00	100.00	98.00	79.50	63.20	38.70
> 20 min	100.00	96.00	83.50	65.15	51.80	30.95
> 30 min	77.00	66.60	52.00	44.65	8.95	0.80
> 60 min	41.00	23.00	15.25	12.00	1.10	0.00
Expansion ratio	2.20	5.50	25.00	30.00	45.00	55.50
Bulk density (mPa·s^{-1})	0.59	0.64	0.73	0.82	0.94	1.09
Relative absorption rate (%)	42.40	48.30	55.10	63.35	72.24	85.25

DISCUSSION

Feeds that are exposed to the elements have higher water activity (Aw) values, facilitating the development of molds and yeasts (Fitri et al., 2022). Fungal contamination can cause economic losses when related to reduced nutritional value, reduced palatability, and health problems related to mycotoxicosis (Abubakar et al., 2016). These factors can affect the health of freshwater fish, causing liver, kidney and eye damage and the formation of neoplasms (Batatinha et al., 2020; Bedoya-Serna et al., 2018).

Starch is the main source of carbohydrates among the ingredients of plant origin used in the formulation of fish feed. Starch is an essential component for expansion and agglutination of feeds for omnivorous fish, as well as high stability for floating foods (Karim et al., 2024). A total of 20% starch is the minimum amount required to ensure good buoyancy of fish feed pellets (Boscolo et al., 2011). However, the production phase and eating habits of farmed fish may vary the nutritional needs of inclusion levels (Coutinho et al., 2018).

Regarding the chemical composition in fish feeds, it must be considered that the maximum moisture allowed by Aquaculture Developing and Control Program (FAO, 1987) of 10 to 13%, the extensive range of variation observed in the artisanal feed (Table 4), probably occurred due to high relative moisture in the storage location. The relationship between moisture and temperature are critical factors in fungal growth and mycotoxin production (Ono et al., 1999). Commercial pelleted feed with an average mineral content of 4.9% showed a content below the limit recommended by the companies, with a maximum content of 9.7–14% (Lall, 1988).

Concerning the nutrient content required by fish, there is a variation between recommended values that differ by age, stage and species of fish (FAO, 1987). Vidal Júnior et al. (1998) determined the protein requirement of juvenile tambaqui at 25.1%. In this sense, the analyzed feeds that demonstrated a protein content between 25 and 30% met the proposed requirements. Thus, the comparison between the chemical composition of commercial and artisanal feeds demonstrated worse nutritional quality, probably due to the lack of extrusion, process that reduces protein denaturation. In relation to lipid levels, they were below the limit of 6–10% recommended by Food and Agriculture Organization of the United Nations (FAO, 1987). The deficiency of this compound prevents the retention and adequate use of proteins.

According to the results of the texture analyzes showed in Tables 6 and 7, the fungi caused hardening and crumbling of the pellets, simultaneously, there was an increase in fracturing and spreadability ($p < 0.05$). Properties related to buoyancy, stability in water and stability during handling are considered critical especially for aquatic foods (Irungu et al., 2019). Adekunle et al. (2012) point out that factors such as these influence the buoyancy of the pellets, significantly reducing their intake by fish. This is because, for the tambaqui to be able to ingest the food offered, the pellets need to float for at least 15 minutes on the surface of the water. Therefore, for artisanal feeds, only control and PA showed adequate floating time. In addition, PC, PD, and PE feeds showed the highest averages of expansion ratio, bulk density, and relative absorption rate.

The expansion ratio and bulk density affect buoyancy, in addition to influencing the durability of the pellets. The increase in the expansion rate after long periods of packaging occurs due to the moistening of the food, which can occur due to poor feed storage conditions, which promotes starch gelatinization (Adeparusi & Famurewa, 2011). Therefore, high averages of this factor may indicate greater loss of nutrients to the water. On the other hand, when evaluating the bulk density, according to Oduntan et al. (2022), the pellets will sink more quickly when the bulk density is lower than 6.4×104 mPa·s^{-1}. In the current study, it was observed that all samples showed lower bulk density, negatively influencing the buoyancy of the feed.

Regarding the water absorption rate, nutritionally poorer diets have greater water absorption, reducing the buoyancy of the feed (Abubakar et al., 2016). Therefore, according to the results found, it can be stated that there were a 50% loss of buoyancy and a considerable increase in the leaching of nutrients from the feed into the water. In other words, instead of the feed serving as food for the fish, a large part of their nutritional content goes straight to the bottom of the fishponds, becoming just a surplus, accumulating together with the organic matter in the sediment.

Therefore, fish feed contaminated by fungi, in addition to being a way of introducing pathogenic microorganisms and mycotoxins into a fish farm, can anticipate the eutrophication process, influencing the physical and chemical variables of water (Bezerra Neto et al., 2023). Terada-Nascimento et al. (2023) found high numbers of fungi in the water of fish farms in the rainy season, including the mycotoxigenic species *Aspergillus fumigatus*, *Penicillium citrinum*, *P. implicatum*, *Fusarium oxysporum*, and *Alternaria alternata.* Furthermore, the authors detected Fumonisins $B_1 + B_2$ (375 to 1,418 µg·kg^{-1}). The water in ponds may have increased turbidity due to the lower levels of dissolved oxygen, which will cause the death of the fish due to any small imbalance or fluctuation in the environment. For example, the greatest biochemical demand for oxygen occurs from dawn, and in the same period there is critical lack of oxygen synthesis (Macedo & Sipaúba-Tavares, 2010).

Surplus fish feed persisting in the sediment can increase nitrogen and phosphorus contents. These factors can cause several problems, such as the risk of increased ammonia content and the proliferation of mycotoxigenic fungi. In addition, it can cause cyanobacteria to bloom (Pinheiro et al., 2023). Ammonia is a very restrictive toxicant for fish life, with many species not supporting concentrations above 5 mg·L^{-1}, and values above 0.01 mg·L^{-1} can be toxic to fish. However, total ammonia is divided into ionized ammonium (NH_4) and non-ionized NH_3, the latter of which has a toxic effect. Above pH 9, with each increase in Log_{10}, the toxic potential increases 10x (Moura et al., 2014).

Another issue worth raising is that fish nutrition in this context is neglected, because fish are unable to ingest the food or it does not meet their nutritional needs because the nutritional quality of the food, when contaminated, is impaired. Faced with this problem, fish farmers suffer critical economic losses. For example, in a fish farm in the state of Rondônia, the cost of feeding the fish can result 81% of the production cost, the loss is so critical that it is difficult to account for the economic loss of an entire production cycle (Martins et al., 2020). Therefore, it is imperative that fish farmers be aware of their commitment to properly store fish feed. This awareness becomes even more necessary due to the high prices of inputs for preparing fish feed, such as corn and soy. Consequently, there is no point in having a balanced diet with high nutritional standards, although, if there is no adequate storage, the feed will lose its original composition and will have sources of contamination for the fishponds water, in addition to the effluents having their contamination load increased. Thus, it can be inferred that fungal contamination can facilitate the introduction of pathogens into the fishponds water or cause an ecotoxicological risk, consequently stretching a public health and environmental problems (Johny et al., 2019).

Terada-Nascimento et al. (2023) found mycotoxigenic fungi in the water from fishponds and Fumonisins in fish feed in 16 fish farms in the Rondônia state. The authors verified a correlation between these fungi and their mycotoxins and erythrocyte changes in the blood cells of tambaqui (159 anomalies) and *Leptodactylus petersii* (299 anomalies). Erythrocytes undergoing apoptosis, micronucleated, undergoing pyknosis and karyorrhexis attested the risk of mycotoxins for farmed fish and aquatic fauna in fish farms.

CONCLUSION

Fungal contamination in fish feeds can negatively influence nutritional, physical and chemical quality, leading to higher production costs, besides accelerating the eutrophication process, because contaminated feed presents a loss of buoyancy and increased leaching. That is, instead of serving as food for the fish, much of the feed goes to the bottom of the pond. Therefore, contaminated fish feeds can represent health, environmental, and economic problem for fish farms. Although such results were obtained, it is suggested that an analysis of economic loss with fungus feed be continued.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

Data will be available upon request.

AUTHORS' CONTRIBUTION

Conceptualization: Nascimento, J.S.T., Dantas Filho, J.V.; **Formal Analysis:** Nascimento, J.S.T., Dantas Filho, J.V., Santos, B.L.T., Adriano, A.C.A., Cama, J.L.V.; **Writing – Original Draft:** Nascimento, J.S.T., Dantas Filho, J.V.; **Formal Analysis:** Nascimento, J.S.T., Dantas Filho, J.V.; **Software:** Dantas Filho, J.V., Cavali, J.; **Data Curation:** Dantas Filho, J.V., Cavali, J., Soares, E.C., Schons, S.V.; **Writing – Review & Edition:** Cavali, J., Soares, E.C., Schons, S.V.; **Supervision:** Schons, S.V.; **Final approval:** Schons, S.V.

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