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Research Article

Sustainable alternative for the use of invasive species of golden mussel (*Limnoperna fortunei*) in the feeding of Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT. The aquaculture of Nile tilapia (*Oreochromis niloticus*) in cages has faced problems with invading golden mussels (*Limnoperna fortunei*), an exotic species that shows uncontrolled dissemination. A possible use of golden mussel flour as a fish feed ingredient is investigated in this study. The assessment as food ingredient includes the description of the raw material processing and the analysis of the performance of Nile tilapia, submitted to diets containing different proportions of golden mussel flour, substituting for the traditional source of calcium in fish feeds. The mussels were collected in a fish farm in the northwest of the State of São Paulo and processed in the laboratory. The diets were prepared with different mussel flour proportions (0, 0.35, 0.68, 1.35, and 2.69%). One hundred fifty Nile tilapia fingerlings weighing 4.69 g were distributed in 15 tanks of 150 L. Neither heavy metals nor total coliforms were detected in the analyses of the golden mussel. The chemical-bromatological composition of golden mussels presented 13.41% crude protein, 1.47% ether extract, 30.58% calcium, and 0.20% phosphorus. Golden mussel flour proved an excellent substitute for limestone, yielding similar results concerning the zootechnical variables and better results regarding body composition variables. Despite the successful use of golden mussel flour as a food ingredient, it may not be the final solution for the problems caused by the mollusk. In any case, controlling its dissemination in nature can be an efficient and sustainable method.

Keywords: *Oreochromis niloticus*; *Limnoperna fortunei*; alternative feed; bivalve mollusk; environmental control; source of calcium; aquaculture

INTRODUCTION

Aquaculture has become a successful food-producing sector that has boosted local and world economies. According to the Food and Agriculture Organization (FAO), 50% of the world's fish products are destined for food production. With the increasing demand for food, it will be necessary to increase food production to supply the world's population (FAO 2020).

The Nile tilapia (*Oreochromis niloticus*) is an option protein source of high nutritional quality for many countries and a potential source of income (FAO 2022). The Nile tilapia was introduced in Brazil in the 1970s, coming from African countries and easily adapted to freshwater. Its zootechnical qualities are rusticity, precocity, and fecundity, which allows it to be bred in different breeding systems which in 10 years, from 2005 to 2015, has made it the most produced fish

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species in Brazil (MMA/IBAMA 2017). Fish breeding in tanks has succeeded over other systems. Tanks or hapas are "floating cages" of various shapes and sizes made of screen or net, enabling free water circulation. They are installed using floats or fixed piles, preventing water column oscillation (Teixeira et al. 2009).

Golden mussels have caused problems in fish farming. Intercontinental navigation brought them to the South American continent in ballast water on vessels (Darrigran & Mansur 2009, Mansano et al. 2022). *Limnoperna fortunei*, or golden mussel, is a bivalve mollusk characterized by its ease of dissemination in freshwater environments. The IUCN Invasive Species Specialist Group (ISSG) reports it as invasive. In Brazil, the Ministry for the Environment created a national plan for the prevention, control, and monitoring of golden mussel (*L. fortunei*) via Ordinance 3639, dated December 10, 2018, whose main focus is the control of golden mussel dissemination in the Brazilian territory. However, this control still needs to be improved, as golden mussel uncontrollably advances in Brazilian rivers.

According to the National Biodiversity Commission - CONABIO Act 5, dated October 21, 2009, in Brazil, the golden mussel is considered an "invasive exotic species", which nowadays is the second major menace to the country's biodiversity. It grows in screens and other metallic surfaces of fish tanks, causing extra weight that can damage the tank structure and decrease fish weight gains, leading to fish mortality. Besides, the mussel causes clogging in water collection systems, hydroelectric plants, and drainage systems (Mansano et al. 2022).

Golden mussels are characterized by early sexual maturation and few predators, which make them an environmental problem. They feed on suspended matter, such as phytoplanktonic and zooplanktonic communities, which decreases density and biomass (Frau et al. 2016). The Emergency Action Plan has resulted from obvious ecological and socioeconomic risk conditions. Due to the increase in aquatic transport, live fish transport, floating platforms, and ponds, the golden mussel has been widely disseminated in Brazilian waters and networks (Belz 2009, Darrigran & Mansur 2009).

The objective of control plans is to refrain from golden mussel dissemination utilizing the vectors involved in its dispersion. Even with these strategies, there are no available methods to eradicate golden mussels, making this task difficult even in places where the invasion has just begun (IBAMA 2020). Certain practices have been adopted to remove the mussels to diminish this species' impact on installations and underwater equipment, such as spraying, physical control, scraping, filtration, and chemical control - but failures still exist. The problem is in the destination of these mussels removed from installations and rivers, once studies of forms of use and destination are few.

Based on a study by Almeida et al. (2006), we envisaged using golden mussels in animal nutrition. We consider *L. fortunei* an alternative source of certain ingredients in feed formulations, which is also interesting from an economic and sustainable viewpoint. Before making available or producing feeds, it is very important to analyze the product to assess food availability and composition properly (Generoso et al. 2008).

Golden mussel flour is calcium-rich and can become a source of nutrients in ideal proportions. During the formation of tissues and bones, chemical elements play an important role in muscle growth and several metabolic and physiological processes essential for fish growth and reproduction. Although elements like calcium can be absorbed directly from water, the main calcium source is food (Kubitza 2011).

According to Canzi (2011), golden mussels are poor in protein and fat but are a good source of mineral elements, such as calcium. This study aimed to evaluate the possible use, adequate, and sustainable destination of the invasive species *L. fortunei* as a food ingredient in the productive performance of Nile tilapia fingerlings, replacing a traditional calcium source used in fish diets.

MATERIALS AND METHODS

The experiment was conducted in the Sustainable Aquaculture Laboratory of Brazil University, Fernandópolis Campus, for 48 days. A total of 150 Nile tilapia fingerlings initially weighing 4.5 ± 0.6 g were tested. The specimens were distributed in 15 tanks of 150 L of useful volume each, arranged in a completely randomized design. Five treatments and three repetitions were applied to the 10 animals in each of the 15 tanks. The experiment was conducted according to ethical guidelines in Brazil's National Council for the Control of Animal Experimentation. The materials and methods (Protocol 1900021) used in the present trial were approved by the Ethics Committee on Animal Use of the Brazil University, Fernandópolis, State of São Paulo, Brazil.

Mollusk collection and flour preparation

Golden mussels encrusted in cages of a fish farm located on the margin of the Paraná River - northwest of the State of São Paulo, southeastern Brazil - were

collected for the experiment. During the collection of mussels, the presence of other mollusks encrusted in the fish farming net tanks was not detected, and only the golden mussels were collected. The Paraná River is considered the eighth longest river in the world (4880 km). It is the second - after the Amazonas River - longest river in South America, crossing five countries before reaching the Prata River Estuary.

The golden mussels were collected between September and December at four different times, i.e. one collection per month. The individuals collected had an average weight of 0.11 ± 0.06 g and an average length of 8.95 ± 5.02 mm. After collection, the mussels were washed (chloride water, 250 mg L^{-1}), placed on plastic trays, and divided into equal quantities. Any seaweed or other contaminant was removed during washing to avoid oscillations in flour quality. They were dried (55ºC) in a forced-air oven (Fanem®, Model Ar320) for 72 h, being stirred every 12 h for a more uniform drying (Fig. 1). After drying, the mussel specimens were ground and stored, and they were processed integrally with their valves. This procedure was done quickly to prevent the material from getting wet. A hammer mill equipped with a 0.5 mm diameter sieve was used for grinding and sieving, resulting in a homogeneous flour easily added to the mixes. The flowchart in Figure 1 summarizes the process to obtain the whole mussel flour.

Experimental diets and water quality

Different proportions of golden mussel flour were used (diet 1: 0%; diet 2: 0.35%; diet 3: 0.68%; diet 4: 1.35%; and diet 5: 2.69%). Different levels of golden mussel flour were added to replace the limestone until it was completely replaced. In turn, the calcium level was kept similar in all diets to balance them. Glutamic acid was added to maintain the same protein level in the diets. Table 2 describes the constitution of the five diets used in this study.

The ingredients of each diet were weighed and bagged, always properly identified on the bags. The bags were sent to the Feed Factory, where the ingredients were ground and extruded to 1 to 2 mm diameter with a Ferraz® model E-62 twin screw extruder. The temperature during the process was maintained between 110 to 120°C. Following extrusion, the feed was dried at 105°C for 30 min. After processing the diets, they were analyzed to determine the bromatological composition (Table 3).

During the experimental period, the diets were administered three times daily (8:00, 12:00, and 17:00 h), *ad lubitum*. The diets were well received, and the fish were soon adapted to them, proving them to be palatable. The diets after processing were kept frozen (18°C) until they were fed to the fish.

The tanks were siphoned daily to remove food remains and feces and maintain water quality. During the experimental period, water quality parameters, such as mean temperature (ºC), were monitored daily. Oxygen, pH, ammonia, nitrite, and nitrate were monitored once a week, determined in a multiparameter photometer for aquaculture "HI83303-01" respectively (Table 4). The values found for water quality were within the ideal conditions for cultivating Nile tilapia, as suggested by Sipaúba-Tavares & Santeiro (2013) and Kubitza (2018).

Laboratory analyses

After 48 days, all the fish were submitted to a 24 h fasting (emptying of the digestive tract) to avoid complications during sedation. The specimens were sedated by immersion in benzocaine diluted in water (250 mg L^{-1}). The fasting specimens were weighed for the biometrics.

The specimens were placed in tanks where continuous water circulation and aeration were maintained. Five specimens were collected per treatment. They were euthanized and later ground (total weight, including viscera) and stored in aluminum containers. The containers were kept in an oven at 55ºC for 72 h. After obtaining this dry matter, the material was sent to the laboratory for grinding in a high-speed blender until a homogeneous powder was obtained.

The dry matter samples were sent to a laboratory for the analysis of protein (Dumas method, using a Leco 528 LC equipment - Etheridge et al. 1998); fat (acid hydrolysis method - AOAC 2016); minerals, and water (AOAC 2016). Diet samples were analyzed for amino acids using liquid chromatography in cationic exchange resin columns and post-column derivation with ninhydrin and an auto-analyzer. Previously, the samples were hydrolyzed with 6N HCl for 22 h at 110°C, according to Moore & Stein (1963). Tryptophan was determined after the enzymatic hydrolysis with Pronase at 40°C for 24 h, followed by a colorimetric reaction with 4-dimethyl-amino-benzaldehyde in sulfuric acid 21.2 N and analyzed at 590 nm using a spectrophotometer. The tryptophan content was calculated according to Spies (1967).

Analysis of the zootechnical parameters

The growth and feeding parameters assessed during the present experiment included: live weight gain (g) (LWG) = final weight - initial weight; food intake (g)

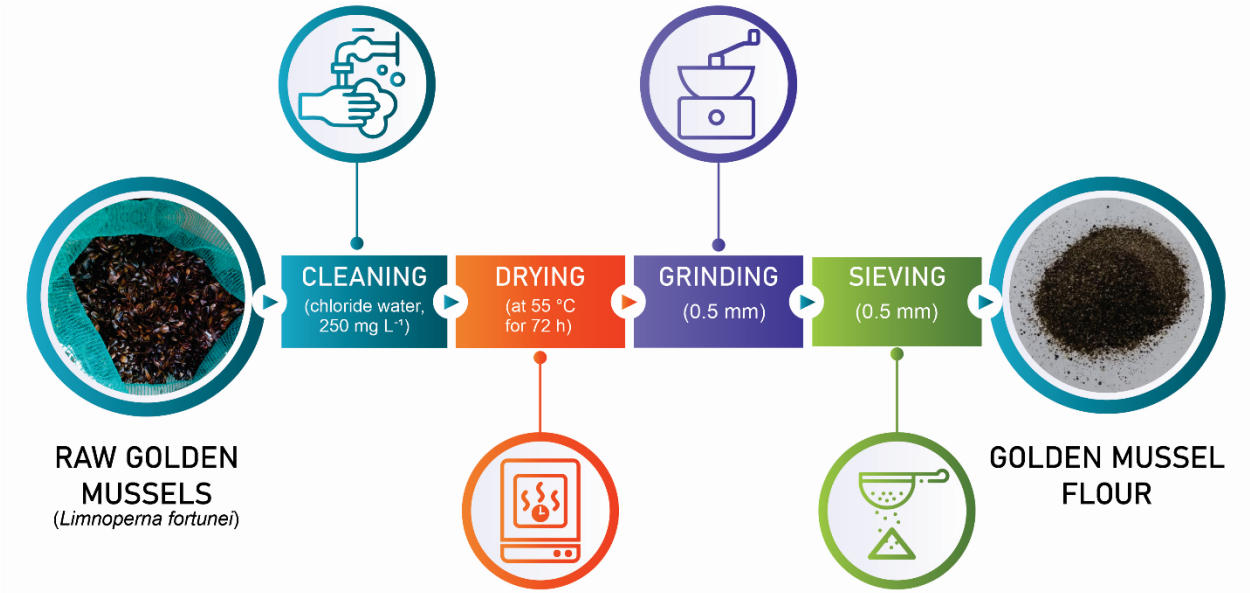

Figure 1. Flowchart summarizing the steps of whole mussel flour preparation. Source: author's archive.

(FI) = (total quantity of feed offered to fish); feed conversion ratio (g g^{-1}) (FCR) = [(feed intake) / (live weight gain)]; food efficiency (g g^{-1}) (FE) = [total weight gain (g) / diet consumption (g)]; specific growth rate (% d^{-1}) (SGR) = [(ln final weight - ln initial weight) × 100/ (time (d))] and deposition of nutrients (g) (DN) = [(live weight gain × nutrient content in the body) / 100].

Statistical analysis

A significance level of 5% was considered in the analysis of variance (ANOVA). Should significance be detected, Fisher's exact test was applied to mean values to compare the treatments. Dunnett's test compared the control treatment (diet 1: 0% inclusion of golden mussel flour) with the other four treatments. Software Minitab (2018) was used in the tests.

RESULTS

The golden mussels used in the diets were collected in the Paraná River. After collecting, they were processed to make the golden mussel flour. The mussel flour was sent to the laboratory for analysis. Its centesimal composition (bromatological, microbiological, and residue analyses) was determined before the formulation and preparation of the experimental diets. The results of the biological quality and centesimal composition analyses are listed in Table 1. It was possible to evidence that its centesimal composition presents characteristics of nutritional ingredients, mainly as a source of calcium in fish feeding.

Table 5 presents the mean values of the zootechnical variables: initial weight, weight gain, diet consumption, apparent feed conversion ratio, food efficiency, specific growth rate and protein efficiency rate. According to the results obtained for the zootechnical variables, no statistical difference was found about the control treatment (diet 1: 0% inclusion of golden mussel flour). We observed that the performance indices remained at the same levels; that is, when the traditional calcium source (limestone) was replaced by mussel flour, the performance was similar, which can be explained by the fact that calcium contents in traditional calcium sources, such as limestone, are close to those of the mussel flour, as attested by the centesimal composition analyses.

Regarding the chemical composition of the Nile tilapia juveniles fed with different golden mussel flour proportions, significant differences ($P > 0.05$) were observed in crude protein, fat, and water contents but not in mineral contents in their bodies (Table 6). An improvement in body composition characteristics is observed, mainly due to increased body protein, using golden mussel flour as a substitute for calcium sources.

DISCUSSION

L. fortunei is a freshwater mytilid of southeastern Asia. Despite being restricted to and native to China, it spread to other Asian countries, such as Laos, Cambodia, Vietnam, Indonesia, and Thailand (Oliveira et al. 2006), typically in freshwater systems, rivers, and

Table 1. Centesimal composition and microbiological of golden mussel flour.

Parameters	Unit	Results	Methodology
Total coliforms	CFU g^{-1}	1.5×10^{1}	Salfinger & Tortorello (2015)
Phenylalanine + tyrosine	%	0.377	Allen et al. (1999)
Phenylalanine	%	0.196	Allen et al. (1999)
Glycine	%	0.689	Allen et al. (1999)
Methionine + cysteine	%	0.223	Allen et al. (1999)
Crude protein	%	13.41	Etheridge et al. (1998)
Threonine	%	0.061	Allen et al. (1999)
Tryptophan	%	0,162	Allen et al. (1999)
Arginine	%	0.179	Allen et al. (1999)
Glutamic acid	%	0.080	Allen et al. (1999)
Heavy metals (such as lead)	ppm	0.328	AOAC (2016)
Mineral matter	%	75.93	AOAC (2016)
Total phosphorus	%	0.199	AOAC (2016)
Total calcium	%	30.58	AOAC (2016)
Kreiss reaction/rancidity		Negative	Instituto Adolfo Lutz (2008)
Humidity and volatiles at 105ºC	%	1.58	AOAC (2016)
Ether extract	%	1.47	AOAC (2016)
Aspartic acid	%	0.042	Allen et al. (1999)
Alanine	%	0.152	Allen et al. (1999)
Cysteine	%	0.003	Allen et al. (1999)
Histidine	%	0.062	Allen et al. (1999)
Isoleucine	%	0.167	Allen et al. (1999)
Leucine	%	0.243	Allen et al. (1999)
Methionine	%	0.218	Allen et al. (1999)
Proline	%	0.353	Allen et al. (1999)
Serine	%	0.101	Allen et al. (1999)
Tyrosine	%	0.181	Allen et al. (1999)
Cystine	%	0.006	Allen et al. (1999)
Lysine	%	0.178	USPC (2016)
Acidity level	NaOH mg g^{-1}	0.510	Instituto Adolfo Lutz (2008)

lakes. This species has been identified in Hong Kong, Taiwan, and Japan. In South America (Argentina, Uruguay, Paraguay, Brazil, and Bolivia), it is found in the Prata and the Guaíba basins. In Brazil, the golden mussel is already found in the main water bodies, in the states of Rio Grande do Sul, Santa Catarina, Paraná, Sao Paulo, Minas Gerais; Mato Grosso do Sul, Mato Grosso; Goiás and Bahia, reaching the Uruguay River basins; Iguaçu River; Tiete River; Big River; Parana River; Paraguay River; Paranaiba River; San Francisco River (CBEIH 2015, Oliveira et al. 2015).

There are no records of predators in the golden mussel's natural environment. However, in areas where the mussels were introduced and with population growth, the following fish species are reported as predators: *Leporinus obtusidens*; *Schizodon borellii*; *Piaractus mesopotamicus*; *Pterodoras granulosus*; *Rhinodoras dorbignyi*; *Oxydoras kneri*; *Pimelodus maculatus*; *Pimelodus albicans*; *Pimelodus argenteus*; *Brochiloricaria chauliodon*; *Hypostomus laplatae*; *Hypostomus uruguayensis*; *Paraloricaria vetul*a; *Megalancystrus parananus*; *Pseudohemiodon laticeps*; *Cyprinus carpio*; *Potamotrygon brachyurus*; and *Micropogonias furnieri* (García & Montalto 2006). Cesar et al. (2003) also described the *L. fortunei* predation by blue crab (*Callinectes sapidus*) in the Prata River Estuary. However, even if these aquatic organisms are sporadic golden mussel predators, they cannot control the mollusk dissemination (IBAMA 2020).

Mussel incrustation in freshwater bodies is an economic and environmental problem for South American rivers. Until the beginning of the 1990s, settlement and proliferation occurred only in marine and brackish waters of neotropical regions. Since the introduction of *L. fortunei*, the recording has extended to freshwater bodies in Argentina, Brazil, Paraguay, and Uruguay (Darrigran & Damborenea 2005), a conse-

Table 2. Formulation of experimental diets used for Nile tilapia juveniles supplemented with golden mussel flour. [1]Moisture (%) 2.0, ash (%) 71.64, choline (mg kg^{-1}) 30,000, magnesium (%) 0.0085, sulfur (%) 1.16, iron (mg kg^{-1}) 25,714, copper (mg kg^{-1}) 1960, manganese (mg kg^{-1}) 13,345, zinc (mg kg^{-1}) 30,000, iodo (mg kg^{-1}) 939, selenium (mg kg^{-1}) 30, vitamin A (IU kg^{-1}) 600,000, vitamin D3 (IU kg^{-1}) 600,000, vitamin E (mg kg^{-1}) 12,000, vitamin K3 (mg kg^{-1}) 631, vitamin E (mg kg^{-1}) 12,000, vitamin K3 (mg kg^{-1}) 631, vitamin E (mg kg^{-1}) 12,000, vitamin K3 (mg kg^{-1}) 631, vitamin E (mg kg^{-1}) thiamine B1 (mg kg^{-1}) 1176, riboflavin B2 (mg kg^{-1}) 1536, pyridoxinE B6 (mg kg^{-1}) 1274, vitamin B_{12} (mcg kg^{-1}) 4000, niacin (mg kg^{-1}) 19,800, pantothenic acid B3 (mg kg^{-1}) 3920, folic acid (mg kg^{-1}) 192, biotin (mg kg^{-1}) 20, vitamin C (mg kg^{-1}) 40,250.

Ingredients	Inclusion golden mussel flour (%)				
	0.00	0.35	0.68	1.35	2.69
Fish meal	9.00	9.00	9.00	9.00	9.00
Corn gluten (60%)	8.00	8.00	8.00	8.00	8.00
Soybean meal	30.00	30.00	30.00	30.00	30.00
Rice broken	9.00	9.00	9.00	9.00	9.00
Corn grain	27.11	27.05	27.01	26.85	26.61
Wheat bran	9.00	9.00	9.00	9.00	9.00
Soybean oil	2.00	2.00	2.00	2.00	2.00
L-lysine	0.40	0.40	0.40	0.40	0.40
DL-methionine	0.15	0.15	0.15	0.15	0.15
L-threoin	0.45	0.45	0.45	0.45	0.45
L-phenylalanine	0.10	0.10	0.10	0.10	0.10
Tryptophan	0.10	0.10	0.10	0.10	0.10
Glutamic acid	0.29	0.25	0.20	0.15	0.00
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00
Limestone	1.90	1.65	1.41	0.95	0.00
Golden mussel flour	0.00	0.35	0.68	1.35	2.69
Mineral and vitamin supplement[1]	0.50	0.50	0.50	0.50	0.50

quence of the proliferation of larvae or *L. fortunei* fingerlings, affecting water sources of water treatment stations, industrial refrigeration systems, hydroelectric power plants, vessels, and the commercial production of fish in cages.

Up to now, it seems impossible to stop golden mussel propagation in natural environments - but propagation can be decelerated. Proper prevention methods can avoid their introduction in installations. Despite available control methods that remove, use, or eliminate mussels from substrates contaminated by man, there are better ecological methods than these because the destination of the removed mussels is not a proper choice. One of the main controls in fish farms is mechanical removal; however, the mussel is usually sent back to the water body or is discarded on the soil or composter - it is not considered a nutritional source or even a product of economic value.

We propose using the golden mussel as a nutritional ingredient and calcium source. We collected discarded golden mussels mechanically removed from a fish farm. This material was processed (dried, ground, and sieved as described in the materials and methods section) and analyzed in the laboratory. The centesimal composition analysis showed that the golden mussel is free of heavy metals or total coliforms in concentrations above the recommended by the Ministry for Agriculture for ingredients of animal origin; on the other hand, the use of the product should be subject to research to analyze the deposition of these elements in the tissues, depending on the collection sites. The process proposed here is very simple, depending only on the collection, washing, drying, grinding, and sieving, not on special equipment.

The chemical-bromatological composition of the golden mussel flour presented 13.41% crude protein, 1.47% ether extract, 30.58% calcium, and 0.20% phosphorus (Table 1). When compared to the calcium contents in limestone (37.7%), calcium carbonate (40.0%), and oyster flour (36.4%) (Rostagno et al. 2017), those of the golden mussel flour are very similar to these calcium sources. It is worth mentioning that mussels' body composition can change depending on the time of year and reproductive period. However, these mollusks reproduce practically all year round in the collection region. According to Boltovskoy et al. (2015), based on studies on the gametogenic cycle in South America, the mature sperm and eggs were recor-

Table 3. Composition of experimental diets for Nile tilapia juveniles supplemented with golden mussel flour. [1]Dumas method in Leco 528 LC apparatus (Etheridge et al. 1998). [2]Digestibility, according to Furuya (2010). [3]Determined by bomb calorimetry (AOAC 2016). [4]Acid hydrolysis (AOAC 2016). [5]Acid hydrolysis and ion-exchange chromatography (High-Performance Liquid Chromatography - HPLC).

Composition (dry matter base)	Inclusion golden mussel flour (%)				
	0.00	0.35	0.68	1.35	2.69
Crude protein (%)[1]	30.18	30.17	30.16	30.18	30.17
Digestible protein (%)[2]	26.38	26.38	26.38	26.38	26.38
Crude energy (cal)[3]	4035	4032	4040	4034	4024
Crude ether extract (%)[4]	5.70	5.65	5.72	5.68	5.67
Calcium (%)[4]	1.32	1.32	1.32	1.33	1.33
Phosphorus (%)[4]	0.68	0.68	0.68	0.68	0.68
Essential amino (dry matter base) (%)[5]					
Arginine	1.82	1.80	1.83	1.82	1.79
Histidine	0.68	0.69	0.66	0.70	0.65
Isoleucine	1.21	1.19	1.22	1.23	1.19
Leucine	2.62	2.63	2.65	2.60	2.65
Lysine	1.65	1.66	1.63	1.62	1.64
Methionine	0.65	0.65	0.66	0.65	0.66
Phenylalanine	1.51	1.53	1.50	1.49	1.54
Threonine	1.50	1.49	1.52	1.48	1.51
Tryptophan	0.38	0.39	0.37	0.38	0.37
Valine	1.36	1.35	1.36	1.34	1.33

Table 4. Mean values of the water quality parameters obtained during the experimental period of 48 days. [1]Mean temperature (ºC) was monitored daily. [2]Ammonia, nitrite, nitrate, oxygen, and pH were monitored once a week.

Ammonia[2]	Nitrite[2]	Nitrate[2]	Oxygen[2]	pH[2]	Temperature[1]
mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}		°C
0.1 ± 0.01	0.04 ± 0.05	0.06 ± 0.05	7.1 ± 1.5	7.8 ± 0.2	25.1 ± 0.6

ded throughout the year, with several intermittent propagation events, resulting in relative continuous and marked reproduction with seasonal peaks in spring and late summer, and larval production during 6 to 10 months throughout the year.

Almeida et al. (2006) obtained 7.38% crude protein in whole golden mussel flour, which is below the values of our study. In turn, Canzi (2011) obtained 12.95% crude protein, closer to our values. Furlan et al. (2007) suggest that the protein content can vary according to the collection site and food availability for these mollusks in the environment. Even if the protein content in the golden mussel flour is low, it must be considered, as it is very close to the protein content in corn grains. The mineral content obtained for the mussel flour was 75.93%, which is considered high, attesting to the value of this ingredient as a mineral element source, especially calcium. Canzi (2011) also found high mineral contents (80.53%) in her study of golden mussels.

The golden mussel flour is calcium-rich but phosphorus-poor. It should not be used as a substitute for dicalcium phosphate but for limestone, which is used only as a calcium source in nutrition practices. According to Fracalossi & Cyrino (2013), individuals fed with low-phosphorus feed showed a worsening in feed conversion, reduced food consumption, and lower weight gains, which can be associated with low calcium-to-phosphorus ratios. Canzi (2011) observed a deficient growth of Nile tilapias fed with golden mussel flour, which resulted from the high mussel flour contents (20%) added to the feed. Another fact of Canzi's study that calls attention is using this ingredient as a substitute for protein and energy ingredients and for dicalcium phosphate, making the diet deficient mainly in phosphorus.

To conduct food microbiology is essential for feed assessment. Petri (2002) calls our attention to Law 6198, dated December 26, 1974, and Decree 76986 of 1976 in Brazil, which establish the general norms on

Table 5. Mean ± standard deviation values of the zootechnical variables for Nile tilapia juveniles fed with increasing golden mussel flour proportions. *A significance level of 5% was considered in the analysis of variance (ANOVA). SGR: specific growth ratio, PER: protein efficiency rate.

Inclusion golden mussel flour	Initial weight (g)	Weight gain (g)	Diet consumption (g)	Feed conversion ($g\ g^{-1}$)	Food efficiency ($g\ g^{-1}$)	SGR (%)	PER ($g\ g^{-1}$)
0.00%	4.93 ± 1.1	41.72 ± 2.5	35.46 ± 1.5	0.85 ± 0.04	1.17 ± 0.06	3.80 ± 0.05	3.88 ± 0.21
0.35%	4.33 ± 0.4	40.46 ± 2.1	32.62 ± 0.7	0.80 ± 0.02	1.23 ± 0.03	3.76 ± 0.05	4.09 ± 0.12
0.68%	4.51 ± 0.6	39.45 ± 1.5	33.24 ± 1.7	0.84 ± 0.01	1.18 ± 0.01	3.74 ± 0.04	3.92 ± 0.05
1.35%	4.49 ± 0.3	43.13 ± 4.5	35.71 ± 2.4	0.83 ± 0.08	1.20 ± 0.12	3.82 ± 0.15	3.97 ± 0.41
2.69%	4.34 ± 0.5	42.05 ± 3.8	35.37 ± 0.9	0.84 ± 0.08	1.18 ± 0.11	3.80 ± 0.07	3.93 ± 0.38
*_P_-value	0.801	0.828	0.112	0.899	0.903	0.816	0.903

Table 6. Mean ± standard deviation body composition of the Nile tilapia juveniles fed with increasing golden mussel flour proportions. *A significance level of 5% was considered in the analysis of variance (ANOVA).

Inclusion golden mussel flour	Mineral matter (%)	Protein (%)	Fat (%)	Water (%)
0.00%	3.68 ± 0.13	12.91 ± 0.09^{c}	6.45 ± 0.22^{a}	74.65 ± 0.55^{c}
0.35%	3.49 ± 0.11	13.49 ± 0.15^{ab}	5.89 ± 0.19^{b}	76.18 ± 0.44^{b}
0.68%	3.37 ± 0.22	13.55 ± 0.13^{ab}	5.56 ± 0.25^{b}	77.41 ± 0.50^{a}
1.35%	3.70 ± 0.09	13.65 ± 0.11^{ab}	5.71 ± 0.27^{b}	76.17 ± 0.37^{b}
2.69%	3.59 ± 0.15	13.73 ± 0.10^{a}	5.95 ± 0.15^{b}	75.44 ± 0.42^{b}
*_P_-value	0.098	0.001	0.006	0.001

inspection and control of animal food production. Both summarize the "good production practices" regarding hygienic, sanitary, and operational procedures applied throughout the production flowchart. Jin & Kirk (2018) point out that most bacteria, among other microorganisms, grow in pH greater than 4.5, which suggests the analysis of the food pH. Regarding total coliforms, considerable levels were not detected in the centesimal composition of the collected golden mussels. Santos et al. (2000) report that the microorganisms that best indicate and represent sanitary quality are those of the thermotolerant coliforms group; salmonella is not tolerated in the case of feeds. It is worth mentioning that for any commercialization of flour of animal origin, the risk of possible contamination of pathogens and its dissemination to organisms of other species or even humans. In this sense, it should always be remembered to consult the official committees of each country for the proper sanitary control and commercialization of these products.

The apparent feed conversion did not significantly vary among the treatments, resulting in less than 1 in response to the diets containing similar nutrient concentrations. Another factor is the density - 10 ind $tank^{-1}$ - which allows easy access to food and better water quality. The age and size of the tilapia fingerlings also influenced, as better food conversions are usually found in this development stage. Good acceptance and adaptation to the feed were observed during the experimental period, resulting in excellent consumption. Considerable results were obtained for the zootechnical variables: weight gain ± 42 g feed conversion ± 0.80 g g^{-1}, and PER ± 3.95 g g^{-1} (Table 5). It was found that the addition of 2.69% mussel flour is positive, as there is no negative interference in the performance of the animals.

Table 6 also shows that the specimens that consumed golden mussel flour yielded a better body composition, with higher protein and water concentrations and lower fat concentrations, which is expected, as there is an inverse relationship between fat deposition and protein and water concentrations. This fact can be explained by the better nutritional balance obtained when adding golden mussel flour. The advantages of golden mussel flour are similar to those of limestone, such as better calcium availability and lower protein and fat concentrations. According to Signor et al. (2007), the chemical composition of the animal body reflects the composition of the nutrients supplied via the feed; in other words, unbalanced food

regarding nutrients can lead to different nutrient (proteins, lipids, etc.) contents in the fish bodies.

Body composition changes along the life cycle, and its use is affected by endogenous (species, size) and exogenous factors, such as time of the year and feed composition (Dumas et al. 2007). According to Bureau et al. (2002), nutritional factors, such as the balance of available amino acids, essential amino acids, protein concentration, and the protein-to-energy ratio of the feed, are important in the deposition of protein and lipids in the tissues. Therefore, seasonal changes occur in body composition during growth, associated with the endocrine state and special physiological stages. In the growth stage, however, new tissues are synthesized and formed (Dumas et al. 2010).

Water quality parameters were analyzed during the experimental period and were maintained within the desired limits. The use of unconventional animal feed can optimize food production, making the use of resources and nutrient recycling possible, thus assuming an important role in sustainable activity.

CONCLUSION

There are no specific programs to avoid golden mussel propagation in the natural environment, but alternative methods can reduce mollusk dissemination. Proper prevention methods can hinder its introduction in installations. This study presents an alternative sustainable way of using golden mussel flour as a food ingredient. It is not the final solution for the golden mussel issue, but it can efficiently control the mollusk population dissemination in the environment.

Golden mussel flour proved to be an excellent substitute for limestone in the case of some constituents of the tilapia diets. The assessment of the zootechnical variables showed that weight gain, feed conversion, and PER did not statistically differ from other types of fish food. On the other hand, positive statistical differences ($P < 0.05$) were observed in body composition for the specimens fed with golden mussel flour. We recommend the addition of 2.69% mussel flour in the fish diet, as it did not influence the growth of the Nile tilapias. Thus, golden mussel flour can substitute for current fish food ingredients, particularly protein content that complements nutritional formulations.

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