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Fish Silage in Black Bass *(Micropterus Salmoides)* Feed as an Alternative to Fish Meal

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ABSTRACT

The objective of this study was to use the residues of fermented sardine to elaborate the acid fish silage and its use in feed for aquaculture. Biological assay was performed by feeding largemouth bass (Micropterus salmoides) fingerlings (initial weight 22g), with extruded diets (41% crude protein; 3,600 kcal/kg digestible energy) containing 0.0, 7.5, 10.0, 12.5, or 15.0% of fish silage in partial substitution to the fish meal. The feed conversion ratio and weight gain for the treatments were: 1.26 and 15.76g; 1.11 and 17.07g; 1.19 and 17.81g; 1.18 and 19.83g; 1.47 and 14.64g, respectively. No significant differences (P<0.05) were detected among the treatments. Results indicated that it was possible to use up to 15% of acid fish silage as partial substitute for fish meal in the formulation of carnivorous fish feed.

Key words: residue, carnivorous fish, protein, fish nutrition

INTRODUCTION

Largemouth bass *Micropterus salmoides* (Percifomes, Centrarchidae) is found in lakes and small rivers in North America, but can also be found in Mexico and some parts of Europe. This species was introduced in Brazil in 1924 in Minas Gerais state, and was called black bass (Godoy, 1954).
Only in the state of São Paulo, where black bass was introduced and completely adapted, the practice of sport fishing has annually yielded gross values of about R$ 500 million (Carvalho Filho, 1997).
As in any animal culture, in aquaculture, feed corresponds to a high percentage of the operational costs, reaching 40 to 60% of the overall. Several studies have been carried out to identify alternative protein sources which would allow for a reduction in the cost of feed (Cheng et al., 2003; Furuya and Furuya, 2006; Portz and Cyrino, 2004).
Fish waste generated by processing and commercialization stations cause serious environmental hazards. The need for the improvement in the residues system is economical and environmentally friend (Maia Junior, 1998; Ferraz de Arruda et al., 2006a).
A viable alternative would be to use the waste

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material in the manufacture of the fish silage, since it does not require high investments. Fish silage is defined as a liquid product produced from the whole fish or parts of it, to which acids, enzymes or lactic-acid-producing bacteria are added, with the liquefaction of the mass provoked by the action of enzymes from the fish (FAO, 2007; Oetterer, 2006).

The manufacturing of silage from the comercialization of fish processing waste aiming to obtain aquaculture feed ingredient has been widely studied over the last few years. Many authors believe that, due to the similarity of this protein source with the raw material, especially amino acids, such as lysine, methionine, and cystine, silage has a high potential of use in aquaculture. Its low cost, especially when compared to fish meal is also attractive (Borghesi et al., 2006a, b; Coello et al., 2000; Fagbenro and Jauncey, 1995, 1998; Ferraz de Arruda and Oetterer, 2005; Ferraz de Arruda et al. 2006a, b; Goddard and Perret, 2005; Lessi et al., 1989; Ristic et al., 2002; Vidotti et al., 2003).

The objective of this study was to use the waste of fermented sardines from a fish industry in Ubatuba-SP-Brazil.

MATERIAL AND METHODS

For the elaboration of silage, waste from the cleaning or finishing of fish products from an anchovy-processing plant in Ubatuba, SP, containing about 30% salt, was used. Silage was produced from 30 kg of waste, which was triturated in a Super Cutter Sire grinder and added to an acid mixture (sulphuric acid and formic acid at the rate of 3:1) for the adjustment of the pH at 4.0. The pH of the silage was monitored and controlled with the help of a digital potentiometer and the addition of acid mixture.

The chemical analyses were carried out to determine the moisture (45.46%), crude protein (15.52%), ash (23.78%), lipids (4.48%), salt (16.89%) and energy (2,500 kcal kg^{-1}) of the acid silage, in accordance with the method described by AOAC (1990).

In order to define the treatments, five rations were formulated where the silage inclusion levels varied in 0; 7.5; 10; 12.5; and 15% through a linear programming application for the formulation of minimum-cost rations (Table 1).

The levels of digestible energy (3,600 kcal kg^{-1}) and protein (41%) were fixed according Portz (2001). The ration´s level of starch was fixed at 12% in order to allow the expansion of the material during the extrusion process (Kearns, 1999).

The ingredients used to the elaboration of the rations were ground in a 1.5 mm sieve. The rations were processed in the form of extruded 3.5 ± 0.5 mm grains, in an experimental Imbramaq MX-50 extruder with extrusion capacity of 50 kg h^{-1}.

After extrusion, each ration was submitted to pulverization with vegetable oil through a pressure pistol, at an amount calculated for the balance of the energy content. Rations were dried in a forced air oven at 45°C for 24 h. Rations were stored at 7°C. A sample from each ration was taken and submitted to the bromatological analysis as a standard for quality control (Table 1).

One hundred and eighty black bass (22 ± 0.05g), conditioned to accept artificial feed (Lovshin and Rushing, 1989), were ramdomly stocked in 60-L cages (15 cages; 12 fish per cage), placed in 1 m^3 plastic tanks (3 cages $tank^{-1}$) in an totally randomized experimental design (n=3), housed in a laboratory supplied by a closed water recirculating system, with aeration and oxygenation systems. Water temperature was maintained at 24 ± 1°C by automatic electric heater. Illumination in the laboratory was provided by eight halogen (daylight) bulbs and maintained by electric timer at 14L : 10D as recommended by Heinen (1998).

Prior to the study, ten fish were submitted to a benzocaine overdose, ground and immediately frozen in a container immersed into liquid nitrogen, and stored in a freezer (-80°C) for later analysis of initial carcass composition.

Fishes were fed until apparent satiation twice a day (7 a.m. and 5 p.m.) for a period of 66 days. Feed consumption was measured by weighing the containers of each diet every three days in a semi-analytical scale of 0.001-g precision.

At the end of the experrimental period, fishes were fasted for 24h, anaesthetized (0.5 g benzocaine 10-L^{-1}) and weighed. Randomized samples of four fishes were taken from each replicate, killed by overdose of benzocaine (3 g 10-L^{-1}), minced and stored as previously described for further analysis of the final carcass composition.

Table 1 - Chemical composition and analyses of the ration.

	Rations				
	T1	**T2**	**T3**	**T4**	**T5**
Ingredients(%)					
Fish meal	32.5	35.0	37.5	40.0	36.5
Soy meal	31.0	29.4	24.7	25.4	34.5
Corn	0.0	0.0	0.0	5.0	2.9
Wheat flour	15.0	15.0	15.0	15.0	15.0
Soy oil	5.9	7.5	11.2	6.4	10.6
Silage	15.0	12.5	10.0	7.5	0.0
Premix	0.5	0.5	0.5	0.5	0.5
Vitamin C	0.1	0.1	0.1	0.1	0.1
*Composition**					
Moisture	13.35	13.19	12.81	10.65	9.54
Crude protein	37.78	37.44	37.27	37.41	37.59
Ash	16.46	16.05	15.94	15.23	13.48
Lipids	32.41	33.32	33.98	36.23	39.44

Where: T1= 15% of silage; T2= 12.5% of silage; T3= 10% of silage; T4= 7.5% of silage and T5= 0% of silage.
*Expressed in: g $100g^{-1}$ of dry matter .

The performance and body composition results were submitted to analysis of variance (ANOVA) and Tukey's test for mean comparison (SAS 1985). The performance parameters were evaluated as recommended by Erfanullah and Jafri (1999), Hung et al. (1989), Moore et al. (1988): Weight gain – WG (%) = (final body weight - initial body weight / initial body weight) x 100; Feed conversion – FC (kg/kg) = food intake / weight gain; Protein retention – PR (%) = [(final body weight x final body protein - initial body weight x initial body protein) / total protein intake] x 100; Protein efficiency rate PER (%) = weight gain (g)/protein in the diet (g); Specific growth rate SGR (%) = 100 [(ln final body weight - ln initial body weight) / experimental days].

RESULTS AND DISCUSSION

As discussed by Ottati et al. (1990), the silage production process is simple, practical and economical, not requiring expensive equipment and procedures, such as those used in the production of fish meal. According to Espíndola Filho (1999), for a decrease in the silage production costs, the use of sulphuric and formic acids (3:1) as propionic substitutes is recommended.
A slight decrease in the protein fraction in the period analyzed was also possible, which was 38.12 % for silages produced with fresh waste and 39.06 % for silage produced with waste from the fermentation process, to 35.64 and 36.32 %, respectively. These decreases indicated the occurrence of protein hydrolysis (FAO, 2007; Oetterer, 2006).
The highest nutritive value of silage is directly related to the storage time (Ferraz de Arruda et al, 2006a; Oetterer, 2006; Raa and Njaa, 1989).
Thus, considering only the total protein analyzed, it would be better to use the silage immediately after its preparation. The mischaracterization observed in the silage in the visual examination, with the formation of a brownish pasty mass, was in accordance with Morales-Ulloa and Oetterer (1997) and Espíndola Filho (1999).
The final pH value of silage produced in this study was 4.12 which was in accordance with Oetterer (2002), who prescribed up to 4.5, an ideal pH to guarantee conservation.
Among the performance parameters (weight gain percentage – WG; feed conversion – FC, protein retention – PR), there was no statistical difference ($P>0.05$) among the treatments (Table 2).
For the moisture, ash, and lipids values, no significant variation ($P>0.05$) was observed, which was an important factor for a possible comparison parameter in posterior fish processing.
There were no significant differences ($P<0.05$) among the treatments in weight gain (WG), Food conversion (FC), Protein efficiency ratio (PER) e specific growth rate (SGR).

Table2 - Performance and composition parameters of the carcass.

	Experimental diets					
	T1	**T2**	**T3**	**T4**	**T5**	**CV**
WG (%)	14.6^{a*}	19.8^{a}	17.8^{a}	17.0^{a}	15.7^{a}	22.5
FC (g/g)	1.4^{a}	1.1^{a}	1.1^{a}	1.1^{a}	1.2^{a}	11.8
SGR (%)	0.7^{a}	0.9^{a}	0.8^{a}	0.8^{a}	0.7^{a}	23.2
PER (%)	3.5^{a}	4.8^{a}	4.3^{a}	4.1^{a}	3.8^{a}	22.5
Carcass composition (%)						
Moisture	78.1^{a}	77.1^{a}	76.6^{a}	77.4^{a}	76.3^{a}	4.7
Protein	15.5^{a}	16.7^{ab}	14.5^{b}	14.2^{b}	14.9^{ab}	6.5
Ash	4.1^{a}	4.0^{a}	3.8^{a}	3.7^{a}	3.6^{a}	8.2
Lipids	2.7^{a}	3.1^{a}	3.4^{a}	3.5^{a}	3.8^{a}	14.0
Nutrient retention (%)						
PR	22.9^{a}	29.0^{a}	36.6^{a}	37.7^{a}	33.6^{a}	17.2

Where: T1= 15% of silage; T2= 12.5% of silage; T3= 10% of silage; T4= 7.5% of silage and T5= 0% of silage.
*Values followed by the same superscript do not differ *P<0.05*.

The results of weight gain were lower than the results obtained by Portz (2001) for the black bass (between 29.20 and 56.87 g), who studied the replacement of fish meal by different by-products (animal and vegetal). The better result obtained by Portz (2001) could be attributed to the diets, which were formulated based on ideal protein concept relating the specific requirements of the essential amino acids for a perfect fish growth, instead of crude protein used in this study that only considered the protein content and, thus, could present unbalance in amino acids, and consequently, low growth and feed efficiency.

Weight gain values also were lower than that obtained with acid fish silage (AFS) by Heras et al. (1994) (42-54 g) and Hardy et al. (1984) working with Atlantic salmon (*Salmo salar*) (37.5–47.9 g) and rainbow trout (*Onchorynchus mykiss*), respectively. Food conversion ratios values were better than that obtained by Fagbenro and Jauncey (1995) (1.45-1.59 g) working with catfish (*Clarias gariepinus*) fed with fermented fish silage co-dried with many by-products (animal and vegetal), Hardy et al. (1984) (1.50-1.62 g) and Stone et al. (1989) (1.33-1.71 g) with rainbow trout (*Onchorynchus mykiss*) and Jackson et al. (1984) (1.55-1.74 g) with Atlantic salmon (*Salmo salar*), all of these working with AFS.

Specific growth rates in this study (0.76-0.92) were lower than that reported by Vidotti (2001) for the pacu (*Piaractus mesopotamicus*) fed with diets containing different kinds of co-dried fish silage and by Fagbenro and Jauncey (1995) with catfish.

The values of protein efficiency ratios (3.57-4.8%) were higher than that obtained by Vidotti (2001) (1.99-2.61%) to the pacu (*Piaractus mesopotamicus*), Stone et al. (1989) (1.28-1.73%), Hardy et al. (1984) (1.61-1.69%) to the rainbow trout (*Onchorynchus mykiss*) and Heras et al. (1994) (1.55-1.57%) to the Atlantic salmon (*Salmo salar*). All of these authors used fish silage as a principal protein source. Fishes are the largest existing vertebrate group and have different dietary habits and specific and differential nutritional requirements. Variability in the results of the parameters studied in the different experiments mentioned could be attributed to the need to consider the specific quantitative and qualitative requirements for the species and development stage during the diets formulation. Besides these factors, silage standardization is necessary to be used as a protein source, delimitating the ideal hydrolysis time in which there is no loss of the protein quality loss, and maximum of *in-vivo* digestibility.

There are few works studying the use of fish silage on fish performance experiments. Larger studies must be developed for the evaluation of the ideal quantity of silage in the elaboration of fish rations. The bioconversion of the wasted material and its consequent use can bring economical advantages for the industries, besides solving a great problem with the elimination of waste, which is a polluting, hardly disposable material as well as for the decrease in feed costs for the carnivorous fish.

Estimating that about 50% of the production cost in aquaculture is due to the feed, a reduction in the cost of feed must result a decrease in the price of fish and facilitate its purchase by the consumer.

CONCLUSION

It is possible to use up to 15% AFS in black bass feed with no harm for the performance parameters of this species.

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RESUMO

O objetivo desta pesquisa foi estudar a utilização de resíduos de sardinha fermentada para elaboração de silagem química e sua utilização em rações para aquacultura. Foi realizado ensaio de ganho de peso utilizando juvenis de “black bass” (*Micropterus salmoides*), com peso inicial de 22 gramas, onde utilizou-se rações extrusadas (41% de proteína bruta, 3.600 kcal/kg de energia digestível) contendo 0,0; 7,5; 10,0; 12,5; 15,0% de silagem em substituição à farinha de peixe. A composição química da silagem de pescado (matéria seca) apresentou valores de proteína bruta de 28,47 g $100g^{-1}$; lipídeo 8,24 g $100g^{-1}$; cinza 60,68 g $100g^{-1}$ A conversão alimentar e o ganho de peso para os tratamentos foram, respectivamente: 1,26 e 15,76g; 1,11 e 17,07g; 1,19 e 17,81g; 1,18 e 19,83g; 1,47 e 14,64g. Nenhuma diferença significativa (*P<0,05*) foi encontrada entre os tratamentos. Os resultados indicam a possibilidade da utilização de até 15% de silagem na substituição da farinha de peixe, em rações para peixes carnívoros.

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