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Elcimar da S Sousa
Technical Support,
Laboratory of Animal Nutrition,
Nilton Lins University. Av. Professor Nilton Lins 3259, 69058-030, Manaus, AM, Brazil

Jessica S da Silva
Technical Support,
Laboratory of Animal Nutrition,
Nilton Lins University. Av. Professor Nilton Lins 3259, 69058-030, Manaus, AM, Brazil

Fabio H de MH Cavalcanti
Doctoral student,
Postgraduate Program in Fisheries Science in the Tropics,
Universidade Federal do Amazonas, Av. Gen. Rodrigo Otávio, 3000, 69077-000 Manaus, AM, Brazil

Iurych NB Bussons
Master student, Postgraduate Program in Fisheries Science in the Tropics,
Universidade Federal do Amazonas, Av. Gen. Rodrigo Otávio, 3000, 69077-000 Manaus, AM, Brazil
E-mail: bussonsinb@gmail.com,
ID: orcid.org/0000-0002-8616-0229

Márcia RF Machado
Professor and Researcher,
Postgraduate Program in Aquaculture,
Coordinator of the Laboratory of Animal Nutrition, Nilton Lins University. Av. Professor Nilton Lins 3259, 69058-030, Manaus, AM, Brazil
E-mail: mmachado@niltonlins.br,
ID: orcid.org/0000-0002-5038-1775

Correspondence
Elcimar da S Sousa
Technical Support, Laboratory of Animal Nutrition, Nilton Lins University. Av. Professor Nilton Lins 3259, 69058-030, Manaus, AM, Brazil
E-mail: mmachado@niltonlins.br,
ID: orcid.org/0000-0002-5038-1775

Use of biological silage in diets for fish of *Brycon amazonicus* (Spix & Agassiz, 1829) fingerlings

Elcimar da S Sousa, Jessica S da Silva, Fabio H de MH Cavalcanti, Iurych NB Bussons and Márcia RF Machado

Abstract
The present work was aimed to evaluate the growth parameters for *Brycon amazonicus* fingerlings subjected to diets with different levels partial replacements of fish meal by biological silage. The experiment was conducted in the Laboratory of the Production of Aquatic Organisms at the Nilton Lins University, during 60 days, 240 fingerlings were used with average initial weight 3.77 ± 0.40 g and average initial length 8.39 ± 0.42 cm, distributed in 12 water tanks of 310 L^{-1} polyethylene. Four experimental diets (0, 10%, 20% and 30%) partial replacement of fish meal by biological silage of fish. The results of weight gain and protein efficiency ratio showed a positive linear regression (p<0.01) for the feed with 10% of partial replacement of fish meal by biological silage. It is concluded that fish meal can be replaced partially to 10% by biological silage, favoring the good performance of the fish.

Keywords: Alternative ingredient, omnivore's fish, performance, nutrition, matrinxã

1. Introduction
The increased production of animal waste has an adverse effect on environment, because its generation rate is much greater than the rate of degradation. However, due to implementation of more stringent environmental laws, who value the environmental management, there has been an awareness of the harmful effects caused by the continuous solid waste dump and the environment [1].
Therefore, it is necessary to the development of technologies that seek to take advantage so fishing waste in feed for fish, for being rich in protein and lipids, seeking in this way sustainable development [2].
However, the waste can be transformed into biological silage, a sustainable alternative once that can be transformed into high-value organic food for animals. The development of the silage is made using a method of preserving fish residue that can be effected by microbial fermentation or by the action of acid. Is a very old practice in communities with scarce resources, lacking in technology, with plenty of fish resources and of by-products from industrial processing [3].
The preservation of the ensiled material is given by the reduction of pH, by the addition of organic acids and/or minerals (silage acid), by the addition of lactic acid-producing organisms (biological silage), besides preventing against microbial action and does not attract insects, the lowering of pH promotes the action of enzymes naturally present in fish and/or added (enzyme silage), which is responsible for the hydrolysis of protein [4-8].
After the waste through chemical-physical processes that results in a nutrient-rich mass, silage can be used in feed for farmed fish feed [9].
Aquaculture among other activities of livestock production is the fastest growing sector in the world [10].
Feed represents the largest part of the operating costs, making food and nutrition studies on aquaculture of extreme importance. Souza *et al* [17] worked with silage biological evaluation on the biomass gain of fingerlings of tilapia (*Oreochromis niloticus*), concluding that adding up to 30% favors the good performance of the fish compared with the average gain of biomass.
However, the objective of this work was to evaluate the performance of fish fed different levels of biological silage, from discarded waste free fairs Manaus city, Amazonas state.

2. Material and Methods

The present study was conducted in the Laboratory of the Production of Aquatic Organisms (LAPOAq) Nilton Lins University, Manaus city, Amazonas stare, Brazil. Experimental procedures used in the research were approved by the Commission of Ethics of Animal (CEUA) use of Nilton Lins University (Protocol nº 001/2015).

The biological silage (SB) was prepared from waste generated in the processing of fish on the Modern Manaus, being used as biological ferment the cabbage, papaya, wheat flour, salt and vinegar (Table 1), according to Lupin [11] methodology.

Fish residues collected were milled into electric grinder meat, sauces, and later the mass was incorporated into the yeast. The silage was prepared and stored at room temperature for seven days, where was held the homogenization and reading of temperature and pH on a daily basis.

Table 1: Ingredients used to manufacture the biological preparation of biological silage.

Ingredients	Amount (%)
Cabbage	41
Papaya	31
Wheat meal	31
Vinegar	8
Salt	3
Total	100

In the preparation of experimental rations, the ingredients were crushed in knife grinder with 0.5 mm sieve, being incorporated into other biology silage ingredients and then pelletised in meat grinder. The pellets were dried in an oven of forced air ventilation to 55 °C, for 24 hours and then were fractionated to obtain 4 and 6 mm beads and stored in freezer to -20 °C. The rations were made in order to contain 32% crude protein (Table 2).

To evaluate the performance, 240 were *Brycon amazonicus* fingerlings, with initial average weight of 3.98 ± 0.29 g, distributed in twelve water tanks of polieitileno of 310 L^{-1} (n = 12 fish per experimental unit), with water recirculation system in a completely randomized design with four treatments (0% (control); 10%; 20% and 30% replacement of fish meal by biological silage of fish) and three replicates per treatment.

During the experiment (60 days), the fish were given diets four times a day, at 9:00 am, 11:00 am, 03:00 pm, 04:00 pm and 05:00 pm, in small amounts until they produce satiety. During the test, different water quality parameters were measured, such as temperature (°C), dissolved oxygen (mg L^{-1}), electric conductivity (µS/cm) and pH, with the help of portable multi-parameter probe YSI 566 (YSI Inc., Yellow Spring, OH, EUA) daily in the morning.

The biometrics were held every thirty days and at the end of experiment, the animals were anesthetized with benzocaine to 100 mg L^{-1}, measured and weighed to determine performance parameters, which were:

Weight Gain (WG, g) = Wf - Wi, where: Wi = average initial weight (g) and Pf = average final weight (g);

Specific Growth Rate (SGR, %.day^{-1}) = 100 x ((ln Wf- ln Wi)/t, where: Wi = average initial weight (g), Wf = average final weight (g) and t = feeding period (days);

Feed Conversion Ratio (FCR) = [total feed intake (g)/total weight gain (g)];

Protein Efficiency Ratio (PER, %) = WG (g) / CP consumed (g), where: CP = Crude protein;

Survival Rate (SUR, %) = 100 x (final number of fish/initial number of fish).

Table 2: Formulation of the experimental diets with the replacement of fish meal by biological silage 0, 10, 20 and 30% of regarding the performance of *Brycon amazonicus* fingerlings.

Levels of biological silage per treatment (%)				
Ingredients (g.kg^{-1})	0	10	20	30
Soybean meal	450.0	313.0	230.0	200.0
Corn Starch	150.0	150.0	150.0	150.0
Corn meal	100.0	130.0	129.8	130.3
Wheat fiber	120.0	150.0	150.0	91.7
Fish meal	100.0	90.0	80.0	70.0
Biological silage	-	100.0	200.0	300.0
Soybean oil	20.0	20.0	20.0	20.0
L-Lysine	10.0	10.0	10.0	10.0
Dicalcium phosphate	10.0	13.0	06.9	05.0
Premix [1]	10.0	10.0	10.0	10.0
Salt	10.0	10.0	10.0	10.0
DL-Methionine	05.0	04.0	03.3	03.0
Proximate composition (dry matter basis) [2]				
Gross Energy (MJ kg^{-1})	14.45	14.46	14.47	14.48
Crude Fiber (%)	3.61	3.58	3.55	3.55
Lipid (%)	4.33	4.41	3.97	3.94
Crude Protein (%)	32.00	32.00	32.00	32.00

[1]Premix (kg-1): Mineral and vitamin mix (Premix Nutrifish Guabi®, Campinas, SP, Brazil): Fe 15,000 (mg); Cu 2500 (mg); Zn 12,500 (mg); I 375 (mg); Mn 12,500 (mg); Se 87.5 (mg); Co 125 (mg); vitamin A 2,500,000 (IU); vitamin D3 600,000 (IU); vitamin E 37,500 (IU); vitamin K 3750 (mg); vitamin C 50,000; vitamin B1 4000 (mg); vitamin B2 4000 (mg); vitamin B6 4000 (mg); vitamin B12 4000 (mg); pantothenic acid 12,000 (mg); biotin 15 (mg); folic acid 1250 (mg); niacin 22,500 (mg); BHT 15,000 (mg).

[2]The nutrient contents were calculated using Super Crac Premium® software.

2.1 Statistical analysis

The results were submitted to analysis of variance (p<0.01), in an experimental randomized design with 4x3 treatments repetitions by One Way ANOVA and in case of difference was applied the polynomial regression test.

3. Results

The results of the water quality parameters were monitored during the experimental period is presented in Table 3.

Table 3: Parameters of water quality during the experimental period (60 days).

Parameters of water quality	
Temperature (°C)	26.82 ± 0.10
Dissolved Oxygen (mg.L^{-1})	4.83 ± 0.25
pH	6.75 ± 0.16
Conductivity (µS/cm)	3.66 ± 1.00

The average values obtained for growth performance parameters with weight gain, feed conversion ratio, specific growth rate, protein efficiency ratio of *Brycon amazonicus* fingerlings fed rations containing various levels of fish meal replacement for biological silage (0% (control); 10%; 20% and 30%), after 60 days of cultivation, are described in Table 4. The parameters with weight gain (WG) and protein efficiency ratio (PER) obtained a significant difference (P<0.01) in the level of 10% of replacement of fish meal by biological silage. No significant difference was observed (P>0.01) for specific growth rate, feed conversion ratio. No mortality among treatments were observed during the experimental period, indicating thus absence of toxic substances and/or harmful to fish.

Table 4: Growth performance parameters of *Brycon amazonicus* fingerlings, fed practical diets containing different levels the replacement of fish meal by biological silage for 60 days[1].

	Levels of biological silage per treatment (%)			
Parameters[2]	0	10	20	30
WG (g)[3]	30.42±1.71a	31.90±1.65b	25.55±1.58a	20.49±1.79a
SGR (% day^{-1})	0.66±0.14a	0.62±0.03a	0.60±0.03a	0.57±0.02a
PER (%)[3]	0.95±0.15a	1.00±0.10b	0.80±0.05a	0.64±0.06a
FCR	1.29±0.07a	1.22±0.03a	1.25±0.22a	1.26±0.13a
SUR (%)	100	100	100	100

[1]The averages Followed by the same letter are not statistically different from each other (P>0.01).
[2] Data are means±SD of four groups of 96 individuals (n=12).
[3] Linear effect (P<0.01) Y= Y = -0.361x + 32.51, R2 = 0.814; Y = -0.011x + 1.016, R2 = 0.814.
Abbreviations: WG = Weight Gain; SGR = Specific Growth Rate; PER = Protein Efficiency Ratio; FCR= Feed Conversion Ratio; SUR = Survival Rate.

However, linear effect was observed (P<0.01) levels of fish meal replacement by biological silage in diets on weight gain and protein efficiency ratio (Figure 1 and 2, respectively), indicating the minimum point of inclusion to 8.77 and 8.48 biological silage in the diet, respectively, occurring an improvement of the average values of these variables to the level of 10% of replacement of fish meal by biological silage in diets.

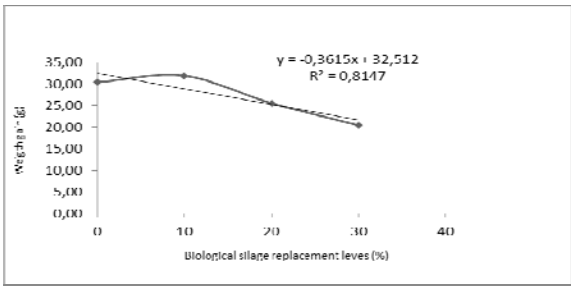

Fig 1: Weight gain (g) of *Brycon amazonicus* fingerlings fed with different levels of fish meal replaced by biological silage.

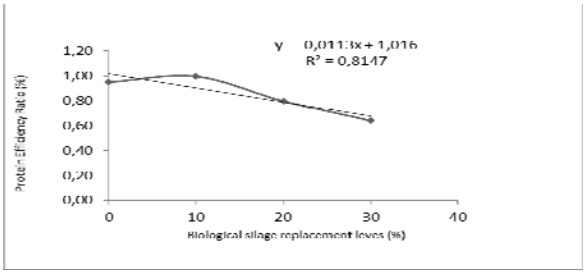

Fig 2: Protein efficiency ratio (%) of *Brycon amazonicus* fingerlings fed with different levels of fish meal replaced by biological silage.

4. Discussion

The parameters of water quality values within the limits considered suitable for *Brycon amazonicus* as for the other species of the Amazon [12, 13]. Good water quality observed during the experimental period favored the survival of *Brycon amazonicus* when fed on this diet. Results corroborate with those observed by Boscolo *et al* [14] working with *Leporinus macrocephalus* juvenile fed with diets containing different levels of inclusion (0, 5, 10, 15%) of biological silage filleting of *Oreochromis niloticus*.

The evaluating of the performance of *Oreochromis niloticus* fingerling fed with diets containing different levels of inclusion (0, 10, 20 and 30%) of fermented residue silage of filleting of *Oreochromis niloticus*, found no significant difference between treatments (P>0.01) for weight gain, and protein efficiency ratio, these results which differ from our work, because it was observed a significant difference to the mentioned parameters [15].

Already Enke *et al* [16] assessed during 75 days the inclusion of 0, 12.5, 25, 37.5 and 50% of silage performance chemistry of *Rhamdia quelen* juvenile, where he observed a quadratic effect (p<0.05), indicating that a diet containing 30-33% of silage provides better performance, does not affect the survival and quality of water. Similar results were obtained by Souza *et al* [17] who used diets containing 0, 10, 20 and 30% of biological silage in feeding of *Oreochromis niloticus* fingerling during 90 days, observed that the additions of up to 30% of silage favor a good performance of the fish with relation to biomass gain, this result differs from this work, as was observed for the weight parameter a decreases in relation to the level of 30% replacement of biological silage. In the present work the level of 10% was obtained an improvement in relation to this parameter. Research focusing on the inclusion of fish silage in feeding of aquatic organisms indicate that there is no significant differences of this inclusion in feed conversion apparent ratio according to Lazzari *et al* [18] and Abimorad *et al* [19]. These results corroborate with those in the present research, where no significant difference was observed (P<0.01) for feed conversion.

Fabgenro and Jauncey [20] also did not observe a significant

difference in feed conversion when compared to commercial diet and fish silage of *Clarias gariepinus* juveniles.

The results for specific growth rate, resemble, with results verified by Vidotti *et al* [21] where there was a significant difference to feed *Piaractus mesopotamicus* fingerling with different levels of inclusion of silage. These results corroborate with Abimorad *et al* [19] which also did not observe significant difference for *Oreochromis niloticus*.

5. Conclusion

The results of growth parameters of fish show that the inclusion of up to 10% of fish residual biological silage does not affect performance and health of *Brycon amazonicus* fingerlings.

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