Scientific Paper

Productive and economic indicators of *Clarias gariepinus*, fed with fishery byproducts ensiled with sulfuric and formic acids

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Abstract

Objective: To evaluate the addition of sulfuric (98 %) and formic acids on the silage of fishery byproducts and its effect on productive and economic indicators of *Clarias gariepinus*.

Materials and Methods: Two hundred seventy fingerlings, of 10,4 ± 0,06 g of average weight, were used, distributed in a complete randomized design with three treatments and three repetitions. The treatments were: concentrate feed for catfish fattening (control), fish silage prepared with sulfuric acid (98 %) and silage elaborated with formic acid. All the animals were individually weighed for calculating the indicators final average weight, feed conversion, protein efficiency and survival. In addition, the costs of the diets were calculated from the international prices of the raw materials. Simple classification variance analysis was carried out through the statistical package INFOSTAT.

Results: No statistical differences were found in final weight (66,3 and 70,7 g), feed conversion (1,18 and 1,15) and protein efficiency (3,09 and 3,18) between the diets with fish silage. These values were higher ($p < 0,05$) than the ones reached with the control (final weight of 56,4 g; feed conversion of 1,5 and protein efficiency of 2,3). The survival was high in all the treatments (94,4; 100 and 96,7 %). The economic analysis showed that the highest profits were obtained with the silages ($ 2 618,8 and 2 428,5 US/t) compared with the control ($ 2 061,15 US /t).

Conclusion: The utilization of silages from fishery byproducts in extruded diets, elaborated with sulfuric (98 %) and formic acids, improved the productive indicators of *C. gariepinus*, with a positive economic effect by decreasing the import of fish meal.

Keywords: formic acid, sulfuric acid, animal feeding, catfish, silage

Introduction

The scarcity of protein raw materials and their high price in the international market generates great uncertainty with regards to the future projection of aquaculture in Cuba. Hence the increasing need to develop new feeding methodologies, as feed represents between 50 and 70 % of the production costs of intensive fish production (Perea-Roman *et al.,* 2018).

A methodology of semihumid feed (74,3 g DM/100 g of feed) was developed, based on silage of fishery byproducts (FS), for the intensive cultivation of African catfish *C. gariepinus*, main species of intensive cultivation in Cuba (Toledo *et al.,* 2013). The FS is a simple technology, of low investment, whose product can be obtained by acidification or lactic fermentation.

Acidification consists in the combination of sulfuric (98 %) and formic acids in the range of 1,5 - 2,0 %, respectively (Toledo *et al*., 2013; Valenzuela and Morales, 2016; Perea-Roman *et al.,* 2018). Nevertheless, due to its availability and cost, sulfuric acid was established as key input in the process. In the Sancti Spíritus province an international project is developed (AID010713 - IPEPAC) with the funding of the Italian agency for Cooperation AlloSviluppo (AICS), aimed at the extension of such feeding methodology, with the adaptation of feed extrusion.

Llanes *et al.* (2017) evaluated two substitution levels (10 and 20 %) of fish meal (FM) by meat silage (MS), elaborated with sulfuric acid (98 %), in extruded diets intended for African catfish. These authors warned about the extreme acidity of MS (pH 2,06), which influenced ration intake.

Perea-Roman *et al.* (2018) reported that formic acid is more advantageous for ensiling fishery byproducts in extruded diets; although its use can be costly, because of its import. The objective of this work was to evaluate the addition of sulfuric (98 %) and formic acids in silage of fishery by products and its effect on productive and economic indicators of *C. gariepinus*.

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Materials and Methods

Location. The bioassay was conducted in the Nutrition Laboratory of the Enterprise of Development of Aquaculture Technologies (EDTA, for its initials in Spanish), in the Cotorro municipality, in Havana.

Animals, experimental design and treatments. The *C. gariepinus* fingerlings were from the young fish area of the El Dique Development and Innovation Unit. They had one week of adaptation in a cement pool of 4,5 m^2, where they received the concentrate feed of catfish fingerlings (36 % crude protein). After this time, 270 fish were fished and selected, of 10,4 ± 0,06 g of average weight, randomly distributed in three treatments with three repetitions, according to simple classification model. The experimental units consisted in nine cement circular tanks of 68 L in which 30 fish were put, and where a water flow of 0,2 L/min was maintained 24 h.

The treatments are shown in table 1 (T1), which corresponded to the formulation of the concentrate feed for catfish pre-fattening, one diet with FS prepared with sulfuric acid 98 % (T2), and another with FS elaborated with formic acid (T3).

Silage preparation. Byproducts from the fileting of tilapias were used, which were ground in a mincer (JAVAR 32, Colombia). The resulting paste was divided into two portions: one was added 2 % sulfuric acid 98 % (p/v), and the other, 2 % formic acid (p/v). Both were stored in two plastic tanks with lid for seven days.

Diet preparation. The meals (fish, soybean and wheat) and wheat bran were ground in a creole hammer mill, to an approximate size of 250 µm. Each diet was mixed in a mixer (*HOBART MC-600,* Canada) during 10 min, and then soybean oil, the vitamin-mineral mixture, and each of the FS in humid form (10 % of inclusion calculated on dry basis and with previous neutralization with 2,5 % of calcium carbonate) were added. Mixing was continued during five minutes. The agglomeration of the diets was performed in an extruder (DGP 70, China), with 3 mm diameter and the pellets were dried in a stove (Selecta, Spain) at 60 °C during 24 h. The control feed was prepared under similar conditions as the experimental ones. The bromatological determinations were made according to the methods described by AOAC (2016). The calculation of digestible energy, which was used for the estimation of the diet energy, was determined according to the calorific coefficients referred by Toledo *et al.* (2015).

Experimental procedure. The values of temperature and dissolved oxygen in water were daily taken with a digital oximeter (*HANNA*, Rumania).

Table 1. Percentage and chemical composition of the experimental diets (g /100 g DM).

Ingredient	T1-control	T2-sulfuric acid	T3-formic acid
Fish meal	10	-	-
Silage (sulfuric acid)	-	10	-
Silage (formic acid)	-	-	10
Soybean meal	40	40	40
Wheat	23	25	25
Wheat bran	20	20	20
Soybean meal	4	3	3
Dicalcium phosphate	2	1	1
P. vitamins and minerals	1	1	1
Total	100	100	100
Dry matter	91,8	88,46	89,11
Crude protein	29,75	27,91	27,39
Ethereal extract	6,21	7,66	7,36
Crude fiber	5,07	4,97	4,81
Ash	6,30	7,18	7,13
Digestible energy (MJ/kg)	11,04	12,11	11,96
CP/DE (g/MJ)	26,94	23,04	22,90

The ammonium content was recorded weekly with a water colorimetric kit (*Aquamerck*, Germany). The diets were offered in two rations at 6 % of the body weight (9:00 and 15:30 h) during 60 days. The rations were adjusted every 15 days.

At the end of the bioassay all the animals were individually weighed on a digital scale (*Sartorius*, Germany) to calculate the following productive indicators:

- Final mean weight
- Feed conversion (FCN) = added feed/weight gain.
- Protein efficiency (PE) = weight gain/supplied protein.
- Survival (S) = number of final animals/number of initial animals x 100.

Economic analysis. It was carried out according to Toledo *et al.* (2015). The cost of the diets was calculated from the international prices of the raw materials for December, 2019 (http/www.indexmundi.com/precios de mercados), plus 45 % for concept of additional expenses (transportation, industrial processing and administrative costs for Cuba). These values were multiplied by the feed conversions to know the feeding costs. To determine the latter 60 % of the total production expenses was considered. The Economy Department of the Enterprise for the Development of Aquaculture Technologies facilitated the value of production ($ 3 400,00 US/t) and of silages.

Statistical analysis. The normality and homogeneity assumptions were proven and simple classification variance analysis was performed through the statistical package INFOSTAT, version 2012 (Di Rienzo *et al.,* 2012). When differences were found ($p < 0,05$), the means were compared by Duncan's multiple range test.

Results and Discussion

During the experimental period, the temperature and dissolved oxygen in the water of the containers varied between 25,7 and 26,9 ºC, and between 5,1 and 6,0 mg/L, respectively. Ammonium remained in 0,01 mg/L through water circulation. According to Toledo *et al.* (2015), these values are considered comfortable for the good productive performance of the species.

In both diets with FS, the intake was fast, which proves the good acceptability by the fish throughout the bioassay, as opposed to the observations made by Llanes *et al.* (2017), who referred higher intake in the control feed with regards to the diet with MS. Thus, in this study, the acid type and silage level (10 % dry basis) did not influence the productive indicators of this stage, which corresponds to pre-fattening (10,0 to 70,0 g of mean weight) of the cultivation.

It is important to state that the pH of the silage with sulfuric acid oscillated between 3,2 and 3,4 during its storage, values that were over the ones obtained in the MS, which showed PH of 1,81 (Portales-González *et al.*, 2015) and 2,06 (Llanes *et al.,* 2017) at seven days of storage. This could prove that the concentration of scales and bones of tilapia by-products could contribute with the buffer effect on silage acidity, which by being later neutralized with calcium carbonate decreases the acidity of the product even more for its incorporation to extruded diets.

Toledo *et al.* (2013) did not observe problems with the acceptability of rations, when using at productive scale 40 % of FS, elaborated with 2 % sulfuric acid 98 % (p/v) in semihumid rations, although the silage was not neutralized with calcium carbonate. This could indicate that, in this type of diet, the water percentage present in the ration decreases the acidity concentration, which does not occur in extruded diets, where extrusion decreases pH.

The best growth and feed efficiency indicators ($p < 0,05$) were obtained with the diets of fish silage (table 2). These results were higher than the ones reported by Llanes *et al.* (2017) with meat silage for this same species, and those obtained in channel catfish (*Ictalurus punctatus*), where it was possible to include only up to 5 % of the fermented silage of tilapia byproducts (Bringas-Alvarado *et al.,* 2018).

Table 2. Productive performance of *C. gariepinus* fingerlings with the experimental diets.

Indicator	T1-Control	T2-sulfuric acid	T3-formic acid	SE ±	P - Value
Final weight, g	$56,4^{a} \pm 4,231$	$66,3^{ab} \pm 3,084$	$70,7^{b} \pm 3,441$	-	0,019
Feed conversion	$1,5^{a}$	$1,2^{b}$	$1,2^{b}$	0,052	0,001
Protein efficiency	$2,3^{a}$	$3,1^{b}$	$3,2^{b}$	0,145	0,000
Survival %	94,4	100	96,7	0,981	0,058

a, b, c: row with different letters indicate significant differences for $p < 0,05$.

Survival was high (higher than 94,4 %) for all the treatments, which indicates that the inclusion of FS in extruded diets does not promote mortality in the fingerlings, and did not influence the productive results of the bioassay.

The results in this study with the FS could be related to a higher availability of partially hydrolyzed protein and of energy, as well as with the acidification offered by silages to the diet, which improves nutrient digestibility and, thus, animal growth (Toledo *et al.,* 2013; Suárez *et al.,* 2018).

Bringa-Alvarado *et al.* (2018) characterized the byproducts of tilapia fileting and found all the essential amino acids and adequate lysine (7,296 g/100 g of protein) and methionine concentrations (3,996 g/100 g of protein), which in formulations for fish must be quantified as they are limitations in most of the protein ingredients (Abdo-de-la-Parra *et al*., 2017).

Abdo-de-la-Parra *et al*. (2017) found the profile of fatty acids, where the highest concentrations were of palmitic C16:0 (25,6 %), palmitoleic C16:1 (6,7 %), linoleic C18:2n-6 (34,3 %) and linolenic acid C18:3n-3 (11,5 %). In addition, they found a proportion of fatty acids n-6/n-3 from 2,3 to 1, very favorable in the rations for African catfish.

Shirai *et al.* (2001) stated that chemical silages show high protein hydrolysis coefficients, due to the activity of digestive enzymes, specifically of the fish proteases, which increase the proteins of low molecular weight. The increase in the content of released peptides and free amino acids can generate higher palatability and, consequently, increase feed intake in carnivorous and omnivorous fish (Valenzuela and Morales, 2016).

It is important to state that FM is the key protein ingredient in feedstuffs for aquaculture, which is due to its high protein content, profile of amino acids and essential fatty acids. However, its high price does not support the development of intensive fish rearing with sweet-water species of low commercial value. Besides, its production in Cuba is not justified due to the little availability of fishery byproducts. Hence the silage that is elaborated with equal raw materials, along with the quality and digestibility of its protein, is an alternative (Llanes *et al.,* 2011).

Regarding the acids, it was corroborated that there were no differences between the utilization of sulfuric or formic acid, regarding the growth and feed efficiency indicators (table 2). Thus, either of the two acids can be used in the elaboration of fish silages to be incorporated to dry diets, as long as they are neutralized before their incorporation to the ration.

The replacement of FM by silages of fishery byproducts in the formulation of high-quality aquaculture feedstuffs can have repercussions on the reduction of feeding costs and, in turn, decrease FM imports. The economic analysis (table 3) showed that the diets with FS were the least costly, because FM is not included in them, which is the most expensive protein ingredient ($ 1 366,94 US/t). Regarding the silage diets, the utilization of sulfuric acid (98 %) was the most economical one as it was purchased in Cuba; while formic acid is imported.

The cost of processing these byproducts through silage techniques with sulfuric acid (98 %) was $ 0,362 US/kg DM, and with the formic, $ 0,816 US/kg DM. These amounts can vary depending on the price of the acids and fishery byproducts, but the trend is that they are lower compared with FM ($ 1,52 US/kg).

In general, feeding costs and total production expenses were lower with the FS diets (table 3), due to their costs, and to the better feed conversions. That is why they provide the highest profits in the production of one ton of whole fish. These results coincide with the report by Perea-Roman *et al.* (2018) in a study on inclusion of fish silage in extruded diets for red tilapia (*Oreochromis* spp).

Table 3. Economic analysis of the *C. gariepinus* production (US $ /t).

Indicator	T1-control	T2-sulfuric acid	T3- formic acid
Cost of the ration	542,8	397,2	506,9
Cost of feeding	803,3	468,7	582,9
Total production expense	1 338,8	781,2	971,5
Profits	2061,2	2 618,8	2 428,5
Saving	-	557,6	367,4

Production value: $ 3 400,00 US/t of whole fish. Profits= production value- total expense.

Conclusions

The utilization of silages from fishery byproducts, elaborated with sulfuric acid (98 %) and formic acid in extruded diets, improved the productive indicators of *C. gariepinus*, with the subsequent positive economic effect, by decreasing the import of fish meal.

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Authors' contribution

- José E. Llanes-Iglesias. Original conception of the research, bioessay execution and manuscript writing.
- Giuliana Parisi. Contribution to the experimental design, bioessay setting up and manuscript writing.

Conflict of interests

The authors declare that there are no conflicts of interests between them.

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