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Supplemented feed with biological silage of fish-processing wastes improved health parameters and weight gain of mice

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ABSTRACT

Wastes from *Merluccius hubbsi* processing were used for biological silage elaborated with *Lactobacillus arizonensis* and for chemical silage performed with 0.18M sulfuric acid and 0.22M formic acid. Mice BALB/c were fed with isoenergetic diets, EFBS and EFCS, containing 36.3% (wt/wt) biological fish silage and 36.3% (wt/wt) chemical fish silage respectively. Promisingly, after 30 day consumption both additives did not provoke lesions in the gut, thinner wall, distension or abnormal vascularization. The higher concentration of lactic acid bacteria (LAB) in the gut of mice fed with EFBS (2.51×10^4 cfu LAB/g EFBS vs. 3.98×10^3 cfu LAB/g EFCS), together with the weight gain (23.8 ± 3.8 g vs. 16.7 ± 3.7 g), feed conversion ratio (4.12 vs. 6.71), protein efficiency rate (0.69 vs. 0.63), villi height (455 μm vs. 418 μm) for EFBS and EFCS respectively, support the probiotic effect of *L. arizonensis*. Nevertheless, both preparations are interesting options to envisage a promising outcome for recycling fish wastes.

1. Introduction

Harbor wastes generate larger amounts of effluents and in the absence of suitable systems for wastes management and treatment, a negative environmental impact is expected. As an example, in Patagonia (south Argentina), fish-processing wastes (e.g. fish heads, frames, and offal) are opencasts deposited, storm-water running off and leachate flows from opencast deposits creating a risk to receiving contaminated surface waters, groundwater or soil. The anaerobic conditions cause undesirable odor mainly generated by gases (e.g. methane and hydrogen sulphide) and volatile fatty acids (Groch, 2001). The negative impact also have touristic implications, since these big deposits promoted the overpopulation of seagulls (birds family *Laridae*), and these birds bite the whale calves provoking blooding animals and dead animals (Yorio and Giaccardi, 2002). Fishmeal production would be the main option to overcome the problem, since it is a valuable source of protein for livestock. However, harbors are situated in areas where the basic infrastructure is lacking; the introduction of sophisticated systems for wastes treatment may not be a viable option due to the costs involved. Therefore, fish silage represents an important option and a source of protein that can be used in large scale for replacing fishmeal.

The biological fish silage for animal feed has been mainly evaluated as a stable substitute of proteins, with low consideration to the benefits derived from the probiotic effect of properly selected LAB. This supplement could be added into the feed to provide additional advantages from the nutritional and sanitary aspects (e.g. increment of digestibility, contribution of vitamins, and protective activity against pathogenic bacteria) (Castellano et al., 2008). In animal production, the preventive use of antibiotics provokes lower yields and it is mainly related to intestinal illnesses. On the other hand, the antibiotics-resistant pathogenic microorganisms and the residual effects of antibiotic in humans have encouraged alternatives, as the probiotics and probiotic-prebiotics combinations (Ndaw et al., 2008). The strain L. *arizonensis* was selected among several LAB as the more suitable for silage of wastes from the processing of *M. hubbsi*, considering the kinetics of acidification and the lower optimum temperature for the process (28 °C), this strain constitute a promising alternative for opencast fish fermentation at locations with temperate to cold climes (Góngora et al., 2012). On the other hand, chemical silage would offer another option for the treatment of fish wastes; giving a stable product whose protein content is similar to that of the raw material, produced at lower cost and energy in comparison to the production process of fishmeal. Herein, we studied the performance of the biological fish silage and the chemical fish silage, performed with wastes from *M. hubbsi* processing, as feed additive on the diet of mice BALB/c.

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2. Materials and methods

2.1. Fish silages

Wastes of *M. hubbsi* (trimmings, heads, frames, fins, skin, and viscera) were purchased immediately after processing from an industrial plant located in Chubut, (Argentine). The samples were transported and kept at 4 °C, processed within 12 h. For biological fish silage (BFS), the material was supplemented with 25 g/L sucrose, sterilized (121 °C, 2 h) and inoculated with 25 mL/L of a 16 h old-culture of NRRL B-14768 *L. arizonensis* performed in Man Rogosa Sharpe (MRS) medium at 30 °C. The fermentation was carried out in a 10 L working volume stirred reactor for 24 h at 29 ± 1 °C. Unsterile material was acidified by adding 0.18 M sulfuric acid and 0.22 M formic acid for chemical fish silage (CFS).

3. Experimental feeds

Isoenergetic (12 MJ/kg) diets, containing constant protein concentration (23 % wt/wt), were designed according to National Research Council (1995). Control feed (CF) was performed with sunflower meal and soy meal as protein source. The compositions of the experimental feed with chemical fish silage (EFCS) and experimental feed with biological fish silage (EFBS) are shown in Table 1.

A mixer was used to include the silage to the dry ingredients and it was pelleted at 55 °C reaching 10% (wt/wt) moisture. The initial silage concentration was 10% (wt/wt) on the humid mixture and it reaches 36.3% w/w after the drying process.

For counting colony forming units of LAB (CFU/mL), the samples were centrifuged at 3000 rpm for 15 min and washed twice with 100 mM sterile phosphate-buffered saline (PBS) (pH 7.0). Dilutions of the suspension (0.1 mL) were inoculated in MRS agar plates and incubated 24-48 h at 37 °C (Kacem and Karam, 2006). The feed (CF, EFCS and EFBS) moisture, crude protein, ethereal extract, ash, crude fiber, calcium and phosphorus concentrations were analyzed according AOAC standard methods (Table 2).

4. Feeding trials

Three weeks old BALB/c mice were maintained between 18 and 20 °C and 60–80% (wt/wt) relative humidity, with a 12 h light–dark cycle. After feeding for 1 week on a basal diet, mice were randomly divided and kept in group cages (n = 5) with males or females and fed with the experimental diets (CF, EFCS and EFBS). Water and feed were administered *Ad libitum* and daily intake was gravimetrically controlled. The animals were weighed using an analytic scale and the cleaning and changed of sawdust bed was carried out every 3 days. After 4 weeks, each animal was placed in an aseptic chamber to collect stool samples (150 mg), suspended in 1.5 mL of sterile PBS (pH 7.2) and properly diluted in the same buffer for LAB counting (Dalloul et al., 2003).

Table 1
Feed composition: control (CF), feed containing chemical fish silage (EFCS) and feed containing biological fish silage (EFBS).

	CF	EFCS	EFBS
	Concentrations (%)		
Wheat middling	29.8	41.2	41.2
Sunflowers meal	35.3	19.7	19.7
NaCl	0.75	0.7	0.7
Sunflowers oil	0	1.35	1.35
Soybean meal (10% wt/wt moisture)	31.4	0	0
CFS (10% wt/wt moisture)	0	36.3	0
BFS (10% wt/wt moisture)	0	0	36.3
Dicalcium phosphate anhydrous	2	0	0
Polivitaminic preparation (Rosenbuch®)	0.75	0.75	0.75

Table 2
Composition of isoenergetic diets (12 MJ/Kg). CF: control feed, EFCS: experimental feed with chemical fish silage, and EFBS: experimental feed with biological fish silage. In brackets: number of the AOAC method used.

	CF	EFCS	EFBS
	Concentrations (%)		
Moisture (*934.01*)	10	10	10
Protein (*981.10*)	23	23	23
Ethereal extract (*922.06*)	14.7	13.7	14
Crude fiber (*962.09*)	9.0	6.7	6.5
Ash (*942.05*)	4.5	4.3	4.2
Free nitrogen extract	38.8	42.3	42.3
Calcium (*927.02*)	0.8	0.9	0.9
Phosphorous (*964.06*)	0.9	0.9	0.9
pH	7.2	4.6	4.8

5. Blood and organs evaluation

Blood samples were obtained by cardiac puncture of animals anesthetized with halothane and sacrificed by cervical dislocation, according to the international guide for the care and use of laboratory animals (National Research Council, 2011). Standard centrifugation procedure was used for hematocrit, while cholesterol (g/L) and uric acid (mg/dL) were measured by enzymatic spectrophotometric methods using commercially available enzymatic kits (Esterase Oxidase and Uricase for cholesterol and uric acid respectively Abbot Clinical Chemistry, USA). The measurements were performed using an automatic analyzer (Alcyon 300, Abbott, USA).

Distension degree of liver and kidneys, content (gas or mucus), serous layer vasodilatation of the stomach and gut were examined for lesions, excessive vasculature or inflammation. The assayed organs were classified in: 0 = without apparent lesions; 1 = slight lesions; 2 = moderate lesions and 3 = severe lesions according to Mann et al. (2012). Each section of the small intestine was weighed and measure.

For gut counting of LAB, 5 mm of duodenum at 2 cm of the pylorus, 5 mm of proximal jejunum at 2 cm of the beginning, 5 mm of distal ileum at 2 cm of the cecum, 5 mm colon at 2 cm of cecum, were cut in aseptic conditions. Each piece was weighed and homogenized following the procedure described above and the suspension inoculated in MRS agar plates (Dalloul et al., 2003). In this work, the villi tallness of any group did not presented differences ($p > 0.01$) among males and females.

6. Diet assessment

Protein efficiency rate (PER) defined as.

$$\mathrm{PER} = \frac{WI}{PI}$$

Where WI: weight increment and, PI: protein intake.

Feed conversion ratio (FC):

$$\mathrm{FC} = \frac{FI}{WI}$$

Where FI: feed intake.

7. Histological evaluation

Jejunum samples were conserved in 10% (vol/vol) formaldehyde. A section (1 cm gut) from each mouse, located at 10 cm of the ligament of Treitz, was extracted and subjected to microscopic assessment. Just about 400 tissue slices (7 μm) stained with haematoxylin and eosin were examined under a light microscope. Villi height was measured in