Physicochemical composition and digestibility of silages from fishery residues in the Atlantic salmon (*Salmo salar*)

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Physicochemical composition and *in vivo* digestibility of chemical and biological silages of fishery residues were determined in Atlantic salmon (*Salmo salar*) (Linnaeus 1758). The chemical silage was prepared with 2 % of sulfuric acid, 98 and 1 % of formic acid (p/v), and the biological with 12 % beet sugar and 3 % yogurt (*Lactobacillus bulgaris* (p/p)). The fishes (141.64 + 28.68 g of average weight and 25.91+ 1.70 cm length) were randomly distributed, according to one-way model, into three triplicate treatments (a diet of reference and two experimental diets with each silage). Chromium oxide was used as inert indicator. Feces were collected by decantation in cylinder-conic tanks, of the type Guelph modified. The chemical composition showed that the protein contents of the silages (52.1 y 38.3 %) diminished ($P < 0.001$) compared with the fresh residues (60.2 %), due to the addition of preservatives in the end product. The digestibility of the nutrients differed according to the silage type. Protein was higher ($P < 0.001$) in the chemical silage (86.1 %), whereas dry matter (80.34 %), lipids (98.65 %), phosphorus (56.5 %), and energy (87.28 %) were higher in the biological. Ash digestibility did not differ between the silages (51.1 and 54.1 %). It is concluded that the silages of fishery residues varied their chemical composition, but did not change their nutritional value, thereby being an alternative protein source in the formulation of rations for the Atlantic salmon.

Key words: *digestibility, fishery silages, salmon, nutritive value*

One of the principal challenges of the salmon industry is eliminating the management mortality, besides discarding legally the residues from the industrial processing. The use of these byproducts through simple and relatively economic techniques, such as the silages based on the acidification of the raw material (Toledo *et al*. 2009), can be a viable alternative. Therefore, they could be recycled as alternative source of proteins for the salmon rations.

Numerous studies proved that fishery residue silages, such as fish meal, can substitute traditional sources of proteins in the feeding of omnivorous species such as pacu (*Piaractus mesopotamicus*) (Vidotti *et al*. 2002a), African catfish (*Clarias gariepinus*) (Llanes *et al*. 2008 and Toledo *et al*. 2009), and red tilapias (*Oreochromis mossambicus* x *O. niloticus*) (Llanes 2010).

The nutritional value of a feed or ingredient is evaluated by the chemical composition and by the level of digestive utilization by the fish (NRC 1993). The objective of this work was determining the physicochemical composition and the *in vivo* digestibility of the chemical and biological silages from fishery residues in the Atlantic salmon (*Salmo salar*, Linnaeus 1758).

Materials and Methods

The bioassay was carried out in the Nutrition Laboratory of the Aquaculture School of the Catholic University of Temuco, Chile.

Preparation of the fish silages and the experimental diets. Sea fish wastes were used (heads, spines, skin, and viscera in lower proportion), marketed in the Fair Pinto de Temuco, Chile. They were ground in a meat mill, at 1cm particle size. The fish paste was homogenized and divided into two equal portions. For the chemical silage, a portion was mixed with 2 % of sulfuric acid, 98 % (p/v), and 1 % of formic acid (p/v). For the biological, it was combined with 12 % of beet sugar (p/p) and 3 % of commercial yogurt (p/p), as culture of lactic acid bacteria (*Lactobacillus bulgaris*). Both silages were put in plastic recipients with caps. The chemical silage was stored at room temperature and the biological, in an oven of dried heat at 40 ºC during seven days (Fagbenro and Jauncey 1993). The pH was measured with HANNA digital potentiometer. Silage samples were collected for the bromatological analysis. Later, both silages were dried at 60 ºC for 48 h in the oven.

For the assay of *in vivo* digestibility, the diet of reference of Glencross *et al*. (2005) (table 1) and two experimental diets were used, prepared with 70 % of the reference and 30 % of each silage (chemical silage and biological silage). The chromium oxide was used as inert marker and it was added in a proportion of 15 g/kg of feed. The proximal chemical composition of the experimental diets is shown in table 2.

For the preparation of the diets, the meals were sieved at 250 µm and mixed in dry conditions in a Kichenaid mixer, K5SS model (10 min). The starch was mixed with distilled water, being subject to boiling (10 min). Later, it was added to the dry mixture, together with the fish oil and the chromium oxide, and it was kept being mixed (20 min). For the pellets, a meat mill RCA of 1HP was used, as well as a matrix with orifices of 3.5 mm. The pellets were dried in an oven at 60 °C for 48 h.

Bioassay. A completely randomized design was performed in nine tanks of cylinder-conic glass fiber of

Table 1. Percent composition of the reference diet (g/100g of feed)

Ingredients	%
Fish meal	65.0
Fish oil	11.0
Pre-frozen starch	15.0
Cellulose	6.5
Mixture of vitamins	0.5
Mixture of minerals	0.5
Chromium oxide	1.5

between means were tested according to Duncan (1955). The data computation system INFOSTAT, version 1, was used to this aim (Balzarini *et al*. 2001).

Results and Discussion

The pH values of the experimental silages during the seven days of storage were from 4.10 to 4.16 for the biological. These values agreed with Llanes *et al*. (2008). For the chemical, they were between 2.73 and 3.01, similar to Vidotti *et al*. (2002b). These values were satisfactory for the preservation

Table 2. Proximal composition of the experimental diets (g/100g of feed). Mean values and standard deviation (n=3)

Indicators	D1 (Reference)	D2 (Chemical silage)	D3 (Biological silage)
Dry matter	96.14 + 0.02	96.97 + 0.20	96.42 + 0.11
Crude protein	47.17 + 0.33	48.02 + 0.47	44.62 + 0.35
Ether extract	18.39 + 0.02	18.42 + 0.07	17.53 + 0.02
Ashes	12.26 + 0.12	13.99 + 0.01	12.53 + 0.03
Phosphorus	01.52 + 0.05	01.53 + 0.02	01.64 + 0.02
Digestible energy (MJ/kg)	18.85 + 0.20	18.20 + 0.20	18.68 + 0.16

the type Guelph modified (three fisheries per treatment). The tanks contained 40 juvenile Atlantic salmons (*Salmo salar*), with average weight of 141.64 + 28.68 g and 25.91 + 1.70 cm of length. The water flow was fitted to maximize the feces sedimentation in the drainage and guarantee its collection in the decantation column. The values of temperature and the concentration of dissolved oxygen were measured in an HANNA Oxy meter.

Fish had one week of acclimatization in the experimental tanks. Later, the feces were collected during five days. The feeding was performed *ad libitum* at two daily times (9:00 am and 5:00 pm). The feces collection was performed the following day, before the first feeding. The feces were frozen in the ultra freezer VWR 5700 at -81 ºC and they were dried in a lyophilizer CHRIST Alpha LD, at 0.060 mbar and -62 ºC. The proximal composition of the samples of silages, diets and feces was determined by triplicate, according to AOAC (1995). The chromium was determined by acid digestion (AOAC 1995) and it was read in 550 µm spectrophotometer of atomic absorption. The values of apparent digestibility (AD) were calculated according to Bórquez *et al*. (2007).

AD dry matter (%) = (AD test – 0.7) x AD reference) / 0.3

AD nutrients (%) = (AD test x Nutrient test – (DA reference x Nutrient reference x 0.7) / (0.3 x Nutrient ingredient)

The data processing was performed through one-way analysis of variance. When necessary, the differences

of the fish residues, and were in accordance with (< 4.5) those of Toledo and Llanes (2007) to guarantee the good quality of the silages in this type of byproduct.

The results of the proximal composition of the silages (table 3) revealed that the processes of acidification and lactic fermentation varied the percent values of nutrients in respect to the raw material. This was previously proved by Gerón *et al*. (2007).

The biochemistry of the fish silages has not been described in detail. A reason for the decrease of nutrients in the biological silages is due to the action of the different preservatives (beat sugar and yogurt) in the formulation. As refereed to by Vidotti *et al*. (2002b) and Gerón *et al*. (2007), the preservatives dilute the concentrations of nutrients in the final product. On the contrary, in a study of Fagbenro and Jauncey (1993) with fermented tilapia residues there was fat increase. This was attributed to the solubility of the lactic acid in the petroleum ether when extracting the fats.

Works of González and Marín (2005) and of Albrecht and Torpoco (2008) proved that the fish silages varied the proximal composition in respect to the storage time, due to the intake of carbohydrates by the lactic bacteria producing the lactic acid, water and volatile metabolites, substances that keep this product in constant change.

Gerón *et al*. (2007) and Santana-Delgado *et al*. (2008) noted that the decline in the protein contents in the chemical silages is due to the hydrolysis and to the autolysis effects on the degradation of proteins and

Table 3. Proximal composition of fresh wastes and silages (g/100g DM)

Indicators	Fresh wastes	Chemical silage	Biological silage	SE ±
Dry matter	32.80[a]	25.98[b]	28.90[c]	1.78**
Crude protein	60.22[a]	52.09[b]	38.26[c]	3.93***
Lipids	22.34[a]	21.10[a]	15.12[b]	1.32**
Ashes	17.38[a]	16.98[a]	11.87[b]	1.12***
Phosphorus	2.16[a]	0.81[c]	1.82[b]	0.26**

SE- Standard error (n=3) **P < 0.01 ***P < 0.001

nucleoproteins. They are transformed into more simple components, such as amino acids and ammonium, which are volatilized during the storage.

The high ash values (table 3) in the silages of fishery residues are given by the presence of large amounts of scales, spines and bones. Likewise, the phosphorus contents in these residues can be beneficial in the rations, because they are in the form of tricalcium phosphate and calcium carbonate (Toledo *et al*. 2009), assimilable by the gastric fishes (NRC 1993).

González and Marín (2005), Gerón *et al*. (2007) and Santana-Delgado *et al*. (2008) reported chemical compositions of silages prepared with different raw materials (residues of sardines, tilapias, complete Spanish mackerel). The compositions reported by these authors differed between themselves and from the results herein. According to Vidotti *et al*. (2002b), these divergences are due to the chemical composition of the different raw materials, which may vary according to species, sex, season, feeding, reproductive status, and even, according to the type of industrial cut during the processing.

Throughout the digestibility assay, the temperature and the dissolved oxygen from the water of the experimental tanks ranged from 12.9 to 14.6 ºC, and from 7.91 to 8.61 mg/L, respectively. The ammonium level was monitored and kept through water circulation in low values of 0.01 mg/L. No mortality was reported and the rations were consumed rapidly.

Refstie *et al*. (2004) and Hevroy *et al*. (2005) agreed with the utilization of hydrolyzed products of fish proteins in the diet of salmons improved the feed intake. This criterion was in accordance with this study, where silages provide free amino acids from the process of hydrolysis that may have attractive effect for fishes (Stone *et al*. 1989).

The apparent nutrient and energy digestibility of the silages (table 4), obtained by the feces collection method through the Guelph system and the chromic oxide as marker, were satisfactory in respect to other reports.

Out of the silages evaluated, the biological had higher total digestibility, lipids, phosphorus and energy, whereas protein was higher for the chemical (P < 0.01). The ash digestibility did not differ between the methodologies under study.

The lowest protein digestibility (P < 0.01) in the fermented residues was given by the rise in the contents of volatile nitrogenous bases (VNB). This process is the result from the oxidative deamination of the free amino acids, non-protein nitrogen components, by part of a number of bacteria causing their reduction and, at the same time, generating ammonium, which leads to negative consequences in the nutritional value of this type of silage. Enes *et al*. (1998) found higher formation of VNB in the biological silage in respect to the chemical. They noted that this was a disadvantage, because it leads to the reduction in the contents of amino acids.

González and Marín (2005) explained that in these silages there is growth of microorganisms capable of multiplying at a temperature lower or equal to that of the environment. They have proteolytic and lipolitic characteristics that play an important function in the process of fish decomposition. The cited authors proved for 60 d progressive increase of VNB, which attained values of 157.4 and 172.9 mg N/100g.

In this study, the protein digestibility values

Table 4. Apparent digestibility of nutrients in the silages of fishery residues in *Salmo salar* (g/100g)

Nutrients	Chemical silage	Biological silage	SE ± Sig
Dry matter	64.16	80.34	2.32**
Protein	86.81	79.34	1.52**
Lipids	95.94	98.65	0.63*
Ashes	51.05	54.08	2.10
Phosphorus	41.29	56.55	2.03**
Energy	76.45	87.28	2.01**

SE- Standard error (n=3) *P < 0.05 **P < 0.01

were in the interval from 75 to 95 %, reported by Köprücü and Özdemir (2005) in ingredients rich in proteins for fishes. This result was similar to that of Sveier *et al*. (1999) with the meal of subproducts from the fillet slicing of herrings (82.1 %) in *Salmo salar*, whose feces were collected by the method of abdominal massage. Besides, they agreed with other studies reporting conventional protein ingredients in carnivorous fishes, such as meals of anchovy (91.4 %) and Menhaden (87.7 %) in coho salmon (*Oncorhynchus kisutch*), through the feces collection system proposed by Hajen *et al*. (1993), cited by Sugiura *et al*. (1998). With feces collection per columns of decantation, Bureaut *et al*. (1999) reported meals of hydrolyzed feathers (81 %), meat and bone (85 %), poultry byproduct (87 %) and blood (82 %) in rainbow trouts (*Oncorhynchus mykiss*). Portz and Cyrino (2004) noted fish meals (87 %) and poultry subproducts (81.5 %) in largemouth bass (*Micropterus salmoides*), with feces collection per column of decantation. Tibbetts *et al*. (2006), with this same collection method, indicated protein concentrates of common vetch (89.8 %), canola (88.8 %), meals of byproducts of poultry (80.2 %), crab (89.4 %) and lupine (89.7 %) in the Atlantic cod (*Gadus morhua*).

In this study, the favorable values in the protein digestibility were due to the little lixiviation of the soluble nitrogenous components in the feces, due to the method of collection (decantation), its consistency and water exposition time. In other methods, such as the abdominal massage, the opposite may occur, because there are contamination problems with undigested material, urine, blood and even sexual products, which may lead erroneously to low digestibility values.

According to Aksnes and Opstvedt (1998), fats are supplied, alone or mixed in the diet ingredients, and have, habitually, digestibility values from 85 to 95 % for fishes. In this study, the digestibility of the lipids (table 4) showed significant ($P < 0.05$) differences between the silages. Nevertheless, values higher than 95 % were found, which could account for the oil characteristics from these byproducts, by having high concentrations of poly-unsaturated fatty acids ω-3 (Vidotti *et al*. 2002b). They permit higher degree of unsaturation, indispensable for higher fluidity, flexibility and permeability of the membranes at low temperatures, essential for sea and cold water fishes such as salmons.

Vidotti *et al*. (2002b) reported that the silages prepared with sea fishes had high levels of long-chain fatty acids and high unsaturation (C22:1 and C22:6 ω3) in respect to those of fresh water. Besides, they are better absorbed than the ingredients with larger amount of saturated lipids.

Bureau (2004) noted that the feeds for rainbow trouts with fish or plant oils (high levels of fatty acids ω-3 and ω-6) were more digestible (6 %) than those containing terrestrial animal fat (high contents of saturated acids), due to the point of fusion of the saturated acids, which is high.

In this assay, the apparent digestibility of the ashes (table 4) was similar between the silages, whereas the phosphorus differ significantly ($P < 0.01$). The fermented residues had the most favorable values.

Studies of Sugiura *et al*. (1998) in coho salmon proved that the phosphorus digestibility (P_8) in animal origin ingredients, with high levels of ashes, could reduce the fractional absorption and increase the fecal excretion of this mineral. Besides, these authors mention an inverse correlation between the intake (μg P_8 / liveweight /d), the apparent digestibility and the gross fractional absorption of P8. Seemingly, the intestinal absorption of P_8 was irregular, specifically the gross absorption is related to the amount ingested. Nevertheless, in this work, fishery residues were used having high ash contents (17.4 g/100g DM), positive phosphorus digestibility and compatible with other meals of fishery origin, such as Menhaden (40.4 %), herring (57.3%) and anchovy (47.4 %), found in the coho salmon (Sugiura *et al*. 1998).

Sarker *et al*. (2007) found that the dietary acidification for gastric species was effective, when increasing the availability of minerals in the fish bones and meal, which proved that by supplementing low levels of citric acid to the diet of the red sea bream (*Pagrus major*). This increased the absorption of phosphorus in the fish meal.

In this research, the effect of the organic acids present in the silages (formic and lactic acids) improved the availability of this mineral (phosphorus), due to the acidifying effect that solubilized the P_8 present in the fishery residues.

The literature about the use of the fishery residues in the diet of Atlantic salmon is scarce. However, works have been found that refer to their beneficial effect on the feeding of these fishes. Berge and Storebakken (1996) fed larvas of *Salmo salar* with a diet containing high levels of fish silage, and obtained favorable growth and survival rates, which they attributed to the supplementation of proteolytic enzymes from the same silage, due to they are scarce in the digestive tract during this life stage of the fishes. Also, Espe *et al*. (1999) noted that the inclusion of 15 % of hydrolyzed protein of fishery silages had positive effect on the productive behavior of these fishes. However, the highest protein digestibility was in the diets containing levels of 30 and 40 %.

The outcomes of this work showed that the fishery silages varied their chemical composition compared with the fresh wastes, but they did not change their nutritional

value. Therefore, they may be an alternative as protein source in the elaboration of the Atlantic salmon rations. Thus, the dependence on the fish meal as primary protein source is diminished, as well as the costs through the removal of the fishery residues.

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