# Utilization of fish silos in the semi-humid diet formulation for red tilapias (*Oreochromis niloticus* x *O. mossambicus*)

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The effect of two semi-humid diets, formulated with silages (chemical and biological) from red tilapia slicing residues, as only animal protein source, was assessed on the feeding of red tilapias (*Oreochromis niloticus* x *O. mossambicus*). Thus, one-way classification models were applied with three repetitions. The physical stability of the diets was determined by dry matter loss and protein lixiviation after water immersion. The *in vivo* digestibility was calculated by the indirect method with chromium oxide III as inert marker. The feces collection was carried out using a siphon at the bottom of the tanks. The growth bioassay was conducted for 60 d, with 270 sex-reversed fingerlings (all-male) of 6.0 + 0.01 g of weight as initial average. Satisfactory losses of dry matter (11.4 and 10.9 %) and protein lixiviation (16.4 and 15.9 %) were found in the experimental rations, which differed from the control (5.1 and 4.7 %). The lowest protein digestibility was reported in the diet with biological silage (86.8 %), differing ($P < 0.01$) from the chemical (89.4 %) and the control (88.7 %). The productive performance showed that there were not significant differences in the final weights (30.0; 29.9 and 29.6 g), the feed conversion (1.5; 1.5 and 1.6) and protein efficiency (1.8; 1.9 and 1.8) in fish fed silages and fish meal. The survival was high for all the treatments (higher than 95 %). It was evidenced that the semi-humid diets, based on silages from tilapia slicing residues, are as efficient in the productive performance of red tilapias as the commercial feeds with fishmeal, which represents a feeding alternative for cultures of this species.

Key words: *feeding, alternative diets, fish silage, tilapia.*

In Cuba, tilapia cultures are intensified due to the stability in concentrated feeds production from imported resources such as cereals (corn and wheat), soybean and, to a lesser extent, fish meal. The latter has little availability and high prices (1 346.00 USD/t) (FAO 2011) at the international market. In order to continue with the support and increase of these cultures, other feeding alternatives are necessary, being based on national raw materials.

The use of fishery byproducts through simple methodologies and low investment such as silages (conservation in acid medium) can be an alternative for the preparation of manufactured rations. This alternative can be added to the abundant residues produced daily in Cuba in fisheries. According to Llanes *et al*. (2011), the nutritional value of the silages from the tilapia slicing on dry basis is very attractive. These authors found between 36 and 43 % of crude protein with high concentrations of lysine, arginine, valine, leucine and digestibility higher than 80 % for red tilapias (*Oreochromis niloticus* x *O. mossambicus*).

The information available in the literature about the utilization of fish silages in tilapia feeding is limited and suggests its use as partial substitute of fish meal (up to 80 %) in Nile tilapia (*Oreochromis nilóticus*) and red tilapia (*Oreochromis spp*) rations (Fagbenro *et al*. 1994 and Botello *et al*. 2011). Nutritional and economic improvements were even reported when the silage substituted 30 % of the commercial feed (Carvalho *et al*. 2006 and Perea *et al*. 2011).

The effect of two semi-humid diets, formulated with silages (chemical and biological), was assessed as only animal protein source in the feeding of red tilapia (*Oreochromis niloticus* x *O. mossambicus*) fingerlings.

## Materials and Methods

The work was conducted in the Laboratory of Nutrition of the Center of Water Preparation "Mampostón". Residues from the tilapia slicing process were used and ground in a meat mill JAVAR 32. The resulting paste was divided into two portions. One was mixed with 2 % of sulphuric acid at 98 % and 1 % of formic acid (p/v) for the chemical silage, both of the commercial brand MERCK. The other was combined with 15 % of final molasses and 3 % of yoghurt *Lactobacillus acidophilus* (p/p) (biological silage, EBL). After 7 d, two balanced semi-humid diets were formulated, according to the requirements of Olvera-Novoa (2002).

The diets were prepared with 40 % silage and 60 % plant feedstuff. They were pelletized in a meat mill at 3-mm diameter. The semi-humid pellets were stored at -10 ºC in plastic containers with covers. Commercial tilapia feed was used as control. The composition of the diets is shown in table 1.

The physical stability of the diets was determined by dry matter loss (DML) and total protein lixiviation (TPL), according to the methodology of Fagbenro and Jauncey (1998). DML was calculated by dry weight percentage difference of the sample after water immersion and it was expressed as DML percentage. Total protein lixiviation was reported as basic remnant percentage:

TPL (% remnant)= (g remnant protein /g remnant

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

| Ingredients | Plant feedstuff | Control Diet | Diet (chemical silage) | Diet (biological silage) |
|---|---|---|---|---|
| Fish meal | - | 20.00 | - | - |
| Chemical silage | - | - | 40.00 | - |
| Biological silage | - | - | - | 40.00 |
| Soybean meal | 58.33 | 40.00 | - | - |
| Corn meal | 11.25 | 10.00 | - | - |
| Wheat bran | 24.84 | 21.75 | - | - |
| Soybean oil | 1.00 | 3.00 | - | - |
| Vitamin-mineral mixture | 1.66 | 1.00 | - | - |
| Common salt | 0.42 | 0.25 | - | - |
| Dicalcium phosphate | 2.50 | 2.00 | - | - |
| Carboxymethyl cellulose | . | 2.00 | - | - |
| Plant feedstuff | - | - | 60.00 | 60.00 |
| Dry matter | 88.10 | 91.90 | 64.40 | 65.10 |
| Crude protein | 31.10 | 35.30 | 23.60 | 22.70 |
| Gross energy (MJ/kg) | 16.20 | 17.40 | 11.90 | 12.10 |

pellets)/ (g nutrient protein /g initial pellets) x $10^2$

The *in vivo* digestibility trial was performed in nine rectangular cement ponds, of 400 L capacity (three per treatment). They had 30 red tilapia juveniles (*Oreochromis niloticus* x *O. mossambicus*) of 61.2 ± 6.23 g of average weight (270 fish as total). The experimental diets with 1 % of chromium oxide III ($Cr_2O_3$) as inert marker were given *ad libitum*. The feces were collected carefully in a siphon at the bottom of the ponds during 6 d and before the feeding (8:00 am and 3:00 pm). Later, they were dried in an oven at 60 ºC for 48 h. Fort he chemical analysis they were ground in a Wiley mill, with mesh of 1 mm. The apparent digestibility (AD) was calculated according to Bureau *et al*. (1999).

AD nutrient (%) $=10^2-10^2$ x (nutrient feces/nutrient diet x $Cr_2O_3$ diet/$Cr_2O_3$ feces).

Digestible protein (DP)= (crude protein percentage in the diet x digestibility)/$10^2$

For the growth bioassay, 270 sex-reversed fingerlings (all-male) of red tilapias (*Oreochromis niloticus* x *O. mossambicus*), of 6.00 ± 0.01 g average weight were used. They were distributed randomly in nine rectangular cement ponds of 700 L (three per treatment). The water flow in the ponds was standardized at 1.2 L/min for 24 h. Every day, the dissolved oxygen and the temperature were recorded with a digital oximeter HANNA.

Given the digestible protein differences between the experimental diets, the feed addition rate (table 2) was calculated according to the feed to be ingested using the requirements of g DP/100 g of liveweight (PV) in tilapias, as established by Llanes *et al*. (2009).

The feed amount/d was determined by the following formula:

Feed amount/d = Requirement (g DP/$10^2$ g LW) / (% DP of the diet) x$10^2$

The diets were given in two daily rations (9:30 am and 4:30 pm) during 60 d, with the aim of adjusting the feed amount. The fish were weighed individually every 15 d.

The following productive indicators were calculated:

Specific growth rate (SGR) = $10^2$ x (ln Final weight, FW – ln Initial weight, IW)/d of culture.

Daily weight increment (DWI) = FW – IW/d of culture.

Feed conversion factor (FCF) = added feed / weight gain. Survival = Final number of fish/number of initial fish x 102.

Protein efficiency rate (PER) = Weight gain /added protein.

Protein retention efficiency (PRE, %) = (Final body

Table 2. Percentages of addition of feeds throughout the experimental period

| Weight (g) | Requirements (g digestible protein /100g peso vivo) | Control diet | Diet (Chemical silage) | Diet (biological silage) |
|---|---|---|---|---|
| 5 | 1.72 | 5.56 | 8.09 | 8.67 |
| 10 | 1.54 | 5.00 | 7.24 | 7.76 |
| 15 | 1.40 | 4.53 | 6.58 | 7.35 |
| 20 | 1.35 | 4.36 | 6.35 | 6.80 |

CP x FW) – (Initial body CP x IW)/added CP.

Percentage of protein in weight gain (PWG, %)= (Final body CP x FW) – (Initial body CP x IW) / FW – IW.

For the calculation of the PRE and PWG, twelve animals were slaughtered at the start of the bioassay. In the end, there were four animals per pond (12 per treatment).

The bromatological composition of the samples of silages, meals, feces and carcasses of the fish, as well as the content of chromium in the feces and the diets, was determined by triplicate according to AOAC (1995). Gross energy was calculated by the net heat coefficients reported by Goda *et al*. (2007).

The average values of the nutritional indicators were analyzed through one-way analysis of variance. The comparison between means was tested according to Duncan (1955) by means of the statistical software INFOSTAT, version 1.0 (Balzarini *et al*. 2001).

## Results and Discussion

Due to the inclusion of agglutinants and to the industrial processes (compression, heating and adherence) for the fabrication of the commercial pellets, the physical stability (table 3) showed lower (P < 0.001) percentage of DML for the control diet compared with the pelletized in a meat mill, and high humidity percentages of the diets with fish silages.

Lower protein lixiviation (P < 0.01) was found for the control (4.7 %), due to higher resistance to the physical impacts of the pellets. This trial was effective for the retention of the nutrients and to hamper greater total protein losses. The diets with fish silages had similar percentages of PMS (table 3) and protein wash (16.4 and 15.9 %), with values that can be considered acceptable for semi-humid pellets, according to the criteria of Fagbenro and Jauncey (1998).

These authors demonstrated that with the cold extrusion, the semi-humid pellets can be improved. After the immersion test, they obtained values of 6.4 % DML and 13.2 % of protein wash.

The results of this work confirmed the agglutinant capacity of the final molasses, the corn starch and the oil of the fish silos, because they contribute to the physical stability of the semi-humid pellets (Fagbenro and Jauncey 1998 and Toledo *et al*. 2009). The results of this study are of great practical importance, because they suggest that simple mixtures of meals and fish byproducts can be processed in the form of pastes or pellets and the losses by dispersion are minimized. Likewise, their quality exemplifies the possibility of their use as alternative for nutritionally complete commercial feeds in tilapia cultures.

The digestibility results (table 3) were satisfactory in respect to the report in the literature. They permit stating that red tilapias use efficiently the nutrients from semi-humid diets with silages of fishery wastes.

The protein digestibility (DCP) differed significantly (P < 0.05) between the treatments. The lowest value appeared in the biological silage. This is due to the rise in the contents of nitrogen volatile bases, as a result from the oxidative deamination of the free amino acids, components of the non-protein nitrogen pool, by a number of bacteria causing their reduction. They, at the same time, produce ammonium, which brings about negative consequences on the nutritional value of this type of silo (Enes *et al*. 1998).

Nevertheless, all the DCP in this study were similar to those for the same protein source (83.5 to 86.6 %) in Nile tilapias (Fagbenro and Jauncey 1998). However, when dehydrating the silage with soybean meal, values of 80.6 % were reported for the same species (Fagbenro *et al*. 1994), and of 76.4 %, in pacú (*Piaractus mesopotamicus*) (Vidotti *et al*. 2002). This shows decline in the bioavailability of some amino acids during the drying process due to the reactions of Maillard.

The energy digestibility did not differ between the treatments (table 3). Its values (80.6, 82.1 and 84.8 %) were next to those of Fagbenro and Jauncey (1998). They can be associated with the high concentrations of unsaturated fatty acids in the oils of these fishery byproducts, which are better absorbed than the saturated from other raw materials of plant origin (Allan *et al*. 2000). Besides, the citations of Goda *et al*. (2007) and Toledo *et al*. (2008) were corroborated. They stated that tilapias utilize frequently the carbohydrates of the ration. This trait is quite favorable because the feeds rich in starch are of lower cost, in general.

During the growth trial, the water temperature in

Table 3. Stability and digestibility of the experimental diets in red tilapias

| Indicadores | Control diet | Diet (chemical silage) | Diet (biological silage) | SE (±) Sig |
|---|---|---|---|---|
| Dry matter loss % | 5.1$^a$ | 11.4$^b$ | 10.9$^b$ | ±0.76 *** |
| Total protein, % remanent | 95.3$^a$ | 83.6$^b$ | 84.1$^b$ | ±1.34 ** |
| Protein digestibility, % | 88.7$^a$ | 89.4$^a$ | 86.8$^b$ | ±0.49 * |
| Energy digestibility, % | 83.8 | 83.7 | 84.7 | ±0.32 |

$^{ab}$Different letters in the same row differ statistically at P < 0.05 (Duncan 1955)
*P < 0.05 **P < 0.01 ***P < 0.001

the ponds varied from 26.8 to 27.6 ºC; the oxygen concentration, from 5.11 to 6.02 mg/L; and the pH, from 7.85 to 8.01. According to Kuanhong (2011), these values are in the ranges convenient for the species culture.

The performance of red tilapias fed semi-humid diets (table 4) showed that growth indicators (FW, SGR, and DWI) were not affected by the type and level of silage substitution. This agreed with the report of Vidotti *et al*. (2002) and Toledo *et al*. (2009), who did not find significant differences in the growth of pacú and catfish (*Clarias gariepinus*) fed diets with fish silage, as only source of animal protein, compared with the results with a commercial ration with fish meal.

The proposed semi-humid feed methodology, with the inclusion of 40 % of fish silage (humid basis), equivalent to incorporating 5.6 % of CP and substituting 45 % of the CP from the fish meal. The SGRs were the same as in the control and as those reported (2.7 and 2.8) for diets formulated with meals from different fishery byproducts (benthic organisms, small pelagic fishes, mixture of different catfish species and wastes from tuna and sardine processing) in Nile tilapias (Goddard *et al*. 2008).

This is in correspondence with the results of Toledo *et al*. (2008), who noted that the performance of Nile tilapia fingerlings is not affected by using rations with high contents of plant protein (mainly soybean) and minimum fish meal to fulfill the sulfur amino acids. Besides, these results suggest that the use of the fishery byproducts through fish silage can be an alternative of protein source to aleviate the little access to the fish meal.

Goda *et al*. (2007) reported lower SGR (1.7 to 1.9) in Nile tilapias fed diets based on soybean cake, integral soybean and corn gluten. This can be attributed to anti-nutritional factors and to the unbalance of some essential amino acids (arginine, histidine, and threonine), regardless the diets were supplemented with L-lysine and DL-methionine.

With the experimental diets, the FCF in humid basis (table 4) was significantly impaired, in 700 and 800 g plus of feed per each kilogram of liveweight increment. This was mainly due to the humidity contents of these diets (35 %) in respect to the commercial ration (10 %). This result was corroborated through the FCF in dry basis, which did not differ between treatments. There were not differences either in respect to Vidotti *et al*. (2002) and Toledo *et al*. (2009), who did not find significant differences in the FCF between the fishes fed silages and fish meal.

This indicator (FCF) reflects the advantages in the utilization of fish silage. The animals fed these rations were more active at the time of the feeding. This is related to the higher palatability provided by the soluble substances available in the silos that stimulate its intake. This was proved in trouts (*Salmo gairdneri*) (Stone *et al*. 1989).

There were not significant differences in the PER (table 4), probably by the high nutritional value of the silages (Llanes *et al*. 2011) and the addition of the same amounts of digestible protein in the treatments (table 2). Also, the action of the endogenous proteases in the fish tissues could have influenced. They are activated in acid medium and increase the solubility of the proteins, which could favor their better absorption and utilization. The PERs (1.9) obtained by Fagbenro *et al*. (1994) in Nile tilapia, and by Botello *et al*. (2011) (2.0) in red tilapias were in correspondence with the records in this study.

Likewise, the PRE and the PWG (table 4) did not

Table 4. Table 4. Nutritional indicators in red tilapias (*Oreochromis niloticus* x *O. mossambicus*) fed experimental diets

| Indicators | Control diet | Diet (chemical silage) | Diet (biological silage) | Sig. |
|---|---|---|---|---|
| Final weight, g | 30.0 + 0.74 | 29.9 + 0.73 | 29.6+ 0.75 | NS |
| Indicators | D1 Reference | D2 Chemical silage | D3 Biological silage | ± SE Sig |
| SGR, %/day | 2.7 | 2.6 | 2.7 | ±0.07 |
| DWI, g/day | 0.4 | 0.4 | 0.4 | ±0.02 |
| FCF, HB | 1.6[a] | 2.3[b] | 2.4[b] | ±0.09** |
| FCF, DB | 1.5 | 1.5 | 1.6 | ±0.07 |
| Survival,% | 96.6 | 95.5 | 96.6 | ±0.04 |
| PER | 1.8 | 1.9 | 1.8 | ±0.08 |
| PRE, % | 28.1 | 28.6 | 27.4 | ±0.04 |
| PWG, % | 23.5 | 23.3 | 22.9 | ±0.03 |

[ab]Different letters in the same row differ statistically at $P < 0.05$ (Duncan 1955) **$P < 0.01$

SGR (specific growth rate); DWI (daily weight increment); FCF, HB and DB (feed conversion factor, humid and dry basis); PER (protein efficiency rate); PRE (protein retention efficiency); PWG (Percentage of protein in weight gain) differ between the experimental treatments, which agreed with Fagbenro *et al*. (1994) for the first indicator. These authors reached values of 29 %.

The favorable results in protein utilization (PER, PRE and PWG) should not be explained only according to the nutritional value of the silages, but also as to the possible capacity of these fishes to utilize efficiently the high contents of glutamic acid as source of non-essential amino acids in this raw material (approximately 13 % of the CP) (Llanes *et al*. 2011).

According to Tacon (1989), part of the dietary portein is utilized by the fishes with energetic purposes. They have certain predisposition to utilize the carbonated chains of the amino acids as energy substrate. Thus, the existence of high rate of glutamic acid in the silos could represent important save in the synthesis of non-essential amino acids out of the essential. Besides, it can be used in the synthesis of fatty acids and in the growth. This would contribute to the better general use of the dietary energy.

The composition of the carcasses (table 5) showed that the fish fed both diets (fish meal and fish silage) had superior ($P < 0.001$) levels of proteins and lipids compared with the start. The ash contents had different performance, decreasing significantly ($P < 0.005$).

There was not influence on the carcasses of the fish due to the type and level of substitution of the silage in respect to the fish meal; although, with the biological silage, there was rise in the deposition of body fat, probably due to the lower protein-energy ratio of this diet (18.7 g/MJ), as compared with the control (20.3 g/MJ), and to the higher feed and energy intake (table 2) of the fish in this treatment.

The ash content in muscle (table 5) did not differ between the treatments. Its decrease in respect to the start can be related to the growth in the juvenile stage. This is characterized by a predisposition of the muscle mass of growing faster than the bone, which provokes decrease in the concentrations of calcium and phosphorus, mineral components of the ashes.

It was evidenced that the balanced semi-humid diets, formulated with silages of residues from the process of slicing the tilapias, as only animal protein source, are as efficient for the productive performance of red tilapias (*Oreochromis niloticus* x *O. mossambicus*) as the commercial feeds with fish meal. Therefore, they represent a feeding alternative for the cultures of this species.


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Table 5. Proximal composition of the carcasses (g/100g)

| Indicators | Starter | D1 Reference | D2 Chemical silage | D3 Biological silage | Sig |
|---|---|---|---|---|---|
| Humidity | 76.80[b] ±0.18 | 73.50[a] ±0.18 | 73.30[a] ±0.19 | 73.50[a] ±0.26 | *** |
| Crude protein | 13.50[a] ±0.20 | 16.20[b] ± 0.20 | 15.90[b] ±0.20 | 15.8[b] ±0.29 | *** |
| Lipids | 2.20[a] ±0.13 | 3.90[b] ±0.13 | 4.10[b] ±0.13 | 4.80[c] ±0.18 | *** |
| Ashes | 4.20[b] ±0.08 | 3.80[a] ±0.08 | 3.80[a] ±0.08 | 3.70[a] ±0.12 | * |

[ab]Different letters in the same row differ statistically at $P < 0.05$ (Duncan 1955)

*$P < 0.05$ ***$P < 0.001$

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