Lat. Am. J. Aquat. Res., 41(3): 468-478, 2013
DOI: 103856/vol41-issue3-fulltext-10

Research Article

Use of tuna industry waste in diets for Nile tilapia, *Oreochromis niloticus*, fingerlings: effect on digestibility and growth performance

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ABSTRACT. During the tuna canning process, about 52~54% of the total weight of the fish is discarded as waste, which can be processed in order to obtain tuna byproducts meal (TBM), or stabilized as tuna silage hydrolysates (TSH). Both products were tested as replacements of soybean meal (SBM), in diets for fingerling male Nile tilapia *Oreochromis niloticus*. Seven test diets were isonitrogenous (35% CP) and isoenergetic (19 kJ g^{-1}): a basal diet contained SBM as main protein source (TSH0); four experimental diets had increasing inclusion levels of TSH replacing 25, 50, 75 and 100% of the SBM protein. The sixth diet contained TBM as sole protein source, and a commercial feed for tilapia was used as reference diet (RD). The diets were fed for eight weeks to triplicate tanks, each with 15 fry tilapia (initial weight of 0.89 ± 0.29 g). Tilapia fed the TBM diet had greater weight gain and feed intake, and lower feed conversion ratios than those fed diets containing with TSH. The RD, TSH25 and TSH50 diets gave the similar growth response. Fish fed diets TSH0, TSH75% and TSH100% showed reduced growth performance.

Keywords: fish feeding, fisheries by-products, silage, rendered proteins, *Oreochromis niloticus*.

Uso de residuos de la industria del atún en dietas para alevines de tilapia del Nilo, *Oreochromis niloticus*: efecto sobre la digestibilidad y el rendimiento del crecimiento

RESUMEN. Durante el proceso de enlatado de atún se desecha aproximadamente el 52-54% del peso total de los peces. Estos desechos pueden ser usados para producir harina de desechos de atún (TBM) o estabilizados como subproductos hidrolizados de atún (TSH). Ambos productos se probaron como sustitutos de harina de soya (SBM), en dietas para alevines de tilapia del Nilo *Oreochromis niloticus*. Seis dietas con igual contenido de proteína (35%) y energía (19 kJ g^{-1}): una dieta basal con SBM como principal fuente de proteína (TSH0); y cuatro dietas experimentales se prepararon con crecientes niveles de TSH en reemplazo de 25, 50, 75 y 100% de la proteína de soya. La sexta dieta fue preparada con TBM como única fuente de proteína y un alimento comercial fue incluido como dieta referencia (RD). Estas dietas se ofrecieron por triplicado durante ocho semanas a grupos de 15 alevines cada uno, con un peso medio inicial de 0,89 ± 0,29 g. Los peces alimentados con la dieta preparada con TBM presentaron mayor ganancia de peso, mayor consumo de alimento y factor de conversión más eficiente que los alimentados con las dietas RD, TSH25 y TSH50. Con las dietas antes mencionadas, se obtuvieron resultados similares entre sí, mientras que los peces que recibieron dietas TSH0, TSH75% y TSH100% dieron las menores respuestas de crecimiento.

Palabras clave: alimentación de peces, derivados de la pesca, ensilado, proteínas prestadas, *Oreochromis niloticus*.

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INTRODUCTION

Tilapia is one of the most important farmed fish crops, and its production continues to increase at an accelerated rate (Fitzsimmons *et al*., 2011). However, since the price of feed may account for an important percentage of the production costs of tilapia farms (Lim & Webster, 2006; Ng & Hanim, 2007), an important challenge facing the tilapia industry is the development of cost-effective feeds.

Fishmeal is the most expensive ingredient in formulated feeds and although research is ongoing on the evaluation of alternative and cheaper ingredients (Kureshy *et al*., 2000; Lim *et al.,* 2008), soybean meal (SBM) has been considered the best protein source to replace fishmeal, due to its wide availability and consistent quality. Despite of its low contets of essencial amino acids, plant proteins may contain anti-nutritional compounds which may affect fish growth (Mbahinzirek *et al*., 2001; Davis *et al*., 2005; Wang *et al*., 2006; NRC, 2011). The effects of SBM in fish diets have been the object of several studies (Kaushik *et al.,* 1995; Refstie *et al.,* 1998). These studies have found evidence that tilapia grows normally when fed diets in which fishmeal is completely replaced with SBM, either supplemented with essential amino acids (El-Saidy & Gaber, 2002; Nguyen, 2008) or minerals (Viola, 1988), or even as the sole protein source (Nguyen *et al.,* 2009).

On the other hand, the demand for SBM as ingredient for compounded animal feeds is increasing (FAO, 2009), and other by-products of the agro industry are also in high demand (Tacon & Nates, 2007), which suggests the importance of investigating the effects of alternative ingredients as protein sources.

In Mexico, annual tuna landings are approximately 134,500 ton, and 52-54% of this amount is discarded as waste during processing by the canning industry. Part of this is converted into a commercially available product (tuna by product meal, TBM). This has considerable potential as feed ingredient for fish culture since substitutions of FM by TBM have been reported with different success in juvenile tilapia (Goddard *et al*., 2008; Saïdi *et al*., 2010; Gümüs *et al*., 2011), common carp fry (Gümüs *et al.,* 2009), rainbow trout (Tekinay *et al.,* 2009), and shrimp (Hernández *et al.,* 2011).

Additionally, there is considerable evidence that, because of their high protein content and positive effects on feed palatability, fish hydrolysates may be desirable ingredients to combine with plant proteins in fish diets (Vidotti *et al*., 2003; Ramírez-Ramírez *et al*., 2008). Fish silages have been used with some success in feeds for tilapia (Plascencia-Jatomea *et al*., 2002; Llanes *et al*., 2010).

This study was designed to determine the effect of partial or complete substitution of soybean meal with tuna silage hydrolysates (TSH), or of its total substitution with TBM, on the growth response of Nile tilapia, *Oreochromis niloticus* fry, as well as on the digestibility and amino acid profile of the experimental diets.

MATERIALS AND METHODS

Experimental ingredients

Soybean meal (SBM) was purchased as defatted, solvent extracted from a local feed manufacturer. Steam-dried tuna by-product meal (TBM) was obtained from the manufacturer (Pescados Industrializados S.A de C.V. (PINSA), located in Sinaloa, Mexico), that provided also the tuna processing waste used for hydrolysis and silage (fresh viscera eliminated before the canning process and the cooked by-products consisting of dark muscle, skin, gills, head, tail and skeleton removed from the eviscerated fish after cooking at 65°C for 160 min). The proximate composition and the essential amino acid contents of the experimental ingredients are shown in Table 1.

Tryptophan not determined

Amino acids essential for tilapia according to Santiago & Lovell (1988) (as percentage of protein): arginine 4.2, histidine 1.7, isoleucine 3.1, leucine 3.4, lysine 5.1, methionine 2.7, phenylalanine 3.8, threonine 3.6 and valine 2.8.

Tuna by-product silage production (TSH)

The cooked by products and the viscera with their proteolytic enzymes were minced (Meat Mincer Torrey Mod 22, Monterrey, N.L. Mexico, with a 5 mm sieve), mixed with sugarcane molasses (180 g kg^{-1} wet basis) as a carbon source, and inoculated with 50 mL kg^{-1} (wet basis) of *Lactobacillus plantarum* (APG-Eurozym) at a concentration of $1x10^9$ cfu (Shirai *et al*., 2001).

Fermentation of 13.6 kg of this mixture was carried out in a 30 L packed bed column reactor at controlled temperature (96 h, 30°C). Preservation of the fermented product was aided by the lactic acid produced and by a reduction in the water activity from the initial 0.98 of the raw material, to 0.94 of the TSH (Ramírez-Ramírez *et al*., 2008). The lactic acid concentration of the hydrolysate was 0.49 mmol g^{-1},

Table 1. Proximate composition (% dry weight) and amino acid profile of feed ingredients included in the diets: defatted soybean meal (SBM), tuna by-product meal (TBM), and hydrolyzed tuna silage (TSH).

	SBM	TBM	TSH
Proximate composition (% dry wt)			
Crude protein (Nx6.25)	44.8	57.2	51.8
Crude fat	1.26	13.9	8.1
Fiber	7.3	0.54	0.23
Ash	7.26	20.8	14.2
NFE	38.8	7.6	25.7
GE (kJ g^{-1})	17.9	20.3	19.8
Essential amino acids (g AA/100 g of protein)			
Arginine	7.6	9.1	6.7
Histidine	2.7	2.0	2.9
Isoleucine	4.5	4.8	5.0
Leucine	7.8	6.6	7.7
Lysine	6.4	6.1	5.9
Methionine	1.3	2.9	2.5
Phenylalanine	3.2	6.1	6.9
Threonine	4.4	4.3	3.8
Valine	4.5	5.8	4.1

and the final pH was 4.4. The TSH was dehydrated by forced air (36 h, 60 ± 2°C). During drying, the silage was turned over periodically to facilitate water loss, and the dry product was ground again and sieved using a sieve of 0.5 mm then stored at 3°C in a refrigerator.

Feed formulation and preparation

Six isonitrogenous and isoenergetic diets were formulated based on the species requirements reported by Jauncey (2000). Five varied in the percentage of substitution of SBM protein with TSH (0, 25, 50, 75 and 100% of SBM; Diets TSH0, TSH25, TSH50, TSH75 and TSH100, respectively). An additional experimental diet was formulated with commercially available TBM as the sole protein source (Table 3). A commercial feed for tilapia (Malta Clayton) was ground, supplemented with 0.5% of chromic oxide and repelleted. This feed served as a reference diet (RD). All diets contained chromic oxide (0.5%), as an indigestible marker for the evaluation of digestibility, and were supplemented with 2% carboxymethyl-cellulose (CMC) as binder.

All ingredients were ground using a laboratory mill and mixed in a Hobart food mixer for 15 min. Hot water (approximately 60°C) was added to get the right consistency for pelleting, and mixing continued for 15 additional min. The resulting mash was passed through a meat grinder to produce pellets of 3 mm, which were dried in a forced air oven for 16 h at 36 ± 2°C. A sample of each diet was retained and stored, in plastic bags at -20°C, until analyses for proximate and amino acid compositions.

Sampling and chemical analyses

Samples of the ingredients, formulated diets, feces and initial and final fish carcasses were analyzed in triplicate using standard methods (AOAC, 1990). Crude protein (total nitrogen x 6.25) was measured using a LECO FP-528 nitrogen analyzer. Crude lipid was determined by petroleum ether extraction using a Micro Foss Soxtec Avanti 2050 Automatic System and crude fiber using a moisture-free defatted sample, using the Fibertec System 2021 (FOSS, Denmark). Dry matter was determined by drying the sample in an oven at 105°C for 16 h and weighing to the nearest 0.1 mg, and the ash content was obtained by incinerating samples in a muffle furnace at 550°C for 12 h. Nitrogen-free extract (NFE) was calculated by difference as:

NFE = (100 - % moisture + % crude protein + % crude fat + % crude fiber + % ash).

Gross energy (GE) was calculated using the energy equivalents suggested by New (1987): protein 5.5 kcal g^{-1}, lipids 9.1 kcal g^{-1}, and carbohydrate 4.1 kcal g^{-1}.

The amino acid composition of the experimental ingredients and diets were determined by HPLC (Varian 9012) analysis using a precolumn Microsorb (4.5x30 mm) packed with octadecyl-silane and a 3-µm, 4.6x100 mm Microsorb Short C18 column, according to Vázquez-Ortiz *et al.* (1995). Amino acid standards were used and α-aminobutyric acid was added as internal standard.

The chromic oxide content of the feed and fecal samples was determined using the acid digestion technique (Furukawa & Tsukahara, 1966).

Fish and experimental procedures

The feeding experiment was conducted indoors in a closed-recirculating water system. This included a settling tank, a bubble bead biological filter and a receiving tank, which provided a continuous water flow of dechlorinated tap water (1.5 L min^{-1}) to the 21 (three for each diet) experimental 100-L circular fiberglass tanks.

Each tank contained 15 tilapia fry with an initial mean weight (± SD) of 0.89 ± 0.29 g. Water temperature and dissolved oxygen were maintained at 27 ± 1ºC and 5 ± 1 mg L^{-1} and the total ammonia and nitrite concentrations, recorded weekly according to Spotte (1979), ranged from 0.06 to 0.3 mg L^{-1} and from below detection level to 0.016 mg L^{-1}, respectively.

Each diet was randomly assigned to three replicate tanks and the experiment lasted eight weeks (56 days). Fish were initially fed at 8% of the biomass of each tank divided into three rations (09:00, 13:00 and 17:00 h), which were adjusted later to limit the amount of unconsumed feed. This was collected one hour after feeding, dried and weighed in order to calculate the mean daily individual consumption.

All fish were weighed every two weeks to calculate their mean body weight and the biomass present in each tank, and adjust accordingly the daily ration. The response variables were determined according to the following equations:

Survival (S %) = 100 × final count initial $count^{-1}$.

Specific growth rate (SGR% d^{-1}) = [(ln final body weight-ln initial body weight) × 100] time (d^{-1}).

Weight gain (%) = 100 {(final weight-initial weight) initial $weight^{-1}$)}

Total feed consumption per fish (on as-fed basis) (TFC; g $fish^{-1}$) = Σ_i^{56} intake on the *i*th day (number of fish on the *i*th $day)^{-1}$;

Feed conversion ratio (FCR) = TFC per fish weight $gain^{-1}$

Apparent nitrogen utilization (ANU%) = 100 (final carcass nitrogen / total nitrogen $intake^{-1}$).

Determination of digestibility

The apparent digestibility coefficients (ADCs) were determined for all experimental diets, as well as for the reference diet.

A total of 153 fish (initial mean body weight 37 ± 3 g; means ± SD) were stocked into six 80-L fiberglass digestibility tanks at 17 fish per tank. After an acclimatization period of 15 days, during which the fish were fed their prescribed diet twice daily to satiation at 09:00 and 13:00 h, feces were collected at 1-h intervals between the morning and afternoon feeds. The feces collected from each tank were gently rinsed with distilled water, oven-dried (60°C) and stored in individual sealed bottles at -4°C until analysis. Sample collection continued during four weeks.

The ADCs of the dry matter and of each nutrient (NUT) of the experimental diets were calculated according to Maynard & Loosli (1969) and Hardy & Barrows (2002):

$$\text{ADC dry matter (\%)} = 100\text{-}100\left[\left(\frac{\%Cr_2O_3 \text{ in feed}}{\%Cr_2O_3 \text{ in faeces}}\right)\right]$$

$$\text{ADC NUT or energy (\%)} = 100\text{-}100$$
$$\left[\left(\frac{\%Cr_2O_3 \text{ in feed}}{\%Cr_2O_3 \text{ in feces}}\right)\left(\frac{\text{\% NUT or energy in feces}}{\text{\% NUT or energy in feed}}\right)\right]$$

Data analysis

The results of growth performance and feeding efficiency were compared with one-way analysis of variance (ANOVA), after verification of the normality and homogeneity of variances using Kolmogorov-Smirnov and Bartlett´s tests, separating the different means using Tukey's HSD tests (Zar, 1984). All statistical procedures were performed using the SigmaStat ver. 3 software package.

RESULTS

Ingredients and diets quality

Considering the tilapia requirements (Santiago & Lovell, 1988), SBM was the only ingredient with methionine deficiency for tilapia feeding. The phenylalanine contents of SBM and TSH were 90% of the requirement (Table 1).

All diets were isonitrogenous (350 g kg^{-1} CP) and isoenergetic (19 kJ g^{-1}) (Table 2) and their essential amino acid (EAA) contents were higher than the

Table 2. Formulation (g kg^{-1} dry weight), and proximate composition of the experimental diets. †Commercial diet, used as reference diet.

	Experimental diet					
	TSH0	TSH25	TSH50	TSH75	TSH100	TBM
Ingredients (g kg^{-1})						
Soybean meal	781.3	585.9	390.6	195.3	0	0
TSH	0	190.2	380.4	570.6	760.9	0
TBM	0	0	0	0	0	616.2
Fish oil (sardine)	35.7	18.1	4.8	0	0	0
Soy oil	35.7	40	40	31.5	18.4	37.5
CMC	20	20	20	20	20	20
Corn starch	95.3	113.8	132.2	150.6	168.7	298.8
Mineral premix†	10	10	10	10	10	10
Vitamin premix†	10	10	10	10	10	10
Vitamin C†	2	2	2	2	2	2
Choline chloride*	5	5	5	5	5	5
Chromic oxide**	5	5	5	5	5	5
Proximate composition (g kg^{-1})						
Crude protein	353	352	357	352	354	353
Crude lipid	81	84	82	83	84	84
Crude fiber	41	33	24	13	7	24
Ash	73	87	83	99	101	124
NFE‖	453.2	452.1	457.1	454.9	457.3	440.7
GE (kJ g^{-1})	19.3	19.3	19.5	19.4	19.5	18.8

†Commercial feed: 361 g kg^{-1} crude protein; 56.2 g kg^{-1} crude fat; 67 g kg^{-1} crude fiber; 74.4 g kg^{-1} ash; 455 g kg^{-1} NFE; GE: 18.33 kJ g^{-1}.
†BASF Mexicana S.A. de C.V. (by cortesy).
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tilapia requirements, with the exception of lysine, methionine and phenylalanine in the 100% SBM (TSH0) diet (Table 3).

Survival, feed intake, growth performance and feed utilization

Fish were observed to be in good health conditions. In all treatments mean survival ranged from 95.5 to 100%, and there were no significant differences ($P > 0.05$) among treatments. The fish were observed to be in good condition of health. Feed consumption was markedly higher for fish fed diet with TBM than that fish fed feeds contained SBM or TSH as sole protein source as well as feeds containing different inclusion levels of TSH as well ($P < 0.05$). However, the fish fed the RD diet showed similar total feed intake to diets contained 25 and 50% of TSH.

The highest growth performance and feed efficiency (SGR 5.4%; FCR: 1.1 and ANU: 42.1%), were in fish fed TBM ($P < 0.05$). In contrast, the fish that received the TSH100 and the TSH0 diets showed slower growth, ($P < 0.05$) (Table 4). The diets RD, TSH25 and TSH50 showed similar results for growth performance.

Significant differences were observed in the whole body protein and fat compositions of the fish ($P < 0.05$). The carcass of fish fed diet TSH25 and TBM had the lowest moisture and the highest protein contents. The crude protein and ash content increased progressively with increasing TSH inclusion, but the diets TSH75, TSH100 had lowest protein content (Table 5). The highest crude fat contents were for fish fed TSH0 and RD diets.

Digestibility

As a general rule, the apparent digestibility increased with increasing levels of TSH until the 50-75% level, and decreased significantly for the 100% inclusion. In

Table 3. Essential amino acid profile (g AA/100 g of protein) of diets containing soybean meal and different levels of TSH or with 100% TBM (TSH0 = SBM). Req = requirement of Nile tilapia fry (Santiago & Lovell, 1988).

Amino acids	Req	TSH0	TSH25	TSH50	TSH75	TSH100	TBM	RD
Arginine	4.2	4.51	5.10	5.80	6.30	6.75	6.89	5.72
Histidine	1.7	1.78	2.62	2.90	2.99	3.09	3.76	2.63
Isoleucine	3.1	3.01	3.98	3.18	3.21	3.10	3.97	3.21
Leucine	3.4	5.82	6.14	6.29	6.96	6.54	6.44	6.32
Lysine	5.1	4.00	5.11	5.38	5.49	5.59	5.88	5.19
Methionine	2.7	2.18	2.30	2.57	2.62	2.60	2.76	2.60
Phenilalanine	3.8	2.47	3.47	3.86	4.12	4.26	4.25	3.79
Threonine	3.8	3.74	3.87	3.92	3.96	3.84	3.97	3.91
Valine	2.8	3.22	3.38	3.92	4.11	4.74	4.84	4.29

Table 4. Growth performance and feed utilization of tilapia fry, fed with the experimental diets for 56 days (mean and standard deviation in parenthesis, n = 3). Values in the same row with the same superscript are not significantly different ($P > 0.05$). WG: weight gain; SGR: specific growth rate; TFC: total feed consumption, and FCR: feed conversion ratio (food on "as fed" basis). ANU: Apparent nutrient utilization.

Mean value†	Experimental diet						
	TSH0	TSH25	TSH50	TSH75	TSH100	TBM	RD
Survival (%)	95.6[a] (3.9)	97.8[a] (3.8)	97.8[a] (3.9)	100[a] (0.0)	95.5[a] (3.9)	97.8[a] (3.8)	97.8[a] (3.9)
Initial weight (g)	0.89[a] (0.29)	0.89[a] (0.29)	0.89[a] (0.29)	0.90[a] (0.29)	0.89[a] (0.29)	0.90[a] (0.29)	0.89[a] (0.29)
Final weight (g)	2.9[d] (0.16)	10.3[b] (0.57)	10.9[b] (0.93)	6.4[c] (0.11)	1.9[d] (0.23)	20.8[a] (0.79)	11.7[b] (0.25)
WG (%)	221[d] (16.0)	1054[b] (63.8)	1131[b] (94.2)	611[c] (19.1)	116[d] (26.8)	2204[a] (73.6)	1228[b] (23.6)
SGR (d^{-1})	1.9[e] (0.09)	4.2[b] (0.10)	4.3[b] (0.15)	3.3[c] (0.03)	1.2[d] (0.20)	5.4[a] (0.07)	4.4[b] (0.04)
TFC (g $fish^{-1}$)	5.0[d] (0.5)	13.1[b] (0.3)	13.2[b] (0.8)	10.5[c] (0.2)	4.3[d] (0.3)	21.4[a] (1.0)	13.7[b] (0.8)
FCR	2.6[b] (0.2)	1.4[c] (0.1)	1.3[c] (0.0)	1.9[bc] (0.0)	4.3[a] (0.6)	1.1[d] (0.0)	1.3[c] (0.1)
ANU (%)	23.2[c] (2.0)	35.9[b] (1.3)	35.0[b] (1.2)	23.0[c] (0.1)	9.2[d] (1.4)	42.1[a] (0.3)	34.8[b] (1.6)

all cases, the highest ADC values were for the 50% inclusion of TSH, and the lowest were those of diet TSH100. Diets TBM and RD had in all cases intermediate values (Table 6).

DISCUSSION

Nowadays, fish feeding faces ethical and ecological controversies related with the use of fishmeal as the main protein ingredient, which forces the industry to identify new options to solve this situation. Conside-ring that the main objective of agriculture and fisheries ought to be the production of feeds for human being, the use of their by-products is seen as a sustainable source of feedstuffs for animal feeding. By now, there are several examples of ingredients derived of the processing of agriculture crops or meat/fisheries industries that have good nutritional value to be included in fish feeds, with the soybean meal or rendered meat meals as ones of the most relevant.

Table 5. Carcass proximate composition (%, wet weight.) of *O. niloticus* fry, fed with the experimental diets for 56 days (mean values and standard deviation in parenthesis). Values in the same column with the same superscript are not significantly different ($P > 0.05$).

Diet	Moisture	Crude protein	Crude fat	Ash
Initial	77.0 (0.1)	13.4 (0.1)	4.5 (0.2)	2.7 (0.3)
TSH0	70.5^{a} (0.4)	18.1^{a} (0.1)	6.9^{a} (0.1)	3.3^{e} (0.01)
TSH25	73.7^{b} (0.1)	15.9^{b} (0.2)	5.6^{b} (0.2)	3.2^{e} (0.1)
TSH50	75.7^{b} (0.5)	15.3^{c} (0.2)	3.9^{d} (0.2)	3.7^{d} (0.1)
TSH75	76.0^{b} (0.1)	14.4^{c} (0.2)	3.8^{d} (0.0)	4.5^{b} (0.1)
TSH100	75.1^{b} (0.7)	13.2^{e} (0.2)	4.7^{c} (0.2)	5.3^{a} (0.3)
TBM	73.8^{b} (0.2)	15.2^{c} (0.1)	5.4^{b} (0.2)	4.2^{c} (0.4)
RD	74.5^{b} (0.1)	14.6^{d} (0.2)	6.8^{a} (0.2)	2.8^{f} (0.2)

Table 6. Apparent digestibility coefficients (ADC%, mean values and standard deviation in parenthesis) of the experimental diets for *O. niloticus* fingerlings (mean weight 37 ± 3 g). DM: dry matter, CP: crude protein, CL: crude lipids, and E: energy. Values in the same column with the same superscript are not significantly different ($P > 0.05$).

Diet	ADC			
	DM	CP	CL	E
TSH0	70.7b (0.45)	85.1d (0.24)	91.6e (0.75)	80.0b (0.22)
TSH25	67.6c (0.50)	89.3b (0.36)	94.5c (0.10)	80.8ab (0.23)
TSH50	77.5a (0.95)	90.0a (0.30)	96.9b (0.29)	85.6a (0.61)
TSH75	76.0a (0.45)	87.9c (0.43)	97.8a (0.24)	84.8a (0.43)
TSH100	51.9d (7.22)	70.2e (5.48)	86.1e (2.37)	58.9c (6.65)
TBM	72.5b (1.10)	84.4d (0.53)	92.8d (1.10)	81.0ab (0.83)
RD	71.4b (0.80)	88.7b (0.36)	86.7e (0.53)	74.9b (1.16)

In the last years the industrial processing of agriculture crops has expanded the options for using agricultural by-products in animal feeding including protein concentrates from soybean (Zhao *et al.*, 2010) for tilapia, rapeseed (Nagel *et al*., 2012) or lupin and pea (Zhang *et al*., 2012), for rainbow trout.

Additionaly, with the advent of the biofuels industry and in addition to the industrial processing of traditional crops, there are several examples of potential ingredients for fishmeal replacement in aquaculture feeds, including microbial by-products like brewer's waste or distiller's dried grains with solubles used successfully in tilapia feeds (Zerai *et al*., 2008; Lim *et al*., 2011) or biofuel yeast protein concentrate for carp and tilapia (Schaffer *et al*., 2010; Omar *et al*., 2012).

Approximately half of the processed seafood is discarded as a waste product, most of the time with negative environmental effects. This by-product is composed of body parts that are not suitable for human consumption and include heads, bones, viscera and skins. Because of its multi specific origin varies highly in its physical characteristics and chemical composition, and it is highly unstable, requiring special preservation methods to maintain its nutritional quality, before it is processed as a meal, or stabilized using other methods including the chemical or microbial silage, resulting in a liquid or semiliquid product that generally contains 31-56% CP (db) and 6-8% crude fat (Ayadi *et al*., 2012).

The potential of using silages in fish feeding have been demonstrated with several studies with different species. Kader *et al*. (2012), determined that it is possible to replace 36% fish meal protein in diets for Japanese flounder, *Paralichthys olivaceus*, with a mixture 1:1 of fermented soybean meal and squid by-product.

In this study, fish fed diets containing TSH gained progressively less weight as the level of TSH increased, and their growth was significantly lower than with TBM. This shows that, although both products came from the same base material, the nutritional value of TSH was inferior compared to TBM. However, the growth performances obtained with diets containing 25 and 50% TSH were similar to those observed with the commercial feed, indicating that tilapia feeds prepared with a blend (1:1, w/w) of soybean and TSH proteins do not affect fish growth. Fagbenro *et al.* (1994), observed this effect feeding tilapia with diets containing up to 75% of a 1:1 mixture of co-dried wet tilapia silage and soybean meal, concluding that fish silage is a suitable protein source for tilapia feeding. In another study, Gonçalves *et al.* (2010), replaced 20% fish meal protein with a co-dried blend of shrimp protein hidrolizate (SPH) and soybean meal (53.5% of the diet), in diets for Nile tilapia, with better results than those obtained with a commercial diet. They conclude that SPH is a promising feedstuff for tilapia.

Digestibility is an important determinant of protein quality in fish feeds, but our results seem to show that it has a poor predictive value when it is used to characterize hydrolysates. This is probably due to the fact that small peptides fragments and free amino acids in hydrolysates are more rapidly absorbed than amino acids from intact proteins, making them easily available for energy production rather than for protein synthesis (Hardy *et al*., 1983; Stone & Hardy, 1986; Fagbenro *et al*., 1994).

Low palatability reduces feed intake, thereby reducing the amount of nutrients available for fish growth. Thus, the poor acceptance of diets TSH0 and TSH100 was the most evident cause of reduced growth, whereas the high consumption of the TBM diet, which might be attributed to the high palatability of its ingredients, as well as to their content of essential amino acids, vitamins and minerals, may serve to explain the fact that the fish fed with this diet had the highest weight gain.

Low consumption and low digestibility could explain the adverse results obtained with the TSH100 diet, whereas the low palatability of diet TSH0 seems the main reason for the low growth observed, since only its fat ingredients had lower digestibility than the diet which gave the highest growth. Inconsistent results still exist on how soybean meal inclusion affects feed palatability, or how the anti-nutritional factors could be the main cause of poor growth and feed utilization in fish (Wilson & Poe, 1985; Bureau *et al*., 1998; Refstie *et al*., 1998; Peres *et al*., 2003). Furthermore, the soybean meal-based diet was deficient in some amino acids essential for tilapia fry, especially methionine and phenilalanine (Santiago & Lovell, 1988; Hertrampf & Piedad-Pascual, 2000), suggesting that a portion of dietary protein for tilapia fry should be from animal/fish sources (Coyle *et al*., 2004).

The inclusion of up to 50% TSH in a SBM-based diet had a positive effect on feed consumption as well as on growth and feed utilization, indicating that this percentage may be used in Nile tilapia practical diets, with results equivalent to fish fed a standard commercial feed. However, when the substitution increased to 75%, feed consumption and growth decreased. The worst result was observed with 100% substitution. These is in agreement with findings by Espe *et al*. (1999), and Plascencia-Jatomea *et al*. (2002), who reported a positive effect of low levels of inclusion of fish silage or shrimp head silage on productive performance of Atlantic salmon and Nile tilapia diets, but found that its efficiency decreased with high inclusion levels, due to a lower feed intake associated with reduced palatability of the diets. This effect was avoided by Olivera-Cavalheiro *et al*. (2007), using a mixture of shrimp head silage with soybean, bovine blood and corn meals, substituting 100% fishmeal without adverse effects on tilapia performance.

Middleton *et al*. (2001), observed that it is possible to include up to 33% of an extrusion, co-processing culled sweet potatoes and poultry mortality silage in diets for hybrid tilapia (*Oreochromis niloticus* x *O. mossambicus*), without adverse effects in the fish performance. This inclusion level was the highest tested, so it is possible that could be increased without deleterious effects.

ACKNOWLEDGEMENTS

Funded by SAGARPA-CONACYT Project Nº12375. PINSA, S.A de C.V. donated the tuna by-product meal and the tuna by-product samples. The authors acknowledge the help of Dr. K. Shirai and the Biotechnology Laboratory, Universidad Autónoma Metropolitana, for processing the tuna by-product silage.

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Received: 26 September 2012; Accepted: 8 June 2013

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