Lat. Am. J. Aquat. Res., 44(2): 327-335, 2016
DOI: 10.3856/vol44-issue2-fulltext-13

Research Article

Total and partial fishmeal substitution by poultry by-product meal (petfood grade) and enrichment with acid fish silage in aquafeeds for juveniles of rainbow trout *Oncorhynchus mykiss*

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ABSTRACT. Aquafeeds are formulated with a high content of fishmeal as protein source. The fishmeal is usually produced from pelagic fish resulting in a reduction of fish availability for human consumption. Thus, it is important to find appropriate protein substitutes in the production of formulated aquafeeds. Several works reported the positive effect of partial addition of poultry by-product meal (PBM) in aquafeeds for different fish species, and from them it is clear that the total substitution of fishmeal is limited by the unbalance of essential fatty acids. In this work, aquafeeds were designed with the total and partial substitution of fishmeal by PBM and enriched with acid fish silage. The designed aquafeeds were evaluated in the rainbow fish *Oncorhynchus mykiss* growth. Six diets were formulated at different levels of substitution of fishmeal by PBM pet feed grade (33, 66 and 100%), with and without addition of acid fish silage (FS) from tuna fish by-products. All treatments were formulated to contain similar protein and energy in a factorial random design with three repeti tions. The diet containing 100% enriched PBM (EPBM) showed the highest specific growth rate (2.0% day^{-1}) (P < 0.001), whereas the lowest growth rate was observed with PBM33 without enrichment (1.76% day^{-1}), after 22 weeks treatment These results demonstrate that the addition of FS in an aquafeed containing only poultry by-product meal as protein source improves the growth of rainbow fish. The positive effect of FS, mainly the role of essential fatty acids, is discussed.

Keywords: *Oncorhynchus mykiss*, poultry by-products meal, trout, aquafeeds, protein.

Substitución total y parcial de harina de pescado por subproductos avícolas (grado mascota) sólo y enriquecido con ensilaje ácido de pescado, en alimentos acuícolas para juveniles de trucha arcoíris *Oncorhynchus mykiss*

RESUMEN. Los alimentos acuícolas son formuladas con alto contenido de harina de pescado que es producida con peces pelágicos (FN por sus siglas en inglés), disminuyendo la posibilidad que sean destinados a consumo humano, lo que motiva la necesidad de encontrar sustitutos proteínicos para formular estos alimentos. Se conoce el efecto positivo de los subproductos avícolas (PBM por sus siglas en inglés) en alimentos acuícolas, aun sin encontrar la substitución total de la FM por un balance inadecuado de ácidos grasos. En este trabajo las dietas se diseñaron con una substitución total y parcial de la harina de pescado por PBM sólo y enriquecido con ensilado ácido de pescado (FS por sus siglas en inglés). Las dietas fueron evaluadas en trucha arcoíris *Oncorhynchus mykiss*. Seis dietas se formularon con diferentes niveles de sustitución de FM; tres PBM (33, 66 y 100%), y otras tres dietas en niveles similares, pero con PBM previamente enriquecida (EPBM) con FS utilizando subproductos de atún en una proporción de 60(PBM):40(FM) (peso: volumen). Todos los tratamientos

Corresponding editor: Patricio Dantagnan

tratamientos se formularon para contener el mismo nivel de proteína y energía. Se llevó a cabo un diseño factorial aleatorizado con tres repeticiones cada uno. Después de 22 semanas, la dieta EPBM100 alcanzó la tasa de crecimiento específico más alta (2% día^{-1}) ($P < 0{,}001$), mientras que la tasa de crecimiento más baja se observó con el PBM33 sin enriquecimiento (1,76% día^{-1}). Aquí se demuestra que una combinación de PBM con FS elaborado con subproductos resulta en un mejor crecimiento en dietas para trucha arcoíris. Se discute el efecto positivo del FS, principalmente por el papel de los ácidos grasos.

Palabras clave: *Oncorhynchus mykiss*, subproductos avícolas, trucha, alimentos acuícolas, proteína.

INTRODUCTION

Fish farming has rapidly intensified in the recent decades where aquafeeds are generally formulated to contain high levels of fishmeal as protein source, favoring the growth rate of aquatic organisms apparently due to their high protein digestibility and amino acid profile (NRC, 2011). However, the use of fishmeal in aquaculture tends to be reduced by the gradual increase in cost and low availability (Tacon *et al.*, 2012). This is due to the growing interest in the fishery sustainability that is trying to redirect the use of small pelagic fish such as sardines and anchovies for human consumption. Besides the increasing demand for fishmeal to supply the growing aquaculture activities, no alternative protein source has been found for aquafeeds formulations to efficiently replace the fishmeal (Tacon & Metian, 2008; Costa-Pierce *et al.*, 2012).

Among the protein sources that can be used to replace the fishmeal in aquafeed formulation, there are oilseed meals (soybean, canola, sunflower, cotton), grains (wheat and corn proteins) and legumes (lupine and peas), among others. The different sources have specific recommendations depending on the species to be fed and on the pre-treatment process as well. Protein source substitution raises some concerns such as protein quality, anti-nutritional factors, digestibility, and carbohydrate content (Gatlin *et al.*, 2007).

An important protein source being an attractive option for fish feed formulations is the renderers' by-products from animal production, such as poultry by-product meal (PBM), which is a protein source with high digestibility (90%) and containing a proper balance of essential amino acids (Steffens, 1994; Yang *et al.*, 2006; Shapawi *et al.*, 2007); meat by-product, from cattle production, such as bone and blood meals among others, are also available, some with less digestibility, but useful to complete the amino acid balance, or to fulfill the crude protein content in aquaculture diets (Bureau, 2006).

There are encouraging results in the use of PBM for tilapia, shrimp and rainbow trout (Steffens, 1994; Cruz *et al.*, 2000; Hernández *et al.*, 2010). In addition, has even been established that a mixture of three parts of PBM and one of fish meal (3:1) resulted in a higher growth rate than that observed with only fishmeal in *Totoaba macdonaldi*, which is a carnivore fish species with high protein requirements (Badillo *et al.*, 2014a). Nevertheless, in the same work a PBM as a sole protein source showed low growth rate and mortality that could be due to limiting essential nutrients like fatty acids. Also, a similar substitution combinations of PBM for fishmeal was performed for rainbow trout (0, 33, 67 and 100% PBM substitution of fishmeal) and using the broken line method, significant differences were observed with a point of intersection in the slope when FM was totally substituted (Pares *et al.*, 2014). The fact that rainbow trout, as well as totoaba, did not performed well with total FM substitution could be attributed to differences in digestibility, and the lack of limiting amino acids and fatty acids.

The aquafeed industry uses in their feed formulations up to 30% PBM in several aquatic species including salmon from Chile (Tacon *et al.*, 2009). Earlier, terrestrial renderers meals (poultry, swine or bovine) used as protein sources in aquafeed were considered low quality ingredients showing low protein digestibility and unable to support normal fish growth (Hardy, 2006). Therefore, their use was limited and generally was not recommended for carnivore fish species. However, this situation has radically changed after several research works that have demonstrated a considerable increase in quality (Hardy, 2006). Nevertheless, so far there is not available literature showing an acceptable growth rate in trout using a PBM as sole protein source when compared to fishmeal.

Fishery by-products are also used as protein source, like the tuna fish by-products that are mainly used to produce fishmeal with lower protein content (close to 58%) with an acceptable digestibility and amino acid profile (NRC, 2011). However acid fish silage is a different preservation process widely utilized in the 80'. This preservation process can be performed chemically by adding a mixture of acids or biologically through an acid lactic fermentation by providing a source of carbohydrates (Jackson *et al.*, 1984; Borghesi *et al.*, 2007). Acid fish silage is prepared by adding a mixture of organic and inorganic acids to drop down the pH to promote the hydrolysis caused by the enzymes contained in the viscera tissues from fish (Viana *et al.*, 1993). The main advantage of acid fish silage process is its low manufacture cost preserving its nutrients with high quality, in addition to be considered an environmental friendly process (Fagbenro & Jauncey, 1993; Viddoti *et al.*, 2003). This product contains a high amount of water, representing a disadvantage for transport and commercialization. Drying procedure reports difficulties due to its high amount of collagen, however, Goddadg & Perret (2005) reported a co-dried product where fish silage is mixed with different proportions of wheat brand meal. Although this process was succesfull, the high fibre content is not desirable in aquafeeds.

With the aim of a partial or total substitution of fishmeal in diets for juvenile rainbow trout *Oncorhynchus mykiss* in this work we have established a co-drying procedure using acid fish silage from tuna fish by-products to enrich the PBM. The effect of the different formulations on fish growth and performance are discussed.

MATERIALS AND METHODS

Feed ingredients and diet preparation

Fishmeal (FM) made with tuna by-products was obtained from a local distributor (Ensenada, Baja California), whereas the poultry by-product meal (PBM) "Petfood grade" was kindly donated by the National Renderers Association, USA. The proximate composition and amino acid profile from protein sources are shown in Table 1. Six diets were formulated to contain similar crude protein and fat (Table 2). In three of them the FM was gradually substituted by PBM at levels 33, 67 and 100% from the protein (PBM33, PBM67 and PBM100). The other three diets were prepared by mixing acid fish silage into the PBM in a proportion of three parts of PBM with two of fish silage (weight/volume) to prepare the enriched PBM dietary treatments and with the same levels as those without silage (EPBM33, EPBM67 and EPBM100). The acid fish silage (FS) was prepared as follows: the chopped tuna fish by-products were mixed with a mixture of acids (2.5% citric and 2.5% phosphoric acids). After three weeks at room temperature, the silage was mixed with the PBM (60:40; FS:PBM v/w) and dried at 50ºC during 24 h and grinded before blended to formulate the different substitution diets as prior described. Each diet was mixed as follows: starch and gelatin were cooked separately and added to the ingredients to produce a homogeneous mixture with 60% moisture content. The resulting mixture in the form of dough was then cold pressed through a meat grinder into 0.2 mm diameter pellets, from which pieces were cut in pieces (0.5 x 0.7 mm) and then dried at 60ºC for 24 h. The feed was then stored at 4ºC until the day used for feeding. The proximate composition and amino acid profiles from diets are shown in Table 3.

Fish, experimental design and biological indices

Three thousand juvenile rainbow trout (4.11 ± 0.29 g) were purchased from Troutlodge Inc. (Orting, WA, USA) transported by airplane to the research facility in Mexico. Fish were randomly distributed in eighteen 550 L fiberglass tanks (100 fish per tank) equipped with biofilter under a recirculation system with 5% water renewal every day with freshwater. A factorial experimental design was evaluated using two factors (PBM without and with FS) and three levels of substitution (33, 67 and 100). For each treatment three replicates were performed. Water was controlled at 18.0 ± 1ºC by cooling the water in a reservoir after filtration through the biofilter. Water quality was registered once a week using a kit for freshwater to estimate ammonia, nitrites (Aquarium Pharmaceutical, Inc. Canada), oxygen and pH.

Fish were fed three times a day (7:00, 13:00 and 19:00 h) to apparent satiation and collecting the unfed feed to calculate the ingestion rate in a parallel tanks the different dietary treatments were immersed a similar time to calculate the feed stability to calculate the fed ingestion. Growth increase was measured every three weeks during 20 weeks. The following indices were calculated to evaluate growth performance as follows:

Weight increment:

% Weight gain = [(final weight – initial weight)/initial weight] x 100 (1)

Specific growth rate:

SGR = (ln final weight – ln initial weight) / time (days) x 100 (2)

Feed intake (FI): FI = [GS/100) - R] (3)

where G represents the amount of feed offered in grams; S is the percentage of feed recovered in the control tanks without animals and R the remnant food (grams) from the tanks after fish were feed to apparent satiation. Mean daily rate of feed intake for each treatment was then calculated and expressed as a percentage of the fish body weight.

Food Conversion Ratio (FCR): FCR = dry weight feed consumed (g)/wet weight gain (g) (4)

Protein Efficiency Ratio (PER): PER = body weight increase, wet weight (g)/protein ingested (g) (5)

Chemical analysis

Proximate composition of diets expressed as dry matter basis, was determined in triplicate samples according to

Table 1. Proximal composition and amino acid profile (AA g 100 g^{-1} of total) from the ingredients used as protein source: fishmeal (FM); poultry by-product meal (Petfood grade) (PBM) and enriched (EPBM) with acid fish silage made from tuna by-products (60:40; weight volume). [1]Nitrogen free extract: NFE = 100 - (% crude protein + % crude fat + % ash).

	Protein source ingredients		
	Fishmeal (FM)	Poultry by-product meal (PBM)	Enriched poultry by-product meal (EPBM)
Proximate composition (dry weight)			
Crude protein	58.5	64.5	65.0
Crude lipids	8.0	15.2	15.5
Ash	25.9	11.5	11.9
NFE[1]	7.6	8.8	7.6
AA (g 100 g^{-1} of total)			
Essential amino acids			
HIS	1.8	1.5	1.8
ARG	3.8	4.4	4.8
THR	2.8	2.7	2.7
VAL	2.3	2.2	2.3
MET	2.3	2.0	1.9
LYS	4.2	4.6	4.1
ILE	2.2	2.3	2.3
LEU	4.4	4.8	4.7
PHE	2.5	2.7	2.6
TAU	0.2	0.3	0.5
Non essential amino acids			
ASP	5.3	5.7	6.0
SER	3.1	3.2	3.4
GLU	7.2	8.3	8.5
GLY	6.2	8.5	8.2
ALA	3.9	4.4	4.4
PRO	3.6	4.1	4.1
TYR	2.8	2.7	2.6

Table 2. Experimental diets with poultry meal by-products ingredient formulation, "Pet food grade" (PBM), and acid fish silage (EPBM) enriched in replacement of fishmeal. [1]Fishmeal locally produced, from tuna fish by-products. [2]Poultry meal donated by the NRA "Pet food grade" (National Renderers Association) USA. [3]Mixture of vitamins and minerals donated by DSM Nutritional Products, Mexico.

Ingredients	Treatment					
	PBM			EPBM		
	PBM33	PBM67	PBM100	EPBM33	EPBM67	EPBM100
Fishmeal[1]	40	20		21	40	
Poultry by-product meal[2]	20	40	59			
PBM with fish silage				40	21	62
Whole corn meal	5.5	5.5	5.5	5.5	5.5	5.5
Fish oil	3.3	2.5	1.8	1.6	2.8	0.1
Starch	18.7	19.5	21.2	19.3	18.2	19.9
Gelatin	6	6	6	6	6	6
Vitamin stay C[3]	0.4	0.4	0.4	0.4	0.4	0.4
Rovimix[3]	3.3	3.3	3.3	3.3	3.3	3.3
Sodium benzoate	0.23	0.23	0.23	0.23	0.23	0.23
Choline chloride	0.09	0.09	0.09	0.09	0.09	0.09
Tocopherol	0.01	0.01	0.01	0.01	0.01	0.01
Cellulose	2.5	2.5	2.5	2.5	2.5	2.5
Total	100	100	100	100	100	100

Table 3. Proximate composition of diets formulated at three different fishmeal substitution levels with poultry by-product meal (PBM)[1] alone and enriched (EPBM) with acid fish silage. The amino acid profile (AA g 100 g^{-1} of diet) is also given. [1]Poultry by-product meal (Petfood grade) kindly donated by the National Renderers Association. [2]Nitrogen free extract; NFE = 100 - (% crude protein + % crude fat + % ash).

	Treatment					
	PBM33	PBM67	PBM100	EPBM33	EPBM67	EPBM100
Proximate analysis (% in dry weight)						
Crude protein	43.2	44.7	45.3	44.0	45.0	45.5
Crude lipids	9.5	10.5	10.6	9.3	9.6	9.9
Ash	16.6	13.5	10.8	17.2	13.5	10.9
NFE[2]	32.3	31.3	32.9	29.5	31.9	33.7
AA (g 100 g^{-1} feed)						
Essential amino acids						
HIS	1.4	1.5	1.5	1.4	1.1	1.3
ARG	1.5	1.6	1.6	1.4	1.6	1.7
THR	2.4	2.5	2.4	2.3	2.6	2.7
VAL	1.9	1.9	2.1	1.9	2.0	2.1
MET	0.8	0.8	0.9	0.9	0.8	0.8
LYS	2.6	2.7	2.6	2.6	2.5	2.8
ILE	1.3	1.3	1.3	1.3	1.4	1.4
LEU	2.9	3.0	3.3	3.0	3.2	3.4
PHE	1.2	1.2	1.4	1.2	1.3	1.4
TAU	0.1	0.1	0.1	0.1	0.2	0.2
Non essential amino acids						
ASP	6.1	5.9	5.5	5.5	5.8	5.2
SER	2.9	3.0	2.8	3.4	3.1	3.2
GLU	5.3	5.5	5.6	6.0	5.5	5.5
GLY	3.5	3.6	4.0	4.2	4.1	3.8
ALA	5.1	5.4	5.5	4.6	5.3	5.3
PRO	3.4	3.8	3.8	3.5	3.8	3.9
TYR	0.8	0.7	0.8	0.7	0.8	0.9

standard procedures (AOAC, 1990). Moisture content of each sample was calculated from samples (2 g) dried to constant weight at 60ºC. Total nitrogen content was determined by the micro-Kjeldahl method, and crude protein was then calculated as % N x 6.25. Total lipid concentration was determined by Soxhlet extraction with petroleum ether, the dissolved fat was dried and crude fat was calculated gravimetrically. Ash content was determined by burning samples to 550ºC for 6 h. Nitrogen free extract was calculated by difference (% NFE = 100 - (% crude protein + % total lipid + % ash + % crude fiber).

Amino acid analysis

The amino acid (AA) profile of defatted raw materials (using Soxhlet extraction according to AOAC, 1990) were analyzed after hydrolysis and derivatized according to Viana *et al.* (2007). In summary, 200 µL of 6 N HCl containing 0.06% phenol was added to 25 mg of protein sources and formulated diets. The mixture was then digested for 24 h at 110ºC, in a closed vial under nitrogen atmosphere to avoid oxidation, to form free amino acids (FAA). The digested samples were then dried under nitrogen and further rehydrated with 1 mL of deionized water. The samples were then filtered through a 0.45 µm Teflon filter. The filtered samples were derived with the Waters AccQ·Tag™ kit and chromatographed in a Waters HPLC equipped with an AccQ·Tag™ C-18 reversed-phase column (3.9x150 mm; from Waters). The samples were monitored with a fluorescence detector (Waters 474 series, Milford, MA, USA). The samples were eluted using the water-acetonitrile gradient recommended by the Waters AccQ·Tag™ system at a constant temperature (37ºC) and monitored with excitation at 250 nm and emission at 395 nm. Standard curves were obtained (from 18.5 to 300 pmol) for the AA quantification using the AA standard solution.

Statistical analysis

A two-way analysis of variance (ANOVA) was used to compare fish response in terms of the overall performance in growth, feed ingestion and protein efficiency ratio among treatments (Zar, 1999). When significant differences among treatments were found ($P \leq 0.05$), Tukey's multiple comparison analysis was performed. SigmaStat 3.5 (Systat Software, Inc. Chicago, IL, USA) was used to perform all statistical analysis.

RESULTS

Fish were cultured and fed three times a day to satiation with the different diets. Growth was monitored every three weeks during 22 weeks. The water temperature was maintained at 18 ±1ºC throughout the experiment and the water quality parameters were continuously monitored. Ammonium varied from 0 to 0.25 mg L^{-1}; nitrites from 0 to 0.25 mg L^{-1} and dissolved oxygen was maintained at ≥6 mg L^{-1}.

The goal was to maintain the same proximate composition of the different diets (Table 3). The crude protein content only varied from 45.5 to 43.2%, while crude lipids varied only from 10.6 to 9.3%, and ash content from 16.6 to 10.8%. The AA profile of each diet is also shown in Table 3 for each dietary treatment.

Significant differences were found in growth rates whereas no effect was registered with the FS enriched preparation (EPBM) when compared with non-enriched ones (PBM) (Table 4). All treatments resulted in survival values higher than 98%. Juvenile trout reached a significant higher weight of 58.1 g (1419% increase in weight) from the initial weight of 4 g in treatment EPBM. The highest SGR was obtained in the treatment EPBM100 (2.0% day^{-1}), which contains PBM and fish silage, and without FM, followed by EPBM66 and PMB100 (1.90% day^{-1}). The lowest SGR was observed in treatment PBM33 (1.76% day^{-1}).

The protein efficiency ratio PER varied between 2.4 to 1.9 being significant lower the treatment PBM100 compared to treatments PBM33 and PBM67 and all the enriched treatments. However, all the enriched treatments resulted significantly similar among them.

DISCUSSION

All fish treatments showed good survival rate and the obtained growth values are within those previously reported, as well water quality during the treatments (Hokanson *et al.*, 1977; Thiessen *et al.*, 2003). The proximate composition obtained in the different dietary treatments resulted in small differences but close to the expected levels, as reported for rainbow trout (NRC, 2011), whereas the lipid content was slightly lower than these reported for optimum growth (Sevgili *et al.*, 2012). Earlier, Parés *et al.* (2014) reported a maximal growth with a diet formulation similar to the PBM67 used in this work. However here, a significant better growth was obtained when the PBM was enriched with FS in a proportion of 2 parts of FS per 3 parts of PBM, even when FM was absent. The resulting EPBM mixture showed slightly higher protein content but lower fat content (45.5 and 9.9, respectively) when compared to the rest of the treatments (43.2 and 10.5, respectively). Small changes were also observed in the amino acid profile content from the different diets, where in general the enriched diets had a tendency to increase the essential amino acids such as Arg, Lys, Phe, Thr, Ser, including Tau. Taurine content in EPMB100 and EPMB67 was twice the amount than those observed in the rest of treatments. Both, fish protein sources (FM and FS) came from the same origin, tuna by-product meal and therefore it could be expected a similar amino acid profile. However, since Tau do not conform structural proteins, its presence in FM could be lower as it might be lost during the FM production procedure where a large amount of water is discharged containing a significant amount of protein (press liquid slurry). Taurine has been recently considered as an essential nutrient for fish, being a derivative from cysteine and incorporated in diets as a free nutrient (Salze & Davis, 2015).

The procedure to prepare FS used here was according Viana *et al.* (1993) with modifications. The innovation here was the co-drying procedure where FS was mixed together with the PBM prior drying with the aim to enrich the amino acid content and facilitate the FS mixture into the aquafeeds.

The maximal growth reached with the dietary treatment EPBM100 resulted also in an optimal SGR for rainbow trout. The SGR varied from 1.76 up to 2.0% $day^{-1}$for the different treatments. These growth rates are similar to those reported by Sevgili *et al.* (2012) where the SGR under different regimes, different protein content levels and in organisms with similar size, in which SGR values of 1.67 to 1.83% day^{-1} were obtained. Here, the growth was maximized with treatment EPBM100 where the FS induced somehow an improvement of the required nutrients compared to previous results (Pares-Sierra *et al.*, 2014). Moreover, when Pares-Sierra *et al.* (2014) used a similar diet (PBM100) but without FS, the growth rate was lower when compared to PBM67, in agreement with our results (PBM33, PBM67 and PBM100) where PBM33 and PBM100 resulted in lower growth than that observed in PBM67. However, when the PBM was enriched with FS (EPBM) the total substitution of FM resulted in an increase in the growth rate.

Table 4. Biological indices in rainbow trout (*Oncorhynchus mykiss*) juveniles after being fed during 20 weeks with diets containing different fishmeal substitution levels with poultry by-product meal (PBM) alone or enriched with acid fish silage. Values are means ± standard deviation (n = 3), different superscripts means significant differences. [1]SGR = (ln final weight – ln initial weight)/time (days)*100. [2]Weight gain (%) = [(final weight – initial weight)/initial weight] x 100; [3]FCR = dry weight feed consumed (g) / wet weight gain (g); [4]PER= g protei n ingested / g body weight increase (wet weight).

	Treatment								
	Poultry by-product meal			Enriched poultry by-product meal			Two way ANOVA		
	PBM33	PBM67	PBM100	EPBM33	EPBM67	EPBM100	PBM	EPBM	Interaction
Initial weight (g)	4.30 ± 0.37	4.08 ± 0.37	3.94 ± 0.08	4.26 ± 0.36	4.06 ± 0.29	4.11 ± 0.30	0.32	0.8	0.8
Final weight (g)	47.62 ± 3^{c}	55.63 ± 4.7^{bc}	50.63 ± 4.7^{bc}	51.90 ± 7.7^{bc}	54.76 ± 2.0^{b}	60.33 ± 0.62^{a}	0.057	0.044	0.126
SGR (% day^{-1})[1]	1.76 ± 0.02^{c}	1.93 ± 0.03^{b}	1.90 ± 0.04^{b}	1.81 ± 0.05^{bc}	1.90 ± 0.03^{b}	2.0 ± 0.05^{a}	<0.001	0.014	0.09
Weight gain (%)[2]	1005 ± 31.9^{c}	1230 ± 6.13^{b}	1217.9 ± 101.3^{b}	1070 ± 40.2^{bc}	1220 ± 28.2^{b}	1419 ± 49.3^{a}	<0.001	0.016	0.09
FCR[3]	0.85 ± 0.04	1.08 ± 0.07	0.89 ± 0.2	0.97 ± 0.02	0.99 ± 0.14	1.00 ± 0.04	0.135	0.77	0.27
PER[4]	2.1 ± 0.09^{b}	2.4 ± 0.04^{a}	1.92 ± 0.05^{c}	2.2 ± 0.5^{ab}	2.2 ± 0.31^{ab}	2.2 ± 0.08^{ab}	0.002	0.173	0.002
Survival (%)	98.0 ± 1.4	98.3 ± 0.7	99 ± 1.4	98 ± 3.5	99 ± 0.7	96.3 ± 2.1	0.65	0.47	0.31

Nevertheless, no significant differences could be observed in the FCR, the PER values resulted in significant differences among treatments. The Per values were lower for the treatment PBM100 which is in accordance to Pares-Sierra *et al.* (2014). However, no differences in protein efficiency were observed among the enriched treatments, differences that show a lower efficiency for the enriched treatments as a result of a higher feed intake. Both indexes were calculated from the feed ingestion measurements, an evaluation that easily is overestimated due to the water stability of diets, especially if diets are not prepared by extrusion (Jobling *et al.*, 2001).

The results shown in this work indicate that the poultry by-product meal together with FS has a superior performance in growth as protein source in aquafeeds for trout. Earlier results from our lab indicated that PBM67 diet contained an EAA profile that appeared to successfully meet the requirements for adequate growth. PBM is known to contain high levels of protein (up to 65%) and a favorable AA profile to supports adequate growth in most cultured fish species, particularly the EAA, with the exception of methionine (NRC, 2011). Also Badillo *et al.* (2014b), using stable isotopes to evaluate the protein retention between FM and PBM, concluded that PBM was slightly higher assimilated than FM. However, when FM was absent a lower growth could be observed, a fact that here was not observed when FS was added. The enrichment of poultry by-product meal with acid fish silage allows a slightly increase of amino acids content, including Taurine, relative to that found in PBM or FM alone.

It is well known for rainbow trout that a diet supplemented with taurine, a nutrient in most aquatic organisms, promotes growth, feed conversion and protein retention efficiency (Gaylord *et al.*, 2006, 2007; Salze & Davis, 2015). The use of acid fish silage in aquaculture feeds is not new and has high nutritional value (Jackson, 1984; Johnson *et al.*, 1985; Viana *et al.*, 1996); however, the high amount of liquid makes its handling difficult even considering its subsequent drying. The use of an additional ingredient as carrier improves the drying process (Godard & Perret, 2005). Using the PBM as carrier to facilitate the drying of acid fish silage yielded a product with potential use in aquaculture. It would be advisable to make a cost-benefit study of this process, and evaluate the use of a vegetable meal with low biological value and high fiber content as well, to improve the AA profile and nutritional characteristics for further diet formulation.

In this study, the partial or total replacement of fish meal for poultry by-product meal type "Petfood grade" in the experimental diets formulated for rainbow trout showed satisfactory growth which is consistent with similar studies in this species (Bureau *et al.*, 1999; El-Haroun *et al.*, 2009). This confirms the potential use of poultry by-products in association to FS as a protein source in aquaculture aquafeed for trout. Thus, we conclude that PBM "Petfood grade" combined with FS can be use as a full replacement for FM to sustain an optimum rainbow trout growth.

ACKNOWLEDGEMENTS

This work was supported by the SAGARPA/ CONACyT 109150 y UABC 15ª Convocatoria. We thank DSM and the National Renderers Association for their kind donation of ingredients. We thank Geraldo Montoya for its assistance during the experiment.

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Received: 3 August 2015; Accepted: 18 January 2016

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