

Crecimiento y poliaminas intestinales en tilapia del Nilo (*Oreochromis niloticus*) y bagre de canal (*Ictalurus punctatus*) alimentadas con aceite de soya y suplementadas con probióticos

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Resumen

El principal objetivo de este trabajo fue comparar el efecto de cuatro dietas en los niveles de poliaminas intestinales y crecimiento de la tilapia del Nilo (*Oreochromis niloticus*, Linnaeus 1758) y el bagre de canal (*Ictalurus punctatus* Rafinesque, 1818) juveniles. La dieta práctica control (dieta C) contiene 2% aceite de pescado y 6 % de aceite de soya. La dieta alternativa (dieta S) contiene 8% de aceite de soya y no aceite de pescado. Las dietas CP y SP corresponden a las dietas C y S, suplementadas con un probiótico multiespecie utilizado en la acuicultura. Poliaminas biogénicas putrescina (Pu) espermidina (Spd) y espermina (Spm) fueron determinadas durante los 120 días de experimentación por HPLC. La media de los niveles (nmol μ L⁻¹) de estas

Growth and intestinal polyamines in tilapia (*Oreochromis niloticus*) and catfish (*Ictalurus punctatus*) fed diets with soybean oil and supplemented with probiotics

poliaminas en intestino de tilapia fueron: Pu, 0.0194 ± 0.0048 ; Spd, 0.0949 ± 0.0266 ; Spm, 0.0561 ± 0.0196 y para bagre: Pu, 0.0144 ± 0.0025 ; Spd, 0.1054 ± 0.0142 ; Spm, 0.1036 ± 0.0143 .

Los datos experimentales mostraron que las dietas S, CP y SP no afectaron el crecimiento y niveles de poliaminas comparado con la dieta C. Una relación fue observada entre los niveles de poliaminas intestinales y el periodo experimental. Los datos experimentales sugieren que el aceite de pescado puede ser totalmente sustituido por el aceite de soya en la formulación de la dieta práctica para tilapia del Nilo y bagre de canal.

Palabras clave: Aceite de soya, *Ictalurus punctatus*; *Oreochromis niloticus*; Poliaminas; Probióticos, Crecimiento

Abstract

The aims of this work were to compare the effect of four diets on intestinal polyamine levels and growth of juvenile Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) and channel catfish (*Ictalurus punctatus* Rafi-

nesque, 1818). The control practical diet (C diet) contained 2% fish oil and 6% soy oil. The alternative diet (S diet) contained 8% soy oil and no fish oil. The CP and SP diets correspond to the C and S diets, supplemented with a multispecies probiotic formulated for aquaculture. Biogenic polyamines putrescine (Pu), spermidine (Spd) and spermine (Spm) were determined by HPLC during a time-course feeding trial conducted for 120 days. Mean levels (nmol μ L⁻¹) of these polyamines in the intestine of tilapia were: Pu, 0.0194 ± 0.0048 ; Spd, 0.0949 ± 0.0266 ; Spm, 0.0561 ± 0.0196 and for catfish: Pu, 0.0144 ± 0.0025 ; Spd, 0.1054 ± 0.0142 ; Spm, 0.1036 ± 0.0143 . Experimental data showed that the S, CP and SP diets did not affect fish growth and polyamine levels compared to the C diet. A relationship between intestinal polyamine levels and experimental period was observed. Experimental data suggest that fish oil may be totally substituted by soy oil in the formulation of practical diets for tilapia and catfish.

Key words: Soy oil, *Ictalurus punctatus*; *Oreochromis niloticus*; Polyamines; Probiotics, Growth.

Introduction

Aquaculture offers a food source alternative of great nutritional value and represents the fastest growing food production sector, with a global expansion of 5% to 10% annually in the last 10 years. In Mexico, a total of 61 species are cultivated, of which 40 are native and 21 are of exotic origin. Tilapia (*Oreochromis* sp) and catfish (*Ictalurus* sp) are the most popular species for fresh water aquaculture in Mexico. In the state of Jalisco, tilapia is the most productive aquaculture species, followed by channel catfish, carp, shrimp, tuna, and trout (INAPESCA 2012). Tilapia and catfish have the following advantages for cultivation in a great variety of aquaculture systems: good adaptability to diverse environmental conditions, easy domestication, breeding, handling, and feed conversion. Both fish species are commercially viable at the national and international levels and represent a healthy, economical and nutritious food alternative. Tilapia and catfish, though predominantly vegetarian species, are omnivorous and capable of feeding on a wide variety of plants and organic matter. The farming of these species in Mexico developed rapidly with improvements in pond management and the identification and control of diseases and prepared food stocks (Mejía-Mojica *et al.* 2013).

The feeding of organisms in the aquaculture sector depends on industrialized formulated diets and represents 50 to 60% of the production cost of aquaculture species (Watanabe 2002). Lipids are the major source of

energy for fish and play an important role in the animal's metabolism, including the biosynthesis of essential fatty acids. Fish oils are the basis for the formulation of commercial feed and are the most common source of lipids with a high content of highly unsaturated fatty acids (HUFAs). At the international level, an entire industrial sector is dedicated to the production of fish oils from small marine species (sardines, anchovies and eels). The capture of fish from marine ecosystems solely to supply the world's aquaculture farms is not sustainable. The growth of the aquaculture sector has caused a progressive shortage of fish meal and fish oil, an increase in the price of this resource and consequently an increase in the price of feed for farmed fish; particularly oil of animal origin constitutes one of the bottlenecks for industrial feed formulations (Gatlin *et al.* 2007; Deng *et al.* 2017). Vegetable oils represent an alternative source of lipids for the formulation of balanced feed for freshwater organisms, soy oil is among the main sources of vegetable oil to be explored. Soy, because of its high oil content, constitutes a sustainable alternative for the formulation of fish feed; 60% of the total soybean lipids are polyunsaturated fatty acids (PUFAs), which are precursors of HUFAs (Torres & Tovar 2009; Zhou *et al.* 2017). HUFAs are physiologically more important than PUFAS because of their critical role in the integrity of cell membranes (Santerre *et al.* 2015). Thus, the substitution of soy oil for fish oil must not be undertaken without experiments in order to preserve the fish health and fish farming productivity.

Adequate nutrition and good water quality are essential factors for the prophylaxis and control of diseases in intensive aquaculture and for successful fish farming. An infectious outbreak of bacterial origin can cause serious economic losses, thus the prophylactic use of antibiotics is common in the aquaculture industry. However, this use has led to the emergence and selection of resistant bacteria (Lara-Flores 2011; Banerjee & Ray 2017). Probiotics are live microbes that may serve as dietary supplements and in adequate amounts confer a health benefit to the host. Probiotics represent an alternative to the excessive application of antibiotics because they strengthen the fish's immune system, increase its resistance to diseases, and may improve fish growth (Welker & Lim 2011; Sankar *et al.* 2017). Additionally, it has been reported that lactic acid bacteria (LABs) act against intestinal pathogens and other microorganisms and compete for cell surface and mucin binding sites (Liu *et al.* 2013). LABs, yeast and probiotic bacteria from the genus *Bacillus* are the most commonly used in the area of aquaculture (Gatesoupe 2016). There is evidence that probiotics improve the efficiency of feed conversion (Welker & Lim 2011). Waché *et al.* (2006) highlight the positive effect of the yeast *Saccharomyces cerevisiae* on the growth of rainbow trout (*Onchorhynchus mykiss*). Several trials have also highlighted the importance of supplementation of fish feeding with probiotics during the larval stage (Lara-Flores 2011). It has also been shown that supplementation of diets with live yeast, *Debaryomyces hansenii*

(1.1%), improves the maturation of the gastric system and the survival of seabass larvae (*Dicentrarchus labrax*). Some authors suggest that this effect is due to the fact that the yeast produces a large amount of polyamines in the intestinal lumen of these fish (Tovar-Ramírez *et al.* 2004; Gatesoupe 2007; Tovar-Ramírez *et al.* 2010).

Polyamines are ubiquitous aliphatic amines of low molecular weight, essential for cell cycle progression and differentiation. The control of the homeostasis of the main polyamines, putrescine (Pu), spermidine (Spd) and spermine (Spm), also involves - in addition to their complex biosynthesis- the processes of retroconversion, degradation and transport (Babbar & Gerner 2011; Kalač 2014; Andersen *et al.* 2015). The intestinal mucosa is a tissue of rapid renewal and is sensitive to the intake and presence of food in the digestive tract. Diet polyamines and those derived from the intestinal microbiota have been reported to be important during the postnatal and adult stages of organisms, including fish (Deloyer *et al.* 2001). These molecules are required for the growth of the intestinal mucosa (Löser *et al.* 1999) and the renewal of intestinal epithelial cells through the modulation of apoptosis and the expression of several genes (Peulen *et al.* 2000; Rao & Wang 2011). Polyamines are also necessary for the biochemical, maturation and immunological and morphological adaptation of the intestines and subsequent growth of the fish. These effects are dose-dependent and it has been shown that

Spm (tetraamine) and Spd (triamine) play a more important role than Pu (diamine) in this process (Deloyer *et al.* 2001). The objectives of this work were to compare the weight, standard length and, levels of intestinal polyamines, of Nile tilapia (*O. niloticus*) and channel catfish (*I. punctatus*), fed for 120 days diets with a base of fish and / or soybean oil and supplemented with a commercial multistrain probiotic.

Materials and Methods

Fish used in the study

The fish were provided as fry by the Aquamol Aquaculture Farm located in Jamay, Jalisco, México, and were acclimated to the laboratory conditions for one month, fed with 1.5 mm commercial pellets, with 42% crude protein. Subsequently, they were distributed randomly in 12 fish tanks of 30 x 45 x 60 cm (25 fish per fish tank), with three tanks per diet. The diets were administered *ad libitum* three times a day. The basal weight at the beginning of the experimental period was 8.96 ± 0.3 g for tilapia and 5.04 ± 1.39 g for catfish; at initiation the standard basal lengths were 26.78 ± 3.71 cm for tilapia and 7.03 ± 0.64 cm for catfish (averages \pm standard deviation).

Description of the diets

Diet C: Commercial pellets with 32% crude protein and 8% lipids, of which 2% are fish oils and 6% soybean oils. **Diet CP:** diet C + probiotics. **Diet S:** alternative diet with 32% of crude protein, 8% of soybean oil, absence of fish oils. **diet SP:** diet S + probiotics. Bacterol-

shrimp-Forte is a commercial probiotic available from Veterinary Pharmacology (FAV, Santiago, Chile). Its formulation contains a mixture of microorganisms including *Bacillus spp.*, *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*. The probiotic was supplied together with the feed in a proportion of 1 g Kg⁻¹ of feed in dry form. The pelleted food was manufactured by Consorcio Súper SA de CV (Guadalajara, Jal, Mexico).

Biometrics

The experiments lasted 120 days; periodically, individual fish were collected to determine the relationship between standard weight and length during the development of the fish, until the end of the breeding stage. The relationship between both measures was analyzed by applying the polynomial regression formula for biological systems as described by Bhujel (2008). The fish were anesthetized with clove oil, sacrificed, and intestine samples were removed by dissection under aseptic conditions (Télliez-Bañuelos *et al.* 2014). During collection, the tissues were kept on ice, and samples were maintained at -20°C until their analysis.

HPLC determination of polyamines

The intestine samples (50 mg) were treated with 0.05 M trichloroacetic acid (Merck, Kenilworth, NJ, USA) in 0.5 N HCl (Mallinckrodt Backer, DF, Mexico), in a 1:2 ratio (w:v). The process of chromatographic separation of polyamines is based on a previous report of Irecta-Nájera *et al.* (2017). The HPLC equipment used contains a autosampler (AS3000,

Spectra-Physics, Stahnsdorf, Germany), a flow pump (515, Waters, Milford, MA, USA) and a C18 reverse phase column (150 mm x 4.6 mm, Inertsil ODS-2, with a 5 μm particle diameter, Varian, Walnut Creek, CA, USA). A flow of 0.5 mL min^{-1} was used. Detection was performed by fluorescence, by post-column derivatization with ortho-phthalaldehyde (Merck, Hohenbrunn, Germany). The polyamines were identified according to their retention time as compared with commercial standards of known concentrations of Pu, Spd and Spm (Sigma Aldrich, St. Louis, MO, USA). 1,7-Diaminoheptane (Sigma Aldrich, St. Louis, MO, USA) was used as the internal standard of the chromatographic process. The data were analyzed with the Galaxie program version 1.9 (Varian, Walnut Creek, CA, USA). Each sample was analyzed in duplicate and the concentrations were expressed in $\text{nmol } \mu\text{L}^{-1}$.

Statistical analysis

To analyze the effects of the diets on growth (weight and standard length), a one-way analysis of variance was performed. The means of the polyamine levels between species were compared using the Student t-test and the Statgraphics Centurion XVI program. To analyze the levels of polyamines over time, the results of the four diets were grouped and the means \pm standard deviations of the levels of each polyamine were calculated using the Sigmaplot 11.0 program. One-way analysis of variance was applied after verifying normality, homogeneity of variance, as well as random distribution of

data error. The post hoc comparison of means was performed using the SNK test or the Kruskal Wallis test. In all cases, a $P < 0.05$ was considered significant (Zar 1999).

Results

Growth of tilapia and channel catfish

Even though some organisms supplemented with the probiotics presented a higher weight for a given fish length, no significant differences ($P > 0.05$) between the growth values of tilapia or catfish fed the different diets, were observed. Figures 1 and 2 show the growth data with the four diets of Nile tilapia and channel catfish, respectively. The polynomial regression equation (PRE) for tilapia was: $Y = 1.081X^2 - 14.03X + 69.80$, with $R^2 = 0.970$; for the catfish the values were: $Y = 0.500X^2 - 4.025X + 5.097$, with $R^2 = 0.979$. No significant differences were found in the PRE of the fish grown under the four different diets.

Effect of the four diets on intestinal polyamine levels

The experimental data indicate that there were no statistical differences in the polyamine levels between the fish fed the four diets (data not shown). Due to this observation, the levels of each polyamine were averaged across the four diets for the analysis of polyamine levels over time. When averaged over the 120 day experimental period, significantly higher levels ($\text{nmol } \mu\text{L}^{-1}$) of Pu ($P < 0.05$) were observed in tilapia (0.0194 ± 0.0048) than in catfish (0.0145 ± 0.0025), while Spd levels showed no differences between the two species. Spm levels ($\text{nmol } \mu\text{L}^{-1}$) were significantly higher ($P < 0.05$) in catfish (0.1037 ± 0.0143) than in tilapia (0.0561 ± 0.0196).

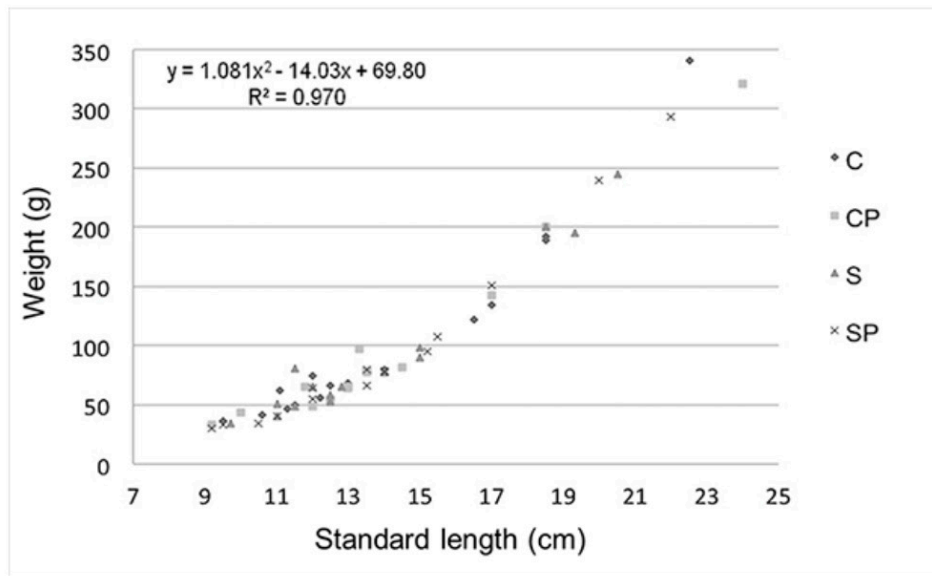


Figure 1. Growth of Nile tilapia represented as weight (g) and standard length (cm), during the experimental period of 120 days. The fish were fed with the control diet C (2% fish oil and 6% soybean oil), the alternative diet S (8% soybean oil and without fish oil) and the CP and SP diets, which correspond to the C and S diets supplemented with probiotics.

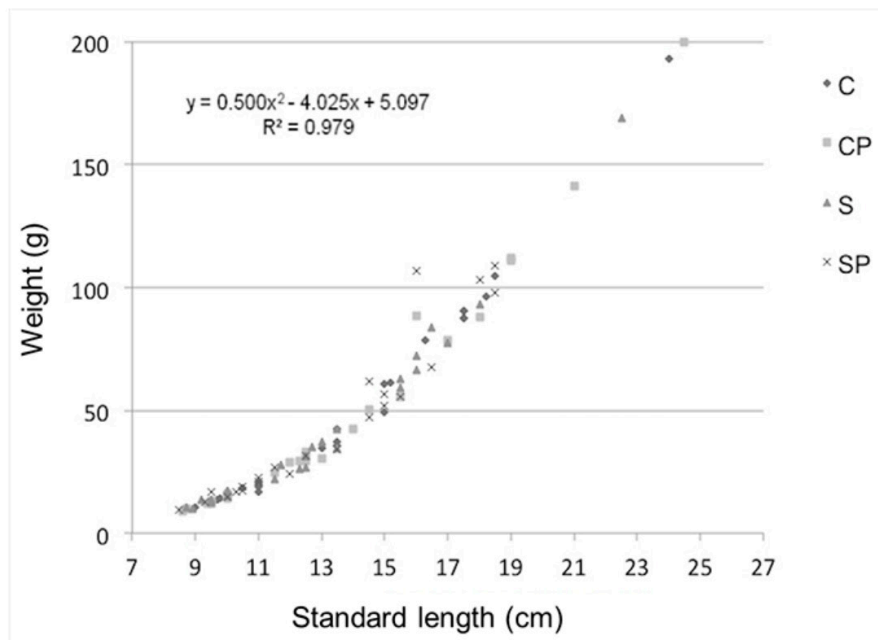


Figure 2. Growth of channel catfish represented as weight (g) and standard length (cm), during the experimental period of 120 days. The fish were fed with the control diet C (2% fish oil and 6% soybean oil), the alternative diet S (8% soybean oil and without fish oil) and the CP and SP diets, which correspond to the C and S diets supplemented with probiotics.

Polyamine levels during the growth of Nile tilapia

Figure 3 shows that the intestinal levels of the three polyamines showed a tendency to diminish over the time course of the experiment; the concentration of Spd ($\text{nmol } \mu\text{L}^{-1}$) was found to be significantly higher ($P < 0.05$) on the first day (0.1732 ± 0.0444) versus day 120 of the experiment (0.0386 ± 0.0139).

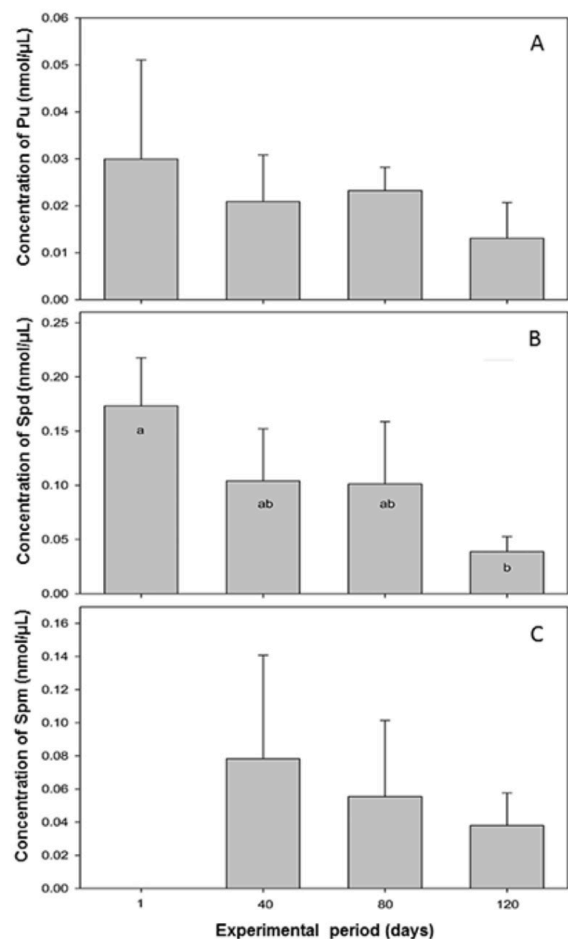


Figure 3: Levels of polyamines in the intestine of Nile tilapia (*Oreochromis niloticus*) during the 120 day experimental period. A: putrescine (Pu), B: spermidine (Spd) and C: spermine (Spm). Note that spermine levels were below the limit of detection at day 40. Each bar represents the mean \pm standard deviation ($N = 8$, expressed in $\text{nmol } \mu\text{L}^{-1}$). The bars with different letters differ significantly ($P < 0.05$).

Polyamine levels during the growth of channel catfish

Figure 4 shows the intestinal polyamine levels of the catfish throughout the experimental time course. In this species, a significant increase ($P < 0.05$) in the intestinal levels ($\text{nmol } \mu\text{L}^{-1}$) of the three polyamines was observed between day 20 (Pu, 0.0229 ± 0.0123 ; Spd, 0.1360 ± 0.0482 ; Spm, 0.1570 ± 0.0310) in comparison with day 120 (Pu, 0.0093 ± 0.0081 ; Spd, 0.0583 ± 0.0119 ; Spm, 0.0651 ± 0.0183).

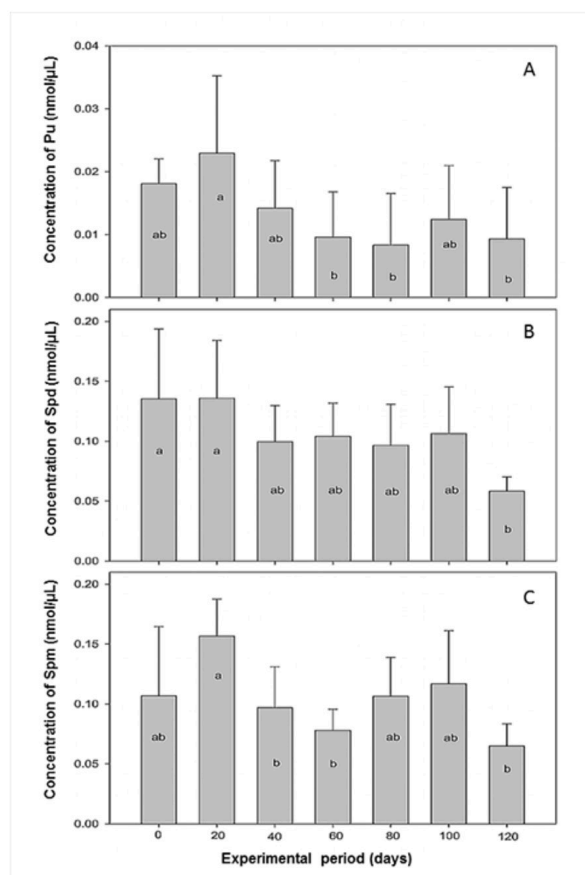


Figure 4: Levels of polyamines in the intestine of channel catfish (*Ictalurus punctatus*) during the 120 day experimental period. A: putrescine (Pu), B: spermidine (Spd) and C: spermine (Spm). Each bar represents the mean \pm standard deviation ($N = 8$, expressed in $\text{nmol } \mu\text{L}^{-1}$). The bars with different letters differ significantly ($P < 0.05$).

Discussion

In this work, we evaluated the levels of biogenic polyamines in the intestines of juvenile tilapia and catfish fed diets with replacement of fish oil with soybean oil and supplemented with a commercial multistrain probiotic. The effect of these diets on the growth of fish was also evaluated as these data are not well reported for both species which are economically important and represent a fast-growing sector of aquaculture worldwide.

The growth curves coincide with expected charts for tilapia and channel catfish respectively (Hai 2015; Mota *et al.* 2015). Interestingly, the total substitution of soybean oil for fish oil did not affect the growth of tilapia (*O. niloticus*) or catfish (*I. punctatus*). This result is similar to that of Jiang *et al.* (2013), who found that replacing fish oil with soybean oil did not significantly affect the growth and muscle fatty acid levels of the freshwater fish *Pelteobagrus vachelli*. The partial replacement of fish oil by different vegetable oils, 80% in rainbow trout (*Oncorhynchus mykiss*), and 50% in Atlantic salmon (*Salmo salar*) did not compromise growth, survival or food use in these species (Rosenlund *et al.* 2001; Caballero *et al.* 2002). Similarly Hunt and Tekelioglu (2008) reported that soybean oil could be used as a partial dietary substitute for fish oil within compound feeds for sea bass (*Dicentrarchus labrax*). In marine species such as *Sciaenops ocellatus*, substitution levels of up to 70-80% fish oil to soybean oil or flaxseed oil were tested without decreasing growth (Lochmann & Gatlin 1993; Tucker *et al.* 1997). However, in a different marine species, *Sparus aurata* (gilthead sea bream), the total replacement of fish oil by these vegetable oils decreased growth, phagocytic activity and activation of the alternative pathway of the complement

system, while diets with a partial substitution (50%) of vegetable oils for fish oil did not affect these parameters (Montero *et al.* 2008). Similarly, the growth performance of turbot fish (*Scophthalmus maximus*), found in marine or brackish waters, was significantly lower when fish oil was totally replaced by soy oil. The negative impact of vegetable oil in these particular species could be related to the unbalanced fatty acid profile and to their poor palatability and digestibility and may affect marine fish more than fresh water fish (Caballero *et al.* 2002). Fish oils are the most common source of lipids with a high content of HUFAs, so the lack of these fatty acids in vegetable oil is a factor that could affect fish growth. In a previous study, in the liver and brain of tilapia and catfish administered these same diets, we showed that the S diet does not significantly increase the transcriptional expression of the $\Delta 6$ -desaturase ($\Delta 6$ -FAD) and elongase (FAE), so these fish probably have the ability to transform PUFAs (of vegetable origin) into HUFAs (of animal origin) (Santerre *et al.* 2015). These earlier results and those of the present work indicate the practical feasibility of feeding Nile tilapia and channel catfish, two omnivorous freshwater species with diets containing soybean oil as a unique lipid source. The large scale implementation of this replacement diet could help reduce the increasing demand for fish oil due to the rapid development of the aquafeed industry and the great pressure that the fish oil industry puts on worldwide natural fishery resources (Du *et al.* 2017). This dietary observation is especially pertinent for tilapia, which has been reported as the second most cultivated fish species worldwide, with production levels increasing rapidly over the last ten years, due to its fitness for aquaculture, marketability and steady price market (Hai 2015).

The supplementation of the C and S diets

with probiotics did not indicate an effect on the growth of tilapia or catfish. Our data differ from those of Apún-Molina *et al.* (2009) who, in a 134-day study, at sub-optimal temperatures (19.9 - 24.82°C), demonstrated that juvenile tilapia experienced significantly better growth when they were fed Bacillus and LABs isolated from tilapia (either separately or together); these results suggest that these probiotics are appropriate for the stimulation of fish growth even at low temperatures. Hai (2015), in a review on the use of probiotics in tilapia aquaculture, highlighted the importance of several factors when using commercial probiotics in aquaculture of tilapia. These factors include: the use of multiple versus single probiotic strains, the administration method (in feed or in water), and the dosage and the duration of use. When evaluating the efficacy of probiotic supplementation of feed, another important aspect to consider is the adherence of the probiotic to the intestinal mucosa: Liu *et al.* (2013) reported the effects of two probiotic strains of Lactobacillus on juvenile tilapia; they used a more adherent strain (*L. brevis*) and a less adherent one (*L. acidophilus*). These authors concluded that the degree to which the Lactobacillus strain adheres to the intestines is an important criterion for the selection of the probiotic strain that will produce the most beneficial effects on survival, growth, feed conversion, immune response and resistance to fish diseases. We have previously reported that the Bacterol-Shrimp diet supplementation did not modify the concentration of LABs in the intestines of tilapia and catfish (Santerre *et al.* 2015) and it is likely that the lack of adherence of the strains used explains our current results with respect to the lack of growth differences between the four diets. That is to say that the effect of the probiotic and its polyamine content may have been only transient because of the

lack of adherence within the digestive system and may not have provided a continuous benefit for fish growth.

Focusing on polyamines, in this current study we found that the levels of these biomolecules in the intestines of tilapia or catfish fed the C and S diets were not significantly different, thus indicating that the substitution of soy oil for fish oil in the feed did not affect negatively the homeostasis of the polyamines of either species. Additionally, the supplementation of the food with the commercial probiotic (CP and SP) did not modify the levels of these molecules either. These results contrast with those of Tovar-Ramírez *et al.* (2004), who studied the role of yeasts in the secretion of polyamines in the intestines of larval stage snook (*Dicentrarchus labrax*), and suggested that dietary yeast activates the expression of genes associated with the immune system, promotes intestinal maturation and increased enterocyte nutrient absorption due to the high secretion of Spd and Spm by the yeasts. Based on the above references, it is suggested that the state of development of the fish at the time of supplementation with the probiotic is an important factor for the observation of beneficial effects on growth, food conversion, immune response and resistance to diseases. It is notable that we began this experiment with juvenile fish as this is the typical stage at which the fish farmer buys their organisms in order to grow them for food production; however, our experimental results and the reviewed literature suggest that it is necessary to supplement the diet with probiotics at an earlier stage which may provide extra levels of polyamines in order to assist in the maturation of the fish digestive system and provide long term benefit for fish growth.

When evaluating the intestinal levels of Pu, Spd and Spm in catfish over time, we observed that

their concentrations were significantly elevated at day 20 of experimentation when compared to day 120. The same tendency was observed in tilapia even though it did not reach significance. This could be due to the fact that by the end of the experimental period the fish had reached full growth and intestinal maturation, therefore decreasing polyamine requirements.

In conclusion, our data indicate that the diet formulated with soybean oil had no adverse effect on the growth and the intestinal polyamine levels in Nile tilapia and channel catfish. Thus it may be possible to replace the dietary fish oil for the less expensive soy oil. For large scale farming this would help reduce the dependency of the aquaculture sector on fish oil, and contribute to the preservation of the balance of the marine ecosystem. On the other hand, as our results did not show a significant effect of the probiotic used, larger follow-up studies will be

required in order to test the effects of other probiotic strains, especially those that adhere to the intestine in early stages of fish development.

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