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Δ6-fatty acid desaturase and fatty acid elongase mRNA expression, phagocytic activity and weight-to-length relationships in channel catfish (*Ictalurus punctatus*) fed alternative diets with soy oil and a probiotic

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ABSTRACT. A time-course feeding trial was conducted for 120 days on juvenile channel catfish (*Ictalurus punctatus*) to study the effects of diets differing in oil source (fish oil or soy oil) and supplementation with a commercial probiotic. Relative levels of Δ6-fatty acid desaturase (*Δ6-FAD*) and fatty acid elongase (*FAE*) expression were assessed in brain and liver tissues. Both genes showed similar expression levels in all groups studied. Fish weight-to-length relationships were evaluated using polynomial regression analyses, which identified a burst in

weight and length in the channel catfish on day 105 of treatment; this increase was related to an increase in gene expression. Mid-intestinal lactic acid bacterium (LAB) count was determined according to morphological and biochemical criteria using API strips. There was no indication that intestinal LAB count was affected by the modified diets. The Cunningham glass adherence method was applied to evaluate phagocytic cell activity in peripheral blood. Reactive oxygen species (ROS) generation was assessed through the respiratory burst activity of spleen macrophages by the NBT reduction test. Probiotic-supplemented diets provided a good substrate for innate immune system function; the phagocytic index was significantly enhanced in fish fed soy oil and the probiotic, and at the end of the experimental period, ROS production increased in fish fed soy oil. The substitution of fish oil by soy oil is recommended for food formulation and will contribute to promoting sustainable aquaculture. Probiotics are also recommended for channel catfish farming as they may act as immunonutrients.

Key words: *Ictalurus punctatus*; Probiotics; Δ6-fatty acid desaturase; Soy oil; Fatty acid elongase; Phagocytic activity

INTRODUCTION

The catfish genus *Ictalurus* is native to running water ecosystems of temperate areas of North America, ranging from the south of Canada to northern Mexico. More than 39 species of *Ictalurus* have been identified, although catfish farming is largely restricted to *Ictalurus punctatus* (channel catfish). The success of channel catfish farming is related to the improvements in overall management, disease control, and diet formulation. Fish oil (FO) obtained from natural resources has been used for decades in fish feed. This is no longer sustainable as FO has become scarce and expensive, and will constrain the future growth of aquaculture (Pike, 2005; Naylor et al., 2009; Bostock et al., 2010; Zhou et al., 2010). Vegetable oils, such as soy oil, are good candidates as alternative sources of oil for fish food formulation, and may help reduce the dependence of the aquaculture industry on FO. However, it is possible that the oil source used to prepare fish food may affect fatty acid (FA) metabolism in the fish. In particular, FO is rich in highly unsaturated fatty acids (HUFAs), while vegetable oils are rich in polyunsaturated fatty acids (PUFAs) (Bell et al., 2002). Fresh water fish are mostly incapable of *de novo* synthesis of HUFAs although they can convert PUFAs to HUFAs to a greater or lesser extent depending on the species (Sargent et al., 2002; Zheng et al., 2004a). Δ6 fatty acid desaturase (Δ6-FAD) and fatty acid elongase (FAE) are critical enzymes in the HUFA biosynthetic pathway (Chen et al., 2014). High levels of HUFAs in fish food may inhibit the expression of Δ6-FAD and FAE, so total removal of FO from the diet may modulate the expression of these genes (Vagner and Santigosa, 2011).

Improvement of disease control in fish farms has included the prophylactic application of probiotics, which are inexpensive and easily accessible, present few side effects, and may produce substances that inhibit colonization by pathogenic microbes (El-Haroun, 2007; Soccol et al., 2010; Cui et al., 2013). Much of the research on alternative oil sources for fish food and food supplementation has dealt with their potential effects on digestion and growth

parameters; however, modification of the diet may also modulate the immune function of the fish and their resistance to pathogens (Pirarat et al., 2011). In fish, phagocytosis is the main nonspecific cellular immune mechanism for destroying extracellular bacteria, viruses, and fungi in the environment. Phagocytic activity is followed by the generation of a series of reactive oxygen species (ROS) in a process called a respiratory burst, recognized as one of the key killing responses of phagocytes during microbial infection (Kokou et al., 2012).

In the present study, we investigated the effect of an alternative diet based on soy oil as the sole source of FAs, and of dietary supplementation with a commercial probiotic. We assessed the consequences of the dietary modifications using a range of parameters including *Δ6-FAD* and *FAE* gene expression at the transcriptional level, growth in weight and length of the fish, gut lactic acid bacterium (LAB) content, phagocytic index, and ROS production in juvenile channel catfish maintained under controlled experimental conditions.

MATERIAL AND METHODS

Experimental fish

Juvenile channel catfish (*I. punctatus*) weighing 1.0 ± 0.2 g were obtained from the Aquamol fish farm in Jamay, Jalisco, Mexico. Phenotypic males were used for all experiments. Fish were transferred to 400-L fiberglass tanks containing a chlorine-free fresh water recirculating aquaculture system and were acclimated to greenhouse conditions for 1 month before the start of the trial. During this acclimation period, they were fed a standard 1.5-mm feed size starter commercial diet (52% crude protein; El Pedregal, Toluca, Mexico).

Experimental design

After the acclimation period, the juvenile fish (6.83 ± 0.15 g) were randomly assigned to 12 aquariums (3 aquariums per diet) with individual water flow of 0.5 L/min. Each tank (30 x 45 x 60 cm) contained 25 fish at the beginning of the experiment. Fish were fed twice daily to apparent satiation. Water temperature was maintained at 27° ± 1°C and the physicochemical parameters of the culture system were maintained within the optimal ranges for the species (Robinson and Li, 2007). The fish were routinely checked for signs of abnormal behavior and visible lesions. They were not vaccinated, and no antibiotics were used.

Diets

During the experiment the fish were fed commercial diets manufactured by Consorcio Super (Jalisco, Mexico). Diets were prepared according to the nutritional requirements of channel catfish (Robinson and Li, 2007), and formulated with the same ingredients (35% crude protein, attractants, minerals, and vitamins) except for the oil composition. Table 1 highlights the differences between the diets. The control diet (C diet) contained 2% FO and 6% soy oil. The alternative diet (S diet) contained 8% soy oil and no FO. The CP and SP diets correspond to the C and S diets but were supplemented with the multispecies Bacterol-shrimp Forte probiotic after diet manufacture. This probiotic is derived from 14 different microorganisms including *Bacillus*, *Lactobacillus*, and *Saccharomyces* at an overall concentration of 5 x 10^8

colony-forming units (CFU/g (Farmacología en Aquacultura Veterinaria, Santiago, Chile). The probiotic was added at 1 g/kg as recommended for other probiotics (Chang and Liu, 2002; Abdelhamid et al., 2009).

Table 1. Formulations of the four diets used to feed channel catfish for 120 days.

	Diet			
	C	CP	S	SP
Fish oil (%)	2%	2%	0%	0%
Soy oil (%)	6%	6%	8%	8%
Probiotic	-	+	-	+

C = control diet; CP = control diet supplemented with probiotic; S = soy-oil diet; SP = soy-oil diet supplemented with probiotic (1 g/kg) (- indicates no supplementation, + indicates supplementation).

Sampling

The experiment was run for 120 days. Every 2 weeks, three fish from each diet (one fish per aquarium) were randomly withdrawn and anesthetized with clove oil (100 mg/L). The fish were blotted dry, weighed, and measured (standard length). Peripheral blood was immediately withdrawn by caudal venipuncture with a 3-mL plastic syringe for the phagocytic assay; the remaining blood was passed into Eppendorf tubes and allowed to clot for 30 min. Serum for opsonization was obtained by centrifugation at 200 *g* (Spectrofuge 24 D, Labnet, USA) for 20 min and was kept at -20°C until needed. Once blood was withdrawn, fish were aseptically and rapidly dissected in order to obtain the liver, brain, and mid-intestine. At the end of the experimental period, the spleen was also obtained. These samples were evaluated as a pool (one pool per diet).

Expression of fatty acid desaturase and fatty acid elongase mRNA in liver and brain

Semi-quantitative reverse-transcription polymerase chain reaction (semi-quantitative RT-PCR) was used to evaluate the expression of *Δ6-FAD* and *FAE*. Total RNA was extracted from 50 mg tissue (liver and brain) using 1 mL Trizol reagent. Complementary DNA (cDNA) was synthesized from 500 ng total RNA using oligo dT_{12-18} and M-MVL reverse-transcriptase (RT) following manufacturer protocols (Gibco-Invitrogen, USA). Quantity and integrity of the RNA was checked by UV spectrophotometry and agarose gel electrophoresis before cDNA synthesis. Specific primers for *Δ6-FAD*, *FAE*, and *β-actin* amplicons were designed from mRNA sequences obtained from GenBank. All gene-specific primers for semi-quantitative RT-PCR detection are listed in Table 2. Primers were synthesized by Integrated DNA Technologies (IDT, USA). Amplification was carried out in 25-µL reaction volumes containing 1X PCR buffer, 3 mM $MgCl_2$, 0.4 mM of each dNTP, 0.4 µM of each primer, and 1 U *Taq* polymerase (Gibco-Invitrogen); cDNA and milliQ water volumes were added according to *β-actin* normalization. Duplicate amplifications were performed using a PTC-100 thermocycler (MJ Research, USA) as follows: 95°C for 2 min; 32 cycles of denaturation at 95°C for 45 s, annealing at 52°C for 45 s, and elongation at 72°C for 45 s; and a final incubation at 72°C for 5 min. PCR products were separated by electrophoresis on 1.5% agarose gels stained with ethidium bromide. Results were analyzed using the Kodak 1D Image Analysis Software, Version 3.5 (Eastman Kodak CO, USA). Values are reported as arbitrary units.

Table 2. Primers used for the semi-quantitative RT-PCR analysis of the expression of Δ6-fatty acid desaturase (*Δ6-FAD*) and fatty acid elongase (*FAE*) genes in brain and liver of channel catfish.

GenBank accession No.	Primer positions on gene (forward/reverse)	Primer sequence	Hybridization temperature (°C)	Amplicon size (bp)
Δ6-FAD (EF125211.1)	26-43 / 129-111	5'-GGTCATTAAGGACCCTGA-3' 5'-GAAGTACTGGTGCTGATG-3'	52	121
FAE (CK417097.1)	141-159 / 283-265	5'-TGTCTGGTGGTTGATTTC-3' 5'-CGTTGGTGATTACAGAGC-3'	52	143
β-actin (DQ399027.1)	291-309 / 491-473	5'-TGCCTATTCCTCCTCTCT-3' 5'-GTGTTGGCATACAGATCC-3'	52	201

Weight-to-length relationship

The relationship between standard length (L, in cm) and weight (W, in g) of channel catfish was investigated by applying a polynomial regression analysis as described by Bhujel (2008) for biological systems.

LAB quantification in mid-intestine

LABs were quantified in order to study the effect of diets on intestinal microflora. Classical microbiological protocols were applied (Ghosh et al., 2014). Briefly, the mid-intestine segments were weighed, washed with 9 mL MRS (de Man, Rogosa and Sharpe; Difco Laboratories, USA), the selective medium for LAB bacteria, cut into pieces and vortexed. Serial dilutions were made in MRS broth and a 250-µL aliquot of each dilution was pipetted onto MRS agar and incubated at 37°C for 48 h. A Quebec counter was used to count the CFU of LAB according to morphology. Then, the bacteria were identified according to their biochemical criteria using API50CHL strips following manufacturer instructions (Biomerieux, France). All cultures were performed in duplicate for each intestinal sample. Data are reported as log of the number of CFU/g intestine, and as means ± SD.

Phagocytic activity of peripheral blood evaluated by the glass adherence method

Phagocytic cells from peripheral blood were used for the functional phagocytic assay. This was performed by a modification of the Cunningham glass adherence method reported by Casas-Solis et al. (2007); in our modified protocol, the antigen consisted of 1 x 10^6 CFU opsonized *Aeromonas hydrophila* (ATCC 7966, USA) (Giron-Perez et al., 2007). The phagocytic index is reported as the average number of *A. hydrophila* particles engulfed per cell, and was calculated by dividing the total number of engulfed particles by the cell count. All experiments were performed in duplicate for each experimental group.

Respiratory burst activity (RBA) assay in spleen macrophages

RBA produced by spleen macrophages was evaluated using the nitro-blue tetrazolium (NBT) reduction test (Tellez-Bañuelos et al., 2009). Briefly, a mixture of 100 µL of spleen macrophages (1 x 10^6 cells/mL) and 500 µL PBS containing 0.1% NBT (Sigma, USA) and 25 x 10^6 CFU opsonized *A. hydrophila* as the antigen (ATCC 7966, USA) was incubated at 25°C for 2 h with occasional shaking. The mixture was centrifuged at 1800 *g* for 5 min. The reaction was

stopped with 500 µL 70% methanol and centrifuged at 1800 *g* for 5 min. The cellular button was dried at 60°C for 15 min, and 450 µL 2 M potassium hydroxide was added and the suspension was sonicated for 15 min. Five hundred microliters of dimethyl sulfoxide (Sigma, USA) was added and the mixture was incubated for 10 min, and then centrifuged at 4000 *g* for 5 min. Optical density was measured with an OpsysMR microplate reader (Dynex Technologies, USA) at 630 nm. The experiment was performed in triplicate.

Statistical analysis

Averaged data (means ± SD) are reported for each study group in a given experiment. Statistical analyses were carried out utilizing one-way ANOVA and the Tukey honestly significant difference *post hoc* test for all pairwise comparisons of the mean responses of the different experimental groups. In all cases, the significance level was set at $P < 0.05$.

RESULTS

Semi-quantitative RT-PCR evaluation of *Δ6-FAD* and *FAE*

We performed semi-quantitative RT-PCR to measure the levels of expression of *Δ6-FAD* and *FAE* in liver and brain during the 120-day treatment period (Figure 1a). Single bands of the expected sizes were obtained (121 bp for *Δ6-FAD*, 143 bp for *FAE*, and 201 bp for *β-actin*). No differences were observed between the four different diets at any sampling time (Figure 1b; $P > 0.05$).

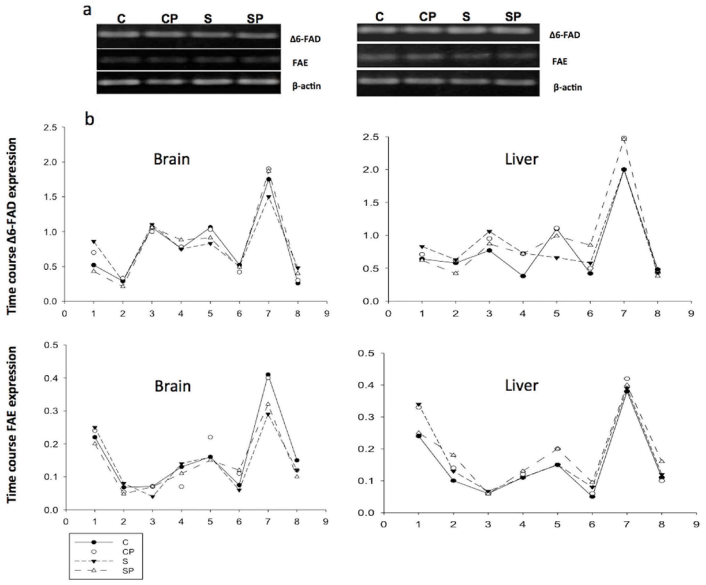

Figure 1. Expression of Δ6-fatty acid desaturase (*Δ6-FAD*) and fatty acid elongase (*FAE*) genes. **a.** RT-PCR products of brain and liver tissue from fish in each treatment group. **b.** Time-course changes in relative levels of *Δ6-FAD* and *FAE* mRNAs at 2-week intervals during the 120-day experiment (8 samples) in the brain and liver of channel catfish in the four diet groups (C, CP, S, and SP). Results are reported as means ± SD. For diet abbreviations, see legend to Table 1.

For all four diets and at any sampling time, *Δ6-FAD* expression was approximately four times higher than *FAE* expression in both liver and brain. A significant increase ($P < 0.05$) in the expression of the genes was seen in both tissues and in all dietary groups at day 105 of treatment (Figure 1b).

Weight-to-length relationship

The relationship between weight (W, g) and standard length (L, cm) of the channel catfish is shown in Figure 2. A polynomial regression analysis $W = 43.720 - (12.406 * L) + (1.072 * L^2) - (0.0123 * L^3)$, $N = 126$, indicated an r value of 0.992. No differences were observed among the r values of the four dietary groups when evaluated individually (data not shown).

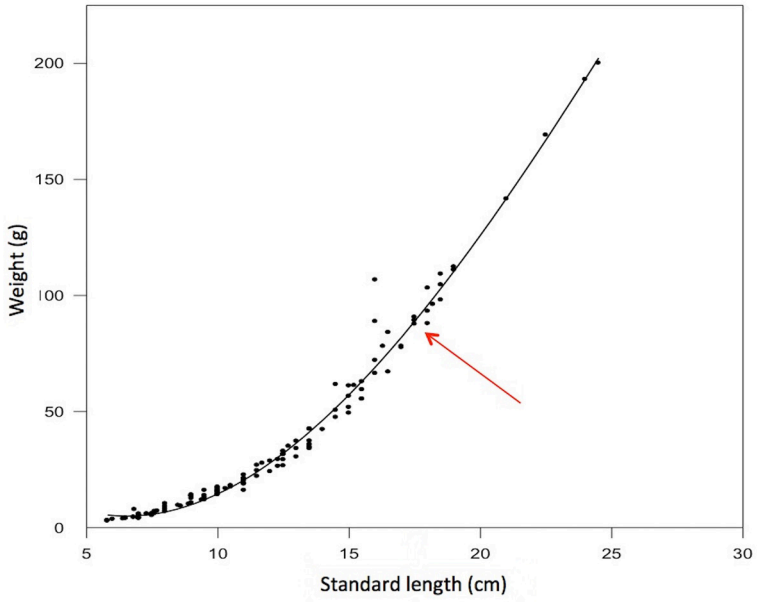

Figure 2. Relationship between weight (g) and standard length (cm) of channel catfish. A polynomial regression analysis was applied and the data were fitted to a regression curve. The arrow indicates the growth burst in the fish, which corresponds to a significant increase in transcriptional expression of *Δ6-FAD* and *FAE* genes.

Intestinal LAB content

The LAB content values (log of the number of CFU/g mid-intestine) for the four diets were as follows: 6.87 ± 0.42 for the C diet; 6.95 ± 0.39 for the CP diet; 7.00 ± 0.30 for the S diet; and 6.95 ± 0.33 for the SP diet. Intestinal LAB count was not affected by either replacement of FO by soy oil or the supplementation of the diet with the probiotic.

Phagocytic activity

Our analysis of phagocytic activity in blood cells did not reveal any significant changes within each treatment group over the time course of the experiment; the data are

therefore reported as means ± SD for each treatment group (Figure 3). Fish that received the SP diet had a significantly higher phagocytic index (13.3 ± 1.98) than fish fed the control C diet (6.92 ± 2.87) or the CP diet (8.06 ± 2.65). However, the phagocytic index of the SP group was not significantly different from that of the S group (9.81 ± 2.35).

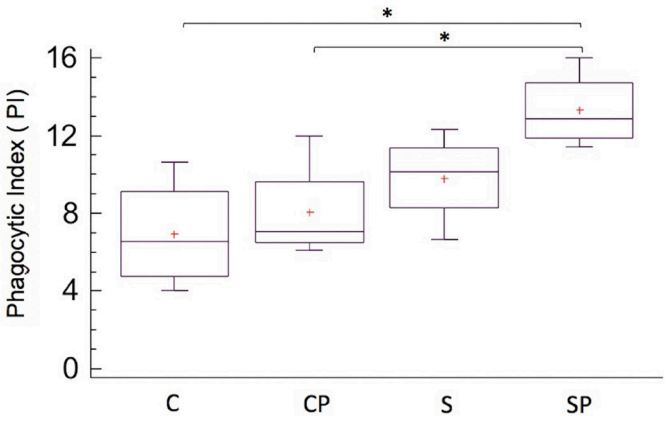

Figure 3. Box-plot representation of the effect of fish oil replacement and probiotic supplementation on the phagocytic index in channel catfish. The graphs show average behavior over the 120-day study. Results are reported as means ± SD; one-way ANOVA and the Tukey test were used for the analysis. The level of significance was set at *P < 0.05. For diet abbreviations, see legend to Table 1.

Respiratory burst activity

RBA was evaluated on day 120 of the treatment period. A significant increase in RBA was found in the spleens of fish fed the S diet compared with fish fed the C diet (Figure 4). Moreover, RBA was significantly lower (P < 0.05) in splenocytes from fish that received the probiotic (CP diet: 0.123 ± 0.029; SP diet: 0.281 ± 0.008) compared to fish that did not receive this supplement (C diet: 0.392 ± 0.023; S diet: 0.503 ± 0.012).

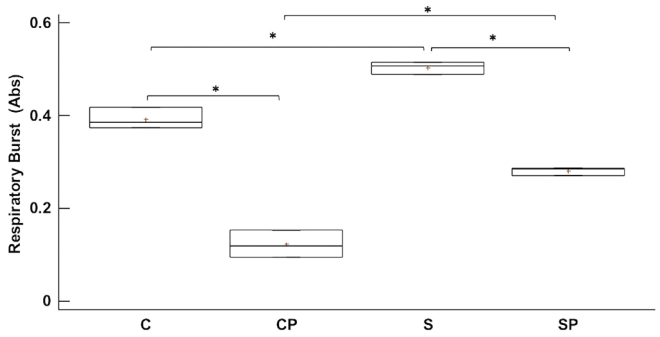

Figure 4. Box-plot representation of the effect of fish oil replacement and probiotic supplementation on respiratory burst activity in channel catfish at the end of the experiment. Catfish were fed four different diets (C, CP, S, and SP) for 120 days. Reactive oxygen species production in the splenocytes of the fish was determined using nitroblue tetrazolium reduction (absorbance at 630 nm). Results are reported as means ± SD. One-way ANOVA and the Tukey test were used for the analysis. The level of significance was set at *P < 0.05. For diet abbreviations, see legend to Table 1.

DISCUSSION

Aquaculture is a fast-growing sector that provides many job opportunities and contributes to meeting the increasing demand of consumers for fishmeal. However, the successful development of this industry could stall as fish farming faces increasing production costs and the constant risk of fish diseases. The present 120-day study evaluated the effect of removing FO from the diet and of supplementing it with a commercial probiotic. Various endpoints were screened to assess the effects of dietary changes, namely, *Δ6-FAD* and *FAE* expression, growth in fish lengths and weights, intestinal LAB contents, and innate immune responses.

Juvenile channel catfish fed a diet without FO showed no significant differences in their weight-to-length ratio compared to fish fed the conventional diet. This indicates that dietary FO can be replaced by soy oil without compromising this growth parameter in channel catfish. A similar conclusion was reached in previous study of darkbarbel catfish (*Pelteobagrus vachelli*) that were fed diets with different amounts of soybean oil for 80 days (Jiang et al., 2013), and was also reported for other species such as *Acanthopagrus schlegeli* (Peng et al., 2008), *Diplodus puntazzo* (Piedecausa et al., 2007), *Epinephelus malabaricus* (Lin and Shiau, 2007), and *Oncorhynchus mykiss* (Caballero et al., 2002).

Probiotics have been proposed as alternative prophylactic agents for the aquaculture industry. Interestingly, another practical use of probiotics in fish diets is the promotion of growth by a variety of mechanisms including modulation of the immune system (Nayak, 2010). Sáenz de Rodriguez et al. (2009) and Garcia de la Banda et al. (2010) reported that growth and nutrient utilization were significantly higher in Senegalese sole receiving probiotics than in fish fed a control diet. However, Merrifield et al. (2010, 2011) conducted a 10-week feeding trial to assess the effect of the probiotic *Pediococcus acidilactici* on rainbow trout and reported no significant improvement in growth performance or other parameters, such as feed utilization and carcass composition, in the probiotic-fed fish compared to the control group. Similarly, under our experimental conditions, the supplementation of channel catfish diets with a probiotic did not affect the weight-to-length ratios during the experimental period. A detailed study of additional growth parameters and a proximate analysis of channel catfish fed the commercial probiotic used here will be necessary before drawing final conclusions on its effect.

The extent to which fish transform PUFAs to HUFAs varies with the species (Sargent et al., 2002; Zheng et al., 2004a). Freshwater species such as carp, tilapia, trout, and salmonoids have a recognized ability to bio-convert PUFAs to HUFAs and this is related to the expression of *Δ6-FAD* and *FAE* (Bell et al., 2002). The elimination of HUFAs from the diet of these species up-regulates the rate of transcription of *Δ6-FAD* (mRNA). However, fish fed diets with a low HUFA content do not always show significant differences in *Δ6-FAD* mRNA levels compared to fish fed diets rich in HUFAs (Vagner and Santigosa, 2011). To the best of our knowledge, the present study is the first report on the transcriptional expression of *Δ6-FAD* and *FAE* in channel catfish. Our data showed no statistical differences in the levels of *Δ6-FAD* and *FAE* mRNAs in the different treatment groups. The lack of a significant effect on expression of the *Δ6-FAD* and *FAE* genes in channel catfish under the C and S diet may be due to the low percentage of FO in the control diet used here (C diet).

Over the time-course of the study, the levels of expression of *Δ6-FAD* and *FAE* followed the same patterns in liver and brain; in both tissues, a significant increase in expression of the genes was observed on day 105 of treatment. This increase coincided with a burst in weight and length and might represent a response to the need of the fish for synthesis, storage, and

distribution of FA to different parts of the body for various physiological functions (Kandemir and Polat, 2007). The relative expression of *Δ6-FAD* was four times higher than that of *FAE*; a similar result has been reported in salmon (Zheng et al., 2004b). The supplementation of the diets with a probiotic did not affect *Δ6-FAD* or *FAE* gene expression.

Lactic acid bacteria are amongst the most widely used probiotics. They are good producers of bacteriocins and organic acids; these compounds have inhibitory effects on the growth of some pathogens in fish and give LAB a competitive advantage over other bacteria (Zhou et al., 2010; Lara-Flores, 2011). Our experimental data showed no statistical differences in LAB count between treatment groups, a finding that is consistent with previous studies in which differences in the intestinal microbial balance only occurred in a few experimental cases (Vine et al., 2004; Varela et al., 2010). In agreement with other authors (Welker and Lim, 2011), we suggest that the LAB present in the probiotic had not settled on the intestinal mucous membrane of channel catfish because of the constant washing of this organ within the natural environment of the fish. It is possible that the effect of the probiotic on growth parameters may be long-term; this is an important consideration for practical application of probiotics in fish farms.

Even if the removal of FO and the supplementation of the diet with a probiotic did not have a significant effect on the measured parameters, they did provide a boost to the innate immune function. Comparison of the phagocytic indexes of fish fed on C or S diets showed that the use of soy oil as the sole source of FA increased phagocytic function in channel catfish. Montero et al. (2003) reported a significantly higher phagocytic activity in gilthead seabream (*Sparus aurata*) fed a diet with FO compared to a diet of 80% soybean oil for prolonged periods. In contrast, our data suggest that freshwater, omnivorous fish species such as channel catfish are better suited to total FO replacement than marine fish. Another interesting observation was that supplementation of the S diet with a probiotic enhanced the positive effect of the S diet on the phagocytic index. This index also increased in the CP diet compared with the C diet, but the difference was not significant. These results are consistent with other reports showing that probiotics stimulate phagocytosis in different fish species (Nayak, 2010; Soccol et al., 2010; Sun et al., 2010). Thus, the SP diet has potential utility in aquaculture of channel catfish as it enhances the defense mechanisms of this species against pathogenic organisms.

The production of ROS was evaluated at day 120 of the experimental period. RBA significantly increased in splenocytes of fish fed with the S diet compared to those on the C diet. This finding differs from that in the study of Montero et al. (2003) in which *Sparus aurata* were fed a diet of 80% soybean oil for 204 days. Vegetable oil had no effect on macrophage RBA in this species compared to fish fed a standard diet. The contrasting outcomes in these two studies may be related to differences in species and treatment duration. The present study also showed that supplementation of the C and S diets with a probiotic significantly decreased ROS values on day 120. ROS are products of the phagocytic process and are highly microbicidal, but when produced in excess they can lead to severe cellular damage (Tellez-Bañuelos et al., 2009). One of the main enzymes that detoxify ROS is superoxide dismutase (SOD), which is abundant in fish tissue (Harikrishnan et al., 2011). In *Epinephelus coioides* given a supplement including *Saccharomyces cerevisiae*, Chiu et al. (2010) reported that SOD activity increased on day 28 of treatment. Furthermore, Sun et al. (2010) showed in the same species that *Bacillus pumilus* and *Bacillus clausii* provoked an increase in SOD concentration on day 60. From the results of the present study, we hypothesize that the decrease in ROS seen

in fish fed diets supplemented with a probiotic might be due to its long-term effect on SOD activity in fish immune cells. Complementary time-course data will be needed to substantiate this hypothesis.

In conclusion, no effects on weight-to-length relationships or transcriptional expression of *Δ6-FAD* or *FAE* were found in channel catfish fed for 120 days on a diet based on soy oil as the sole source of FA or a diet supplemented with a commercial probiotic. The results presented here indicate that channel catfish might easily be transferred from a commercial diet including fish oil to a diet based on soy oil as a source of FA without compromising their growth, but still maintaining their health. This is encouraging for catfish farming as the large-scale removal of FO from catfish meal would help to lower fish food cost. In addition, it would contribute to the sustainability of aquaculture and help to preserve the balance of the marine ecosystem. Our results also indicate that the use of probiotics is beneficial to channel catfish farming. The probiotic used here acted as an immunonutrient by enhancing immunity in the catfish. Its long-term use may reduce the incidence of infectious fish disease and improve the overall health of channel catfish in aquaculture. Ongoing experiments will allow us to characterize classical growth parameters and FA content in order to give a more thorough assessment of growth performance.

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