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LONG TERM EFFECTS OF EARLY PROBIOTIC INTAKE ON *Solea senegalensis* CULTURE

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Introduction

Lactic acid bacteria (LAB) are one of the most studied probiotic bacteria in terms of their beneficial effects on aquatic species health. *Pediococcus acidilactici* CNCM MA18/5M, is a LAB strain which is registered in the European Union for use in feed for aquaculture. Previous studies have focused on the positive effects of this probiotic on a variety of species with commercial interest such rainbow trout (Ramos et al., 2013), tilapia (Standen et al., 2013) or blue shrimp (Castex et al., 2010) among others.

The current experiment aims to explore the potential beneficial effects of a prolonged probiotic supplementation (up to 150 days post hatching (dph)) with this strain on growth performance in *Solea senegalensis* culture.

Material and methods

This experiment was conducted in accordance with the Spanish and European law on animal experimentation and was specifically approved by the Bioethics Committee of the University of Cantabria (authorization number 2021-02).

Embryos were obtained by *in vitro* fertilization (IVF) (Rasines et al., 2013) using F1 *Solea senegalensis* gametes from only one couple. General culture protocol and feeding regime were based on Cañavate and Fernández-Díaz (1999) with slight modifications. One dph, the batch of siblings was homogenously split into 6 tanks (Volume: 200 L; density: 50 larvae L^{-1}). Two experimental groups (3 tanks/group) were created differing in the feeding program: 1) the control group (CTRL) in which live food (rotifer and artemia metanauplii) was enriched with a commercial product (Easy Dry SELCO®, Inve Aquaculture) and 2) the experimental group (PROBIO) in which live food was enriched (10^{11} CFU mL^{-1}) with *Pediococcus acidilactici* MA 15/5M (Bactocell®, Lallemand). Once weaning was over (90 dph), the PROBIO group maintained the probiotic supplementation in the pellets. The probiotic was included in the inert food of this experimental group using the oil coating technique (20 % w/w vegetal oil).

Growth was evaluated taking into account three temporal frames: 1) Live-inert cofeeding (3 dph; beginning of feeding-90 dph; end of weaning), 2) Inert feeding (90 dph-150 dph), and 3) Complete trial (3 dph -150 dph). Specific Growth Rate, SGR $((\ln W_f - \ln W_0)*100/T_f-T_o)$ was used as variable to evaluate fish growth. Statistical analysis was performed using SPSS 21.0. Normally distributed variables were analyzed using the student's t-test. Values with $p < 0.05$ were considered to be statistically significant. Data are expressed as mean ± SEM.

Results and Discussion

The prolonged dietary supplementation with *P. acidilactici* CNCM MA18/5M from the beginning of larval feeding (PROBIO) equaled CTRL group in terms of growth ($p = 0.372$) after the first temporal frame (live-inert cofeeding) (Table 1). These results report evidence on the capacity of the bioencapsulated probiotics in live food to provide similar growth rates upon the end of weaning when compared to tanks in which live feeding was enriched using a commercial enrichment product (SELCO®) in *S. senegalensis* specimens. In the second temporal frame involving inert feeding exclusively (90 dph-150 dph) the statistical analysis did not report significant differences ($p = 0.0720$) between CTRL and PROBIO (maintaining probiotic supplementation with probiotic-coated pellets) although a higher mean SGR value was registered in this experimental group (Table 1). Overall, taking into account the complete trial, up to the last evaluated sampling (150 dph), a significant increase ($p = 0.037$) was reported in the growth values in the PROBIO tanks (Table 1). It is our purpose to maintain constant probiotic supplementation during more months to monitor the evolution of growth, as well as other indicators such as gonad development. So far, our data provide clues indicating the potential positive effect of constant probiotic supplementation in the culture of Senegalese sole, which may be the starting point for the development of new specific feeding programs based on this approach.

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Table 1. Growth performance of *S. senegalensis* cultured with the two experimental feeding programs: control (CTRL) and long-term supplemented with *P. acidilactici* CNCM MA18/5M (PROBIO) during the three studied temporal frames in the culture. Data are expressed as mean ± SEM. Mean values with different superscript letters in the same column were significantly different among treatments ($p< 0.05$)

	SGR (%)		
Experimental Group	**Live-inert cofeeding (up to 90 dph)**	**Inert feeding (90-150 dph)**	**Complete trial (up to 150 dph)**
CTRL	10,839 ±0,057[a]	2,925 ±0,147[a]	7,495 ±0,089[a]
PROBIO	10,929 ±0,068[a]	3,541 ±0,207[a]	7,807 ±0,048[b]

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Acknowledgments

Authors would like to acknowledge FCJ2018-037566-I grant, PROBISOLE project (Fundación Biodiversidad; PLEAMAR2020-FEMP), Lallemand Animal Nutrition S.A. and STOLT Sea Farm S.A.