Probiotic *Shewanella putrefa*ciens Pdp11 enhances stress resistance in just weaned *Solea senegalensis* fry

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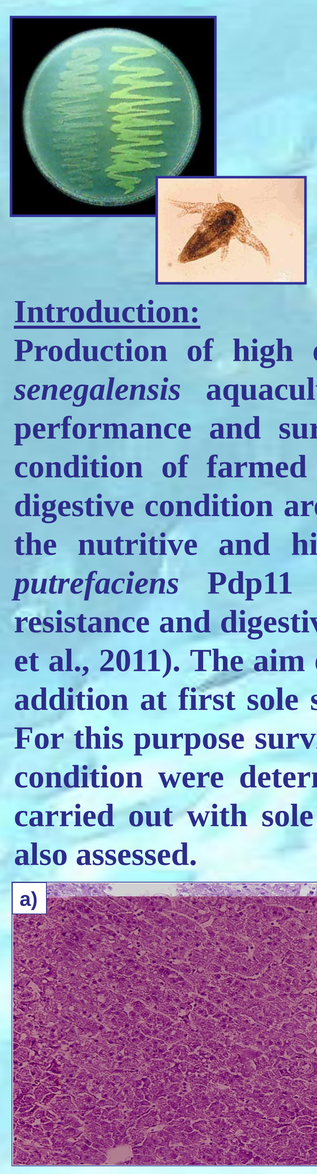

Introduction:

Production of high quality juveniles is still a bottleneck in *Solea senegalensis* aquaculture. Stress resistance together with growth performance and survival are parameters that assess physiological condition of farmed fish. Likewise RNA/DNA ratio and liver and digestive condition are interesting tools when choosing a diet based on the nutritive and histological condition detected in specimens. *S. putrefaciens* Pdp11 incorporated in inert diet improved stress resistance and digestive condition of sole juveniles (García de la Banda et al., 2011). The aim of this study is to evaluate the influence of Pdp11 addition at first sole stages (2-21 dah) on just weaned *S. senegalensis*. For this purpose survival, growth performance and liver and digestive condition were determined. A food deprivation stress challenge was carried out with sole fry where mortality and RNA/DNA ratio were also assessed.

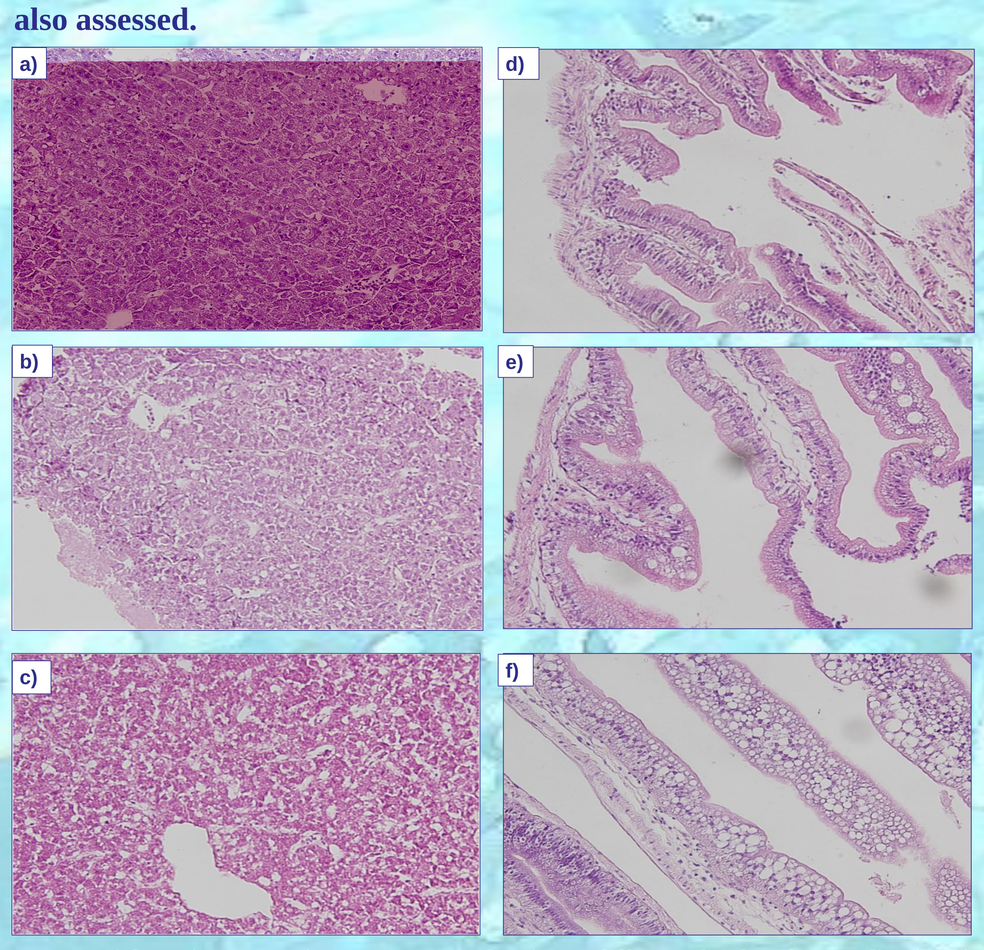

Figure 1. Light microscopy images (X500) showing different levels of vacuolization in *S. senegalensis* fed the two experimental diets in liver a)-1, b)-2 and c)-3 and intestine d)-1, e)-2 and f)-3 *

Table I. Growth performance , survival and lipid vacuolization* in liver and intestine at the end of the weaning period in *S. senegalensis* fed two experimental diets. (Mean SEM). Different letters denote significantt differences (P<0.05)

	Length (mm)		Dry weight (mg)		Survival (%)		Intestine		Liver	
Control	31.8	0.2	58.9	2.0[b]	97.6	0.3	2.17	0.21	1.67	0.19
Pdp11	32.8	0.2	64.4	1.4[a]	98.1	0.7	2.60	0.21	1.60	0.13

*0-absence,1-low-vacuolization,2-middle-vacuolization, 3- abundant vacuolization ,4- plenty of vacuolization

Material and Methods

S. senegalensis larvae were distributed into 280 l tanks by triplicate. (19.3 ± 0.5 ºC). Illumination and feeding regime was based on Lobo et al. (2014). Phytoplankton and rotifers were supplied (2-9 dah) and cofeeding was carried out with *Artemia* metanauplii (Origreen, Skretting) and dry feed (Larviva, Biomar) since 10 dah. *S. putrefaciens* Pdp11 was daily incubated in TSA (1.5%NaCl) at 22ºC, collected and suspended in a PBS solution (pH 7.2) and finally supplied to live feed ($2.5*10^7$ cfu mL^{-1}) 3 hours prior to larval feeding. Two experimental groups were established: Control fish and Pdp11 fish fed with Pdp11 (2-21 dah). Growth performance and survival were determined at the end of the weaning period. For histological purposes 3 specimens per replicate were anesthetized (clove oil) and sacrified (66 dah). Digestive tract and liver sections were included in paraffin and stained with haematoxylin-eosin, after fixation in prediluted (100mL L^{-1}) buffered formalin. Microscopic sections were examined under a Leica DM400B microscopy equipped with a digital camera Leica DF350FX. Lipid vacuolization in digestive was determined in the mucosa and submucosa on the proximal, media and distal sections. Lipid vacuolization level was quantified according to 5 categories: 0) absence, 1) low, 2) middle, 3) abundant and 4) plenty of vacuolization. A stress challenge was performed by triplicate with 50 sole fry (66 dah) per group. Specimens were maintained in food deprivation conditions. RNA/DNA ratios were determined in 3 specimens per replicate as Caldarone et al. (2001). Mortality was controlled daily and the end of the trial was considered when all the replicates of at least one group reached 95% of mortality.

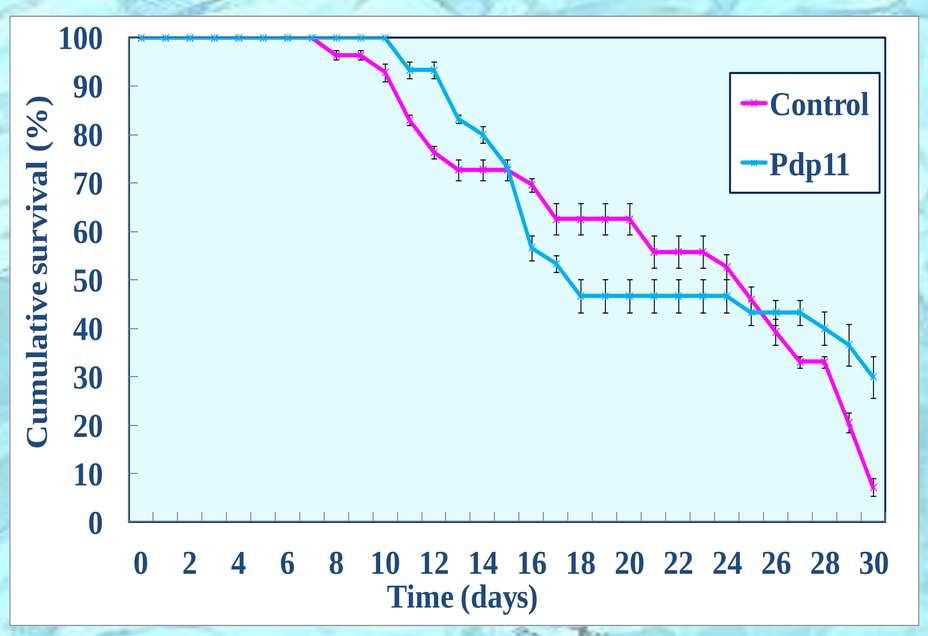

Figure 2. Control and Pdp11 sole fry (66 dah): a) Cumulative survival (%) after food deprivation test

Table II. Evolution of RNA/DNA ratios in S. senegalensis of the two experimental diets along the food deprivation test. (Mean SEM) Different letters denote significative differences (P<0.05)

Stress challenge day	Control		Pdp11	
0	2.10	0.04	2.04	0.06
5	1.20	0.08	1.16	0.02
9	1.16	0.03	1.15	0.03
14	1.07	0.06	1.09	0.03
19	0.96	0.02	0.97	0.02
23	0.83	0.04	0.96	0.02
28	0.65	0.04	0.63	0.03

Results and Discussion

S. putrefaciens Pdp11 administration at first stages (2-21 dah) promoted a better growth performance and improved stress resistance in just weaned *S. senegalensis* as was reported in common sole by Avella et al. (2011). Lipid vacuolization levels were not high (1.60-2.60) in any of the studied groups. Probiotic addition didn t affect enterocyte epithelia integrity. In this way the slightly higher lipid vacuolization detected in the digestive of Pdp11 group might indicate a greater lipid transport for probiotic fry. This better competence might be related to the enhanced lipid transport registered in their enterocytes. No significant differences were detected in RNA/DNA ratios between groups along the stress challenge. Further studies should be carried out to determine the *S. putrefaciens* Pdp11 mechanisms to enhance sole larviculture.

References:

Avella et al., 2011 Aquaculture 315(3-4): 384-393 Caldarone et al., 2001 http://www.nefsc.noaa.gov/nefsc/publications/ crd/crd0111/crd0111.pdf 63 García de la Banda et al., 2011 V Workshop “Cultivation of Soles”, Faro (Portugal). Lobo et al., 2014. Fish Physiology and Biochemistry 40(1): 295-309.

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