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PREVENTION OF VER IN SOLE USING PTS AND PTSO-SUPPLEMENTED DIETS

J. Gémez-Mata*, R. Leiva-Rebollo, E. García-Rosado, A.M. Labella, D. Castro

Department of Microbiology
Faculty of Sciences
University of Malaga
29071 Malaga, Spain

Introduction

Viral encephalopathy and retinopathy (VER), caused by nervous necrosis virus (NNV), is one of the main viral diseases affecting Senegalese sole (*Solea senegalensis*) in Southern Europe aquaculture. This virus (genus *Betanodavirus*, family *Nodaviridae*) is classified into four species: SJNNV, RGNNV, TPNNV and BFNNV (Sahul Hameed *et al.*, 2019). In addition, highly virulent reassortants (RGNNV/SJNNV) have been identified in VER outbreaks in sole (Oliveira *et al.*, 2009). Recently, NNV reassortant strain SsNNV19 was isolated from farmed Senegalese sole during an NNV outbreak in Southwestern Spain. Various prophylactic methods are being developed to reduce NNV infections in sole, including vaccines, supplemented functional feeds, and a combination of both treatments, among others. The *Allium* spp extracts propyl propane thiosulfinate (PTS) and propyl propane thiosulfonate (PTSO) are key compounds for the control of pathogenic microorganisms in livestock (Liu *et al.*, 2013), and their potential use in the aquaculture industry have been tested (Cabello-Gómez *et al.*, 2022). In this study, different sole feed supplemented with PTS or PTSO were developed, and its VER protective capacity was evaluated by measuring post-infection mortality and the study of host oxidative stress and inflammatory response after the feeding regime.

Materials and methods

Sole (0.5g) were divided into three groups and fed with different WinFlat (SPAROS) based feed formulations: Control (WinFlat), PTS (WinFlat + PTS), and PTSO (WinFlat + PTSO) at 6% of biomass for 30 days. At the end of the feeding period, six fish from each experimental group were sacrificed, and gene expression related to oxidative stress in the liver and inflammatory response and apoptosis in the intestine were analysed by qPCR. After feeding, the fish were experimentally infected with the SsNNV19 strain by intramuscular injection at 10^5 TCID₅₀/g of fish. Mortality was recorded for 30 days.

Results

In Senegalese sole specimens fed with PTSO-supplemented diet, downregulation of the *hsp90* (-3.32) and *nos* (-2.89) genes was observed, as well as upregulation of the *sod* (1.17) gene in the liver. In the case of the specimens fed with PTS-supplemented diet, only the differential expression of *sod* (1.035) gene was observed, being upregulated (Figure 1).

In the intestine, no significant differences were observed in the expression of genes related to the inflammatory process. However, in specimens fed with both supplemented diets (PTSO and PTS), upregulation of *casp9* (0.88 and 0.83) gene, related to the cell apoptosis process, was observed (Figure 2).

During the first days of the challenge, mortality registered in fish fed with the supplemented diets was lower than in the control group, but from 9 dpi onward, mortality increased similarly in all groups, ending with 100% mortality at 19 days post-challenge.

Conclusions

Dietary supplementation with PTS and, in particular, PTSO modulates the expression of genes related to oxidative stress in the liver, which could lead to an improvement in the health status of Senegalese sole specimens.

In intestinal samples, no induction of genes related to the inflammatory response was observed after 30 days of feeding with the supplemented diets. Nevertheless, the induction of the gene encoding caspase-9, associated with apoptosis, in both diets could indicate a certain degree of intestinal toxicity of these compounds.

Although these compounds do not confer direct protection, they may be used in conjunction with other strategies, such as vaccination, since the mitigation of oxidative stress, together with the reduction of early mortality, can potentiate the efficacy of vaccines.

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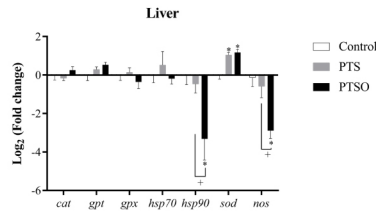


Fig1. Differentially expressed genes related to oxidative stress in liver samples of Senegalese sole fed with PTSO and PTS-supplemented diets. Values are mean \pm SD (n = 6). * denote significant differences between control and treatment group ($p < 0.05$). + denote significant differences between treatments ($p < 0.05$).

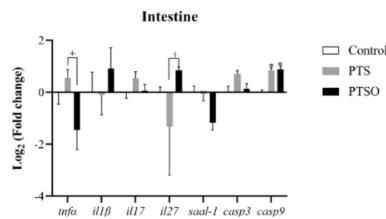


Fig2. Inflammatory response and apoptosis related gene transcription in the intestine of Senegalese sole fed with PTS and PTSO. Values are mean \pm SD (n = 6). * denote significant differences between control and treatment group ($p < 0.05$). + denote significant differences between treatments ($p < 0.05$).

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