

MODULATION OF THE INTESTINAL MICROBIOTA AND EXPRESSION OF GENES OF SENEGALESE SOLE (*SOLEA SENEGALENSIS*, KAUP 1858) BY DIETARY ADMINISTRATION OF OXYTETRACYCLINE

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ABSTRACT

There are few antimicrobials are authorized to be applied in aquaculture industry to treat infectious diseases. Oxytetracycline (OTC) is one of the first choice drugs for nearly all bacterial diseases in aquaculture. It has been demonstrated the effect of OTC on the intestinal microbiota of farmed fish, and it is important because it plays an important role for nutritional and physiological functions and is implied in the innate immune response. The objective of this study was to evaluate the effect that the dietary administration of OTC could exert on the intestinal microbiota and on expression in liver of Senegalese sole specimens of genes related with immunity. The results obtained in this study have demonstrated that the administration of OTC produced an increase of the expression in liver of genes related to apoptosis. Important modulations of the intestinal microbiota of *Solea senegalensis* specimens were exerted by OTC alone and correlation between these changes and those detected in the gene expression have demonstrated by Principal Component Analysis.

INTRODUCCIÓN

The oxytetracycline (OTC) is an antibiotic widely used in the aquaculture, principally in the treatment of infection. The problems derived by the use of antimicrobials regards to the increase of resistance and environmental impact are well known, but it is scarce the information about the effect on the intestinal microbiota of fish and its correlation with the physiology of the specimens. The objective of this study was to correlate changes produced on the intestinal microbiota of *Solea senegalensis* specimens treated with OTC and the expression in liver of genes related to apoptosis, oxidative stress and immunological response at 3 and 10 days of treatment.

MATERIALS AND METHODS

OTC
(40mg kg⁻¹)



INTESTINE

ANALYSIS OF INTESTINAL MICROBIOTA

DGGE and SEQUENCING

LIVER

Samples obtained at 3 and 10 days

ANALYSIS OF EXPRESSION OF GENE
Complement component 3 and 7 (C3 and C7, respectively)
heat shock protein (GP96),
NADPH oxidase (NADPHox)
c-lisozyme (c-LYZ)
caspase6
transferrin (TF)
hepcidin (HP)
ferritin (FerrM)

qPCR

ANALYSIS OF THE CONTRIBUTION OF VARIABLES

Sequences obtained by sequencing
Level of expression of analyzed gene

PCA

RESULTS

Related microorganism	N° accession Genbank	Similarity %	Control		OTC	
			3 d	10d	3 d	10 d
23. <i>Allivibrio fischeri</i> OT162	AB239367	100			5.31	8.56
22. <i>Pseudoalteromonas atlántica</i>	AB049728	98		8.06	5.31	9.8
36. <i>Vibrio alginolyticus</i> NRIL-SS41	AY357910	100	4.71		7.61	
19. <i>Pseudoalteromonas</i> sp OC-2	AY669164	99		4.72	5.31	
29. <i>Pseudomonas acephalatica</i> ind01	F1178096	98			5.31	15.5
1. <i>Vibrio harveyi</i> N8	DQ166244	99	5.46	6.69	7.07	14.22
3. <i>Vibrio ichthyocentri</i> SF11070701B	JQ904784	99	6.95	7.6	7.76	14.45
12. <i>Vibrio ichthyocentri</i> HQ010223-1	DQ003270	99	8.36			
24. <i>Vibrio ichthyocentri</i> strain H5	KC884596	99		5.24	5.31	
4. <i>Vibrio parahaemolyticus</i> M2-31	KC210810	98	9.43	10.09	4.81	14.10
13. <i>Vibrio parahaemolyticus</i> XG409	DQ948037	100	5.46	5.23		
18. <i>Vibrio splendidus</i>	AY620972	99		6.39	5.13	11.34
5. <i>Vibrio</i> sp B2-6	JX134442	99		7.2	8.06	
49. <i>Vibrio</i> sp B2-6	JX134442	99				
8. Uncultured <i>Vibrio</i> sp P-F2	HQ897566	97	6.26	4.72	4.81	5.89
14. Uncultured verrucomicrobia clone T7_11	FM206239	99	7.38	10.79		
7. Uncultured beta proteobacteria T7_182	FM206221	98	7.27			
11. <i>Mycoplasma microti</i> IL37	FJ609188	91	7.06			
28. Uncultured bacteroidete T7_119	AM407893	97		9.5		
30. <i>Lactobacillus fermentum</i> 3F9	AM117178	98			8.01	
6. Uncultured planctomyete	EU330396	99	5.7	7.6		
9. Bacterium NT4_C27	HM630215	100	6.26	8.06	6.08	5.89
10. Bacterium YO00334E05	FJ835362	100	6.26	4.55	4.66	

Table 1

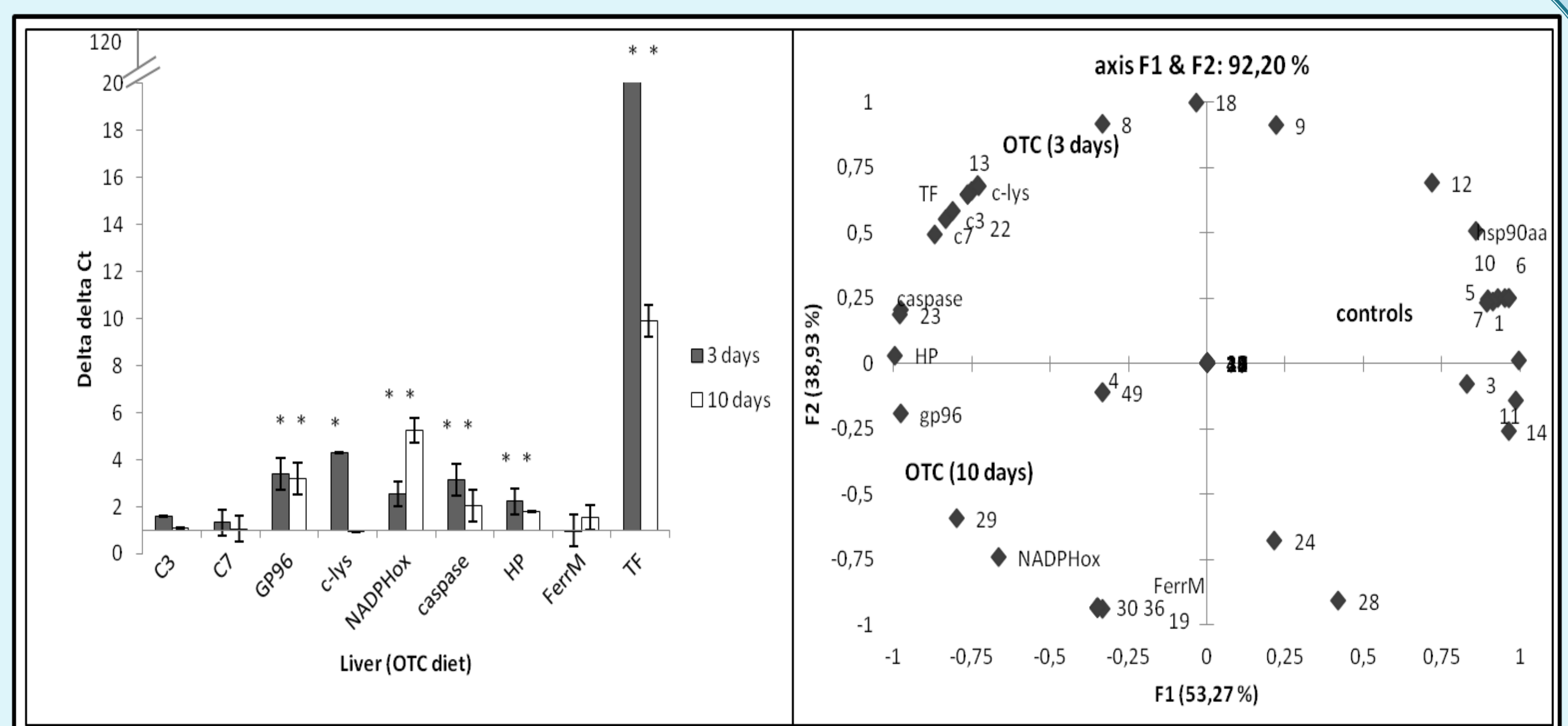


Figure 1. Relative expression levels of in liver of *Solea senegalensis* control specimens and specimens receiving OTC for 3 and 10 days.

Data are expressed as the mean fold difference (mean SEM, N=4) from the calibrator group (liver). Values with the superscript are significantly different (p<0.05).

Figure 2. Principal component analysis (PCA) applied to evaluate the relationship among the intestinal microbiota and liver gene expression of *Solea senegalensis* control specimens and specimens receiving OTC for 3 and 10 days

Table 1: Principal microorganisms detected in the intestinal microbiota of treated and control fish and its abundance.

DISCUSSION

The study of patterns of intestinal microbiota by DGGE showed important differences between control and OTC diet. This result is in concordance with other authors, who observed that the comparison of DGGE patterns between control fish and treated with OTC in the profile of the intestinal microbiota.

Gene related with the immune system such as c-LYZ or gene related with oxidative stress (GP96, NADPHox and caspase 6) were up-expressed at 3 and 10 days. These results showed that OTC could stimulate process implied cell death. Also, iron metabolism was affected and an up-regulation of the gene encoding for hepcidin and transferrin.

PCA was realized to study the relation between intestinal microbiota and gene expression. This method showed a clear separation along the F1 component axis of individuals that received a control and OTC diet and a strongly correlation between certain gene and some species of the intestinal microbiota. Gene such as caspase, GP96 and NADPHox were correlated with species Planctomyetes and Verrucomicrobia. First group is associated with aquaculture water systems and fish farm sediments while Verrucomicrobia is indicative of intestinal dysbiosis (Green et al., 2013).

Also, gene related with the iron metabolism expressed in liver are related with some species of intestinal microbiota. Previous studies show that when there is a reduction of absorption of iron in the intestine perhaps by certain microbial species, the organisms increase the caption of iron in liver (Dunn et al., 2006).

CONCLUSION

These results showed important changes in the species of intestinal microbiota and establishment a relationship between these species and the genetic expression in the host.

References

Dunn LL, Rahmanto YS, Richardson DR (2006) Iron uptake and metabolism in the new millennium. Trends Cell Biol; 17:93–100.
Green TJ, Smullen R, Barnes AC (2013) Dietary soybean protein concentrate-induced intestinal disorder in marine farmed Atlantic salmon, *Salmo salar* is associated with alteration in gut microbiota. Veterinary Microbiology, 166: 286–292.