Effect of Next Enhance® 150 and Previda® on growth performance and the intestinal transcriptome of gilthead sea bream (*Sparus aurata*)

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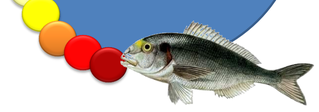
INTRODUCTION

Carvacrol and thymol are **essential oils** from oregano and thyme extracts that can improve fish performance, enhance populations of beneficial gut bacteria, inhibit the growth of some pathogens and improve gut barrier structure and function. In this study we tested the effect of Next Enhance® (NE) 150, a patented combination of carvacrol and thymol, as a feed additive for gilthead sea bream. In a first trial, the best dose of NE 150 was established after measuring growth performance, plasma biochemistry, total antioxidant capacity, respiratory burst (RB) and haematology. In a second trial, the effect of **NE 150-100 ppm alone or combined with a prebiotic (Previda®)** was further studied analysing **the transcriptomic profile** of the **anterior and posterior intestinal segments**.

NE® 150
Several doses
9 weeks



Initial BW: 27 g

0 ppm

50 ppm

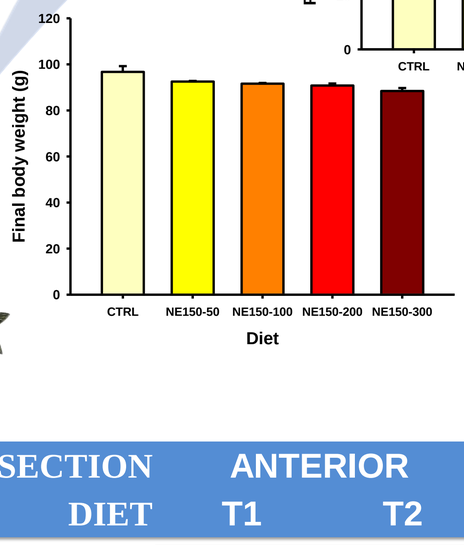
100 ppm

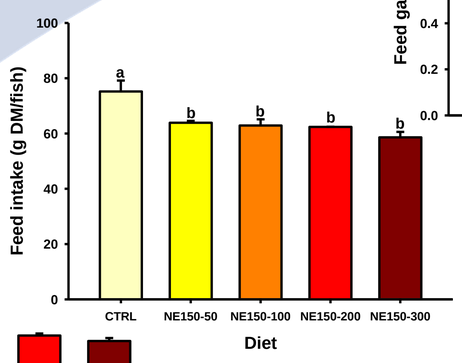
200 ppm

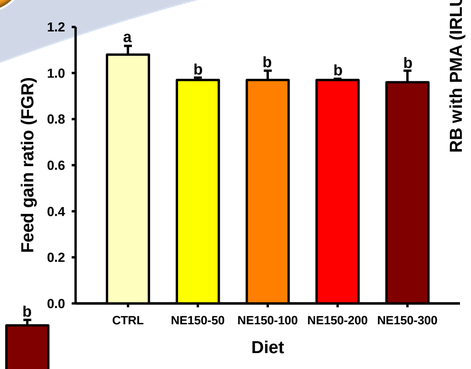
300 ppm

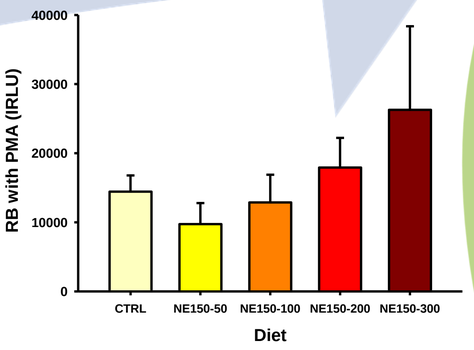
- **FGR: improved**
- **Feed intake: decreased**
- **RB: increased**

Threshold dose: 100 ppm









NE® 150 at 100 ppm
11 weeks

Alone (T1)

Combined with 0.5% Previda® (T2)

Initial BW: 24 g

FGR: improved
No changes in:
- SGR
- Haematology
- Plasma antioxidant capacity

TRANSCRIPTOMIC ANALYSIS: 90-gene PCR-array
Based on our transcriptomic database:
www.nutrigroup-iats.org/seabreamdb

INT. SECTION			ANTERIOR		POSTERIOR	
		DIET	T1	T2	T1	T2
CELL SIGNALING & INTESTINAL ARCHITECTURE	Cell diferentiation & proliferation	BMPR1a	1.08	0.96	0.85	0.75**
		IHH	0.91	0.97	0.58	0.37**
		SHHA	0.89	0.92	0.68	0.76*
		GLI1	0.90	0.81	0.73**	0.82
		WLs	0.80	0.93	0.65**	0.86
		Myc	0.92	0.92	0.64*	0.78
		CTNNB1	0.84	0.78**	0.82	0.87
		Tcf4	0.77**	0.79	0.57**	0.72**
		GFI-1	1.44**	1.22	0.89	0.97
	Cell to cell communication	ITGB1BP1	0.85	0.74*	0.90	0.98
		CLDN12	0.91	0.80	0.76*	0.75**
		CLDN15	0.90	0.98	0.78	0.73*
		TJP1	0.84	0.85	0.68*	0.69**
		DSP	0.82**	0.76**	0.92	0.92
		GJB4	0.87	0.86	0.65**	0.71**

INT. SECTION			ANTERIOR		POSTERIOR	
		DIET	T1	T2	T1	T2
IMMUNOSURVEILLANCE	Interleukins & Cytokines	IL-1β	0.47*	0.64	0.64	0.68
		IL-6	0.23**	0.39*	0.12**	0.25**
		IL-8	0.74	0.38**	0.95	0.67
		IL-10RA	0.66	0.63*	0.84	1.22
		TNFα	0.92	0.81	0.76	0.64**
		CD276	0.91	0.93	0.76	0.78**
	Pathogen Recognition Receptors	TLR1	0.69	0.71	0.72*	0.95
		TLR2	0.90	0.92	0.78*	0.94
		TLR5	0.53	0.67	0.55**	0.60**
		TLR9	1.14	0.80**	0.57**	0.91
		CLEC10A	0.68*	0.79	0.53**	0.60**
		LGALS1	0.94	1.14	0.69**	0.93
		CSL2	0.5	0.24*	0.49	0.5
		FCL	18.02**	6.34	3.26	1.83
		VIM	0.89	0.99	0.65**	0.62**
ENTEROCYTE MASS, CELL DAMAGE & ACTIVITY	Enterocyte Markers	ALPI	1.09	0.76**	0.91	0.72
		FABP2	1.36	3.33**	1.10	0.71
		FABP6	0.3	0.09*	1.52	2.45*
	ER Chaperone	CANX	1.02	0.74	0.85	0.77**
	Mitochondrial Activity	mtHsp10	1.37*	1.12	1.13	1.28
		ECH	1.98**	1.55**	1.21	1.28
		HADH	1.51*	1.16	1.30*	1.01
		Tim44	0.96	0.71**	0.73	0.68*

For each intestinal segment, gene and experimental diet, the fold change is referred to the control diet. **Red tones** correspond to **up-regulation** and **green tones** correspond to **down-regulation**. The intensity of the colour stands for the degree of the change. ** stands for statistically significant changes at $P < 0.05$ and * at $P < 0.1$ with Student-t test.

CONCLUSIONS

At 50 ppm fish grew well with improved FGR and slightly reduced RB. The threshold dose of NE150 was established at 100 ppm, as higher doses may induce low growth performance profiles due to the lower feed intake and a very high inflammatory status (high RB). When using the 100 ppm dose alone (T1) or combined with previda (T2), the decrease in feed intake was confirmed and FGR was equally improved. No significant changes in haematology and plasma antioxidant capacity were detected. The intensity of the changes and the number of genes that were significantly regulated were higher in PI than in AI. At PI, both treatments invoked a clear down-regulation of genes involved in cell differentiation and proliferation, some involved in cell to cell communication, interleukin IL-6, and several PRR. By contrast, up-regulation was mostly found for genes related to enterocyte mass, cell epithelial damage and mitochondrial activity at AI. The changes were of the same order for T1 and T2, except for fatty acid-binding proteins 2 and 6 (FABP2, FABP6) and the PRR fucolectin. Thus, the combination of NE150 and Previda induces an anti-inflammatory status and changes in the absorptive capacity of the intestine.

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