EXPRESSION OF GENES INVOLVED IN FAT-SOLUBLE VITAMINS METABOLISM ARE ADAPTED TO DIETARY CONTENT IN RAINBOW TROUT *Oncorhynchus mykiss* DIETS.

Ignacio Fernández[1*], Fátima de Vicente[1], Francisco J. Toledo-Solís[2,3], Silvia Beato[1], Francisco J. Alarcón[3], Juan B. Ortiz[4], Carmen Sarasquete[4], Pedro López de la Cuesta[5], Ana M. Larrán[1]

[1]Agro-Technological Institute of Castilla y León (ITACyL), Segovia, Spain; [2]Consejo Nacional de Ciencia y Tecnología, Mexico City, Mexico; [3]University of Almeria, Almería, Spain; [4]ICMAN/CSIC, Cádiz, Spain; [5]ITACyL, Valladolid, Spain; *Email: fermonig@itacyl.es

INTRODUCTION

Fat-soluble vitamins (FSVs) play essential roles in vertebrate's development and homeostasis. Although their minimum nutritional requirements have been fixed (at least in salmonids; NRC 2011) through unifactorial nutritional-dose-response trials (Fernández et al., 2018), little is known on their metabolism. Furthermore, the different FSVs have genes involved on the processes of assimilation and transport from intestine to liver and target tissues in common (Fernández et al., 2018). How these genes are modulated in response to dietary FSVs content might help to further develop fully nutritionally balanced diets.

OBJECTIVE

The fish growth performance, the histopathological status of the digestive system, the content of FSVs in fish liver and the expression of genes involved in the metabolism of FSVs were explored in rainbow trout (*O. mykiss*) juveniles fed with diets containing different levels of FSVs.

MATERIALS AND METHODS

Seven experimental diets (isonitrogenous, isolipidic and isoenergetic) were specifically formulated to contain: the reference content on vitamin A (VA), D (VD), E (VE) and K (VK) (NRC 2011) and/or 10 or 0.2 times the reference content of vitamins A, E and K. Twenty-one fish (94.04 ± 0.81 g and 20.28 ± 0.09 cm) per 500 L tank were randomly allocated. Experimental diets were tested in triplicate. Fish were daily hand-fed (3% of daily feed intake) during 90 days (Figure 1) and liver and intestine samples were taken.

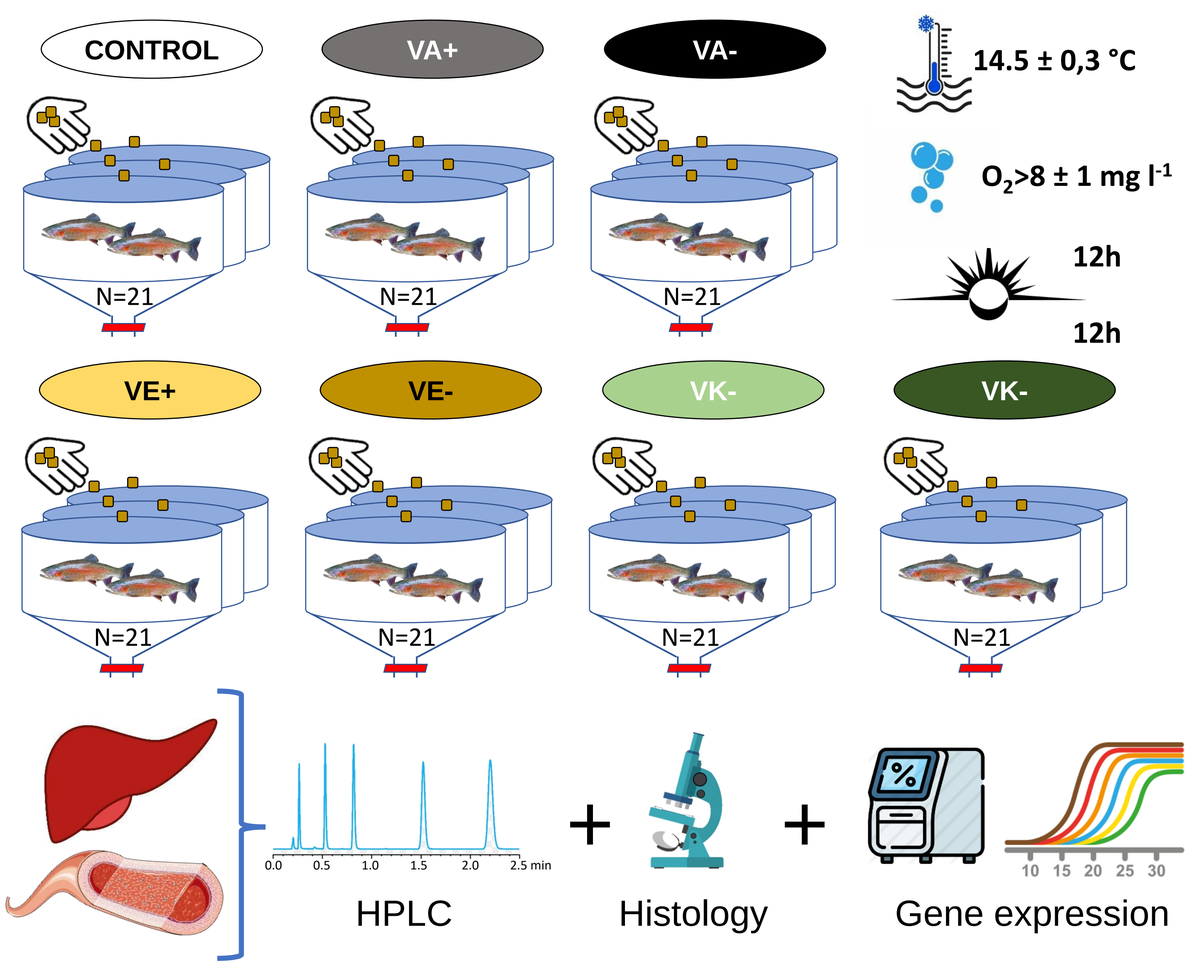

Figure 1. Experimental design and analysis performed. Control, diet containing the reference content (RC) of each vitamin as in NCR (2011); VA+, diet containing the RC of each vitamin but 10 times the one of VA (as retinol acetate); VA-, diet containing the RC of each vitamin but 0.2 times the one of VA (as retinol acetate); VE+, diet containing the RC of each vitamin but 10 times the one of VE (as α-tocopherol); VE-, diet containing the RC of each vitamin but 0.2 times the one of VE (as α-tocopherol); VK+, diet containing the RC of each vitamin but 10 times the one of VK (as menadione sodium bisulfite (MSB)); and VK-, diet containing the RC of each vitamin but 0.2 times the one of VE (as MSB). At the end of the trial, liver and intestine were sampled for HPLC, histology and gene expression analyses.

RESULTS AND DISCUSSION

Only slight differences in particular growth performance indexes were found after 90 days of feeding (Table 1). Reduced VE content lead lower specific growth rate (SGR) and hepatosomatic index (HIS). Lower dietary VK content decreased SGR, HSI and viscerosomatic index, while increased VK content decreased condition factor, SGR and HSI.

Table 1. Rainbow trout growth performance fed 90 days with experimental diets.

	Control	VA+	VA-	VE+	VE-	VK+	VK-
BW	617.5 ± 13.4	596.5 ± 7.2	599.3 ± 5.3	619.3 ± 37.0	599.7 ± 40.8	621.7 ± 23.3	619.9 ± 27.4
FL	34.5 ± 0.2	34.2 ± 0.3	34.1 ± 0.1	34.5 ± 0.5	34.2 ± 0.5	35.0 ± 0.4	34.8 ± 0.2
CF	1.50 ± 0.02^{a}	1.48 ± 0.03^{a}	1.50 ± 0.02^{a}	1.50 ± 0.02^{a}	1.48 ± 0.03^{a}	1.44 ± 0.00^{b}	1.46 ± 0.03^{ab}
SGR	2.11 ± 0.03^{a}	2.07 ± 0.02^{a}	2.08 ± 0.00^{a}	2.02 ± 0.05^{a,b}	1.96 ± 0.07^{b}	1.99 ± 0.04^{b}	1.97 ± 0.05^{b}
FCR	0.95 ± 0.02	0.95 ± 0.02	0.93 ± 0.01	0.94 ± 0.02	1.00 ± 0.06	0.98 ± 0.03	1.00 ± 0.05
HSI	1.27 ± 0.07^{a}	1.23 ± 0.08^{a}	1.13 ± 0.03^{a}	1.28 ± 0.04^{a}	1.03 ± 0.11^{b}	1.06 ± 0.01^{b}	1.06 ± 0.03^{b}
VSI	15.33 ± 2.05^{a}	13.52 ± 0.51^{a}	13.43 ± 1.85^{a}	12.48 ± 1.50^{a}	13.18 ± 1.26^{a}	12.62 ± 0.89^{ab}	11.51 ± 0.52^{b}

Different superscript letters within each row denote significant differences among experimental groups (ANOVA, $p < 0.05$; n = 3). BW, body weight (g); FL, furcal length (cm); CF, condition factor; SGR, specific growth rate (% d^{-1}); FCR, feed conversion ratio; HSI, hepatosomatic index (%); VSI, viscerosomatic index (%).

The content of FSVs in fish liver reflected that of experimental diets: the higher dietary content the higher abundance in the liver (Table 2).

Table 2. Levels of vitamins in livers from rainbow trout fed experimental diets.

	Control	VA+	VA-	VE+	VE-	VK+	VK-
VA	8.07 ± 0.51^{ab}	11.11 ± 1.64^{a}	4.45 ± 2.20^{b}	21.43 ± 18.18	6.37 ± 7.03	9.41 ± 1.76	9.00 ± 2.10
VE	759,65 ± 44.10^{b}	1146.39 ± 704.42	1169.98 ± 1.13	7075.48 ± 1894.43^{a}	334,46 ± 108.56^{c}	439.47 ± 135.11	510.36 ± 217.67
VK	3.68 ± 1.19^{b}	6.682 ± 4.70	5.53 ± 1.13	5.12 ± 3.04	8.92 ± 3.09	9.40 ± 3.24^{a}	4.88 ± 0.58^{b}

Vitamin levels are reported in mg kg^{-1}. VA, vitamin A (Retinol); VE, vitamin E (α-tocopherol); VK, vitamin K (phylloquinone). For statistical analysis, only high and low dietary levels of each vitamin were compared with Control diet. Different superscript letters within each row denote significant differences among experimental groups (ANOVA, $p < 0.05$; n = 3).

While VA+, VE+ and VK+ diets increased goblet cells density compared to VA-, VE- and VK- diets, respectively (Figure 2); VK+ diet also increased the thickness of muscularia and serosa layers compared when compared to VK- diet (not shown).

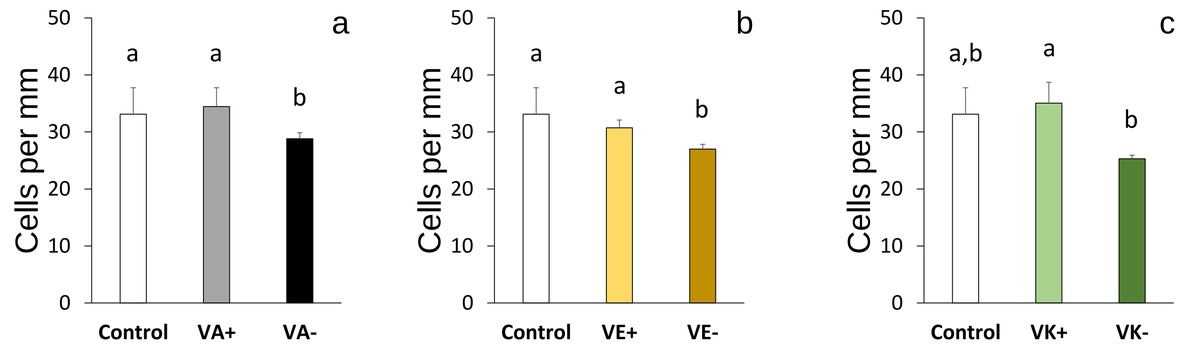

Figure 2. Density of goblet cells at proximal intestine in fish fed Control and VA (a), VE (b) and VK (c) diets. Different superscript letters at the top of each bar denote significant differences among experimental groups (ANOVA, $p < 0.05$; n = 3).

The expression of some genes involved in the uptake of FSVs has been evaluated (Figure 3). *Niemann-Pick C1-like protein 1* (NPC1L1) has been upregulated by the increase of VA dietary content but downregulated in fish fed high VK dietary levels. Expression of *scavenger receptor class B member 1* (SRB1) was not altered by dietary VA or VE content, but reduced in fish fed diet containing high levels of VK. Finally, the expression of *cluster determinant 36* (CD36) was only upregulated by high dietary VA content.

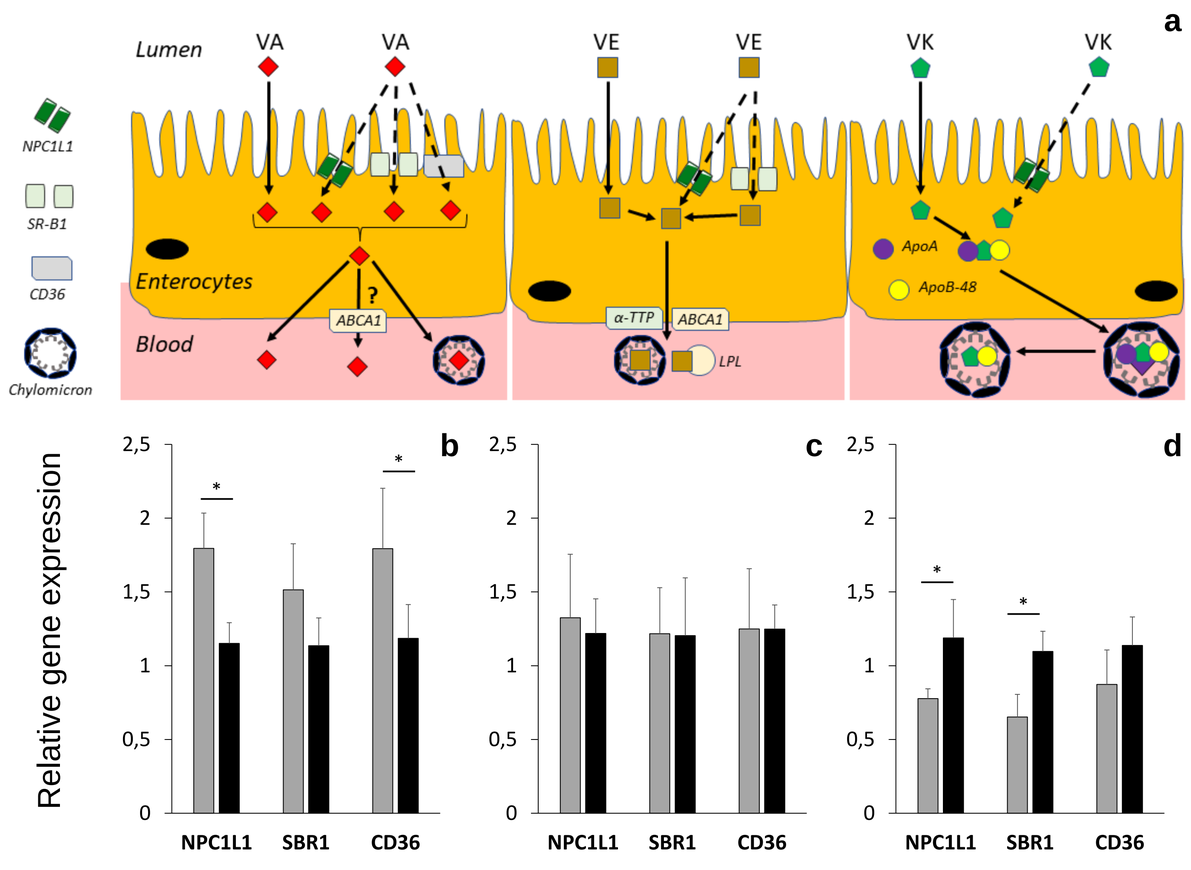

Figure 3. Genes involved in the uptake of FSVs and its expression. Schematic representation of FSVs uptake from intestinal lumen (a): solid line, passive transport; dash line, hypothetical active transport. Gene expression of NPC1L1, SBR1 and CD36 at the intestine of fish fed diets containing high (grey bars) or low (black bars) levels of VA (b), VE (c) and VK (d). Asterisk denotes significant differences among experimental groups (Student T-test, $p < 0.05$; n = 3).

CONCLUSIONS:

- ✓ In ongrowing specimens of rainbow trout, the FSV dietary content affect fish growth performance.
- ✓ Reduced dietary VA, VE and VK levels reduced the density of goblet cells
- ✓ Genes involved in intestinal uptake of FSVs were differentially expressed depending the FSV considered.
- ✓ NPC1L1, SBR1 and CD36 genes seems to be relevant for defining fully nutritionally balanced diets in FSVs.

More info at:

REFERENCES

Fernández et al., 2018. In "*Emerging Issues in Fish Larvae Research*" 159–208.

ACKNOWLEDGEMENTS

Research work funded by MET2VI (RTI2018-099029-A-I00) project from ERDF-MICIU-AEI. FJTS acknowledges CONACYT postdoc fellowship (2019–000012-01EXTV-00292) from México. IF acknowledges Ramón y Cajal (RYC2018-025337-I) contract from MICIU and European Social Funds "from Spain.

European Union
European Regional Development Fund