FISH MEAL SUBSTITUTION BY *Tenebrio molitor* MEAL IN RAINBOW TROUT *Oncorhynchus mykiss* DIETS AFFECTS FAT-SOLUBLE VITAMINS METABOLISM: IMPLICATIONS FOR FUTURE FORMULATIONS

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INTRODUCTION Aquaculture sustainability largely depends on the implementation of alternative raw materials in aquafeeds to replace fish oil and fish meal (FM). Among the different alternatives considered, insects have recently attracted the attention as suitable alternatives (Nogales-Mérida et al., 2018). Different species of insects have been evaluated, but we recently found mealworm (*Tenebrio molitor*; TM) as the most promising to replace fish meal up to 50% in rainbow trout (*Oncorhynchus mykiss*) diets (Melenchón et al., 2021). High levels of FM substitution by insects' meal might impact fish physiology, but the nutritional causes are not fully understood.

OBJECTIVE Here, we explored the effects on fish growth, histopathological status of the digestive system, the content of fat-soluble vitamins (FSVs) and the related gene expression when 50% of FM is replaced by TM meal (either defatted or not) in rainbow trout diets.

MATERIALS AND METHODS Three experimental diets (isonitrogenous and isoenergetic; Table 1) were specifically formulated with FM (Control), 50% replacement of FM by defatted TM meal (TD50) or non-defatted TM meal (T50), all of them including an equal amount of mineral and vitamin premix. Diets were tested in triplicates. Twenty-one fish (46.2 ± 0.21 g and 15.9 ± 0.12 cm) per 500 L tank were randomly allocated in 9 tanks connected to a recirculating aquaculture system (RAS). Fish were daily hand-fed to apparent satiation (up to 3% of daily feed intake) during 68 days (Fig. 1).

Table 1. Ingredients and proximate composition of experimental diets.

	Control	TD50	T50
Fish meal LT	35.90	18.00	18.00
Defatted TM meal	0.00	18.00	0.00
TM meal	0.00	0.00	18.00
Wheat gluten	10.50	10.10	11.90
Soy concentrate	15.50	15.80	17.00
Fish oil	12.20	12.20	9.00
Soy lecithin	1.30	1.30	0.50
Wheat meal	16.60	16.60	17.0
Premix	2.00	2.00	2.00
Binder (guar gum)	2.00	2.00	2.00
Hemoglobin	4.00	4.00	4.00
Proximate composition (% on dry matter)			
Crude protein	44.85	44.38	44.55
Crude fat	17.69	17.20	17.25
Carbohydrates	20.81	21.75	22.41
Ash	5.13	3.65	3.64
Energy (MJ kg^{-1})	16.8	16.9	17.0

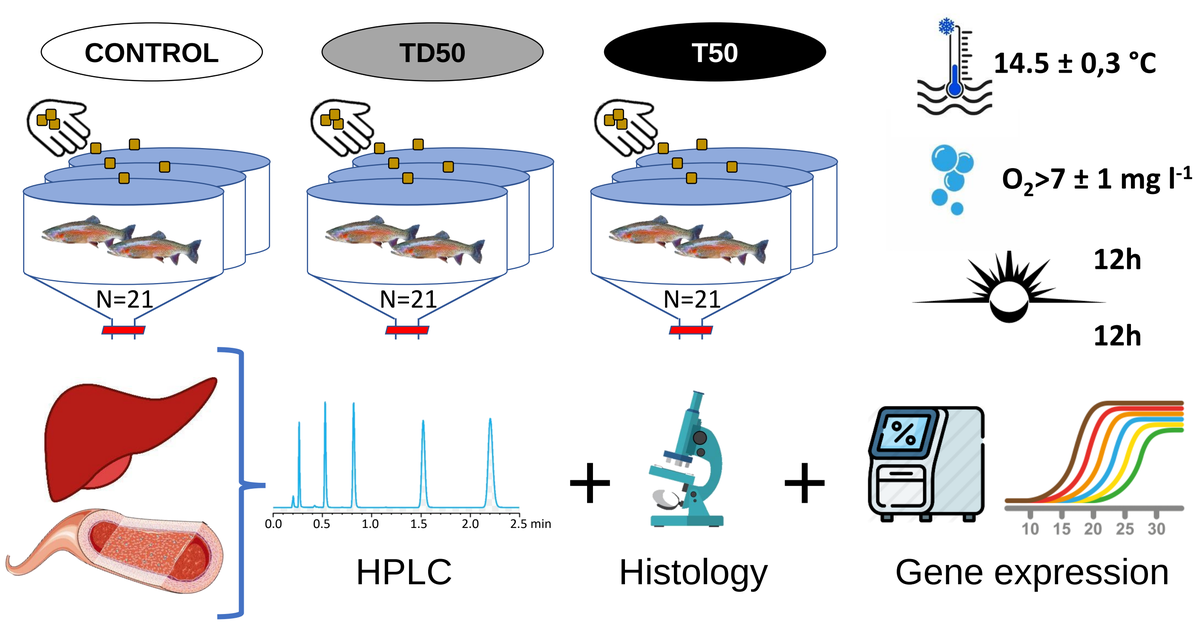

Figure 1. Experimental design and analysis performed. Control, diet with FM as main protein source; TD50, diet where the 50% of FM was replaced by defatted *Tenebrio molitor* (TM) meal; and T50, diet where the 50% of FM was replaced by non-defatted TM meal in rainbow trout diets. At the end of the trial, liver and intestine were sampled for HPLC, histology and gene expression analyses.

RESULTS AND DISCUSSION While 50% of FM was replaced by defatted TM meal did not affect fish growth performance, non-defatted TM meal reduced body weight and weight gain, and increased feed conversion rate when compared to fish fed Control diet (Table 2).

Table 2. Growth performance of rainbow trout fed experimental diets during 68 days.

	Control	TD50	T50
BW	261.57 ± 1.69^{a}	259.18 ± 3.96^{ab}	251.27 ± 5.15^{b}
FL	26.38 ± 0.21	26.49 ± 0.02	26.03 ± 0.27
CF	1.42 ± 0.03	1.39 ± 0.02	1.42 ± 0.02
WG	466.65 ± 3.78^{a}	462.32 ± 10.21^{ab}	442.77 ± 9.85^{b}
SGR	2.55 ± 0.01	2.54 ± 0.03	2.49 ± 0.03
FCR	0.79 ± 0.01^{a}	0.79 ± 0.00^{a}	0.82 ± 0.00^{b}
HSI	0.99 ± 0.03	1.06 ± 0.04	1.01 ± 0.04
VSI	9.96 ± 0.47	10.17 ± 0.48	9.78 ± 0.36

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; WG, weight gain (%); SGR, specific growth rate (% d^{-1}); FCR, feed conversion ratio; HSI, hepatosomatic index (%); VSI, viscerosomatic index (%).

No major histopathological alterations were found in the digestive system. Only fish fed T50 diet showed a slight increase on the width of the serosa layer when compared with fish fed Control diet (Figure 2).

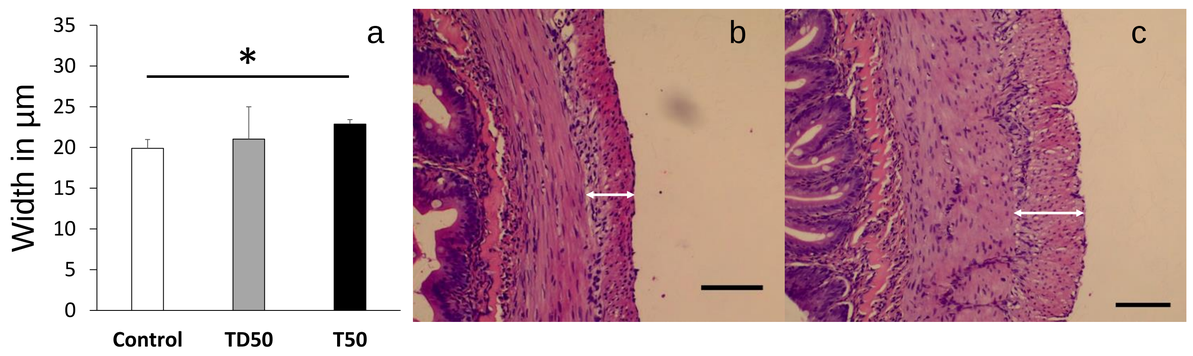

Figure 2. Width of serosa layer in fish fed experimental diets (a) and micrographs showing serosa layer from Control (b) and T50 (c) fish. Asterisk denotes significant differences among experimental groups (T-test, $p < 0.05$; n = 3). Scale bar = 50 µm

Regarding FSVs content in fish livers, vitamin A (VA) was only significantly reduced in fish fed TD50 diet when compared with Control group. In contrast, independently of being defatted or not, a 50% FM substitution by TM meal led to reduced vitamin K (VK) levels in fish liver. No differences in vitamin E (VE) were reported.

Table 3. Levels of vitamins in livers from rainbow trout fed experimental diets.

	Control	TD50	T50
VE	559.90 ± 134.81	459.90 ± 20.42	439.32 ± 65.45
VK	6.96 ± 1.33^{a}	2.68 ± 2.42^{b}	2.71 ± 0.77^{b}
VA	20.48 ± 2.33^{a}	13.11 ± 1.27^{b}	18.91 ± 7.98^{ab}

Vitamin levels are reported in mg kg^{-1}. VE, vitamin E (α-tocopherol); VK, vitamin K (phylloquinone); VA, vitamin A (Retinol). Different superscript letters within each row 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). Expression of *Niemann-Pick C1-like protein 1* (NPC1L1), *scavenger receptor class B member 1* (SRB1) and *cluster determinant 36* (CD36) genes was up-regulated in fish fed TD50 diet. In fish fed T50 diet, CD36 expression was down-regulated compared with that of fish fed Control diet (ANOVA, $p < 0.05$).

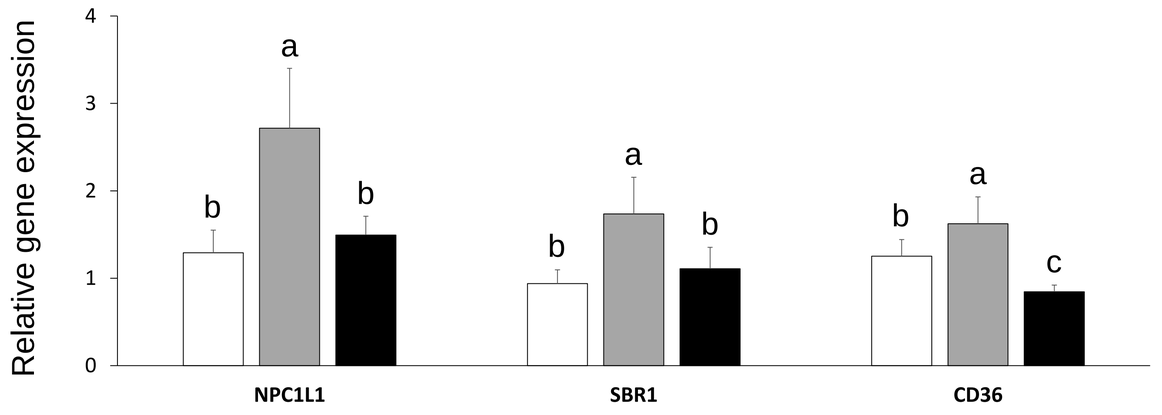

Figure 3. Expression of genes involved in the uptake of FSVs at the proximal intestine. Gene expression of NPC1L1, SBR1 and CD36 at the intestine of fish fed Control (white bars), TD50 (grey bars) and T50 (black bars) diets. Different superscript letters denote significant differences among experimental groups (ANOVA, $p < 0.05$; n = 3).

CONCLUSIONS:

- ✓ 50% replacement of fish meal with non-defatted meal (T50) from *Tenebrio molitor* reduced fish growth and feed conversion rate
- ✓ Fish fed diets containing *Tenebrio molitor* meals showed reduced vitamin A and K content in liver
- ✓ 50% fish meal replacement with defatted *Tenebrio molitor* meal can be achieved without compromising fish growth performance and histopatological status
- ✓ Fat-soluble vitamins supplementation might be recommended to fulfill their nutritional requirements during the outgrowing phase, and to cope with the reduced uptake at intestinal lumen.

More info at:

REFERENCES

Melenchón et al., 2021. *Aqua. Nut.* 27, 491–505.
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