A MULTIPARAMETRIC TOOL FOR SCREENING AND IMPROVING THE USE OF ALTERNATIVE RAW MATERIALS IN RAINBOW TROUT (*Oncorhynchus mykiss*) DIETS

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INTRODUCTION

Aquaculture sustainability has been deeply questioned. It requests increasing amounts of fish oil and meal (FO and FM, respectively) as main raw materials for aquafeeds. During the last decades, a large effort has been focused to identify alternative raw materials (e.g. soybean, insect, algae and single cell's meal among others) to substitute FO and FM (Turchini *et al.,* 2018). Soybean (*Glycine max*) meal (SBM) is still one of the main alternatives currently used in commercial fish diets. However, for European aquaculture it has to be imported from third countries (e.g. USA and Brazil). Thus, the identification of locally produced crops able to partially or totally substitute SBM is urgently needed to reduce both the European SBM dependency from third countries, as well as the aquafeed's carbon footprint.

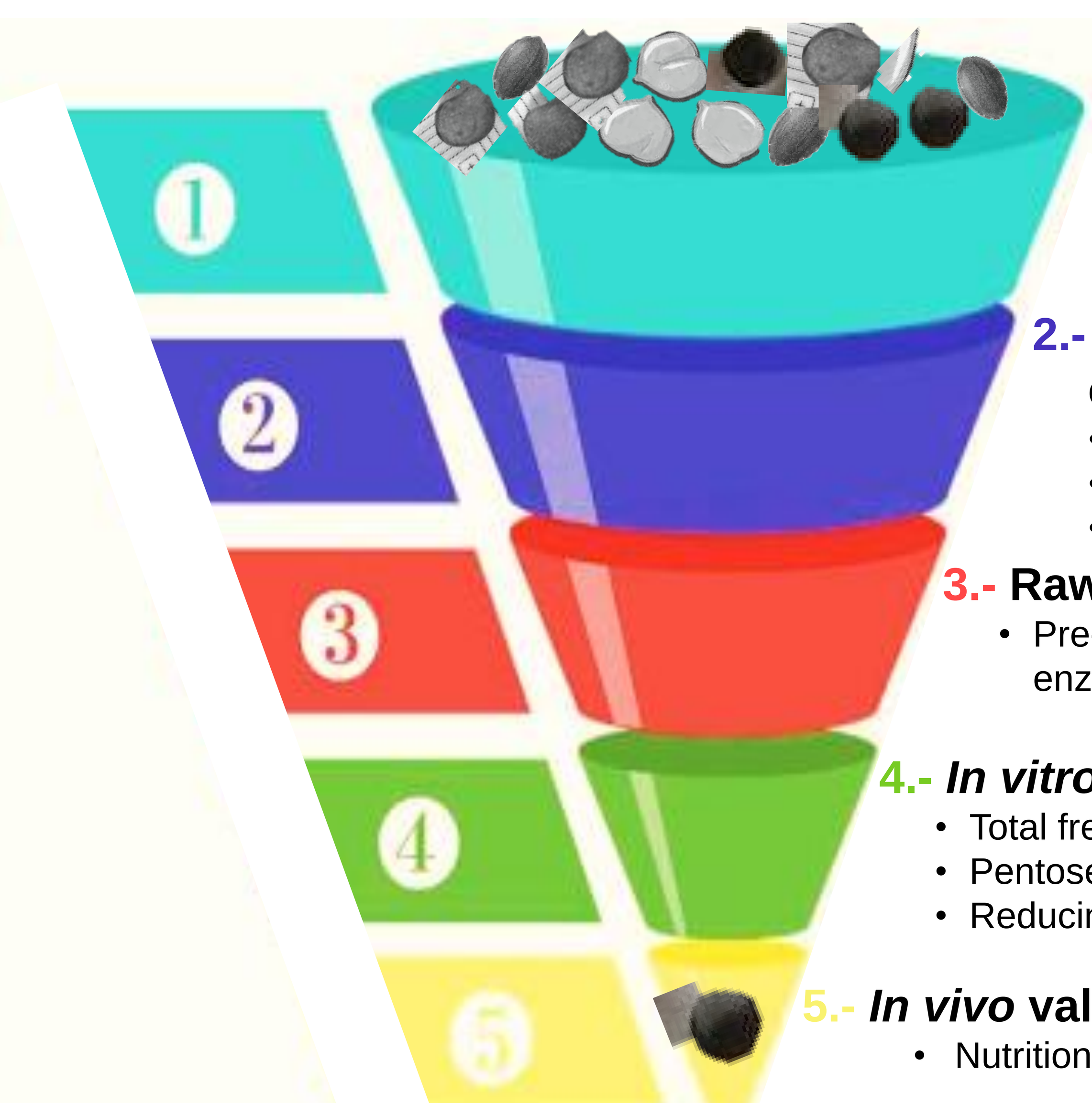
OBJECTIVE

A fifth-step screening protocol, including *in vitro* and *in vivo* assays, is proposed to evaluate different alternative vegetable protein sources locally produced in Europe for rainbow trout (*Oncorhynchus mykiss*) diets.

MATERIALS AND METHODS:

In a first step, a complementary characterization of each raw material was done, including the assessment of buffer capacity, inhibition of digestive enzymes, soluble protein content, etc. Also, the presence of some nutritionally limiting factors (e.g. non-starch polysaccharides and phytate), their stability/activity, as well as their *in vitro* digestibility after treatment with an exogenous enzyme (Rovabio® Phy) was evaluated (Fig. 1).

STAGE: **EVALUATION PROCEDURES:**



1.- Raw material selection
- Nutritional
- Economical
- Environmental factors

2.- Nutritional value characterization
- TBC, IAP activity
- SP, Phytate
- TSP, Poly

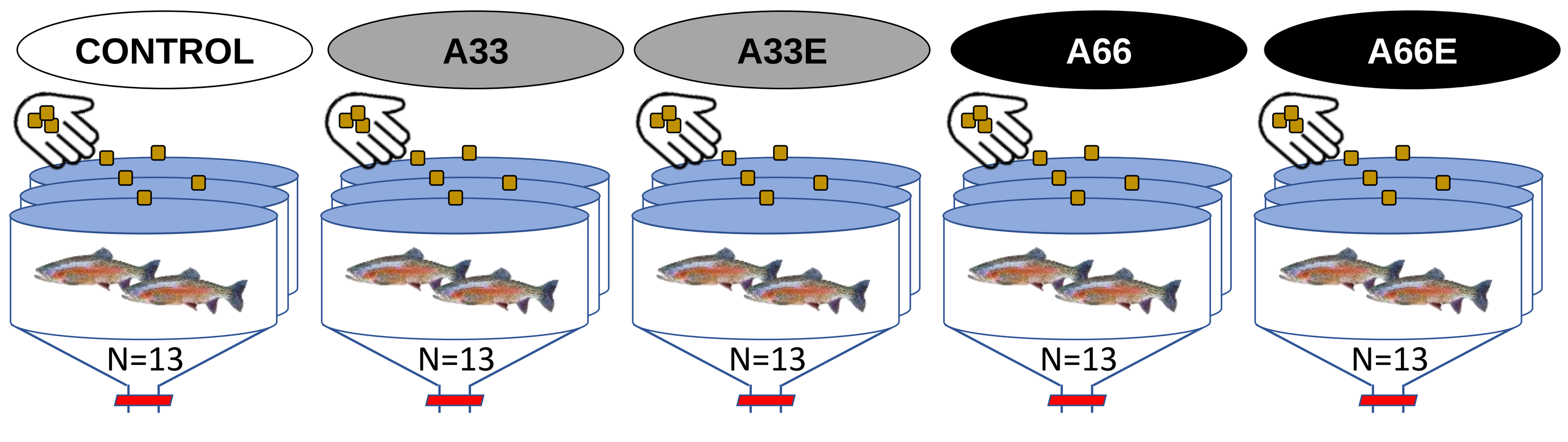
3.- Raw material improvement
- Pre-treatment with exogenous enzyme

4.- *In vitro* digestion evaluation
- Total free amino acids
- Pentoses
- Reducing sugars

5.- *In vivo* validation
- Nutritional *in vivo* trial

Figure 1. *Schematic representation of the steps followed for the characterization, selection and validation of potentially interesting alternative raw materials for soybean meal replacement. TBC, Total buffer capacity; IAP, inhibition of alkaline protease; SP, Soluble protein; TSP, Total soluble phosphorus; Poly, Phenolic compounds.*

In a second step, the most promising raw material to partially replace SBM in rainbow trout diets was evaluated *in vivo.* Five experimental diets (iso-nitrogenous 43%, -lipidic 18%) were formulated: one diet with FM and SBM as main protein sources (Control), and 4 diets where SBM was partially (33% or 66%) replaced by Narbonne vetch (*Vicia narbonensis*) meal (NVM) (the selected raw material) previously treated with exogenous enzyme Rovabio® phytase (A33E and A66E) or not (A33 and A66). Thirteen fish (38.04 ± 0.07 g and 15.10 ± 0.07 cm) per 500 L tank were randomly allocated. Diets were tested in triplicates during 63 days. Fish were daily hand-fed (3% of daily feed intake) (Fig. 2).



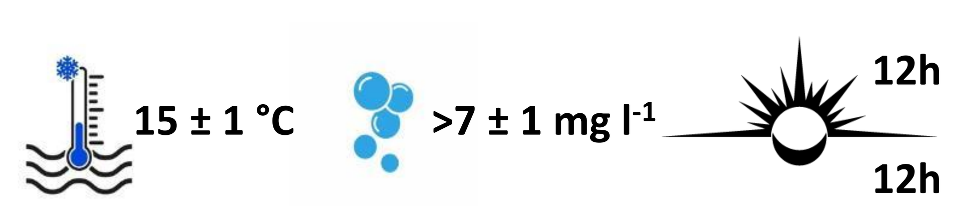


Figure 2. *Experimental design to validate the replacement of SBM by NVM treated and not treated with an exogenous enzyme (Rovabio® Phy) in rainbow trout diets.*

RESULTS AND DISCUSSION

1 Nine different meals obtained from cultivars of Narbonne vetch, red vetchling *(Lathyrus cicera)* and green pea *(Pisum sativum)* meals (NVM, RVM and PM, respectively) were previously selected.

2 Complementary characterization of the nutritional value showed as NVM variant ZV-156 when autoclaved (AC) or not, RVM and GPM were the most promising meals. Afterwards, NVM/ZV-156 and NVM/ZV-156AC were selected based on the total soluble phosphorus (TSP) and phenolic compounds (Poly) content (Table 1).

Table 1. *Total buffer capacity (TBC), inhibition of alkaline protease (IAP) activity, soluble protein (SP) and phytate contents, total soluble phosphorus (TSP) and phenolic compounds (Poly) content in the different meals evaluated.*

Meal/Code	TBC	IAP activity	SP	Phytate	TSP	Poly
NVM/ZV-156	92.35 ± 0.00^{f}	47.12 ± 4.64^{cd}	4.32 ± 0.09^{bc}	4.90 ± 0.22^{a}	0.47 ± 0.01^{b}	0.13 ± 0.005^{a}
NVM/ZV-156 HP	95.09 ± 0.00^{c}	49.35 ± 3.02^{bcd}	4.38 ± 0.01^{bc}	4.33 ± 0.24^{bc}	-	-
NVM/ZV-156 G	92.31 ± 0.00^{g}	43.65 ± 0.96^{d}	4.60 ± 0.01^{ab}	4.43 ± 0.09^{abc}	-	-
NVM/ZV-156 AC	95.24 ± 0.00^{b}	33.29 ± 1.86^{e}	0.98 ± 0.13^{g}	3.96 ± 0.03^{c}	0.42 ± 0.03^{bc}	0.11 ± 0.001^{b}
NVM/ZV-145	89.02 ± 0.00^{h}	50.17 ± 3.47^{bcd}	4.20 ± 0.01^{c}	3.21 ± 0.10^{d}	-	-
NVM/ZV-151	81.63 ± 0.00^{i}	69.00 ± 1.80^{a}	3.31 ± 0.17^{e}	3.07 ± 0.03^{d}	-	-
NVM/Agropal	92.31 ± 0.00^{g}	53.00 ± 2.24^{bc}	4.84 ± 0.05^{a}	4.73 ± 0.31^{ab}	-	-
RVM	94.75 ± 0.00^{d}	56.10 ± 1.76^{b}	2.95 ± 0.12^{f}	1.69 ± 0.28^{f}	0.39 ± 0.02^{c}	0.08 ± 0.002^{c}
GPM	93.18 ± 0.00^{e}	50.24 ± 2.23^{bcd}	2.69 ± 0.12^{f}	2.26 ± 0.02^{e}	0.38 ± 0.01^{c}	0.09 ± 0.004^{c}
SBM	248.06 ± 0.00^{a}	24.18 ± 3.54^{f}	3.76 ± 0.19^{d}	4.15 ± 0.25^{c}	0.66 ± 0.04^{a}	0.12 ± 0.001^{a}

*TBC in µmol H+ per g sample * ΔpH; IAP activity in %; SP in mg per g of sample; Phytate in mg per g of sample; TSP in g per 100 g of sample; Poly in g per 100 g of sample. NVM, Narbonne vetch meal; RVM, red vetchling meal; GPM, green pea meal; SBM, soybean meal; HP, meal subjected to high pressure; PG, meal subjected to germination; AC, Autoclaved meal. Different superscript letters within each column denote significant differences among meals (ANOVA; Tukey test; $p < 0.05$; $n = 3$).*

3 Pre-treatment with Rovabio® Phy, removed phytate and improved nutrient availability in meals (Fig. 3). NVM/ZV-156 was selected for the next stage.

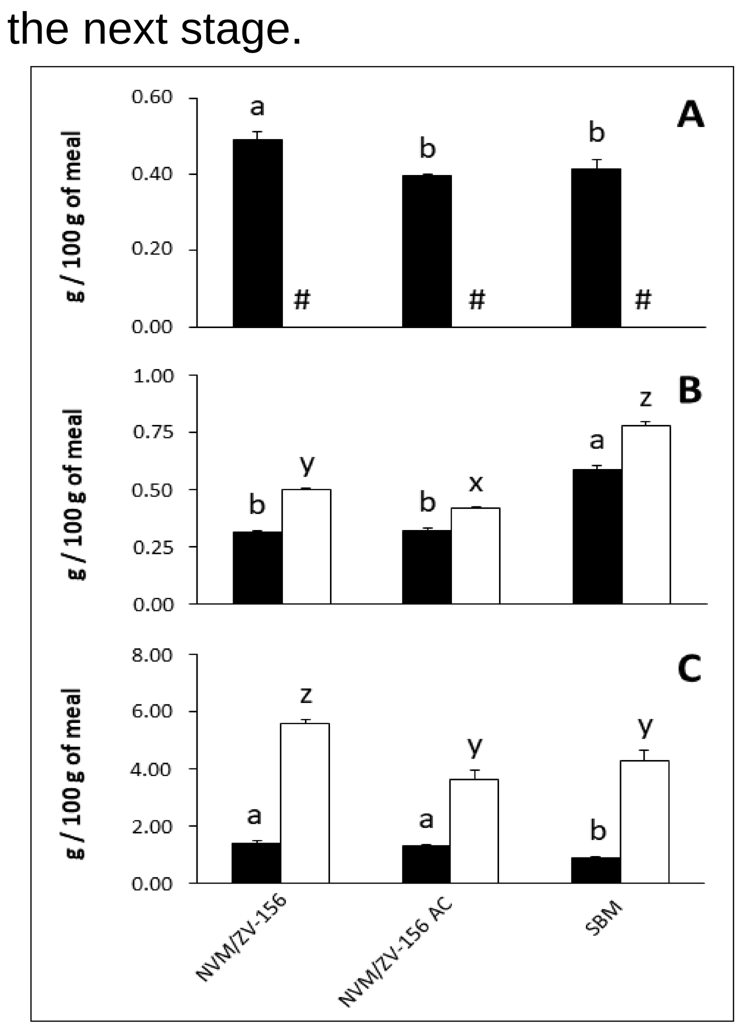


Figure 3. *Phytate (A), pentoses (B) and reducing sugars (C) availability in the different screened meals before (black bars) and after (white bars) treatment with exogenous enzyme Rovabio® Phy. NVM, Narbonne vetch meal; SBM, soybean meal; AC, Autoclaved meal. # indicates absence of phytate. Different superscript letters denote significant differences among meals (ANOVA; p<0.05, n=3).*

4 *In vitro* digestion of NVM/ZV-156 and SBM treatment with Rovabio® Phy increased the bioavailability of amino acids, pentoses, and reducing sugars.

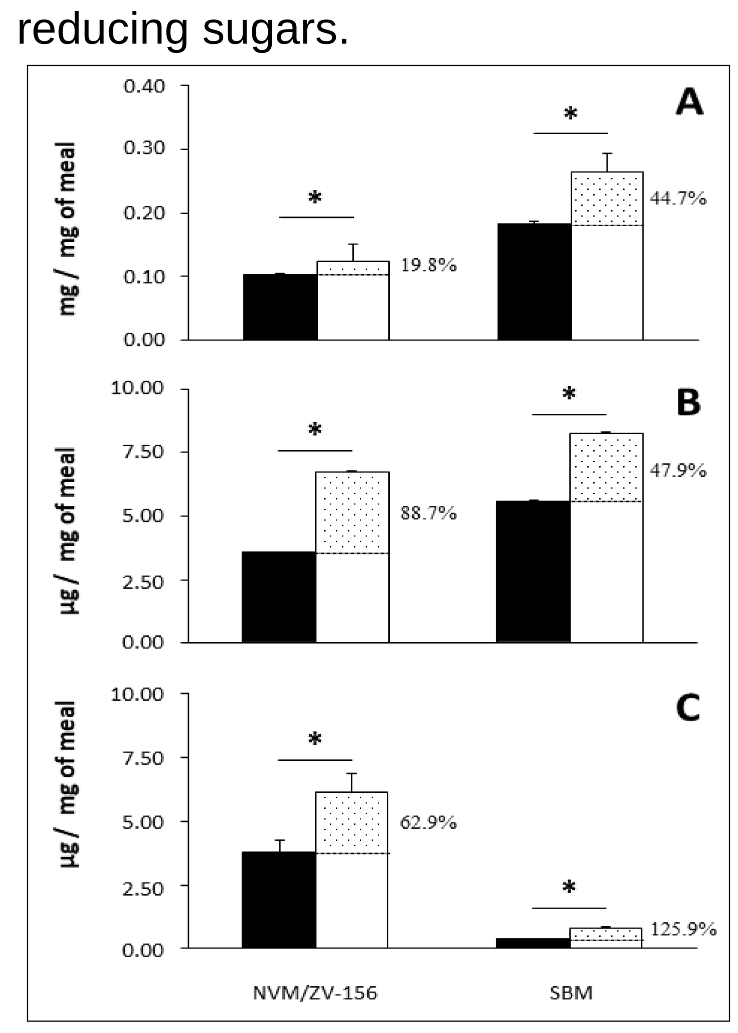


Figure 4. *Amino acids (A), pentoses (B) and reducing sugars (C) released after in vitro hydrolysis from Narbone vetch meal (NVM) and soybean meal (SBM), previously treated (white bars) or not (black bars) with Rovabio® Phy. The hatched fragment in the white bar indicates the percentage of increased nutrient release with Rovabio® Phy. Asterisks denote significant differences before and after Rovabio® Phy treatment (T-test; p<0.05, n=3).*

5 High replacement percentages (66% NVM/ZV-156), treated or not with the enzyme Rovabio® Phy, reduced the growth of trout (Table 2). A 33% replacement of SBM in rainbow trout diets (A33E) can be achieved with NVM/ZV-156 treated with the enzyme Rovabio® Phy, being growth performance not significantly different from the fish fed the Control diet.

Table 2. *Growth performance of rainbow trout fed experimental diets where soybean meal was partially replaced by Narbonne vetch meal treated or not with Rovabio® Phy.*

	CONTROL	A33	A33E	A66	A66E
BW	223.18 ± 6.78	204.98 ± 1.85*	211.39 ± 2.45	196.46 ± 5.39*	198.57 ± 6.84*
FL	24.88 ± 0.27	24.09 ± 0.08*	24.62 ± 0.26	23.97 ± 0.22*	24.09 ± 0.09*
WG	487.80 ± 18.15	437.48 ± 5.60*	455.73 ± 7.03	416.92 ± 13.30*	422.19 ± 18.52*
SGR	2.81 ± 0.05	2.67 ± 0.02*	2.72 ± 0.02	2.61 ± 0.04*	2.62 ± 0.06*
FCR	0.81 ± 0.01	0.86 ± 0.01*	0.84 ± 0.01	0.91 ± 0.02*	0.91 ± 0.02*
HSI	1.10 ± 0.01	1.20 ± 0.02	1.23 ± 0.04	1.34 ± 0.10*	1.33 ± 0.07*

BW, body weight (g); FL, furcal length (cm); WG, weight gain (%); SGR, specific growth rate (%); FCR, feed conversion rate; HSI, hepatosomatic index (%). Asterisk within each row denote significant differences between Control and each experimental group (ANOVA, $p < 0.05$; $n = 3$).

CONCLUSIONS:

- ✓ The present methodology allows to perform a preliminary screening of alternative raw materials to replace FM or SBM.
- ✓ Treatment with exogenous enzyme Rovabio® Phy improves the use of alternative vegetable protein sources.

REFERENCES

Turchini et al., 2018. *North Am. J. Aquac. 81*, 13–39.

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