

# IN VITRO DIGESTIBILITY OF ALTERNATIVE PROTEIN SOURCES

# TO REPLACE FISH MEAL IN AQUACULTURE DIETS

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## **INTRODUCTION & AIM**

- Aquaculture production has increased considerable over the past decades, supplying 49% of worldwide fish demand by 2023.
- Aquaculture requires a protein supply between 45–65% in dry weight of diet, mostly provided as fish meal from herring, anchovy and other species.
- Alternative sustainable sources such as edible insects, plant meals or microalgae can provide protein to replace fish meal in aquaculture diets.
- The digestibility of novel proteins can be evaluated through in vitro digestion methods, using enzyme extracts from fish stomach, intestine and caeca pylori.

• The aim of this work is to evaluate in vitro digestibility of novel protein sources from insect (Tenebrio molitor), sunflower (Helianthus annuus) and microalgae (Nanochlorosis gaditana), employed as dietary ingredient for the aquaculture rearing of European seabass (Dicentrarchus labrax).

### METHODOLOGY

- Enzyme extracts were recovered from seabass stomach, intestine and caeca pylori after grinding and homogenization at 4°C with 1:5 solid/water. Aqueous extracts were centrifuged and filtered to remove fats and insoluble debris, recovering and freeze-drying supernatants, used as enzyme extracts.
- Protein suspensions at 1.8 g protein·100 mL<sup>-1</sup> were transferred to a jacketed reactor for simulated digestion (2 h gastric phase + 2 h intestinal phase)
- Protein digestibility was evaluated as the degree of hydrolysis (DH), monitored during reaction by the pH-stat method.
- Fish meal presents the highest digestibility among the protein sources, followed by insect protein from *Tenebrio molitor*. Sunflower and microalgae were poorly digested. This may be attributed to the presence of fibre and other insoluble components in the sunflower meal, as well as the resistance of microalgae membranes against enzyme attack.

RESULTS

• Further research is needed to evaluate if protein digestibility can be improved by (i) physical pretreatments (e.g. ultrasound, homogenization) of the protein meals and (ii) partial substitution of native protein by enzymatic hydrolysates.







**Fig. 2**. Evolution of the degree of hydrolysis (DH,%) during the simulated gastrointestinal digestion fish meal, insect meal, microalgae, and sunflower seed meal employing enzyme extracts from European seabass (*D. labrax*)



**Fig. 1**. Methodology employed for enzyme extraction and simulated gastrointestinal digestion in batch reactor with control of pH and temperature

### CONCLUSIONS

- Insect protein can be used as protein ingredient to replace fish meal in aquaculture diets. Moreover, its digestibility could be improved by partial hydrolysis of the insect meal.
- In vitro evaluation methods allow evaluating the digestibility of protein ingredients prior to zootechnical experiments.

Fig. 3. Molecular weight distribution of the protein digests, obtained by Size Exclusion Chromatography

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