



## SUBMISSION

2<sup>nd</sup> International GRAB-IT workshop

### **Louisiana red crayfish meal: a novel functional feed ingredient for organic fish diets?**

Type of submission:

Paper for poster presentation

I wish to be included in the best paper award competition (*only for full length papers*)

NO

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## Abstract

Red swamp crayfish is one of the most dangerous invasive alien species in Europe. This study investigates the nutritional value of a flash-dried whole meal as a novel possible ingredient in finfish diets. Based on the analysed nutrient composition and digestibility measured in rainbow trout, the flash-dried red swamp crayfish meal results to be a suitable complement or a partial alternative to conventional vegetable and/or animal protein sources in fish diets. Moreover, the high concentration of astaxanthin (a carotenoid with several positive physiological roles) makes it even more attractive also as a natural and functional ingredient for organic aquafeeds.

## Introduction

Feed-based aquaculture is expected to grow faster in the near future, generating a demand for aquafeeds of some 87 million tons in 2025, that will sharpen competition for food resources with other livestock sectors and finally with human population. Therefore, economic and ecological sustainability of aquaculture will depend to a great extent on the ability to save poorly renewable resources or heavily depleted ones, furthermore increasingly contended with other sectors, by using novel nutrient-rich raw materials or feed ingredients that are currently little studied or valued, possibly by finding them outside the human food chain.

The Louisiana Red swamp crayfish (*P. clarkii*) is one of the most invasive alien species (IAS) in Europe. It was imported in Europe in 1970 for aquaculture purposes, and now it is spread in the wild (Drake, 2009) due to its high fecundity and tolerance to extreme conditions. In Italy, its diffusion caused alterations of freshwater environments impairing species biodiversity. To limit crayfish invasions, several EU authorities have issued eradication campaigns, but crayfish removed from water bodies are usually destroyed by incineration. The SUSHIN project (supported by a consortium of ten banking foundations - AGER) investigated a new ingredient derived from crayfish, to be used in future feeds, in order to face both (1) the need of reducing the dependence of the sector on the exploitation of natural resources converted into fish meal and fish oils, in alternative to conventional vegetable protein sources, and (2) the requirement of a natural ingredient with high nutritional value and high in carotenoid (astaxanthin) to be used in finishing diets for improving skin or flesh pigmentation of certain cultured fish species. This latter point fits pretty closely one of the challenges of organic feedstuffs set by the EU rule N° 775/2008, i.e. the need to substitute synthetic pigments with natural ones. Thus, under this latter perspective crayfish meal could represent a new solution for organic aquafeeds.

Based on the above traced directions, the present study was aimed at evaluating the nutritive value of a flash-dried meal obtained from whole red swamp crayfish to establish its suitability to use as a functional feed ingredient in conventional and organic fish diets.

## Methods

In 2017 sampling campaigns aimed at collecting a sufficient biomass of *P. clarkii* for laboratory analysis and digestibility fish trials were set up in two protected areas of Latium: Circeo National park and Regional Natural reserve of Tevere-Farfa. A total of 400 kg of crayfish (corresponding to ~ 23.000 individuals) were removed, frozen and stored. Crayfish meal (RSCM) was obtained by a flash drying technique.

The nutrient apparent digestibility coefficients (ADCs) of RSCM, have been measured in rainbow trout (*Oncorhynchus mykiss*) with the indirect method and compared to that of a mixture of highly purified vegetable protein sources (VEG), such as corn gluten and soybean protein concentrate, which is being commonly used in aquafeeds. From a basal diet mash, including acid insoluble ash (1.5%) as an inert marker, the two test diets (RSCM and VEG) were obtained by extruding into 3 mm pellets a mix of the basal diet and test ingredients at a 70:30 w:w ratio. The digestibility trial

has been performed in nine 65L tanks and supplied with 3 L h<sup>-1</sup> of well water at a temperature 13.5 ± 1°C. Twenty trout juveniles (body weight 16.9 ± 2 g) were randomly distributed into each tank and adapted to the experimental condition and diets for one week before faecal collection. The apparent digestibility coefficients (ADCs) of dry matter (DM), organic matter (OM) and crude protein (CP) of the diets were measured in triplicate. The ADCs of nutrients were calculated by difference relative to those of the basal diet.

The proximate composition of crayfish meal, diets and faecal dry samples was determined according to the AOAC (1995). The fatty acid composition of RSCM was performed by Gas chromatographic analysis on a GC 6890 N (Agilent, Inc. California, USA) instrument with CP-Sil88 column (100m, 0.25mm x0.2 µm). Amino acids analysis was performed by HPLC Waters 2695 system with fluorescence 2475 analysis using the AccQ-Fluor reagent kit from Waters (Milford, MA, U.S.A, 1993). Total carotenoids and astaxanthin concentration was obtained by Parisenti et al. (2011); briefly the sample was extracted with acetone/hexane solvent, the hexane layer, with carotenoids, was read at 470 nm and calculated using astaxanthin as standard, express in mg/100g. The same extraction was used for quantify the astaxanthin by injection in HPLC, express in mg/100g.

## Results

The RSCM studied here showed a crude protein level of 44.4% dry weight, a well-balanced amino acid profile, coupled with a high level of ash (29.7%) and low level of lipid (8.9%). The ratio between ω-3 and ω-6 fatty acids (0.8) was very high and better when compared with that of other animal and vegetal protein-rich meals, compares more favourably with that of fishmeal. Crayfish meal contains also chitin (7.2 %) and it is a relevant source of carotenoids (81.4 mg/100g, mostly astaxanthin, 59.0 mg/100g)(Tab.1).

When compared to the VEG mix, the RCM resulted in reduced dry matter digestibility (76 vs 87%, P<0.05) but in the same crude protein bioavailability (98 vs 97%, P>0.05) and in higher Energy digestibility (98 vs. 91%, P<0.05).

**Table 1 Proximate composition (on a d.w. basis), fatty acid and amino-acid profiles and pigment concentration of crayfish meal**

Proximate composition		<b>Protein</b>	<b>Moisture</b>	<b>Fat</b>	<b>Ash</b>	<b>Chitin</b>	<b>Other</b>
	%	44.4	90.9	8.7	29.7	7.2	0.9
Fatty acids		<b>SFA</b>	<b>MUFA</b>	<b>PUFA</b>	<b>ω-3</b>	<b>ω-6</b>	<b>ω-3:ω-6</b>
	%	36.8	46.8	16.4	7.3	8.7	0.8
Amino-acids		<b>Ala</b>	<b>Arg</b>	<b>Asp</b>	<b>Cys</b>	<b>Glu</b>	<b>Gly</b>
		4.1	1.3	4.6	0.3	9.0	2.7
		<b>His</b>	<b>Ile</b>	<b>Leu</b>	<b>Lys</b>	<b>Met</b>	<b>Phe</b>
	g/100g	1.1	1.9	3.5	2.3	0.8	2.1
		<b>Pro</b>	<b>Ser</b>	<b>Thr</b>	<b>Tyr</b>	<b>Val</b>	
		1.4	1.1	1.8	1.2	3.2	
Pigment		<b>Total carotenoid</b>	<b>Astaxanthin</b>				
	mg/100g	81.4	59.0				

## Discussion and Conclusion

No much information exists on the nutrient composition of *P. clarkii* besides, to our best knowledge, this is the first study where the nutritive value of a dry meal obtained from red swamp

crayfish has been estimated in a fish species in order to establish its potential as an aquafeed ingredient.

The nutrient composition and digestibility of a meal obtained from specimens collected in areas of high naturalistic value, showed that RSCM possess a high nutritive value to fish making it a suitable candidate feed ingredient to complement or partially replace conventional ones in fish diets.

Moreover, being a source of astaxanthin, a high value keto-carotenoid pigment, RSCM has the potential to be included in fish diets as an innovative natural ingredient to boost and improve colour appearance of most aquatic animal or their food products, which represents an important quality criterion for the marketing of aquaculture products. Moreover, the wide range of other beneficial physiological roles of astaxanthin in fish, such as improved growth, survival, reproductive capacity, stress tolerance, disease resistance and immune-related gene expression (Lim et al., 2017), makes crayfish meal even more attractive also as a sustainable, natural and functional ingredient for conventional and organic aquafeeds.

## References

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