Enzyme pretreatment of plant ingredients used in diets for mullet

F. Javier Moyano, Francisca P. Martínez, Waldo G. Nuez-Ortín,
University of Almería and Adisseo

Mugilidae (mullets) are a group of fish showing an increasing interest for aquaculture due to their rapid growth, resistance to a wide range of environmental conditions and omnivorous profile. Over the past few years, the culture of these species, particularly of the grey mullet (*Mugil cephalus)*, is considered a priority within the current strategies of European aquaculture. Besides specific research aimed to complete its reproduction in captivity, the culture of grey mullet requires the development of suitable species-specific diets. In this sense, several studies have demonstrated the possibility of using high amounts of plant by-products with a limited nutritive value in its feeds, even during the early stages of development. This represents important advantages, like a higher diversity of offer and availability, as well as positive environmental and economical features.

Enzyme additives in plant-based diets

From a nutritional point of view, plant by-products also present limitations, linked both to their amino acid imbalances and to the presence of a wide variety of antinutritional compounds like alkaloids, lectins, digestive enzyme inhibitors, indigestible carbohydrates (mainly non-starch polysaccharides; NSPs) and phytate.

The use of enzyme additives may be a powerful tool to counteract the potential negative effects derived from the presence of phytate and NSP, and thus increase the nutritional value of plant ingredients. The addition of carbohydrases capable of totally or partially hydrolyze NSPs has shown positive effects on the nutritive use of feeds since in many cases these compounds form a matrix that hinders the access of digestive enzymes to the protein and starch present in cereal and leguminous seeds. Similarly, the use of phytase has shown positive

INGREDIENT (in g/100 g d.w.)	FEED
Fishmeal 67/10	10.00
Soybean meal 47	18.83
Defatted rice bran	10.00
Brewer's spent grain	20.00
Soybean protein concentrate	8.00
Corn gluten meal 60	8.00
Guar meal (Korma)	11.16
Fish oil	3.25
Sunflower oil	2.60
Soy lecithin	0.65
Squid hydrolysate	1.50
Vitamin/mineral premix	0.05
Taurin	0.30
Yeast	3.00
PROXIMATE COMPOSITION (in g/100 g)	
Crude protein	38.00
Crude fat	3.49
Digestible carbohydrates (starch + oligosaccharides)	10.56
NSP	27.73
Ash	6.21
Phosphorus	0.85
Phytate P	0.35

Table 1. Ingredients and proximate composition of the diets used in the experiment.

effects on diets for different fish species due to its ability to improve the whole nutritional use of different ingredients and to reduce phosphorus discharge into the environment.

Almost all the published works dealing with the use of enzyme additives in fish feeds have tested their inclusion in the feeds using some protected forms or applied post-extrusion via oil top coating or spraying, resulting, in most cases, in a limited effectiveness and somewhat contradictory results.

We present some results obtained within a project aimed to develop feeds for the grey mullet, *Mugil cephalus*, formulated with very high contents of plant ingredients. Such ingredients were pre-treated by solid-state hydrolysis (SSH) with an enzyme mixture before the preparation of the feed pellets to reduce the contents in NSPs and phytate. SSH operates with a percentage of solid substrate greater than 15%, so little or no free water is present. However, the hydrolysis is performed under optimal conditions for the enzymes, so their activity is not affected either by the high temperatures reached during feed preparation or by the biochemical conditions present in the gut of the fish.

Use of enzymes prior to pelleting

The diets were designed to include a low amount of fishmeal (10-15 g/100 g) but a high amount of plant-based ingredients (>70% in weight) including 300 g/kg of by-products like brewer's spent grain and rice bran (Table 1).

SSH of the mixture of plant ingredients was carried out after milling them to a mesh size of 0.5 mm, being after mixed with citrate buffer (pH 5.0, 0.1 M; 1:2 w/v) to obtain a moist mass providing the optimal conditions for the action of the multienzyme complex. The product used was Rovabio®, a mixture of xylanases, glucanases, arabinofuranosidases, as well as phytase, provided by Adisseo. It was added to the mixture by spraying four hours prior to mixing in with the rest of the ingredients used in the feed.

Two feeds (enzyme-treated and control without enzyme pre-treatment) were prepared and tested on triplicate groups of juvenile mullets of 12 g initial weight, maintained in the facilities of CTAQUA (Cadiz, Spain), that were fed during six weeks on the experimental diets. The effect of the multienzyme complex on the chemical profile of the mixture of plant ingredients was evaluated by measuring reducing sugars, pentoses and phytate present prior to and after the treatment.

As indicated in Figure 1, the amounts of potentially bioavailable reducing sugars and pentoses were significantly increased (by 35% and 25%, respectively) while the amount of phytate was reduced by 36%. These increases in the potential bioavailability of nutrients were reflected in the growth and feed efficiency measured in the fish.

Enzyme pretreatment increases nutritional value of feeds

Due to the high contents in plant by-products, values of FCR and SGR in the control diet were quite unfavorable,

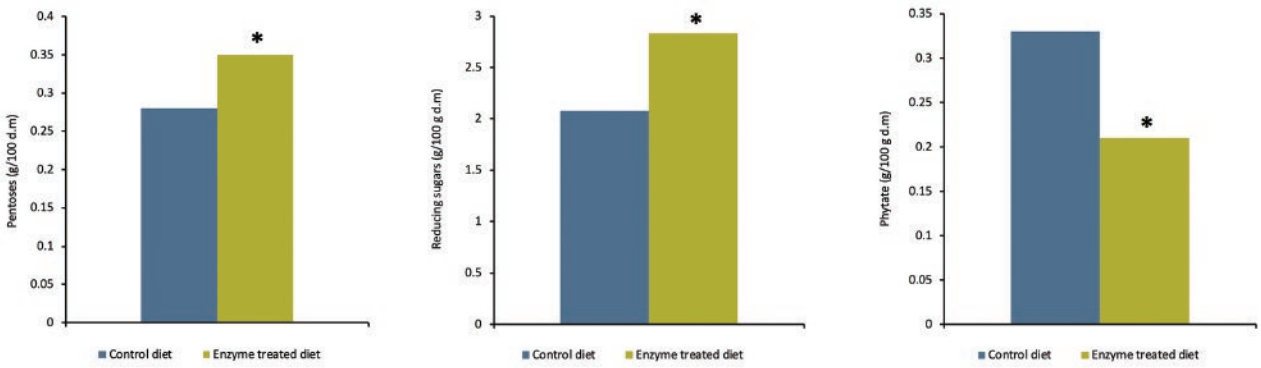

Figure 1. Differences in nutrient content of experimental feeds (g/100 d.m). Statistical comparisons between feeds (3 samples per feed) with or without enzyme treatment are detailed in asterisk. Values showing the asterisk differ significantly with p <0.05.

Parameter	Control diet	Enzyme treated diet
Initial weight (g/fish)	11.89 ± 0.55[a]	12.38 ± 0.16[a]
Final weight (g/fish)	13.21± 0.49[a]	15.36 ± 0.04[b]
Increase (g/fish)	1.33 ± 0.28[a]	2.98 ± 0.15[b]
Feed consumption (g/fish)	8.65 ± 0.45[a]	9.23 ± 0.17[b]
FCR (g feed/g fish)	5.89 ±1.48[a]	3.11 ± 0.19[b]
SGR (g/100 g fish/day)	0.25 ± 0.05[a]	0.50 ± 0.03[b]

Table 2. Zootechnical indicators obtained in the nutritional assay performed on mullet juveniles.

but the use of the enzyme mixture significantly improved it by nearly 50%. Such results could be explained considering several positive effects associated to the use of the enzymes under optimal operative conditions such as: a) the partial hydrolysis of NSP could enhance the bioaccessibility of the fish digestive enzymes to fractions of nutrients that otherwise could be hardly digested (the so called "cage effect"); b) the reduction in the amount of phytate also had very positive effects, not only through increased availability of phosphorus but also of some fractions of protein and minerals that form complexes with phytate; and c) the use of citrate buffer at pH 5.0 to develop the SSH also could enhance solubilization of some minerals, like Fe or Mn.

Also, from a practical point of view, the use of enzyme pre-treatment by SSH adapts the more suitable operative conditions (dose, reaction time, etc) to the specific features of different plant ingredients. Since the enzyme mixture is used prior to pelleting, inactivation due to thermal processing should eliminate any further undesired effect.

Conclusions

The results obtained suggest the utility of the enzyme pre-treatment of ingredients in diets for *M. cephalus* and may help in the future development of commercial feeds based on the use of high amounts of by-products for this species.

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More information:

Fco. Javier Moyano
Professor
University of Almería, Spain
E: fjmoyano@ual.es

Francisca P. Martínez
MsC in Biotechnology
University of Almería, Spain

Waldo G. Nuez-Ortín
Lead scientist aquaculture
Adisseo, Belgium