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In vitro protein hydrolysis by digestive enzymes of *Thunnus thynnus*

Z. Essed, I. Fernández, F.J. Alarcón, M. Díaz and F.J. Moyano
Departamento de Biología Aplicada, Universidad de Almería, 04120 Almería, Spain

SUMMARY – This work presents the preliminary results of some experiments oriented to evaluate, by using the pH-stat system, the ability of *T. thynnus* digestive proteases to hydrolyse different protein sources.

Key words: Inhibitor, pH-stat, proteases, protein sources, *Thunnus thynnus*.

RESUME – *"Hydrolyse* in vitro *de protéines par les enzymes digestives de* Thunnus thynnus*". Ce travail présente les résultats préliminaires de quelques expériences visant à l'évaluation, en utilisant le système pH-stat, de l'aptitude des protéases digestives de* T. thynnus *à hydrolyser différentes sources de protéines.*

Mots-clés : *Inhibiteur, pH-stat, protéases, sources de protéines,* Thunnus thynnus*.*

One of the most important challenges in the rearing of tuna is to develop a kind of feed capable of substituting the general use of fresh fish. Nevertheless, the current rearing system is based on the growing of wild animals, which makes it difficult their adaptation to inert diets. But if rearing is developed from the earliest stages, such adaptation could be required. Research for tuna aquafeed should follow the same phases as those arranged for other currently cultivated marine species. On these grounds and drawing on the information contained in a previous study on bluefin tuna digestive proteases (Essed *et al.*, 2002), the aim of this work is to assess by *in vitro* techniques tuna ability to hydrolyse different proteins in order to determine which ones could be the most suitable for its feeding. Thus, the following steps were carried out in the present work: (i) the determination of the degree of hydrolysis (DH) of proteins by digestive proteases of *T. thynnus*; (ii) a comparative study of DH with the same proteins using the digestive enzymes of another carnivorous fish, seabass (*Dicentarchus labrax*); and (iii) the evaluation of the inhibitory effect of raw sources on digestive proteases of *T. thynnus*.

Digestive tracts of tuna were obtained from 250-300 kg fish supplied by Tuna Farms of Mediterráneo, S.L. (Murcia). Seabass were given by Predomar, S.L. (Almería). Samples of digestive tract were homogenised in distilled water (1:10 w/v). Supernatants obtained after centrifugation were stored at –20ºC until use. Acid and alkaline protease activities were measured according to Alarcón *et al.* (1998). As protein substrates, it was employed several animal and plant meals typically used in the elaboration of commercial aquafeed and provided by Proaqua, S.A. (Palencia). Casein was employed as internal control. DH was determined by pH-stat titration following the method described by Alarcón *et al.* (2002).

Degree of hydrolysis of proteins by *T. thynnus* digestive proteases

The degree of hydrolysis measured in protein samples using stomach and pyloric ceca digestive extracts is detailed in Table 1. Mean values of DH obtained with plant proteins did not exceed 5%, whereas those obtained with animal proteins in all cases exceeded 6% and even reached 10.62%.

Comparative study with seabass

Results in Fig. 1 indicated that the action of digestive proteases of both species determines a similar profile of protein hydrolysis. However, except for a commercial aquafeed, final values of DH for *T. thynnus* were 25% higher than those for *D. labrax*.

Table 1. Values of degree of hydrolysis obtained after enzymatic digestion of different protein sources†. Data are mean of duplicate measurements (values in brackets were SD)

CS	MM	FM 1	FM 2	BM 1	FM 3	FM 4	CF	BM 2	PM 1	SC	PM 2	SM2
11.62 (0.13)	10.62 (0.39)	8.90 (0.03)	8.72 (0.36)	8.58 (0.48)	8.56 (0.19)	7.62 (0.16)	7.09 (0.25)	6.04 (0.78)	5.06 (0.29)	4.9 (0.06)	4.67 (0.19)	4.53 (0.06)

†CS: casein, MM: meat meal, FM: fish meal, BM: blood meal, CF: commercial fish feed, SC: soybean concentrate, PM: plant meal, SM: soybean meal.

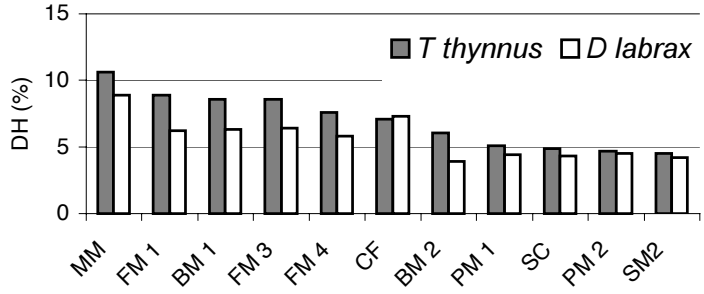

Fig. 1. Degree of hydrolysis of proteins after digestion by *T. thynnus* and *D. labrax* digestive proteases.

Inhibition of *T. thynnus* digestive proteases by protein sources

The presence of protease inhibitor within plant protein was tested by incubation of tuna extracts with a solution containing increasing amount of plant meal. Inhibition values obtained depend of type and amount of meal employed in the assay. High concentrations of meal in the inhibition assay determine high inhibition values. At the same assay concentration (75 mg of meal per unit of protease of activity), commercial soybean meal inhibits only 4% of tuna digestive protease activity whereas a concentrate of the same source reduces 27% of protease activity (Fig. 2).

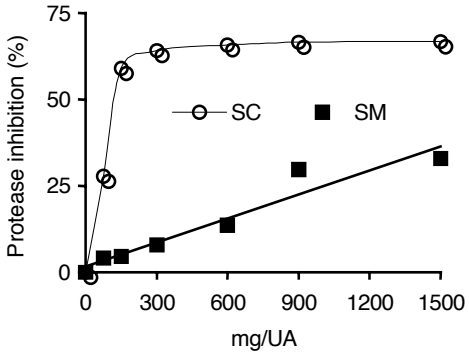

Fig. 2. Inhibition of *T. thynnus* alkaline proteases using different relative concentrations of SC and SM.

The use of an *in vitro* digestibility test indicates that *T. thynnus* digestive proteases hydrolyse several proteins in an equal or higher degree than other carnivorous fish like seabass. The effect of soybean protease inhibitor contained in soybean meal seems to be lower than on other marine fish. This fact could allow the employment of significant amounts of such protein in future aquafeeds for the rearing of this species. However, more investigations are requested for this topic.

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