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Development methodology for measuring *in vitro* digestibility of feeds Mediterranean fish species - Assessment of the nutritional value and growth rate

ABSTRACT

The determination of a diet digestibility in different environmental conditions and under different physiological systems is the first step to evaluate the bioavailability of any living organism.

This study was essential to develop and improve the pH-stat method for determining the digestibility of dietary protein *in vitro* on seabass and study the effects of environmental and biological parameters on the degree of autohydrolysis (BL) and the degree of enzymatic hydrolysis (DH) using statistical and differential calculus form developing equations. Has been study the influence of concentration of the diet, from 9 different concentrations of dietary protein per unit of enzyme activity of total alkaline protease (0.5, 0.75, 1, 1.5, 3, 5, 10, 30, 50 $\mu\text{g} / \text{U}$) on degree of enzymatic hydrolysis and autohydrolysis. The influence of pH degree (pH 8, pH 7 and pH 6) on degree of enzymatic hydrolysis and autohydrolysis using titrisoft program and the influence of temperature (25 ° C, 20 ° C and 15 ° C) using a water bath on degree of enzymatic hydrolysis and autohydrolysis. To study the influence of salinity level of enzymatic hydrolysis and autohydrolysis prepared artificial seawater salinity 35 ‰, which contained the following amounts of salts: 468 mmol NaCl \ L, 10,4 mmol CaSO₄ \ L, 9,97 mmol KHSO₄ \ L, 33,3 mmol MgCl₂ \ L, 7,73 mmol NaSO₄ \ L. The Preparation of artificial brackish water of salinity 15 ‰ was achieved by dilution. To study the effect of acid pre-digestion on degree of enzymatic hydrolysis was at pH 2 and pH 3 and study the effect of the enzyme profile on degree of enzymatic hydrolysis was performed using extracts of seabass pyloric caeca that had fed on locust bean, pea and chickpea.

In total, there were 81 combinations (curves) of different parameters. The results showed that, such decrease in concentration of the substrate influenced positively degree of autohydrolysis and degree of enzymatic hydrolysis statistically, for the reduction of protease inhibitor in the test mixture. The increase of the pH level had a positive degree of autohydrolysis and degree of enzymatic hydrolysis statistically, the number of splitting peptide bonds with negative load during the hydrolysis is higher at level PH 8 than in PH7 than in PH6. The temperature has positively influenced on degree of autohydrolysis and enzymatic hydrolysis statistically that the catalytic property of temperature on the degradation of proteins, then the reduction in salinity affect positively on degree of autohydrolysis and degree of enzymatic hydrolysis statistically as a result of increased electrostatic forces. Thus the various salt ions forming an electric field to bind the charged carboxyl terminus of

peptide chains. The previous acid digestion for one hour at pH 2 and pH 3 did not significantly improve the degree of enzymatic hydrolysis due to the low concentration of substrate per U total acid proteases. The different enzyme profiles of sea bass influences the degree of enzymatic hydrolysis. The locust bean has higher DH than pea and chickpea.

The ninth grade growing equation is as follows :

$$Y = f(x) + f(pH) + f(T^{\circ}C) + f(S\%) + f(TRP) + f(CHTR) + f(CPA) + f(CPB) + f(P) =$$

$$(21,21 * e^{(-0,09631 * X)} + 141,2 * e^{(-1,919 * X)} + 5,428) + (-23,10 + (1,296 + 23,10) / (1 + 10^{(5,955 - pH)})) + (-6,783 + (0,2908 + 6,783) / (1 + 10^{(19,45 - T^{\circ}C)})) + (-12,20 + (0,5186 + 12,20) / (1 + 10^{(S\% - 15,07)})) + (10^{(-1,998 + 1,153 * \log(TRP \text{ U/mg protein.}))}) + (-23,84 + 9,082 * \log(CHTR \text{ U/mg protein.})) + (10^{(0,3626 + 0,8114 * \log(CPA))}) + (10^{(-1,846 + 6,399 * CPB)}) + (2,424 - 0,4080 * P).$$

Further experiments improved pH-stat method for determining the digestibility of dietary protein experimental diets of different treatment methods pulses (Extrusion and Dehulling) and dietary protein source (plant and animal) in 4 different ratios of concentration of substrate per U enzyme activity of total alkaline proteases seabream (1,2µg, 1,8µg, 1,6µg και 20 µg diet proteins / U) and the correlation values of the variable parameter NTP which resulted from the growth equation, one time used autohydrolysis experiment data and second time used the enzyme hydrolysis experiment data, with results indicators diets with dietary protein digestibility *in vivo* in sea bream, the results were positive correlation indicating the accuracy of pH-stat. The extrusion of chickpea and pea did not statistically different results, the Dehulling of the chickpeas gave statistically negative results due to the reduction of content proteins.

Finally, using the pH-stat method for determining the digestibility of dietary protein feeds prepared one of which contained a recombinant protease enzymes (CHTRI 10,225 U, CHTRII 36,456 U and TRPII 23,727 U) that has produced from the bacteria e coli in different concentration ratios of substrate / U (0.5, 0.75, 1, 1.5, 3, 5, 10, 30, 50 µg / U). The purpose of the enrichment of feeds by recombinant protease enzymes is to increase the rate of digestibility.

Enrichment of diet with recombinant enzymes had a negative effect on degree of hydrolysis of feeds proteins *in vitro*. Also had statistics negative effects on the weight gain of fish, specific growth rate and the Feed conversion ratio. Possible causes of this negative effect is the degradation of endogenous enzymes by the added enzymes, the age of fish, the quantities of the recombinant enzymes, experimental period. the results The correlation coefficient of the range of the variable parameter NAE, which resulted from growth equation with biological parameters (weight of fish, SGR & FCR) of seabream *in vivo* experiment, were

positive. The effect of the addition of each recombinant enzyme separately in degree of enzyme hydrolysis of the feed has been investigated. CHTRII gave better grades enzymatic hydrolysis with statistical differences from other recombinant enzymes but not in relation to pyloric caeca extract from sea bream. The degree of autohydrolysis of CHTRI and TRPII did not differ statistically but the degree of proteins autohydrolysis was higher their stats. In conclusion, the pH-stat method has proved economically and provides results quickly available and easy to perform. The use of non-linear equations is the ideal method of treatment results and correlation with other biological parameters.