TECHNICAL RESEARCH REPORT

HEAT STABILITY - A MAJOR HINDRANCE TO THE USE OF **PROTEASES IN AQUACULTURE FEED**

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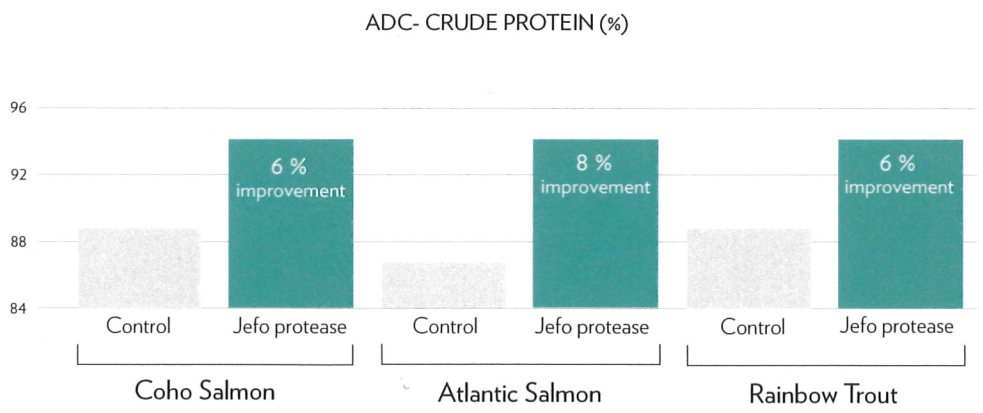
Use of enzymes in animal feed has recently increased due to fluctuating supply, price, and variable quality of the raw materials. Like any other group of enzymes, heat stability of proteases has remained a concern for aquafeed manufacturers. Commonly found proteases usually start losing their activity at, or slightly above 60°C, and destroyed at temperatures higher than 90°C, severely limiting their use in aquaculture feed.

More than 15 years ago, Jefo developed a unique protease complex produced from single bacterial species unrelated to the commonly used *Bacillus sp*. The enzyme complex has successfully been used in poultry feed for more than ten years.

Use of a heat resistant species and 15 years of technological improvement resulted in a remarkably stable protease complex able to withstand extreme manufacturing conditions in aquafeed production. *In-vitro* experiments showed that ~70% of the activity of Jefo protease remains intact after high temperature (120°C) extrusion and expansion. However, the moisture level in feed or mash plays a significant role in retaining the enzymatic activity. At moisture level ≥20%, a significant loss of activity may occur during high temperature (>90°C) processing of feed.

Several *in-vivo* trials with salmonids fed extruded feed (>120°C) showed significant improvement in apparent digestibility of crude protein (+6-8%, see the figure below), total carbohydrate (+21-34%), and gross energy (+6-8%). A growth trial with tilapia fed extruded feed also showed significant improvement in weight gain and feed conversion (FCR).



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Jefo

HEAT STABILITY OF **JEFO PROTEASE FOR AQUACULTURE**

Trials performed at the College of Aquaculture and Life Science, Shanghai Ocean University, China, by professor Leng, in 2011.

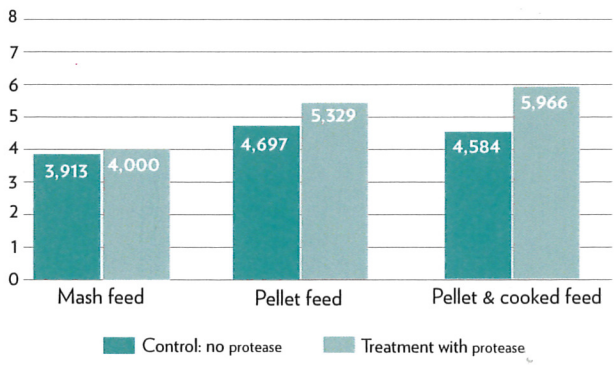
METHOD

The method used determines the quantity of free amino acids liberated by the activity of the protease in the feed, before and after heat treatment. In this case, after pelleting, the feed was submitted to a heat treatment at 95 °C for 25 minutes, this is a common practice for shrimp feeds, to get a harder pellet that will not disintegrate rapidly when in water.

TRIAL 1

Feed containing 20% fish meal was added with 30% water, pelleted at 65 °C, then cooked at 95 °C for 25 minutes, feed without protease and with protease added. The level of free amino acids (indicator of protease activity) was measured in the mash feed, in the pelleted feed and in the pelleted and cooked feed. In this case we can see that the pelleting of the feed triggered an enzymatic activity, so did the cooking, without destroying the enzyme activity. In this trial, the cooking at high temperature increased the protease activity slightly compared to the pelleted feed.

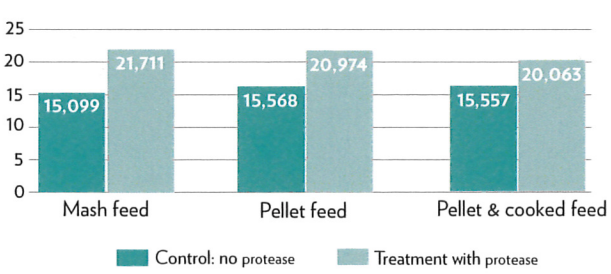
Table 1: Result of free amino acids in µg/ml



TRIAL 2

Feed added with 30% water, pelleted at 65 °C, then cooked at 95 °C for 25 minutes, feed without protease and with protease added. In this second trial extra fish meal was added to the feed (1:1) as a protein substrate to enhance the quantity of free amino acids liberated by the protease activity. The level of free amino acids (indicator of protease activity) was measured in the mash feed, in the pelleted feed and in the pelleted and cooked feed. In this case we can see an important protease activity at all phases of processing, and the level of free amino acids in the cooked feed containing the protease was reduced by 7.6% only, when compared with the mash feed supplemented with the protease.

Table 2: Result of free amino acids (µg/ml) generated after 2 hours at 40°C incubation.



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