Animal Co-product Hydrolysates: a Source of Key Molecules in Aquaculture Feeds

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Abstract

Changes in production technology and marketing and changes in feed ingredients are key structural transformations necessary for the aquaculture sector to grow. Today, with improved genetic techniques novel genetic lines are being bred for maximum efficiency over a shorter production period with lower feed conversions. Thus, the correct amount of micro-nutrients present in their diet is crucial. Fishmeal has always been the main source and the preferred choice of nutritionists for quality protein, above all in the formulation and especially in feeds for the youngest ages. Though, with the market volatility of fishmeal, the aquaculture feed industry is looking for cheaper sources of protein to substitute the fishmeal and this has become a priority. Additional renewable and sustainable protein alternatives are needed. Animal byproducts are well accepted as aqua feed ingredients these days due to short supplies and escalating cost of fishmeal. Protein content in animal byproducts is higher and their complement of indispensable amino acids is superior to those of plant origin. In addition, animal Co-Product Hydrolysates (ACPH) can meet the many nutritional needs of aquaculture worldwide as a protein alternative in aqua feeds. ACPH's can help reduce pressure on natural fisheries stocks and provide sustainability to the growing demand for aquatic products.

Keywords: *co-products, feeds, ingredients*

Nates, S., *et al.* 2013. Resultados Preliminares del Cultivo Piloto Comercial de Pargo *Lutjanus guttatus* Criado en Laboratorio y Engordado en Jaulas Flotantes en el Noroeste Mexicano.. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., Alvarez-González, C. (Eds), Contribuciones Recientes en Alimentación y Nutrición Acuícola, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, pp. 42-47.

Introduction

Changes in production technology and marketing and changes in feed ingredients are key structural transformations necessary for the aquaculture sector to grow. Today, with improved genetic techniques novel genetic lines are being bred for maximum efficiency over a shorter production period with lower feed conversions. Thus, the correct amount of micro-nutrients present in their diet is crucial. On the other hand, the rapid growth of aquaculture worldwide has become increasingly dependent upon the use of external feed inputs, and in particular upon the use of compound aquafeeds. Pressures to reduce fishmeal consumption for sustainability reasons, combined with economic reasons, require intensive research efforts to find candidates for fish meal replacement. However, formulating low fish meal aquaculture feeds requires the use of combinations of several ingredients since most feedstuffs have been shown to have significant nutrient and functional limitations and cannot be used individually at very high levels in the diets of most aquaculture species. Fishmeal has always been the main source and the preferred choice of nutritionists for quality protein, above all in the formulation and especially in feeds for the youngest ages. Though, with the market volatility of fishmeal, the aquaculture feed industry is looking for cheaper sources of protein to substitute the fishmeal and this has become a priority. Additional renewable and sustainable protein alternatives are needed. Animal byproducts are well accepted as aqua feed ingredients these days due to short supplies and escalating cost of fishmeal. Protein content in animal byproducts is higher and their complement of indispensable amino acids is superior to those of plant origin. In addition, animal Co-Product Hydrolysates (ACPH) can meet the many nutritional needs of aquaculture worldwide as a protein alternative in aquafeeds. ACPH's can help reduce pressure on natural fisheries stocks and provide sustainability to the growing demand for aquatic products.

Methods

Animal Co-Product Hydrolysates result from controlled enzymatic digestion of byproducts from the meat processing industry. Technically, it is feasible to generate ACPH from most kinds of slaughterhouse waste such as scrape meat, offal, feathers, and blood as well as from rendered animal byproducts such as meat and bone meal, poultry meal, feather, and blood meal. Protein materials are partially defatted by hexane extraction prior to hydrolysis. Hydrolysis is conducted in a thermostat reaction vessel with constant stirring adequate to prevent the settling of the substrate in the vessel. Alkaline hydrolysates are produced with 0.1 g CaOH/g substrate, at 85 °C. During enzymatic hydrolysis, pH is monitored continuously and maintained through the addition of 8M NaOH. Alkaline hydrolysis reactions are terminated by sparging with CO_2 until the pH drops to 9, followed by neutralization with sulfuric acid. Enzymatic reactions can also be terminated by raising the reaction temperature to 90 °C for 10 minutes. Residual solid material is removed by centrifugation followed by filtration. To test the antioxidant activities of ACPH's, two hydrolysis methods have been adopted (Kalambura e*t al.*, 2008), including the alkaline hydrolysis by a strong base and an enzymatic hydrolysis by a commercial enzyme protease at a specific pH condition. Most results have showed that alkaline hydrolysis produced better antioxidant hydrolysates than the enzymatic hydrolysis (Bo Li, F. *et al. 2*007 y Jingbo, L. *et al.*, 2010).

Results and Discussion

Hydrolysis improves the nutritive value of feed ingredients that are produced from slaughterhouse waste (Table 1). Enzymatic digestion of the raw material breaks the protein chains into peptides that are better absorbed in the gut. Enzymatic hydrolysis of poultry meal with endo- and exopeptidases shows the feasibility of hydrolyzing poultry by-products so significant amounts of short-chain peptides and free amino acids can be produced. High levels of digestible protein characterize poultry protein hydrolysates with a digestibility index above 95%. Feather hydrolysates produced by bacterial keratinases have been tested as additives in aquaculture feeds and several species of bacteria with high keratinolytic activity has been isolated from feather meal broth. In recent studies, it has been established that pepsin digestibility and amino acid content of fermented feather meal (FFM) can be far better than those of commercial feather meal. The microbial cells could also potentially supply carotenoid pigments to FFM, whereby the ingredient may be useful in animal feeding not just as a source of protein but also that of pigments. The short-chain peptides and free amino acids produced as a result of hydrolysis along with nucleotides that are rich in meats confer excellent attractability and palatability properties to ACPH. Poultry liver hydrolysates have been added to animal feeds at levels as high as 6% and have been found to enhance palatability. Spray-dried hydrolysates produced from poultry byproduct meal can contain up to 70% protein with compounds that have molecular weights ranging from 5,800 to 12,000 Da. We have found that inosine is the dominant nucleoside in poultry meal, a molecule that is believed to enhance diet attractability in several fish species, including largemouth bass, turbot and mackerel. Among monophosphate forms, adenosine monophosphate (AMP) dominated and similar trends have been seen in fishmeal hydrolysates. It is known that alkali-hydrolysates and enzyme-hydrolysates from meat and bone meal, blood meal and feather meal have similar ash and protein content as the parent materials, but with concomitant liberation of bioactive peptides that are encoded within the protein. It has been demonstrated that ACPH prepared under alkaline hydrolysis can be a source of antioxidants with activities comparable to BHT. Results from previous studies have also shown the presence of antioxidant, carnosine, a histidine containing dipeptide, in poultry byproducts (Manhiani, P. 2011 y Manhiani, P. *et al.*, 2013). Carnosine levels in poultry products ranged from 0.95-102.3 mg/g (wet basis) (Table 2). Carnosine levels in Meat and Bone meal ranged from 500 to 1,800 ppm, while in fishmeal they can be as low as 5 ppm. Soy and other plant proteins don't contain carnosine. The results of adding carnosine on growth performance of Nile tilapia showed that the body weight and body length of tilapia in the group supplemented with carnosine were higher than that in the control group, and diets supplemented with carnosine could increase the levels of GH, IGF-1 and T_3 in their serum indicating that diets supplemented with carnosine could improve antioxidation in muscle. Anti-microbial peptides have also been identified in PBM and FeM hydrolysates. These include cysteine rich antimicrobial peptides. Other potential molecules that can be found in cattle, chickens and turkeys include "Galanin", which has previously been reported to elicit feeding in satiated animals, and "Defensins" that show antimicrobial activity against bacteria and fungi, but at this point we don't know if these molecules are being present in animal by-products. Compared with fishmeal, dried porcine blood co-products and bone protein hydrolysates are poor in methionine and lysine (Sun, Q.

and H. Shen, 2011). However, blood by-products are rich in microelements, which can improve Ca and Cu retention in aquaculture species, especially shrimp in which it has been reported that inadequate supplementary dietary copper level can result in significant growth depression. Porcine plasma hydrolysates are also effective inhibitors of lipid oxidation as well as metal chelating and reducing agents.

Table 1. Effect of hydrolysis on animal byproducts

Effect of hydrolysis	Resulting benefit
Digestion of protein	Improved digestibility, absorption and assimilation of peptides
Increase in the proportion of low molecular compounds like short-chain peptides, free amino acids and nucleotides	Enhanced attractability and palatability
Production of bioactive peptides	Antioxidant and anti-microbial activities

Table 2. Carnosine levels in different tissues of stress and non-stress broilers (from Manhiani, 2011)

Organ	Treatment	Calcium content ppm	p-value	Carnosine Content (wb)[2, 3]	p-value	Carnosine Content (db)	p-value
Breast	**Non-stress**	6.74±0.07 [a]	P=0.001	1.85±0.24 [1, a]	P=0.005	7.52±0.97[a]	P=0.0005
	Stress	11.03±0.13 [b]		17.39±1.33[b]		70.89±5.39[b]	
Thigh	**Non-stress**	10.50±0.37 [a]	P=0.006	11.10±1.02[a]	P=0.001	44.47±4.08[a]	P=0.002
	Stress	13.99±0.19 [b]		21.25±1.25[b]		85.12±5.01[b]	
Brain	**Non-stress**	8.48±0.38 [a]	P=0.002	10.16±1.53[a]	P=0.82	45.44±12.37[a]	P=0.54
	Stress	13.78±0.24 [b]		10.27±2.77[a]		55.12±14.88[a]	

1. All values are in Mean± S.E.M (N=5)
2. wb= wet basis; db= dry basis
3. Carnosine content is expressed in mg/gm of the original sample.
4. Fisher's Least Significant Difference Test was used to compare mean values; [a-b] similar letters indicate that the means values are not significantly different ($p \geq 0.05$); while different letters indicate that the mean values are significantly different ($p \leq 0.05$).

Conclusions

While we need to keep in mind the ability of bioactive peptides and nutraceuticals to exert a physiological effect *in vivo*, these examples of key molecules found in animal by-product hydrolysates show the potential for use as functional ingredients in aquaculture feeds.

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