



# Protein hydrolysates from animal processing by-products as a source of bioactive molecules with interest in animal feeding: A review<sup>☆</sup>



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## ABSTRACT

Industrial processing of livestock, poultry and fish produces a large amount of waste in a solid or liquid form that can either be destroyed or be used to make compost, biogas or other low-added value products. However, the by-products from animal processing industries have a potential for conversion into useful products of higher value, such as protein hydrolysates, with interesting applications in animal feed. Low amounts of animal protein hydrolysates included in aqua-feeds may enhance growth rate and feed conversion of farmed fish and crustacean. Animal protein hydrolysates may also be incorporated in diets to enhance the nonspecific immunity of fish. As well, these hydrolysates can be used as a good source of amino acids for newly weaned animals. Protein hydrolysates from animal by-products including antimicrobials, antioxidants, opioid-like and/or other interesting bioactive molecules have promising and interesting applications on companion and production animals. By-products from animal processing industries are therefore a promising source of bioactive peptides of considerable interest for animal care, always within the framework of the existing legislation. Possible drawbacks and future trends of the use of animal by-products and/or production of protein hydrolysates from those materials are also discussed.

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## 1. Introduction

Industrial processing of livestock and poultry generates significant amounts of waste including viscera, meat, fat or lard, skin, feet, abdominal and intestinal contents, bone, feather and blood. Data on the amount of waste are relatively sparse and vary from around 33% to around 43% (w/w) of the live weight (Hamilton, 2004). The by-products derived from slaughter and processing of cattle, pigs and broilers may represent about 49, 44 and 37% of the total live weight, respectively (Meeker, 2009). In the specific case of chickens, blood represents about 2–6% of the total bird weight, and feathers could be up to 10% (Jamdar & Harikumar, 2005; Lasekan, Abu Bakar, & Hashim, 2013).

Fish processing industries also generate large amounts of waste that can represent as much as 57% (w/w) of the weight of the total catch after filleting (Meeker, 2009). This waste is mainly composed of muscle-trimmings (15–20%), skin and fins (1–3%), bones (9–15%), heads (9–12%), viscera (12–18%) and scales (5%) (Rustad, Storrø, & Slizyte, 2011; Torres, Chen, Rodrigo-García, & Jaczynski, 2007). In the

particular case of cephalopods, waste resulting from processing may amount to as much as 60% of the total live weight of the animal.

In general terms, the current volume of animal by-products generated from the industry is nearly 54 billion pounds annually (Meeker, 2009). Non-utilization or underutilization of animal by-products not only leads to loss of potential revenues but also leads to increasing cost of disposal of these products. For that reason, industry has begun to develop various technologies to make use of this waste, mainly in the form of low value-added products, at the same time reducing the costs derived from its disposal. In global terms, the rendering industry processes approximately 60 million tonnes per year of animal by-products (Hamilton, 2004), from which 25 million tonnes are processed in North America and 15 million tonnes in European Union. Argentina, Australia, Brazil and New Zealand collectively process another 10 million tonnes of animal by-products per year. However, the reality is that considerable amounts of animal by-products are hardly recovered in most of countries, and in many cases it comes up against limitations imposed by existing legislation. In this regard, the outbreak of Bovine Spongiform Encephalopathy (BSE) in Europe during the 1990s led to ban the use of slaughterhouse waste material as a fodder ingredient as being a probable transmission pathway. Although BSE has been almost eradicated worldwide, the reality is that the use of animal by-products is nowadays restricted and they are mainly used as either animal feed ingredients or fertilizers, or mostly disposed of. In Europe, only those by-products belonging to the third category (EC Regulation No. 1069/2009) can be

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used, after technological processing, for the production of low value-added products (animal feeds, silages, fertilizers, etc.), or derived products of greater added value (cosmetic, sanitary or veterinary medicinal products), as expressed in the Article 33. Legislation in the United States is also restrictive regarding the use of animal by-products for various purposes (Liu, 2002).

The animal by-products are mostly rich in high-quality protein than can be hydrolysed by proteases to obtain bioactive peptides with promising therapeutic, functional and/or nutritional applications, as has been mainly reported in dairy hydrolysates (Choi, Sabikhi, Hassan, & Anand, 2012). Protein from feathers, bristles, horns, beaks or wool can be only enzymatically hydrolysed after destruction of the keratin structure, either by acid or base treatment, by the use of specific microorganisms or enzymes, by thermochemical pre-treatment, or else by steam flash explosion (Eslahi, Dadashian, & Nejad, 2013; Lasekan et al., 2013; Mokrejs, Svoboda, Hrnčirik, Janacova, & Vasek, 2011; Zhang, Yang, & Zhao, 2014).

The protein hydrolysates derived from animal by-products have potential applications in food technology as flavourings, functional ingredients, or else as a good source of amino acids (Cho, Baik, Choi, Hahm, & Kim, 2010; Guérard et al., 2010; Kumar, Nazeer, & Ganesh, 2012; Qiao, Tong, Zhou, & Zhu, 2011; Zhang et al., 2013). In addition, animal by-products hydrolysates include peptides that are claimed to be potential health enhancing nutraceuticals for food and pharmaceutical preparations (Khan et al., 2011; Lasekan et al., 2013; Rustad et al., 2011; Senevirathne & Kim, 2012; Toldrá, Aristoy, Mora, & Reig, 2012). All these properties make animal protein hydrolysates of interest for manufacture of products for human or animal feed. In the specific case of pets and production animals, the objectives of the use of animal by-products hydrolysates should be focused on strategies to optimize animal welfare and/or food production (e.g. increasing milk production in dairy cows, increasing survival rates in aquaculture species, or increasing weight gain in post weaning pigs). However, the functional and/or nutritional applications of those hydrolysates for pets or production animals have not been widely studied.

The present review focuses on the current and potential applications of bioactive protein hydrolysates from animal processing by-products for aquaculture species, farm animals and pets. Furthermore, possible drawbacks and future perspectives of the use of these hydrolysates in animal feeding will be discussed.

## 2. Application of animal protein hydrolysates in aquaculture

### 2.1. Application of seafood protein hydrolysates in aquaculture

The world's harvest of seafood has increased considerably in recent years, reaching about 158 million tonnes in 2012, of which 66.6 million (42.1% of the total) came from aquaculture (FAO, 2014). This increase is mainly due to a gradual increment in aquaculture output and for the coming decades it is expected that this production will be decisive to compensate for the stagnation of fishing catches and to satisfy the growing demand for seafood and fresh-water fish. The satisfaction of future demand for fish farming will largely depend on the availability of cheap and good quality feed in the sufficient quantities. Soybean meal is currently used as a protein source in aqua-feeds to reduce costs (Uran et al., 2009), although it contains antinutritional substances as phytic acid or lectins that affect growth and feed efficiency. In contrast, fishmeal does not contain antinutritional factors and is a much more appropriate protein source than soybean meal in aqua-feeds. In fact, aqua-feeds use to contain a minimum level of fishmeal in order to ensure an optimal content of amino acids and other nutrients needed for fish growth and flesh quality. However, the variations in the catches of wild pelagic fish (mainly anchovy) and the growing demand for fishmeal has pushed prices up during the last years (FAO, 2014; Tacon, Hasan, & Metian, 2011). This fact, and also the running

costs and the thermal damage induced by the drying process on protein quality and overall protein digestibility, have increased the interest in fish silage, protein hydrolysates and fermented products as alternatives of fishmeal in aqua-feeds. Fish silage has been used particularly in the feeding of fish farming (De Arruda, Borghesi, & Oetterer, 2007). However, production of silage requires the use of acids that might potentially destroy essential amino acids, particularly tryptophan, and reduce the nutritional value of the silage (Plascencia-Jatomea, Olvera-Novoa, Arredondo-Figueroa, Hall, & Shirai, 2002). Protein hydrolysates are produced with proteases under mild conditions and could be much more suitable than silage as a source of good quality protein in aqua-feeds (Santos et al., 2013).

#### 2.1.1. Effect of seafood protein hydrolysates on fish growth

The enhancement of growth rate is a particularly important economic parameter, as it can significantly reduce the time required to produce market-size fish. To increase growth rate, researchers have primarily used transgenic fish species that overexpress growth hormones, and have also experimentally treated fish with recombinant growth hormones. The administration of these hormones as a food supplement is known to be a viable method to enhance growth rates and feed conversion in cultured fish. However, the production and use of hormones is expensive and difficult, and there is concern over the safety of hormone-fed fish destined for human consumption. In this context, some aqua-feeds supplemented with seafood protein hydrolysates stimulate fish growth and are a cheap and natural alternative to growth hormones. This growth promoting effect can be largely attributed to the flavouring characteristics of diets including protein hydrolysates, which promotes a higher feed intake (Carvalho, Sá, Oliva-Teles, & Bergot, 2004; Chotikachinda, Tantikitti, Benjakul, Rustad, & Kumarnsit, 2013; Grey, Forster, Dominy, Ako, & Giesen, 2009; Ho, Li-Chan, Skura, Higgs, & Dosanjh, 2014; Kotzamanis, Gisbert, Gatesoupe, Zambonino Infante, & Cahu, 2007; Refstie, Olli, & Standal, 2004). As well, it is important to note that these diets are highly digestible and that facilitates the fast passing and absorption of peptides and amino acids through intestinal membrane (Aksnes, Hope, Høstmark, & Albrektsen, 2006; Wilson & Castro, 2010; Zheng, Liang, Yao, Wang, & Chang, 2012). Aksnes, Hope, Jönsson, Björnsson, and Albrektsen (2006) suggested that diets supplemented with seafood protein hydrolysates may contain low molecular weight compounds beneficial for growth and feed performance. Other theories suggest that protein hydrolysates contain molecules with ability to stimulate production of insulin-like growth factors (IGF-I and IGF-II) which may enhance fish growth. In this field, incorporation of fish protein hydrolysates in fish feed has been related to increments in growth performance, plasma IGF-I levels and liver IGF-I mRNA expression, whereas partial or total replacement of fish meal by plant-protein sources has been associated with low growth performance, low feed intake and low plasma IGF-I levels (Aksnes, Hope, Jönsson, Björnsson, & Albrektsen, 2006; Gómez-Requeni et al., 2004; Zheng, Liang, Yao, Wang, & Chang, 2013). These differences ascribed to the protein source result in changes in the plasma levels of the essential amino acids Lys and Met, nucleotides, anserine and taurine, and lead to significant differences in IGF-I and Growth Hormone (GH) regulation (Espe, Hevrøy, Liaset, Lemme, & El-Mowafi, 2008; Hevrøy et al., 2007).

During the last decades, extensive efforts have been focused on the use of microdiets as first feeds for most marine fish larvae, in such a way to completely or partially substitute live food organisms which production is costly and time consuming (Kolkovski, 2001). Microdiets represent a considerable saving in production costs and infrastructure, as well as offering nutritional consistency and off-the-shelf convenience. The formulation and manufacturing of microdiets have been improved during the last years and several commercial microdiets can be found in the market. However, marine fish larvae fed microdiets have not, at this stage, matched the growth and survival performances demonstrated by larvae fed live feeds such as rotifers and *Artemia*

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