Commentary

Fish silage hydrolysates: Not only a feed ingredient, but also a useful feed additive [Δ]

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ABSTRACT

Background: Processing of fish and shellfish may result in substantial amounts of by-products and unless they can be used as food, the most realistic option in most cases is the production of preserved feed ingredients. If large volumes are available, reduction to fishmeal and fish oil is the preferred technology. However, fresh by-products are most often available in insufficient quantities to justify production of fishmeal. Preservation by acid silage is, however, a simple and inexpensive alternative.

Scope and Approach: The purpose of this paper is to highlight that silage preservation of by-products using formic acid produces a protein hydrolysate that may function as a useful feed additive and not only an important feed ingredient. The fast growing global aquaculture industry is particularly in need of high quality feed ingredients and the focus in this paper is therefore on including acid protein hydrolysate in diets for fish and shellfish.

Key findings and Conclusions: The proteins in acid silage are largely hydrolysed to free amino acids and short-chain peptides. Studies have shown that moderate amounts of protein hydrolysate may successfully be included in fish feed and in some cases this leads to improved performance. In addition, the formic acid in the hydrolysate may contribute to the growth and well-being of fish, in particular under unfavourable microbiological conditions. This may encourage fish processors to preserve by-products using acid silage and feed producers to incorporate the products in the feed.

Keywords: Fish by-products, formic acid silage, peptides, growth promotor

1. Introduction

In 2012, 76.2 % of the 91.3 million tonnes (Mt) wild caught fish and all of the 66.6 Mt fish produced in aquaculture were estimated to have been used for human consumption (FAO 2014). These figures also includes crustaceans and other invertebrates and the word fish in this paper is used in accordance with this. The term "human consumption" is, however, not precise since fish are often processed to different degrees before being sold to wholesalers or retailers. Such processing, which mainly occurs on-board fishing vessels in industrial scale fisheries and in land-based processing facilities, may consist of deshelling, gutting, beheading, filleting, skinning and trimming. The fillet yield is species-dependent and is most often in the range of 30 – 50 % (Rustad, Storro, & Slizyte, 2011). Some of the by-products such as heads and off-cuts, may in certain cases be used for human consumption while the majority has traditionally been regarded to be of low value or as a problem and used as feed for farmed animals, as fertilizers or discarded (Olsen, Toppe, & Karunasagar, 2014). Although it is quite often suggested that by-products may be turned into high-value products we believe that these in most cases are not commercially viable and the most realistic utilization of by-products is to convert them into preserved feed ingredients if they cannot be used directly as food (Olsen *et al.*, 2014). The rapidly growing global aquaculture industries are in particular in need of high quality feed ingredients to reduce the amount fishmeal and fish oil produced from pelagic species in formulated feed (Tacon, Hasan, & Metian, 2011).

By-products from processing of fish especially when containing viscera, deteriorate very rapidly and will create unacceptable local pollution if not preserved properly at land-based processing sites. In addition, rapid preservation is also necessary if the raw materials is going to be used as high quality feed ingredients. Discarding of by-products from processing at sea does not usually create any problems unless it occurs close to land. This should however be avoided since it is a waste of resources. Unfortunately, older fishing vessels processing the catch on-board do not, in most cases, have facilities or space to preserve the by-products. Perhaps on-board processing vessels built in future should include equipment for preserving all the products, not only those intended for human consumption.

Use of fishmeal and oil technology is the traditional way of producing feed ingredients from pelagic fish and today the products are mainly used in feed for farmed fish. It has been estimated that 35 % of the available fishmeal in 2012 was based on fish processing residues (FAO, 2014). This technology is, however, a multistep, energy-demanding process which requires large amounts of fresh raw materials daily over a long period to justify the costs of establishing and running such a factory (Naylor *et al.*, 2009; Raa & Gildberg, 1982; Tatterson, 1982). It has been known for a long time that fresh by-products available in smaller amounts may instead be preserved by silage technology using short-chain organic acids. The proteins present in the silage will, to a large extent, be hydrolysed by endogenous acid proteases to small peptides and free amino acids (Espe *et al.*, 2015). The silage or the separated oil and protein hydrolysate may later be included in feed for farmed animals and fish (Gallardo *et al.*, 2012; Jackson, Kerr, & Bullock, 1984; Petersen, 1953; Raa & Gildberg, 1982; Tatterson, 1982; Whittemore & Taylor, 1976). Published works suggest that short chain organic acids like formic acid and peptides/amino acids when included in the feed may contribute to improved performance and growth of farmed animals, and possibly also of fish and crustaceans (Dibner & Buttin, 2002; Gilbert, Wong, & Webb, 2008; Martinez-Alvarez, Chamorro, & Brenes, 2015; Partanen & Mroz, 1999).

The objective of this Commentary is to draw attention to the fact that protein hydrolysate formed during the formic acid silage process is not only a simple way of providing important feed ingredients, but also that the short-chain organic acid, peptides and free amino acids in the hydrolysate may function as useful feed additives.

2. A brief overview of silage technology

Acid preservation is a simple and inexpensive way to preserve processing by-products and can be carried out virtually at any scale (De Arruda, Borghesi, & Oetterer, 2007; Raa & Gildberg, 1982; Tatterson, 1982). The raw materials are minced and acidified most commonly today with 2 – 3 % formic acid to reduce the pH to 4 or below preventing microbial growth. To stop lipid oxidation, an antioxidant, so far most often ethoxyquin, is mixed in the silage which can then be stored for an extended time (Arason, 1994; Raa & Gildberg, 1982). Combinations of organic acids like propionic acid and formic acid or an organic acid and a mineral acid may also be used (Arason, 1994; Hardy, Shearer, & Spinelli, 1984). However, if only mineral acids are used, the pH has to be around 2 in the silage to stop microbial growth and this requires increasing the pH by adding a base before including it in feed (Arason, 1994; Tatterson, 1982). After acidifying the by-products, a temperature dependent autolytic liquefaction will occur due to the action of endogenous proteolytic enzymes, mainly pepsins, present in the viscera. Without the presence of stomach containing viscera in the by-products, the autolysis will go on at a much slower rate, unless acid proteases are added (Raa & Gildberg, 1982). In 2014, 258,150 tonnes of by-products from processing of farmed and wild fish were preserved by silage technology in Norway (Richardsen, Nystøyl, Strandheim, & Viken, 2015). This silage production using formic acid with added antioxidant is carried out at many local fish processing plants along the coast and subsequently the silages are collected by trucks or boats and transported to a few centralized plants. Here, the volumes are large enough to economically separate the silage into an oil product and an aqueous phase containing hydrolysed proteins. The protein hydrolysate has a high water content and it is therefore evaporated to a dry matter content of 45 – 50 % before it is included in a formulated dry feed. According to one of the producers, about 4 - 5 % formic acid is found in the concentrated protein hydrolysate obtained from salmon by-products using silage technology (B. Dulavik, Hordafor, Norway, per. comm.). The oil and the concentrated protein hydrolysate from Atlantic salmon are used in feed for pigs, poultry and fish other than salmon while the products from wild whitefish by-products is used in feed for salmon (Olsen *et al.,* 2014).

One drawback with fish silage is the high water content which makes it difficult to use it directly in dry or moist feed (Madage, Medis, & Sultanbawa, 2015). The silage may however be used locally after drum-drying or co-drying with other feed ingredients like soybean-, feather- or poultry by-products meals or cereal brans (Dong, Fairgrieve, Skonberg, & Rasco, 1993; Goddard & Perret, 2005; Hardy *et al.*, 1984; Madage *et al.*, 2015; Nwanna, Balogun, Ajenifuja, & Enujiugha, 2004).

Fish silage may also be produced by fermentation using lactic acid bacteria like *Lactobacillus plantarum*, as a starter culture. However, since the fish by-products do not contain carbohydrates, a fermentable sugar such as molasses or fruit processing waste must also be added (Bower & Hietala, 2008; Dong *et al.*, 1993; Fagbenro & Jauncey, 1995). The lactic acid produced during the fermentation will reduce the pH in the silage and prevent growth of spoilage bacteria (Faid, Zouiten, Elmarrakchi, & Achkari-Begdouri, 1997). This is a more complicated silage production process than direct acidification since a starter culture must available, but it might be suitable in countries where fermentable sugars are readily available (Hernandez, Olvera-Novoa, Smith, Hardy, & Gonzalez-Rodriguez, 2011; Plascencia-Jatomea, Olvera-Novoa, Arredondo-Figueroa, Hall, & Shirai, 2002). The level of free fatty acids has been reported to be much higher in oil obtained from fermented silage than in oil from acid silage and this may limit the use in feed (Vidotti, Pacheco, & Goncalves, 2011).

3. Use of protein hydrolysate in fish feed

The successful use of fish protein hydrolysates from acid silage in aquaculture feed has been reported in several studies. Espe *et al*. showed that when less than 15 % of the fishmeal in fishmeal-based diets for Atlantic salmon (*Salmo salar*) was replaced by silage protein hydrolysate improved growth was obtained while higher inclusion levels lead to reduced growth (Espe, Sveier, Høgøy, & Lied, 1999). Studies on Japanese sea bass (*Lateolabrax japonicus*) suggested better growth when a similar amount of fishmeal was substituted with acid silage hydrolysate. Improved nonspecific immunity was also indicated in the same work (Liang, Wang, Chang, & Mai, 2006). More recently, Goosen *et al*. reported that low amounts of protein hydrolysate from acid silage in feed for Mozambique tilapia (*Oreochromis mossambicus*) resulted in excellent growth and possibly also increased phagocytic activity (Goosen, de Wet, & Gorgens, 2016). In the work of Ridwanudin & Sheen, it was observed that 50 % of fishmeal in the feed for orange-spotted grouper (*Epinephelus coioides*) could be substituted with 10 or 20 % acid silage protein hydrolysate combined with poultry by-product meal without affecting the growth (Ridwanudin & Sheen, 2014).

Several feeding trials have been carried out substituting fishmeal with different levels of protein hydrolysates produced from fish or fish by-products using commercial enzymes active at approximately neutral pH. In general, these studies showed that a low or moderate amount of hydrolysates may successfully be used in feed and in some cases result in improved feed intake, growth and other performances (Aksnes, Hope, Høstmark, & Albrektsen, 2006; Goosen, de Wet, & Gorgens, 2014; Hevrøy *et al.*, 2005; Khosravi *et al.*, 2015; Nguyen, Perez-Galvez, & Berge, 2012; Refstie, Olli, & Standal, 2004; Zheng, Xu, Qian, Liang, & Wang, 2014). The use of commercial enzymes in hydrolysing fresh by-products is, however, in most cases not an option locally at processing plants due to relatively small amounts of raw materials, the cost of enzymes and the cost of preserving the hydrolysates for example by drying. The cost of producing such hydrolysates will probably also limit the application in feed except perhaps in larval feed.

The mechanisms behind the positive effects of fish protein hydrolysate are not fully understood, but at least in diets containing a high content of plant proteins, a concentrated hydrolysate based on fish will supply free amino acids and non-amino acid nitrogen compounds with feed attractant properties. It is an excellent source of essential amino acids and taurine that is not found in plant based materials (Espe, Ruohonen, & El-Mowafi, 2012). It has also been suggested that the presence of a limited amount of free amino acids and short peptides may result in a more gradual absorption of the total amino acids in the feed (Refstie *et al.*, 2004). A recent *in vitro* study showed that free amino acids and short-chain peptides obtained from acid silage made from salmon by-products might have potential to improve health and welfare of farmed fish during stressful periods (Espe *et al.*, 2015).

4. Effects of short-chain organic acids in feed

Use of formic acid is not only a simple way to preserve by-products from processing of fish, but the presence of this acid in the protein hydrolysate used in feed may also contribute to the improved well-being and growth of the farmed animals and fish. Short-chain organic acids like formic acid, are among the candidates that may be used as growth promotors in feed for poultry and pigs instead of banned non-therapeutic antibiotics (Defoirdt, Boon, Sorgeloos, Verstraete, & Bossier, 2009; Dibner & Buttin, 2002; Khan & Iqbal, 2016). Short-chain organic acids have apparently been applied in diets for pigs for many years (Dibner & Buttin, 2002) and recent published feeding trials confirm this (Eisemann & van Heugten, 2007; Opheim, Strube, Sterten, Øverland, & Kjos, 2016). A main mechanism behind the growth promoting properties is the antimicrobial effects in the upper part of the gastrointestinal track of animals. The short-chain organic acids are weak acids with a pKa between 3 and 5 and the undissociated form may diffuse through the cell membranes of microorganisms. Once inside the cells, the organic acids will dissociate resulting in a lower intracellular pH which affects enzyme catalysed reactions and transport systems. The protons produced have to be exported out of the microorganisms and this use of energy has often been regarded as the major antimicrobial mechanism. However, the accumulation of anions inside the bacteria may also be involved (Defoirdt *et al.*, 2009; Dibner & Buttin, 2002; Ricke, 2003; Ringø *et al.*, 2016). It has been suggested that pathogenic Gram-negative bacteria with more accessible cell membranes, are affected more by short-chain organic acids than Gram-positive microorganisms like lactic acid bacteria. The latter group is also favoured by the slightly more acid conditions in the feed. (Dibner & Buttin, 2002; Ringø *et al.*, 2016). In addition, organic acids in feed for pigs may also improve absorption of certain minerals like calcium and phosphorus (Partanen & Mroz, 1999).

The number of studies published on the effects of including short-chain organic acids in aquaculture feed have been increasing in recent years. The results obtained have, however, not been consistent and this may be because in some cases different acids are used or that in other studies salts of the acids are applied thus providing no extra protons in the feed (Ringø *et al.*, 2016). Other factors that may influence the results are, for example, the buffering capacity of the feed and environmental microbial conditions (Dibner & Buttin, 2002).

Chuchird *et al*. reported no effect on growth of Pacific white shrimp (*Litopenaeus vannamei*) fed a diet 0. 3 or 0.6 % formic acid added. However, improved survival was observed in the formic acid groups when challenged with *Vibrio parahaemolyticus* (Chuchird, Rorkwiree, & Rairat, 2015). Tiger shrimps (*Penaeus monodon)* given a feed containing 2 % of a commercial organic acid mixture had lower cumulative mortality than the control after exposure to *Vibrio harveyi* (Ng, Koh, Teoh, & Romano, 2015). In a separate experiment it was reported that shrimps fed a diet with or without the organic acids had similar growth, but the organic acid group had apparently improved feed utilization. In another study by Koh *et al*., it was demonstrated that when a dietary organic acid blend was fed to red hybrid tilapia (*Oreochromis* sp.) higher resistance against *Streptococcus agalactiae* was obtained (Koh, Romano, Zahrah, & Ng, 2016). Prior to the challenging test, the diets with the organic acid blend resulted in significantly higher phosphorus digestibility, but no significantly better growth was observed. Other studies have also shown that short-chain organic acids in the feed of organisms in aquaculture may protect against pathogenic bacteria. A study by Defoirdt *et al*. showed that 20 mM of different short-chain organic acids protected the brine shrimp *Artemia franciscana*, that are used as live feed for fish larvae, against *Vibrio campbelli* (Defoirdt, Halet, Sorgeloos, Bossier, & Verstraete, 2006). Recently, researchers reported improved growth and disease resistance against *Aeromonas sobria* when Nile tilapia (*Oreochromis niloticus*) was given a diet containing a commercial mix of formic acid, propionic acid and calcium propionate (Reda, Mahmoud, Selim, & El-Araby, 2016). In a feeding trial with Mozambique tilapia conventional fish oil in the diet was replaced with fish silage oil made from rainbow trout by-products (Goosen, de Wet, Gorgens, Jacobs, & de Bruyn, 2014). Inclusion of the silage oil had antimicrobial effects in the feed and gastrointestinal tract and it was suggested that this was due to the presence formic acid in the crude silage oil.

In a paper on farming of South African abalone, it was reported that a combination of 1 % formic acid and 1 % acetic acid in the feed significantly increased growth performances during a 4 month feeding trial (Goosen, Gorgens, De Wet, & Chenia, 2011). Gao *et al*. included a mix of sodium formate and sodium butyrate in the feed for rainbow trout in a 50 days feeding trial, but could not find any improvement in growth rate or feed utilization (Gao, Storebakken, Shearer, Penn, & Overland, 2011). Similarly, when sodium salts of acetic, propionic and butyric acids were included in feed for Atlantic salmon no significant effects were observed on specific growth rate, mortality or digestibility of macronutrients (Bjerkeng, Storebakken, & Wathne, 1999).

Potassium diformate (KDF) is a complex of formic acid and potassium formate and was the first compound approved by the European Union in 2001 as possible a non-antibiotic growth promotor in feed (Zhou *et al.*, 2009). The results from studies on use of KDF in feed for farmed fish have however also been divergent. Castillo *et al*. investigated the effects of KDF, calcium lactate and citric acid in feed for juvenile red drum (*Sciaenops ocellatus*) (Castillo, Rosales, Pohlenz, & Gatlin, 2014). They concluded that all 3 additives seemed to improve growth performance and suggested that at it least in part could be due to increased activity of digestive enzymes. Zhou and co-workers included KDF in diets for hybrid tilapia (*Oreochromis niloticus x O. aureus*), but did not find any effects on growth or feed conversion (Zhou *et al.*, 2009). It did, however, affected the gut microbiota in a different way than a control diet containing antibiotics. Recently, researchers included KDF in feed for Nile tilapia and found that 0.2 and 0.3 % significantly improved growth performance during a 60 day feeding trial. At the end of the trial, all groups of fish were challenged orally with *Aeromonas hydrophila* and the recorded mortally was lower in the groups with dietary KDF than the control (Elala & Ragaa, 2015).

There are limitations on how much formic acid that can be given to fish without resulting in negative effects on growth and health status. Mach *et al*. fed formic acid silage, based on whole fish or whole crabs, as the major protein source to juvenile cobia (*Rachycentron canadum*). The crab silage had to be preserved by as much as 8.5 % formic acid because of the high buffering capacity of the shells while only 2.5 % was needed for the fish. The cobia given a fish silage diet resulted in almost similar growth as cobia fed raw fish and crabs during a 6 week feeding trial. However, the cobia fed with a crab silage diet or a mixture of crab and fish silages hardly grew at all and also experienced liver damage and substantial mortality (Mach, Nguyen, & Nortvedt, 2010).

5. Conclusions

In the era of Blue Growth there is increasing awareness that discarding of by-products from processing of fish is a waste of resources and therefore unacceptable. This also applies to unavoidable by-catch not fit for humans that is caught during harvest of targeted fish species. Preservation of such fresh raw materials by the use of formic acid is a simple and inexpensive technology that can be applied on virtually any scale. The acid silage or the oil and protein hydrolysate obtained from the silage, are useful ingredients when included in moderate amounts in feed for farmed animals and fish. The presence of free amino acids and short-chain peptides in the protein hydrolysate may also function as a feed additive promoting growth performance, not only as a source of amino acids. Similarly, the formic acid in the hydrolysate could contribute to the growth and well-being of fish and animals, in particular under unfavourable microbiological conditions.

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