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Investigation of pharmaceuticals in processed animal by-products by liquid chromatography coupled to high-resolution mass spectrometry

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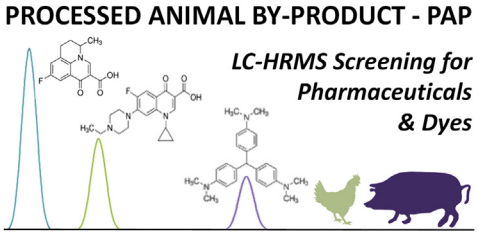
HIGHLIGHTS

- Investigation of pharmaceuticals in commercially processed animal proteins (PAP).
- Broad screening for around 1000 permitted and prohibited residues in salmon feed.
- Pharmaceutical agents and marker dyes in PAPs might be introduced in novel aquafeeds.
- Current interest for control authorities in food safety and public health.

GRAPHICAL ABSTRACT



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ABSTRACT

There is an on-going trend for developing more sustainable salmon feed in which traditionally applied marine feed ingredients are replaced with alternatives. Processed animal products (PAPs) have been re-authorized as novel high quality protein ingredients in 2013. These PAPs may harbor undesirable substances such as pharmaceuticals and metabolites which are not previously associated with salmon farming, but might cause a potential risk for feed and food safety. To control these contaminants, an analytical strategy based on a generic extraction followed by ultra-high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS) using quadrupole time-of-flight mass analyzer (QTOF MS) was applied for wide scope screening. Quality control samples, consisting of PAP commodities spiked at 0.02, 0.1 and 0.2 mg/kg with 150 analytes, were injected in every sample batch to verify the overall method performance. The methodology was applied to 19 commercially available PAP samples from six different types of matrices from the EU animal rendering industry. This strategy allows assessing possible emergent risk exposition of the salmon farming industry to 1005 undesirables, including pharmaceuticals, several dyes and relevant metabolites.

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1. Introduction

Estimated global production of farmed salmon (including *Salmo salar, Oncorhynchus kisutch, Oncorhynchus tshawytscha*) in 2010 was approximately 1.8 million metric tonnes with expected production of around 2.9 million metric tonnes in 2020 (Tacon and Metian, 2008). Furthermore, consumption of all species of farmed fish is expected to exceed that of feral fish (FAO, 2014). Traditional commercial feed for farmed salmon and rainbow trout are based on marine feed ingredients extracted from pelagic fish stocks. Concern of pressure on feral fish stocks and limited fish meal and fish oil availability to supply a rapidly growing aquaculture industry has led to the development of aquafeeds in which marine resources are replaced with alternative feed ingredients to develop more sustainable marine aquafeeds (Tacon and Metian, 2008; Torrissen et al., 2011; Waagbo et al., 2013). On a global basis, processed animal products (PAP) from the rendering industry constitute one of the largest sources of high quality animal protein available for animal feed production (Toldra et al., 2012). The use of PAPs such as feather meal, poultry by-product meal, pork meat and bone meal, and poultry and pork blood meal have been shown to be relevant nutritional replacements of fish meal for many cultured fish species including salmonids (Bransden et al., 2001; Rosenlund et al., 2001; Yanik et al., 2003; Rahnema and Borton, 2007; Wilson et al., 2007; Friesen et al., 2008; Poppi et al., 2011; Burr et al., 2012). However, following the peak outbreaks of transmissible spongiform encephalopathies (TSE) in the UK in the early 1990's, the use of PAPs in all animal feed was banned in the European Union (EU) in 2001 (EC, 2001; EC, 2003a). Following a bovine spongiform encephalopathies (BSE) risk assessment by the European Food Safety Authorities (EFSA) (EFSA, 2011), the EU set out a working plan for the re-authorization of the use of non-ruminant PAPs in animal feed, initially for aquafeeds in 2013 (EC, 2013a).

In EU, the use of veterinary drugs is regulated according to EU legislation. Permitted residue levels of pharmaceutical substances (EC, 2009a) and mandatory monitoring activity (EC, 1996) have been established for all food producing animals. These regulations affect the legal addition of pharmaceuticals to animal feed, including the prohibition of substances in feeds that have hormonal or thyreostatic action as well as β-agonist (EC, 2003b). In addition, the supplementation of coccidiostats or histomonostats as feed additive causing unavoidable carry-over in non-target feed is regulated in the EU (EC, 2009b). In Norway, the use of pharmaceuticals in Atlantic salmon farming is under strict control and all sales of pharmaceuticals in animal farming have to be reported to the Norwegian Food Safety Authority. Open and reliable statistics on the consumption of therapeutic agents in aquaculture is only available from some nations. As an example, the registered use of pharmaceuticals in Norwegian aquaculture in 2014 included the antimicrobials florfenicol and oxolinic acid, the anti-parasitic agents azametifos, cypermetrin, deltametrin, diflubenzuron, teflubenzuron, emamectin, praziquantel and hydrogen peroxide, as well as the anaesthetic agents benzocaine, metacaine and isoeugenol (available at http://www.fhi.no/artikler/?id=114175) (Grave et al., 2008).

The use of PAPs in salmon feed can potentially introduce new chemical undesirables that have not been previously associated with farmed Atlantic salmon. Of special interest are pharmaceuticals such as antibacterials, which are used in all sectors of farming, including poultry and swine production (Toldra et al., 2012). Other pharmaceutical agents used in terrestrial farmed animals include antiparasitic agents such as coccidiostats, which are added to poultry feed to cope with protozoa as well as enhancing animal growth (Ruff, 1999; Chapman, 2014). It should be kept in mind that the number of pharmaceuticals used in terrestrial animals is more diverse than those used for fish, and that there are substantial differences in the prescribing patterns of veterinary agents between countries and regions (EMA, 2011). Earlier screening studies on feather meal from the USA (10 samples) and China (2 samples) for 59 pharmaceuticals, showed the presence of six classes of antimicrobials including fluoroquinolones, tetracyclines, folic acid antagonists, and streptogramins (Love et al., 2012). Two of the main antibacterials were enrofloxacin and ciprofloxacin, and studies in EU also showed the occurrence of these substances in poultry and pork PAPs (Berntssen et al., 2014). In addition to potentially direct adverse health effects of pharmaceutical residues in food and non-compliance with food legislation, concern has been raised for the development of resistant pathogenic microorganisms when exposed to low non-clinical residue levels (Reig and Toldra, 2009; Blazquez et al., 2012; Gillings, 2013).

The list of prohibited and allowed antibiotics in the EU includes many substances which may co-occur in samples of animal origin. This requires the use of comprehensive screening methods for detection of these substances (Bohn et al., 2013; Nácher-Mestre et al., 2013; Masiá et al., 2014; Turnipseed et al., 2014; Boix et al., 2014). Therefore, generic sample treatment is advisable to cover as many compounds as possible during the experimental process. High resolution mass spectrometry (HRMS) allows the acquisition of full-spectrum accurate-mass data using analyzers such as quadrupole time-of-flight (QTOF). The coupling of liquid chromatography (LC) with QTOF MS is nowadays one of the most efficient analytical tools to face the investigation of large number of medium-high polar organic contaminants and residues in food-safety and related fields (Ibañez et al., 2012). The present study reports a wide-scope qualitative screening approach for 1005 permitted and prohibited pharmaceutical residues (also including metabolites) and dyes in commercially available EU produced PAPs with potential use in aquafeed.

2. Material and methods

2.1. Reagents and chemicals

150 reference standards were purchased from Acros Organics (Geel, Belgium), Aventis Pharma (Madrid, Spain), Bayer Hispania (Barcelona, Spain), Cerilliant (Round Rock, TX, USA), Fluka (Buchs, Switzerland), Dr. Ehrenstorfer (Augsburg, Germany), Fort Dodge Veterinaria (Gerona, Spain), National Measurement Institute (Pymble, Australia), Riedel-de Haën (Seelze, Germany), Sigma Aldrich (St Louis, MO, USA), Vetoquinol Industrial (Madrid, Spain), and Witega (Berlin, Germany). All reference materials had purities higher than 98% (w/w), except for marbofloxacin and pefloxacin, which had purities higher than 93%.

HPLC-grade water was obtained from a MilliQ water purification system (Millipore Ltd., Bedford, MA, USA). HPLC-grade methanol, HPLC-supragradient acetonitrile and acetone for residue analysis were purchased from Scharlab (Barcelona, Spain). Formic acid (HCOOH, content > 98%), ammonium acetate (NH_4Ac, reagent grade) and sodium hydroxide (NaOH, reagent grade) were supplied by Scharlab. Leucine enkephalin was purchased from Sigma Aldrich.

2.2. Samples

Commercially available PAPs were studied in this work. A total of 19 available non-ruminant PAPs from 6 different types of matrices were provided by the European Fat Processors and Renderers Association (EFPRA). The samples had been produced in different rendering factories. All PAPs were produced according to EU regulation for PAPs intended for use as feed ingredients (EC,