The utilisation of European processed animal proteins as safe, sustainable and circular ingredients for global aquafeeds

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Woodgate, S.L., Wan, A.H., Hartnett, F., Wilkinson, R.G. and Davies, S.J. (2022) 'The utilisation of European processed animal proteins as safe, sustainable and circular ingredients for global aquafeeds', *Reviews in Aquaculture*.

27 February 2022

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Running head: Safe processed animal by-products for aquaculture

Abstract

The global increase in seafood demand has resulted in significant growth in aquaculture production in a wide range of aquatic species. Consequently, this has led to an industry-wide need to find sustainable feed ingredients that would meet the nutritional requirements used in aquafeeds. The associated dependency on plant by-products as the major alternatives have brought concerns to aquaculture through associated carbon footprint, increase deforestation and arable land use to meet the demands of plant proteins and oils, and the constraining effects of plant by-products has on farmed aquatic animal growth and health. Animal by-products (ABP) are produced as a direct consequence of terrestrial animal production and the associated meat processing industries. The link between feeding meat and bone meal (MBM) and bovine spongiform encephalopathy (BSE) outbreak in Europe during the 1980s, resulted in a ban of its use animal feeds. This led to a radical overhaul of the rendering industry, including the use of only low-risk ABP and the development of rendering processes to reduce the risk of prions that causes BSE to enter the food chain. The resulting processed animal proteins (PAPs) are considered safe to be used in farmed animal feeds. This review examines how ABP production has changed due to the BSE outbreak, leading to the current commercially available PAP products for aquafeed use. We evaluated how these products can be effectively used as viable protein sources in aquaculture and examine their limitations and the potential advancements that could lead to a more circular food production system.

Keywords: Animal By-Products, Processed Animal Proteins, Aquafeed, Feed Ingredient Legislation, Feed Safety, Alternative Proteins.

1. Introduction

Intensively produced carnivorous finfish species (e.g., salmonids, seabass, and seabream) require high protein content diets, which needs to have a balanced amino acid profile, be energy-rich, and use highly digestible ingredients. To meet the protein demand from aquaculture, wild-caught low valued fish (e.g., anchovy, smelt, sand eels and pollock) are processed into fishmeal and oils to produce these highly nutritional aquafeeds. Fishmeal is often considered as the 'gold standard' for providing the protein component in many aquafeed formulations. This is related to the product being stable with a high biological value for protein and essential amino acids and readily accepted by the feeding fish due to its excellent palatability attributes. However, this has attracted a negative image to the aquaculture industry due to the impact on wild fish stocks and marine biodiversity[1]. Couple this with the yearly increase in global fish aquaculture production, which was estimated to be >80 million tonnes in 2016[2], there is an urgent need to address different strategies for more advanced feed formulations for aquaculture to alleviate the use of marine ingredients and address the fish in: fish out ratio often raised as a key issue for sustainably expanding aquaculture production levels[3].

Alternative proteins such as plant-based proteins including various legumes such as soybean meal and cereals have helped mitigate the use of marine-based proteins in contemporary fish diets. This has subsequently led to global aquaculture to further expand[4]. However, with increasing demand for plant proteins for animal feed production, has also led to environmental impact concerns over deforestation, land use displacement, and eutrophication[5,6]. Furthermore, sustainability issues are associated with the use of fossil fuels and non-renewable fertilisers, such as phosphorus[7,8].

Plant proteins have also been critiqued for their negative impact on animal health and growth in farmed animals, including fish[9]. These adverse effects are often caused by antinutritional factors found in plant by-products that include saponins, phytic acid, tannins, lectins, glucosinolates, erucic acid, and protease inhibitors. The compounds act to hinder nutrient bioavailability by inhibiting nutrient digestion and absorption through the gut but can also compromise fish intestinal health and overall animal welfare[10]. Recently, Cottrell *et al.* (2020) reported how global adoption of novel aquaculture feed ingredients (e.g., insects, algae, single-cell proteins) could substantially reduce the demand for fishmeal and fish oil by 2030[11]. Although the authors provided a range of examples, they failed to include an important feed ingredient sector that provides a reliable and substantial source of high-quality protein, animal by-products (ABP).

For many years, processed ABPs have offered an invaluable and viable protein source to the animal feed sector including aquaculture feeds for numerous species. In aquaculture, there has been a long history of using ABPs such as poultry meat meal, feather meal, MBM and whole dried blood as well as plasma proteins in several fish species including gilthead seabream, rainbow trout, Australian silver perch, African catfish, tilapia, as well as many others[12-17]. In a recent review on the risk assessment of aquaculture feeds, Glencross *et al.* (2019) reported on a spectrum of viable feed commodities including processed animal proteins (PAPs) from land animals[18]. The review highlighted their distinction from plant protein sources such as soybean meal. However, the rendering industry in particular Europe has made significant advances in processing technology that was not elaborated further, such as improving PAP value, efficacy, and safety for use in aquaculture.

Processed ABPs offer nutrient profiles that are competitive in their contribution towards meeting the fundamental requirements of farmed fish species. As shown Table 1, it illustrates a range of animal proteins that have the potential for use in aquafeeds. The table is split into two sections, pre- and post- 2002 as the terminology for animal proteins altered when Regulation (EC) 1774/2002 (EU 2002b) was enacted. Prior to 2002, the term PAP did not exist and most of the European animal proteins were called meat and bone meal (MBM). Post-2002, PAP is the description assigned to processed animal proteins (approved for use in animal feeds). Animal proteins may include balanced protein and good essential amino acid profiles, with additional bioavailable macro and trace elements including Ca, P, Mg, Fe, Mn, Zn, and Cu[19]. In particular, the essential amino acid (EAA) profile within poultry by-product meal conforms more favourably to fishmeal than soybean meal, making it a highly desirable replacement for fish and soybean meal. The amino acid composition and related nitrogenous nutrients in feedstuffs for animal diets were comprehensively reviewed by Li & Wu (2020)[20]. Of note, poultry by-product meal, and spray-dried poultry plasma were highlighted as substantial sources of carnosine and anserine that display antioxidative activity and could protect high lipid aquafeeds prone to lipid oxidation[21-22].

Table 1: Chemical composition of meat and bone meal (MBM, Pre-2002), and processed animal proteins (PAPs, post-2002, g kg^{-1}).

Nutritional composition	**Pre-2002**	**Post-2002**					**Soybean meal[26]**	**Fishmeal (LT94) [26]**
	Meat and Bone Meal[23-24]	**Poultry PAP25[,25,26]**	**Porcine PAP[27]**	**Hydrolysed Feather meal[28]**	**Blood meal[26]**	**Haem meal [6]**		
Dry matter*	935	947-941	964-978	930	890	908	947	926
Protein	529	564-620	416-616	850	800	909	500	730
Lipid	175	107-166	96-118	60	10	28	8	119
Ash	250	170-256	183-437	28	44	31	73	133
Calcium	57	-	52-160	-	-	-	-	-
Phosphorus	28	-	30-77	5	2	-	-	-
Amino acids								
Threonine	15.9	22.4-25.6	11.4-20.7	48	38	33.1	19.0	31.0
Cysteine/Cystine	6.2	5.6	1.5-5.3	41	14	-	-	-
Valine	20.1	24.9-28.6	-	55	52	84.7	25.4	31.6
Methionine	7.1	10-14.3	4.9-9.3	5	10	7.4	6.9	19.5
Isoleucine	13.3	18.2-21.6	-	41	8	5.5	21.3	25.6
Leucine	30.8	38.0-43.5	-	71	103	123.3	36.4	50.4
Tyrosine	10.2	16.9	-	3	10	-	-	-
Phenylalanine	17.0	22.9-23.1	-	432	51	65.4	24.4	27.7
Lysine	25.7	37.2-38.3	19.7-32.5	21	69	82.8	30.9	52.5
Histidine	8.9	11.4-14.7	-	17	31	69.0	7.7	17.3
Arginine	36.2	40.7-41.7	-	65	24	36.9	36.5	41.4

[23]MAFF (1986); [24]Wang and Parsons (1998); [25]Liland *et al.* (2015); [26] Davies *et al.* 2019.[27]Van Krimpen *et al.* (2010); [28] Stone, 2009. * wet weight.

This review will uniquely focus on the effect of how the neurodegenerative disease called bovine spongiform encephalopathy (BSE) directly impacted the European rendering industry. It will explain the subsequent development of safeguards against its reoccurrence, and the re-introduction of valuable and safe protein sources back into animal feeds. The review will further focus on the impact of PAPs in aquafeeds within primarily a European perspective, but mindful of global significance and its wider impact. Furthermore, the objective of this review will be appraising the value of ABPs in aquafeeds and their nutritional effects on different farmed finfish species. Overall, how ABPs can form part of a circular economy in seafood production, but also maintain consumer confidence.

2. Rendering and Animal By-Products

2.1. The rendering industry

Rendering is a global industry that has been in existence since humans started to harvest animals to provide meat for food[29]. Rendering is the term used to describe the processing of animal by-products (ABP) from land farmed animals. In Figure 1, the diagram describes the rendering process, whereby ABPs are indirectly heated using high-pressure steam (within closed tubes or discs) produced by the combustion of mainly fossil fuels, such as oil, coal, or natural gas. The heat produced evaporates the water and sterilise the material biomass, followed by the separation of the (protein-rich) solid fraction from the liquid phase/rendered fat. The solid material is a high protein product, typically termed as MBM (meat and bone meal), and the liquid (>40 °C) is rendered fat, commonly termed as tallow. However, it is important to understand the terminology differences between on one hand the EU, and on the other, the ‘non-EU’, i.e., the rest of the world. In the former, PAP is an exclusive EU term that is used to describe processed animal protein (usually with a prefix identifying ingredient or species), that is suitable for use in animal feed, In the latter, MBM is a global term used to describe animal protein derived from mammalian species. However, poultry derived protein meals are termed either as poultry meal, poultry meat meal or poultry offal meal. While blood meal and (hydrolysed) feather meal is typically described as such.

In the early development of the rendering system as an industrial process most operated as batch systems, whereby all the heating occurred within a batch cooker[30]. As processes began to consolidate, starting in the early 1960s, continuous, more efficient systems were developed by equipment manufacturers[31]. The continuous systems, in principle, improved the efficiency of heat treatment of the ABP to achieve, evaporation and sterilisation. Some of the different processing equipment designs that were developed and adopted within Europe to achieve the principles shown in Figure 1 are described by Woodgate and Van der Veen, (2004)[32]. To put the production of animal proteins into context, the total global production of animal protein was estimated at approximately 15 million tonnes[33], which compares with other proteins used in animal feeds, such as soybean meal (SBM) 315 million tonnes[34] and fishmeal, 6 million tonnes[35].

2.2 Evolution of the European rendering industry

Since the first emergence of bovine spongiform encephalopathy (BSE) in the UK in 1986, many changes have occurred within the rendering and animal by-products industry, particularly in the EU. The role of rendering in relation to the BSE epidemic, the development of EU animal by-product legislation and the reintroduction of rendered products into animal feeds are described by Woodgate and Wilkinson (2021)[36]. This paper describes the BSE epidemic in detail and places the rendering industry in context through the direct and indirect legislative changes resulting from Europe from 2001/2.

In brief, the changes that occurred in Europe as a consequence of the BSE epidemic can be summarised as follows; In 1996, a link between BSE in cattle and Creutzfeldt-Jakob disease in young adults was confirmed[37]. The assertion that BSE was linked to a human disease resulted in the recognition that the rendering industry was intricately linked to the food supply chain. As a result of the evolution of evidence from research and public information, a number of landmark EU regulations were approved:

(i) Regulation (EC) 999/2001[38], known as the 'Transmissible Spongiform Encephalopathy' (TSE) Regulation. This regulation immediately prohibited the use of all types of animal proteins in all feeds for animals (including fish species) raised for human consumption

(ii) Regulation (EC) 178/2002[39], known as the 'Food Law' regulation. This law relates to the understanding that the rendering industry is effectively a link in the food chain including the use of feeds for aquatic species.

(iii) Regulation (EC) 1774/2002[40], known as the 'Animal By-Products' (ABP) regulation. This regulation effectively limits the level of risk (ABP) that can be used in the production of animal protein for use in animal feeds, including aquafeed.

The first regulation addresses the types of ABP and animal feed-related issues, the second general food legislation, and the third with the categorisation of ABP and their associated processing standards. All three regulations were designed to complement each other in order to eradicate BSE in the first instance, and then by future amendment of the regulations, allowing for a limited reintroduction of animal proteins into animal feeds.

EU's regulation 999/2001[38] enacted the EU 'feed ban' that 'temporarily' prohibited the use of all animal proteins (excluding fishmeal) in the diets of all animals used for food production. This regulation also contains the legislative mechanism for the re-introduction of animal proteins into the food chain as and when risk analysis determines that safe conditions have been met. This comprehensive regulation also introduced the concept of specified risk material (SRM), as tissues from ruminant animals (cattle, sheep, and goats), considered to contain high levels of BSE or scrapie infectivity which present an enhanced risk of TSE transmission. Accordingly, the products derived from SRM and deadstock ABP were permanently prohibited as ingredients in farm animal feeds.

Regulation (EC) 178/2002[39] set out three main themes underpinning subsequent ABP risk reduction, i.e., 'safe sourcing', 'safe processing', and 'safe use' in the new legislation. This regulation also laid down the legal basis for the formation of the European Food Safety Authority (EFSA) and confirmed that the rendering industry was an important part of the 'food chain'. In practice, the regulations that followed focused on the risk associated with different categories of ABP, processing methods, and the use of rendered products, such as disposal or use in animal feeds, including their utilisation in aquaculture feeds within Europe. EU Regulation 1774/2002[40], laid down the definition of the three categories of ABP (including fishery by-products): Category 1, 2, and 3 (Table 2).

Table 2: Definition and summary of animal by-products (ABP), EU Regulation 1774/2002[40].

Category	Sources	Disposal/Processing	Derived product applications
1	> Bovine & Ovine Specified Risk Material (SRM), > Fallen stock including SRM. > Animals/organs containing residues of veterinary drugs	> Rendering followed by -incineration or, -combustion of products	> Energy > Biofuel
2	> Dead stock: All species including aquaculture. > Slaughterhouse ABP's rejected at post-mortem	> Validated rendering process > Anaerobic digestion/compost*	> Biofuel > Fertiliser*
3	> From animals passing ante-mortem inspection as 'fit for human consumption' and slaughtered in a slaughterhouse	> Anaerobic digestion/compost** > Validated rendering process	> Biofuel > Fertiliser** > Pet food > Animal feed***

*Subject to pre-processing by sterilisation under (3 bar) pressure

**Subject to digestion/composting validation conditions

***Subject to EU Regulations. Currently non-ruminant PAP approved for Aquaculture

This EU regulation also describes the processes that should be applied to yield the categorised product, the possible uses of processed products, and introduced the term 'derived products' to describe the products produced by an approved process. As a result of this regulation, an important terminology change occurred within the EU. Regulation EC No 1774/2002[40] defines that the protein meal derived from Category 1 and 2 ABP is termed meat and bone meal 'MBM'. The protein meal from Category 3 ABP is termed 'Processed Animal Protein' (PAP). Interestingly, the same processing principles (and indeed process equipment) being used for processing terrestrial ABPs is also applied to marine fisheries products, i.e., fishmeal and oil[41,42]. Accordingly, the EU regulations consider that the term PAP applies both to the animal proteins derived from both terrestrial and aquatic species. However, to try and avoid confusion between different types of PAPs, the feed industry adopted the term ‘land animal proteins’ (LAPs) to differentiate these proteins from the PAP produced from fishery by-products. In Table 3, it summarises the important differences in the terminology used to define and differentiate PAPs of different origins, including the source of ABP (e.g., by-product, blood, feather) and species of origin (e.g., Chicken, Turkey, Duck [Poultry-mixed], Porcine). In addition to the terminology definitions, Regulation EC No 1774/2002[40], also contained information on process validation and hazard analysis and the use of critical control points (HACCP) analysis to ensure compliance. Importantly, this regulation approved, subject to strict controls, such as no intra-species recycling, the use of PAPs in animal feeds in the future.

Table 3: Commercially available processed animal proteins (PAPs).

Animal Proteins	**Processed Animal Proteins (PAPs)**		
Source	Land Animal By-Product		Fishery By-Products
Type of PAP	Ruminant PAP	Non-ruminant PAP	
	Bovine PAP	Poultry PAP*	Fishmeal (mixed)* Fishmeal (species specific)*
	Ovine PAP	Porcine PAP*	Hydrolysed fishmeal*
	Mixed ruminant PAP	Mixed non-ruminant PAP*	
	Blood meal PAP	(Poultry) Blood PAP*	
		(Porcine) Blood PAP*	
		Blood Haemoglobin PAP*	
		Blood Plasma PAP*	
		(Poultry) Feather PAP*	

*Approved for use in aquafeeds in Europe

2.3 The rendering industry as part of the food chain

The BSE epidemic highlighted the position of rendering and rendered products in the human food chain by way of their use in feedstuffs for an animal raised for human consumption including within fish feeds. The focus for the re-authorisation of the use of rendered products in animal feeds therefore centred on two aspects: safety and risk. The assertion was that PAPs were safe to be used in animal feeds if they were subject to the conditions laid down in EU regulations 999/2001 and 1774/2002.

In 2005, a quantitative risk assessment (QRA) conducted by European Food Safety Authority (ESFA), confirmed that ruminant protein within animal feeds was the main risk factor in animal feeds. Accordingly, the QRA determined that if animal proteins were to be included in animal feed, in compliance with EU regulations, the risk of a new BSE epidemic was negligible if no ruminant protein was present, and the intra-species recycling of non-ruminant protein was maintained[43]. Also, in 2005, blood PAPs derived from non-ruminants was re-authorised in the EU, partly because of their low-risk nature and because blood meals did not contain any bone fragments, the key regulatory control at the time. Since 2009, the official controls of animal ingredients in animal feeds used a light microscopy method for determination of feed contamination by determination of the presence or absence of bone fragments, (EC) No 152/2009[44]. This method was able to distinguish between bones of aquatic and land animal species, such that fishmeal could be discriminated from (non-approved) LAP's and therefore approved for use in animal feeds. Although feather meal (PAP) was considered to be a very low-risk feed ingredient, it continued to be prohibited because of the presence of poultry bone fragments, which, could not be discriminated from ruminant bones by the official method. Nonetheless, it is interesting to note that the utilisation of animal fats in animal feeds was never prohibited, largely due to the TSE inactivation trials which concluded that rendered fats contained no TSE infectivity[32]. Consequently, rendered fats such as poultry fat, beef tallow and pork lard remained as potential ingredients for use in all compound feeds, including those for fish, and could therefore legally be used in aquafeed diets.

Beginning in 2009, Regulation (EC) 1069/2009[45] and Commission Regulation (EC) 142/2011[46], updated Regulation (EC) 1774/2002[40], but essentially the purpose of the 2009 and 2011 regulations remained the same as the 2002 regulation. In 2013, non-ruminant PAPs were re-authorised for use in aquaculture feeds in Europe, by legislation that followed the successful development and validation of a polymerase chain reaction (PCR) technique for the determination of ruminant protein in complete feeds[47]. A regulation was subsequently approved by Commission Regulation (EC) No 51/2013[48], which confirmed the PCR test for ruminant protein as an official method. At the same time, Commission Regulation (EC) No 56/2013[49] authorising the use of Category 3 non-ruminant PAPs in aquafeeds was also approved as the main criteria was to prevent ruminant proteins from entering the aquafeed sector of the food chain. Table 4 summarises the relevant EU regulations relating to the categorisation, processing and uses of ABP and the controls for PAPs in animal feeds with obvious implications to aquaculture. In 2018, an updated QRA confirmed that if animal proteins were allowed back into non-ruminant feeds, the risk of additional BSE cases in cattle was reduced even further than shown in the 2005 QRA[50]. Although this risk assessment does not impact aquafeeds directly, its results should add confidence to the safety considerations for using non-ruminant PAPs in aquafeeds.

Table 4: Summary of EU regulations relating to the categorisation, processing, and uses of ABP and controls for processed ABP products used in animal feeds.

EU Regulation	Description	Ref
Council Directive No 90/667	Laying down the veterinary rules for the disposal and processing of animal waste, for its placing on the market and for the prevention of pathogens in feedstuffs of animal or fish origin and amending Directive 90/425/EEC.	51
Commission Decision No 92/562	On the approval of alternative heat treatment systems for processing high-risk material.	52
Commission Decision No 94/382	On the approval of alternative heat treatment systems for processing animal waste of ruminant origin, with a view to the inactivation of spongiform encephalopathy agents.	53
Commission Decision No 96/449	On the approval of alternative heat treatment systems for processing animal waste with a view to the inactivation of spongiform encephalopathy agents.	54
Regulation (EC) No 999/2001	Laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.	38
Regulation (EC) No 178/2002	Laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.	39
Regulation (EC) No 1774/2002	Laying down health rules concerning animal by-products not intended for human consumption.	40
Regulation (EC) No 1069/2009	Laying down health rules as regards animal by-products not intended for human consumption and repealing Regulation (EC) No 1772/2002 (Animal by-products Regulation).	45
Commission Regulation No 142/2011	Laying down health rules as regards animal by-products and derived products not intended for human consumption	46
Commission Regulation No 51/2013	Amending Regulation (EC) No 152/2009 as regards the methods of analysis for the determination of constituents of animal origin for the official control of feed.	48
Commission Regulation No 56/2013	Amending Annexes I to IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.	49

Commission Regulation No 2017/1017	Amending Regulation (EU) No 68/2013 on the Catalogue of feed materials.	55

2.4 Global response to the use of animal proteins in aquafeeds

Animal proteins have been used since the early days of aquaculture feed on a global-wide scale in numerous countries practising intensive fish farming particularly salmon, trout, and other carnivorous fish species. Notwithstanding the BSE epidemic in ruminants, concerns about the potential for a TSE type disease in farmed fish persisted. However, as far as fish are concerned, some investigations have been undertaken to determine if prions or related proteins could result in fish from the use of any contaminated animal material as feed. A review by Málaga Trillo *et al.* (2011) detailed the presence of prion proteins in aquatic species and transmission studies within fish models[56]. Only in infected gilthead seabream (*Sparus aurata*) was there any detection of lesions in brain tissue, although there was no manifestation of any pathological symptoms or behavioural changes in these fish.

Since the BSE epidemic, animal proteins in Europe were generally eliminated from animal feed formulations during the 1990s, because of consumer concerns even before an official feed ban was enacted in 2001 in the form of the TSE regulation (EU Regulation 999/2001[38]). Export of all animal proteins (PAPs) to 3rd countries (i.e., outside the EU and associated countries that do not follow EU legislation) was prohibited until 2003, when a regulation requiring a bilateral agreement between the exporting and importing countries, allowed the export of non-ruminant PAPs to 3rd countries to resume (EU regulation 1234/2003[57]). The requirement for a bilateral agreement for exports of non-ruminant PAPs was repealed in 2016 (EU regulation 2016/27[58]). Since 2016 an export health certificate signed by the exporting country's Animal Health Authority became the key animal health control.

However, in many countries such as the USA, Canada, Mexico, Brazil, Argentina, and Australia, changes to veterinary or feed regulations were less restrictive than in Europe. From a global perspective, the only ingredients prohibited in animal feeds were ruminant derived proteins that were banned for use in feeds for ruminants. In general, porcine, and poultry-based PAPs continued as approved ingredients for other farmed species with little if any restriction on their use in aquaculture Nonetheless, leading high street retailers both in the EU and in 3rd countries supplying the EU, has stated that the supply chain from aquafeed producers to farm must be bio-secure and compliant with EU regulations in this respect, (John Williamson, JW Nutrition Ltd, *Pers comm*). It should be especially noted that in the UK there is much more consumer awareness of these issues since the epidemic originated in the nation. For these reasons, despite access to these invaluable high-quality protein sources for aquaculture, the main constraint is the reluctance of the retailers to sell farmed fish that has been fed diets with any LAPs. These included restrictions on certain raw materials in rendered products to be used in animal feeds and the requirement to validate conditions for the processing of products to be used in animal feeds. However, the USA[59] and Australia[60], both took two different approaches in managing the risk of PAPs, where risks are viewed somewhat differently from those in Europe. The former uses a federal regulation to prohibit certain 'high risk' organs, (e.g., brain, spinal cord) from entering the food chain. While the latter uses a 'Standard for the Hygienic Rendering of Animal Products' endorsed by the Australian government but administered by an independent accreditation body.

From a global perspective, the World Organisation for Animal Health's 'Office des Epizooties' (OIE), have played a major role in setting animal health and feeding regulations around the world. The core focus for regulatory impact is via the Terrestrial Animal Health Code, (TAHC) which is reviewed and updated annually by member delegates, according to disease risk criteria[61]. With regards to BSE, it defines three BSE risk categories (negligible, controlled, and undetermined) and recommends the controls required in each country, zone or compartment for animals, processing and use of products or commodities according to the

BSE risk category. As such, the continued review and updating of the OIE TAHC offers the opportunity to harmonise many of the different international animal health standards. This chronological narrative of the impact of rendering, ABP's and their use in the animal feed industry during the last 40 years have highlighted historical deficiencies, vital research, and the development of a new modernised industry that can supply approved and safe animal proteins into the aquafeed industry with confidence.

2.5 Evolution of low carbon rendering and circular economy

Since the publication of the Livestock's Long Shadow[62], the livestock industry has taken steps to understand their industry's output of greenhouse gases (GHG) emissions, and to initiate mitigating steps to reduce the industry's impact. Determining a life cycle assessment (LCA) of animal feed ingredients has become an important tool to use in attempting to reduce the impact of livestock farming (including intensive aquaculture) on global warming. Initiatives such as the Food and Agriculture Organization's (FAO) programme for 'Livestock Environmental Assessment and Performance' (LEAP) have been initiated and joined by industry groups and non-governmental organisations[63]. Many different methodologies for the determination of LCA and GHG emissions have been proposed and care must be taken to only compare data that are using the same allocation rules. Examples of different allocation criteria for LCA studies include waste/product, mass, energy, and economic[64].

In practical terms for the rendering industry, the separation of animal by-products according to risk according to criteria set by the EC and the OIE gave the stimulus to consider the concept of producing PAPs by using low carbon processing techniques. The first step was for the rendering industry (initially in the EU from 2002 onwards) was to separate itself into two separate 'sub-sector' businesses:

1) **Disposal**: The highest risk ABP (EU Category 1 and 2) was banned for use in the human food chain (via animal feed into food-producing animals) and was required to be 'disposed of'. This requirement caused the development of a range of new technologies for the use of derived rendered products (fats and protein meals) as biofuels.

2) **Feed chain**: The lowest risk ABP (EU Category 3) was considered 'in principle' to be safe for use in animal feeds after approved processing and subject to strict conditions regarding the use of the derived products in animal feeds. However, it is important to note that other opportunities also exist for the production of non-animal feed products from Category 3 ABP, such as pet foods and organic fertilisers.

Subsequently, a two sub-sector industry evolved, which dealt with the disposal and feed chain options in totally separate processes, (Figure 2). Although the ABP and derived products from each sector are required to be kept separate, the key common aspect for both processes are their requirement for steam energy. The rendered fat produced by the disposal sector can therefore be used as a fuel for the rendering process in the feed sector. As rendered fat is produced from animal by-products, the CO_2 emissions produced from its combustion are considered to be biogenic, and therefore carbon neutral. This is because biogenic CO_2 is associated with a short carbon cycle, in which carbon absorbed from the atmosphere by plants during photosynthesis is returned to the atmosphere when rendered fat is burnt to produce energy. As such, feasibility studies confirmed that the rendered fat produced by the rendering process could be used as a substitute biogenic fuel which could replace the use of fossil (oil) fuel in steam raising boilers[65].

In practice, EU rendering industry data confirms how the two separate sectors are working. Of 17 million tonnes per annum of ABP, 30 % was processed by the disposal sector and 70 % by the feed chain sector. Of the latter, a total of 2.6 million tonnes of PAPs was produced of which nearly 300,000 tonnes were used in aquafeed[66]**.** Studies of the two sub-sector businesses working as described in Figure 3, have confirmed that the use of Category 1 RF as a fuel in Category 3 rendering plants could reduce the GHG emissions associated with Category 3 rendered products. The GHG emissions of Category 1 RF are -0.77 kg CO_2^e kg^{-1}. Consequently, when used as a fuel in Category 3 rendering plants, the GHG emissions of Category 3 RF and PAP are 0.15 and 0.15 kg CO_2^e kg^{-1}, respectively[67].

If, as suggested by Ramirez *et al.* (2012), the same allocation criteria and measurement units are used to compare animal and vegetable 'by-products', it was concluded that using Category 3 rendered products can have the potential to significantly reduce greenhouse gas (GHG) emissions[67]. For example, Category 3 rendered fat (RF) with GHG emission of 0.15 kg CO_2^e kg^{-1}, has the potential to replace other fat sources, such as palm oil (GHG emissions of 2.1-2.6 kg CO_2^e kg^{-1}) and rapeseed oil used (GHG emissions of 2.2-17.1 kg CO_2^e kg^{-1}) in oleo-chemical and aquafeed industries[67-68]. Category 1 RF could also be used as an energy source for generating power or heat for other industries, e.g., heating recirculating aquaculture systems. Most importantly, Category 3 PAPs have the potential to replace soybean meal and other major plant by-products (e.g., cereals and legumes) in farmed animal diets and especially fish and crustaceans (shrimp). Soya has GHG emissions of 0.72 kg CO_2^e kg^{-1} [69]. As suggested by Parker (2018), a comprehensive life cycle analysis of specific PAPs will need to be carried to appraise the saving effect on GHG, nutrient recycling, energy, and economic efficacy[70].

In a review of feed ingredients, Blonk (2019) compared a range of animal and plant ingredients by calculating their combined carbon footprint (CFP) and land-use change (LUC) value (kg CO_2 eq per tonne protein)[71]. Of the ingredients analysed, three examples are worthy of comparison: palm kernel meal (6,100), soybean meal (9,350), and poultry PAP (2,150), which illustrate that poultry PAP has potentially significant advantages over the vegetable ingredients. In a separate study, Campos *et al.* (2020) compared products from different poultry ABP processing methods[72]. The authors concluded that a poultry meal and a hydrolysed feather meal (HFM) could reduce the impacts of animal feed production since poultry meal (1050) and HFM (650) have lower 'global warming' impacts, in terms of kg CO_2 eq per tonne protein than fishmeal (1600-1900). Overall, it appears that PAPs are feed ingredients that can offer the potential to lower the overall environmental impact in animal production, which now consumers are ever more conscious of when purchasing food products.

As part of this school of thought, is the concept of the circular economy. Recognised and promoted by many institutions including the EU (e.g., European Commission's Circular Economy Action Plan)[73], there is a need to move away from a linear production, consumption, and disposal to a circular based system, i.e., restorative, and regenerative[74]. The outcome of such a system is meant to reduce and mitigate human impact on the natural environment through resource savings, emissions, and effect biodiversity. The rendering industry fits within the remit of circularity, where it is regenerating waste from one industry for other industries for feed and energy. A recent expansive discussion research and analysis piece discuss the possible opportunities and limitations of a circular[75]. Certainly, LCAs can be used to measure the impacts of circular models. Besides the recycling of proteins into aquafeeds, ABP and PAPs allow the saving of other nutrient resources, e.g., macro and trace metals. For instance, the use of poultry meal in aquafeeds allows a possible average of 10.1 g kg^{-1} of phosphorus to be spared from non-renewable sources, i.e., mined ore deposits[76,77]. It has been estimated that in 2018, ~500,000 tonnes of poultry meal were produced within the EU[66]. As such, it is possible to infer that the use of poultry meal in aquafeed could mitigate the need for 5,050 tonnes of phosphorus (i.e., 10.1 g kg^{-1} x 500,000 tonnes). Although, the true appreciation of the saving would need to be adjusted to take into account the differences between the true phosphorus digestibility in poultry meal and the phosphorus salts/derivatives typically used in aquafeeds, e.g., monoammonium phosphate.

2.6 Developing safe, quality, and trusted PAPs.

Although, as thoroughly discussed within this review, the BSE outbreak occurred because the industry was recycling the by-products back into the same system. As such, the development of circular systems needs to be mindful of its possible impacts, not just the positives. PAP products intended for use in the food chain resulted in further demands for new standards of processing and of products, including safety, quality, and traceability, accreditation, and species purity. The absolute requirement for species pure PAPs became a requirement to ensure compliance with the intra-species recycling ban (EU Regulation 1774/2002[40]. In practical terms, purity of source is achieved by the processing of feather, blood or 'single species' ABP, such as porcine, bovine, ovine, avian (i.e., chicken, turkey, and duck) in separate process lines. However, processing of the pure ABP does not offer a guarantee of species purity PAP, if cross-contamination controls are not effectively managed. As a confirmation that species purity is achieved, a laboratory test for the species of origin is required. Consequently, research focussed on species-specific methods, including polymerase chain reaction (PCR), for detecting species-specific DNA in the PAP. The challenge of using partially degraded DNA as the starting point for method development meant that any reference materials also had to be produced under the same conditions[78]. The development and validation of a PCR method for the detection of ruminant PAP in animal feeds[47] paved the way for changes in legislation allowing the re-introduction of non-ruminant PAP into aquafeeds (Regulation 56/2013[49]). In practice, as the ban on intra-species recycling does not apply, testing the non-ruminant PAP for ruminant protein confirms adequate safety.

In practice, the legislative framework that regulates the use of processed animal proteins in animal feeds in the EU led to the further refinement of processing plants. Adequate heat treatment in the process to ensure that the PAPs are safe and validated for use in animal feed is a requirement of the processing regulations. However, excessive heat maintained over a period of time in the absence of water can cause amino acid 'bridging' and condensation reactions, adversely reducing nutritional quality in terms of digestibility[79]. A balance must, therefore, be found between the demands for product sterilisation and the requirement for highly digestible protein above 90 % digestibility coefficient. This is particularly important for ingredients in feeds for carnivorous high-value aquatic species dependent on high-performance nutrient-dense feed formulations[80].

The rendering industry have re-earned some of the public trust through national and international legislations. One important step that had helped to restore the public's acceptance of PAPs in animal feeds was the publication of the EU Feed Catalogue (Regulation 2017/1017[55]). It refers to PAPs, and the inclusion of a statutory declaration requirement if such materials were incorporated into the animal feed. It also states what permissible PAPs can be used in feeds for different farmed species. An updated qualitative risk assessment[50] has recently re-confirmed the negligible risk associated with the use of animal proteins in animal feeds, in compliance with EU legislation (Commission Regulation

(EC) No 51/2013[48]). EU Regulation 2017/1017 together with other related legislation establish the standards of PAPs for safe use in animal feeds. Confidence in PAPs for use in animal feeds has been further enhanced by the recent publication of Commission Regulation 2021/1372[81]. This regulation approves the use of poultry PAP in porcine feed and the use of porcine PAP in poultry feed and although there is no direct link to aquafeeds, this regulatory position on the use of PAPs in animal feeds may encourage wider uptake of PAPs in aquafeeds.

In terms of increasing the safety criteria for animal proteins used in the food chain, hazard analysis & critical control point (HACCP) has become an obligatory requirement in most regulatory approvals for the processing of ABPs. The World Renderers Organisation (WRO) HACCP guidelines have been used throughout the rendering industry as a basis for producing process plant HACCP schemes[82]. Rendering associations throughout the world have developed their code of practice, based on these guidelines, which are refined according to the country or regional needs. For example, the North American Rendering Association (NARA) Industry Code of Practice (2010)[83] and the Australian Renderers Association (ARA) Code of practice for the hygienic rendering of animal products (2017)[84]. Some countries, such as Brazil, possess a two-tier system. Firstly, a national law obliges renderers to follow self-control, including the publication of a HACCP plan. Secondly, the HACCP plan is approved by a competent authority veterinarian and in some countries, products used for animal feeds are accredited by an independent body, such as GMP$^+$. Although, fraud could still exist in food production supply chain as exemplified by the horse meat scandal in 2013 that had impacted many European countries[85].

In more recent times, lab-based traceability tools have been developed and tested as effective at identifying the dietary history of the final fish product. For example, the use of carbon and nitrogen ratio isotopes within the different amino acids of the fish muscle was able to discern the dietary feeding history of a farmed fish [86-88]). While the study of protein composition (proteomics) in the feed ingredient and feeds, or even the final fish product could be another means of identifying their origin[89]. Overall, these tools could be deployed into the aquaculture industry by validating production processes.

The development of traceability technologies is not exclusive to PAPs but could be deployed as an integrated traceability system for other feeds ingredients such as the origin of the fishmeal and oil components (e.g., organic, or non-organic), distinguishing non- and genetically modified plant proteins, or whether certain ingredients are used, e.g., seaweeds, insects, and algae[86]. And when society and production systems move to a circular based economy, traceability would be vital to the concept success. One such possibility is applying blockchain technology and Internet of Things to create an integrated traceability framework that links the different stages of the supply chain in data monitoring/collection, tracking, and verification/validation[90]. The deployment of both IT and lab-based technologies into the aquaculture market will safeguard consumers, increase consumer trust, create industry-wide transparency, protect farmers and feed manufacturers from fraud, and produce sustainable feeds and seafood products.

3. ABPs available to aquaculture

Under European law, only category 3 by-products may be used to produce PAPs for aquaculture and aquafeed purposes. The legal terminology for Category 3 by-products includes but are not limited to carcasses and scraps of animals and are, in principle, fit for human consumption, but are not intended for this purpose[40]. Many different PAPs have been trialled on many different aquatic species, Table 5 shows the reported effects of dietary inclusions of ABPs and PAPs from recent studies that are available to farmed fish species reflecting their inclusion level, trial length, and impact on their growth performance and feed utilisation.

546 **Table 5:** The effects of dietary processed animal proteins have on growth performance and feed utilisation in farmed finfish.

Fish	*Species*	Inclusion (%)	Duration (weeks)	Growth effect	Reference
Hydrolysed feather meal					
Cuneate drum	*Nibea miichthioides*	3.5, 10.5	8	↓ SGR, BW @ ≤10.5 ↑ FCR @ ≤10.5	91
European seabass	*Dicentrarchus labrax*	5, 7.5, 12.5	18	↔ WG, FCR & PER	92
Hybrid Clarias Catfish	*Clarias macrocephalus x Clarias gariepinus*	4.4, 8.8, 13.3, 17.7	8	↔ WG, FCR @ 4.4 ↓ WG @ ≥8.8	93
Hybrid tilapia	*Oreochromis niloticus x Oreochromis aureus*	6, 12	8	↑ WG, SGR @ 6 ↓ WG, SGR @ 12	94
Indian major carp	*Labeo rohita*	10.42, 20.83, 39.06, 52.08	9	↔ SGR, WG, FCR @ ≤20.83 ↓ SGR, WG, FCR @ ≥39.06	95
Japanese Flounder	*Paralichthys olivaceus*	12, 25, 37, 50	8	↔ WG, PER, FE @ ≤25 ↓ WG, PER, FE @ ≥37	96
Nile tilapia	*Oreochromis niloticus*	5, 10, 15	8	↔ WG, PER, SGR @ ≤10 ↓ WG, PER, SGR @ 15	97
Nile tilapia	*Oreochromis niloticus*	9.9, 15	6	↔ WG, SGR @ 9.90 ↓ WG, SGR @ 15	98
Rainbow trout	*Oncorhynchus mykiss*	10, 20, 30, 40	12	↔ WG @ 10, 20 ↓ WG @ 30,40	99
Rainbow trout	*Oncorhynchus mykiss*	6, 12, 18, 24	12	↔ WG ↑ FE	100
Rainbow trout	*Oncorhynchus mykiss*	10.5, 21.1, 31.6	11.42 (80 days)	↓ WG	101
Rainbow trout	*Oncorhynchus mykiss*	15	20	↔ WG, FE	14
Tench	*Tinca tinca*	14.8, 21	17.14 (120 days)	↑ FCR ↓ WG, SGR	102
Poultry meal					

African catfish	*Clarias gariepinus*	22.76, 34.42,	8	↓ WG, SGR, PER ↑ FCR	103
Australian snapper	*Pagrus auratus*	20.5, 25.1, 30	16.4 (115 days)	↓ WG, SGR ↔ FCR @ 20.5, 25.1	104
Australian snapper	*Pagrus auratus*	36, 48, 61, 73	7.14 (50 days)	↔ WG, FCR @ 36 ↓ WG, FCR @ ≥48	105
Black sea turbot	*Psetta maeotica*	21.20, 43.20, 64, 85	8.57 (60 days)	↔ BW, FCR, PER, SGR @ 21.2, 43.2 ↓ BW, PER, SGR @ 64, 85 ↑ FCR @ 64, 85	106
Black sea bass	*Centropristis striata*	25.1, 31.3, 37.6, 43.9, 50.1, 56.4, 62.7	8	↔ WG ≤ 50 ↓ WG ≥ 50	107
Chinook salmon	*Oncorhynchus tshawytscha*	10, 20, 30	20	↔ WG, SGR @ 10, 20 ↓ in WG, SGR @ 30	108
Cobia	*Rachycentron canadum*	12, 24, 36, 48, 60	8	↔ WG, SGR, PER @ 12, 48, 60 ↑ WG, SGR @ 24, 36 ↔ PER, FCR @ 24, 36	109
Cobia	*Rachycentron canadum*	7.55, 15.10, 22.65, 30.20	10	↑ WG, FER, PER, SGR @ 15.10, 22.65 ↔ WG, FER, PER, SGR @ 7.55, 30.20	110
Gibel carp	*Carassius auratus gibelio*	21.47, 25.97, 30.48, 34.98, 39.49, 43.99, 48.5, 53	8	↑ WG, SGR	111
Gilthead seabream	*Sparus aurata*	50.25, 67.	12	↔ WG, SGR, PER, FE @ 50.25 ↓ WG, SGR, PER, FE @ 67	12
Gilthead seabream	*Sparus aurata*	18, 36	15.71 (110 days)	↓ FW, SGR ↑ FCR	112
Golden pompano	*Trachinotus ovatus*	25.10, 32.60, 40.20, 47.70	8	↓ WG, BL ↑ FCR	113
Grass carp	*Ctenopharyngodon Idella*	7.50, 15, 22.5, 30	8	↔ WG, FCR @ 7.5 ↓ WG, FCR @ 15.00	114

				↑ WG, FCR @ ≥22.50	
Humpback grouper	*Cromileptes altivelis*	36.1, 54.2, 72.2	8	↔ FW, WG, SGR, FCR @ ≤54.2 ↓ FW, WG, SGR, PER @ 72.2 ↑ FCR @ 72.2 ↓ PER @ 36.1	115
Japanese sea bass	*Lateolabrax japonicus*	9.70, 19.40, 29.10, 38.70, 48.20	8	↑ WG @ ≤38.70 ↓ FCR @ ≤38.70 ↔ WG, FCR @ 48.20	116
Largemouth bass	*Micropterus salmoides*	16.8	11	↔ FW, FCR, SGR, PER	117
Nile tilapia	*Oreochromis niloticus*	26.5	8	↔ WG, SGR, FCR, PER	118
Nile tilapia	*Oreochromis niloticus*	14.6	10	↑ WG, PER, SGR ↓ FCR	119
Nile tilapia	*Oreochromis niloticus*	15, 20, 26	17.14 (120 days)	↔ WG, SGR, FCR, PER	120
Rainbow trout	*Oncorhynchus mykiss*	63.81	8	↔ WG, FCR	221
Rainbow trout	*Oncorhynchus mykiss*	13.25, 26.5, 39.75, 53	10	↔ WG, SGR, FCR, PER @ ≤26.5 ↓ WG, SGR, PER @ 39.75 & 53.00 ↑ FCR @ ≥39.75	222
Red drum	*Sciaenops ocellatus*	4.51, 9.01, 13.52, 18.02	6	↔ WG	123
Redbelly tilapia	*Coptodon zillii*	43.2, 85	6.42 (45 days)	↑ FW, SGR, PER @ 43.20 ↓ FCR @ 43.20 ↓ FW, SGR, PER @ 85	124
Spotted rose snapper	*Lutjanus guttatus*	15.11, 30.63, 45.94	12	↔ WG, SGR @ 15.11 ↔ FCR ↓ WG, SGR @ ≥ 30.63	125

Striped bass	*Morone chrysops x M. saxatilis*	17.20, 26.68	24	↔ WG, FCR @ 17.20 ↓ WG, FCR @ 26.80	126
Sunshine bass	*Morone chrysops x Morone saxatilis*	9.70, 17.50, 26.40	16	↔ WG, SGR, FCR	127
Totoaba	*Totoaba macdonaldi*	22.50, 45, 67.20	12.29 (86 days)	↑ WG @ ≤45 ↓ WG @ 67.20	128
Blood meal					
Channel catfish	*Ictalurus punctatus*	3.40, 6.80	9	↑ WG ↔ FCR @ 3.4 ↓ FCR @ 6.8	129
Gilthead sea bream	*Sparus aurata*	5, 10	34.57 (242 days)	↔ SGR @ 5 ↓ SGR, PER @ 10	130
Japanese eel	*Anguila japonica*	5.96, 11.92, 23.84, 35.75, 47.67,	6	↔ WG, FCR, SGR, PER @ ≤, 23.84 ↓ WG, SGR, PER @ ≥35.75 ↑ FCR @ ≥35.75	131
Largemouth bass	*Micropterussalmoides*	3.83, 7.66, 11.50	12	↔ FW, SGR, PER, FER @ 3.83 ↓ FW, SGR, PER, FER @ 7.66, 11.50	132
Rohu	*Labeo rohita*	30	7	↑ BW, FCR	133
Nile tilapia	*Oreochromis niloticus*	27.30, 54.60	12	↑ WG, SGR ↓ FCR	134
Nile tilapia	*Oreochromis niloticus*	12.50, 25.50	14.28 (100 days)	↓ WG, SGR ↔ FCR	135
Palmetto Bass	*Morone saxatilis x M. chrysops*	4.00, 9.50, 18.50	12	↔ WG, PER, FE @ 4.00, 9.50 ↓ WG, PER @ 18.50 ↑ FE @ 18.50	136
Rainbow trout	*Oncorhynchus mykiss*	5.70, 11.40, 22.70	12	↔ WG	137

Meat & bone meal					
Channel catfish	*Ictalurus punctatus*	5	23.42 (164 days)	↔ FW, FCR	138
Gibel carp	*Carassius auratus gibelio*	14.05, 46.63	8	↑ FW, SGR ↓ FE, PER @ 46.63	139
Gilthead seabream	*Sparus aurata*	40.9, 61.5	12	↔ WG, FE ↓ PER @ 61.5	140
Hybrid Striped Bass	*Morone chrysops x M. saxatilis*	15, 25, 30, 35, 40, 45	7	↑ WG ↔ FCR	141
Japanese flounder	Paralichthys olivaceus	9, 18, 36, 44	7	↓ WG, PER ↓ FE @ ≥ 36.00	142
Juvenile Red Drum	*Sciaenops ocellatus*	5.34, 10.68, 16.02, 21.35,	6	↔ WG, FE @ 5.34 ↓ WG, FE @ ≥10.68	143
Nile tilapia	*Oreochromis niloticus*	6.23	12	↓ WG, PER ↑ FCR	144
Nile tilapia	*Oreochromis niloticus*	2.3, 4.5, 7	9.28 (65 days)	↔ WG, SGR @ 2.30 ↑ WG, SGR @ ≥4.50 ↓ FCR @ ≥4.50	145
Rainbow trout	*Oncorhynchus mykiss*	12, 24	12	↔ WG, FE	14
Yellow croaker	*Pseudosciaena crocea*	10.86, 21.72, 32.58, 43.44, 54.3	8	↔ WG, FCR, SGR ≤ 32.58 ↓ WG, FCR, SGR ≥ 32.58	146

All studies are aimed at the replacement of fishmeal with PAPs. BW, body weight; FCR, feed conversion ratio; FE, feed efficiency; FW, final weight; PER, protein efficiency ratio; FER, Feed efficiency ratio; WG, weight gain, SGR, specific growth rate. ↔, no change; ↑, increase; ↓, decrease.

Clearly, there has been much scope for the application of ABP and PAPs in aquaculture. In Table 5 broader work is presented concerning use in fish diets over two decades with an emphasis on PAPs affecting growth performance and feed utilisation for commercially important fish species particularly in tilapia, carp, catfish, and rainbow trout. Experimental work has covered in both tropical and temperate fish, as well as freshwater and marine. This also includes studies on high-value marine species relevant to the European market such as seabass and seabreams. Most of these investigations have centred on poultry by-product meal, feather meal, bloodmeal and MBM including from bovine sources demonstrating good overall performance for these species at varying inclusion levels. Investigations for Atlantic salmon were limited due to restrictions on the use of PAPs throughout this period especially in Europe.

3.1 Mammalian ABP

Mixed or species-specific mammalian ABP is typically processed by the indirect heating of the minced ABP in batch or continuous systems, using method 1, described in Annex IV, Chapter 3 of Commission Regulation EC 142/2011[46]. Despite most attention from the aquaculture industry is focused on poultry by-products, there has been substantial research completed outside of the EU with MBM produced from mammalian species, e.g., cattle, sheep, and pigs. There have been mixed reviews about the effective digestibility of these products, varying significantly between fish species. For example, Allan *et al.* (2000) found that the apparent digestibility of MBM incorporated into the diets of Australian silver perch (*Bidyanus bidyanus*) was only 55.4 %, which was lower than the use of fishmeal (76.8-93.9 %)[147]. Furthermore, the study found that the availability of specific amino acids in MBM products, such as lysine and methionine were reduced by up to 35.1 and 55.5 %, respectively when compared to the conventional fishmeal. MBM has also been seen to significantly reduce growth rates in large yellow croaker (*Pseudosciaena crocea*) when comprising more than 45 % of the protein content. However, results showed that at a level of 45 % or less there were no significant effects on the growth rate of this species[146]. For Bureau *et al.* (2000), they tested three dietary levels of MBM in rainbow trout (*O. mykiss*)[14]. These authors found that the inclusion of up to 24 % MBM (providing ~25 % of total digestible protein) in the diet was the most feasible level without compromising growth. Although, a small reduction in feed efficiency was noted when compared to the control diet. The authors explained that this was primarily due to various imbalances with respect to amino acid profile and protein digestibility in comparison to a higher fishmeal reference diet being used in the study. It should therefore be cautioned that formulation of iso-nitrogenous and iso-caloric diets (i.e., based on gross nutrient profiles) may not include accurate digestibility data on protein, individual amino acids, and energy values. This could potentially lead to inaccuracy of the true nutrient value of diets leading to an imbalance in the comparison of different feed ingredients. While Lochman *et al.* (2012) reported that 5 % MBM levels in diets for channel catfish (*Ictalurus punctatus*) fed for 164 days did not alter growth indices or feed conversion ratio (FCR)[138]. Although, this low level is unlikely to have disrupted the available amino acid balance or overall digestibility of the diet for this species. For gilthead seabream (*S. aurata*), the use of MBM (0, 40.9, and 61.5 % in the diet) did not affect weight gain or feed efficiency. Although there was a drop in the protein efficiency ratio (PER) at 61.5 % MBM inclusion due to lower digestibility and essential amino acid profile differences to the control diet[140]. It is worth noting that this high inclusion level has its worth in academic interest but not deemed to be a practical level in diet formulations for farmed seabream or in many other farmed fish species.

It is widely accepted that there is significant variation in quality and composition of MBM between different processing plants primarily, due to the different amounts of which animals go into the manufacturing of the meal. This variation can have a significant impact on the protein content and amino acid profile[148]. Overall, this makes MBM a much less suitable product for application in the aquafeed market. As mentioned earlier in this review, the BSE outbreak, and the subsequent legislation regarding the prohibition of MBM use in Europe in 2001, has resulted in limited research being carried out on mammalian ABP use in aquafeeds, and in particular published by EU researchers.

It is important to note that porcine PAPs are approved for use in EU aquafeeds (as a non-ruminant PAP) by Commission Regulation (EC) No 51/2013[48] but the use of porcine PAPs in the European aquaculture industry (e.g., salmonids fish) is limited due to the social stigma left by the BSE outbreak. However, research has shown that porcine meals can be successfully included in diets in several fish species including the replacement of the fishmeal component in Nile tilapia (*Oreochromis niloticus)* diets. Hernandez *et al*. (2010), concluded that pet food-grade porcine meal at a dietary inclusion level of 34 % was able to achieve a similar weight gain to that of the dietary control consisting of sardine fishmeal[118]. Using a high quality freshly prepared sardine fishmeal offers an excellent highly digestible reference diet ingredient for a more sensitive evaluation of the test ingredient in question. While Li *et al.* (2020) also concluded that channel catfish fingerlings (*Ictalurus punctatus*) could be fed with a diet containing up to 32 % porcine meal[150]. The authors showed weight gain was not significantly affected compared to the control dietary treatment group. FCR however increased from 1.39 in the basal control group to 1.54 in the experimental diet (a 21 % increase). It should be argued that this level of inclusion was quite conservative in these experimental diets for catfish.

3.2 Non-Mammalian ABP

Non-mammalian ABP such as feather and poultry meal are mainly processed by the indirect heating of the ABP in batch or continuous systems, using methods 3, 4, 5, or 7 described in Annex IV, Chapter 3 of Commission Regulation (EC) 142/2011[46]. A major lesson learned by the requirement to characterise rendering systems for the BSE investigations was that future research on the use of animal proteins in feeds should usefully characterise PAPs in such studies. This would have the clear benefit of allowing a more rigorous comparison of products, such as poultry meals or feather meals which could have been processed by many of the different systems operating globally.

3.3 Poultry meal

Poultry meals are typically produced in different quality grades that include pet food grade, feed grade, low ash etc. Each of these grades of 'poultry meal' has different characteristics mainly related to their component animal by-products and their nutritional qualities for target markets, such as pet foods or aquafeeds. Typically, mixed species poultry ABP comprises of the heads, feet, carcass, and internal organs (but not feathers), which are minced and normally processed through continuous rendering methods (EU methods 3, 4 and 7, section 2.3). The resulting dried poultry meal can typically contain protein levels of 56-62 % protein and 11-17 % lipid content (Table 1). The application of poultry meal combined with a small quantity of blood meal (2.5 %) in aquafeeds has previously been shown to be effective at replacing 50 % of the fishmeal component in the Atlantic salmon diet, without reducing growth performance[149]. Complementary use of such proteins in this manner would enhance the synergy of the essential amino acids offering superior biological value.

Poultry meal was effectively demonstrated in a study by Zapata *et al*. (2016) in the emerging candidate marine fish species, totoaba (*Totoaba macdonaldi*) by replacement of fishmeal in experimental diets[128]. It was found that when 67 % of the fishmeal was replaced using poultry meal a mean weight gain of 50.5 g was achieved compared to 23.3 g found in the control dietary group. On top of this, survival increased from 76 % in the control group to 89.3 % fish receiving the test diet. However, when 100 % of fishmeal was replaced by poultry meal, weight gain was significantly reduced by 38 % (14.4 g) compared to the control group, along with a survival rate of only 52.4 %. It is more than likely total substitution of fishmeal with poultry meal would deviate from the 'ideal' protein that is found in fishmeal due to its comparable amino acid profile to the fish's muscle composition.

Many of the past research studies show that poultry meal yields higher growth performance indicators (e.g., weight gain and specific growth rate, SGR) than other rendered products such as MBM [109, 110, 124]. This is likely attributed to a more consistent amino acid profile and protein content in the poultry meal when compared to variable fluctuations in MBM products[148]. Lysine and methionine are often the two key factors in feed ingredients that can affect fish growth. Likewise, these amino acids are typically found at much lower concentrations in MBM than in poultry meals[151] (Table 1). This is primarily due to the fact that poultry meat meal has a lower ash content and more uniform protein quality due to its consistent and defined avian source. Regardless, the use of poultry meals can also impact the performance of farmed fish through amino acid imbalance. For example, African catfish (*Clarias gariepinus*) fed with diets that contain poultry meal inclusion levels of 22.76 and 34.42 % produced lower weight gain and SGR, and higher FCR values than no poultry meal inclusion. When 1 % lysine was added to the poultry meal inclusion diet, the negative impact on growth performance indicators was reversed. This infers that there is a restoration of a more balanced essential amino acid profile in the diet for the catfish[103]. Likewise, the growth performance of black seabass (*Centropristis striata*) was also compromised when poultry meal replaced over 50 % of the fishmeal component[107]. In contrast, Zhou *et al.* (2011) 10-week feed study on cobia (*Rachycentron canadum*) found there was an enhanced PER when poultry meal was used at an optimal level[110]. This level is calculated as 30.75 % and on either side of this inclusion level, PER showed a decreasing response. As such, it can be inferred from the authors modelling that there is a defined ratio between the major proteins of fishmeal and poultry meal that leads to an 'ideal protein' balance diet. This type of response was also shown in the earlier investigation by Nengas *et al.* (1999)[12]. The authors reported that a similar synergistic response was found when a specific inclusion level of PBM had gave better growth and feed conversion than a fishmeal control diet for gilthead seabream.

3.4 Feather meal

Mixed poultry (e.g., chicken, turkey, duck, and goose) feather meal is typically produced by steam pressure processing of raw feathers in batches or through continuous production systems. This process normally involves the use of pressures between 3 and 6 bar that is applied to the feather biomass for periods between 20 and 60 minutes, followed by drying in batch or continuous driers to evaporate the biomass moisture content from ~65 % to ~ 8 %[152]. Feather meals can contain up to 85 % protein, but there have been concerns about whether all the protein is digestible due to >90 % of the protein being in the form of scleroprotein, i.e., keratin[28]. This is primarily due to the inability of keratin to dissolve in water or for endogenous proteolytic enzymes like protease to break the complex structural protein down[153].

When feather meal is formulated into test aquafeeds, many of the fish feed studies observed either an equal or enhanced growth performance with an effective inclusion level of up to 25-30 % (Table 5). However, beyond this level, negative growth performance is often observed. This could be attributed to deficiencies in particular to lysine and methionine (Table 1), or it could be simply due to the high percentage of structural fibrous keratin protein found in feathers, which has a poor digestibility. This low digestibility can be particularly pronounced in fish that possesses a relatively short gastrointestinal tract, where there is insufficient capacity to digest feather meal due to quick transit time of digesta and inefficient enzyme interaction. As such, there is potentially scope for considering exogenous enzyme supplementation of various proteases to assist feather meal degradation and assimilation in fish. For example, a dose-response feeding study conducted on juvenile olive flounder (*Paralichthys olivaceus*) using feather meal (0, 12, 25, 37, and 50 % dietary inclusion) was found that up to 25 % of feather meal could maintain similar weight gain to 0 % diet[96]. Beyond this inclusion level, the fish had up to 50 % lower weight gain and general performance. In this study, the control diet contained 80 % of white fishmeal that is higher in ash content than regular low-temperature brown low-ash fishmeal and this could influence the outcome of such assessments of feather meals for juvenile fast-growing fish. The feed conversion efficiency FCR and protein efficiency ratio PER of flounder fed on the 12 % feather meal diet were almost the same as in the control group, however, these efficiencies decreased as the incremental inclusion of feather meal in the diet increased from 25, 37 to 50 %. It should be noted that supplements of crystalline essential limiting amino acids to the feather meal diet elevated its nutritive value to a considerable degree. This further addition of an amino acid blend containing L-tryptophan, L-methionine, and L-lysine-HCl into the test diet resulted in reversing the negative impact of feather meal. These effects included higher growth performance (e.g., final weight and weight gain) and feed utilisation (e.g., FCR and

PER) when compared to no amino acid supplementation. There was little difference in the proximate whole-body composition together with the haematological and haemato-chemical parameters among the treatment groups tested. Feather meal was shown to be a suitable partial alternative for fishmeal in the diet of juvenile Japanese flounder under these experiential conditions. This is an example of a carnivorous species, and the findings are likely typical for related fish such as sole, turbot and even halibut and need further validation.

It is also important to be mindful of how PAPs and in particular feather meals can degrade during the aquafeed extrusion process. Jasour *et al.* (2017) demonstrated that the aquafeed extrusion temperature can negatively increase protein oxidation in the feather meal component in the extruded diet[154]. With a difference in an extrusion temperature of 30 °C, i.e., 100 °C to 130 °C, the measured protein hydroperoxides, total carbonyls, lanthionine, methionine racemisation (conversion of L- to D-form) in the feed had all significantly increased. This subsequently has led to an increase in FCR when the higher temperature feather meal extruded diets were fed to rainbow trout.

3.5 Blood meal

Blood meal is traditionally produced by heating the liquid blood to ~95 °C to coagulate the blood proteins, which are then separated from the liquid portion by centrifugation. The coagulated blood (normally called blood crumb) is dried to a moisture level of <5 % in either indirect (disc) driers or a ring drier using direct hot air to evaporate the moisture[31]. A spray-dried blood meal is produced by a different method, which involves chilling the liquid blood followed by centrifugation to produce two products: haemoglobin (red blood cell) and plasma. Each is further concentrated using reverse osmosis to remove the excess water, before being spray-dried (>500 °C for 5-10 seconds) to a moisture level of <5 %[155]. The nutritional composition of the various blood meals and derivative products are highly dependent on the temperature employed during drying. High temperatures can cause damage to proteins by cross-bridging of specific amino acids such as lysine and methionine[153] and reducing the overall digestibility of the ingredient in fish such as rainbow trout[156-157]. However, the new generation of drying technologies such as spray, ring, and disc drying has greatly improved blood meal quality and allowed higher inclusion levels to be used in fish diets, particularly farmed salmonids, e.g., rainbow trout[158].

As found in Table 5, blood meal is highly effective at replacing fishmeal in many farmed fish species. For example, Saeed et al. (2005) working with *Labeo rohita* fingerlings reported growth performance as measured by SGR and FCR in a trial study of over 12 weeks[134]. Bovine sourced blood proved to be effective in this study with results showing that blood meal performed efficiently as a feedstuff with a partial replacement of fishmeal in *Labeo rohita*. The test diets produced no adverse effects on the growth performance or the survival in these fish. Saeed *et al*. (2005) also confirmed the advantage of blood meal inclusion in diets for rohu (*Labeo rohita)* where a 30 % level produced superior bodyweight of fish, but with a higher FCR compared to a control, blood meal free group[133]. This might have been explained by the elevated feed intake in carp due to improved palatability that often reduces FCR but allows increased growth rates. In comparison with other plant derived by-products, blood meal offered more scope for inclusion and was better accepted by rohu under the prevailing experimental conditions. Kirimi *et al.* (2016) conducted a study where Nile tilapia (*O. niloticus*) were fed on diets containing 12.5 and 25 % blood meal respectively. These authors found that at 12.5 % inclusion, weight gain was not significantly different from the control group fed mainly with fishmeal[135]. Ding *et al.* (2019) working with freshwater largemouth bass (*Micropterus salmoides*) reported effective inclusion of nearly 10 % chicken haemoglobin powder showing no fundamental changes in performance but may enhance palatability at supplemental levels[132]. However, there were reductions in feed intake, growth (e.g., SGR) and protein utilisation efficiency at around 30 % fishmeal replacement. Some positive findings were reported for marine species such as gilthead seabream by Martinez-Llorens *et al.* (2008) when fed 5 % blood meal inclusion but reduced performance growth and feed efficiency was obtained at 10 % inclusion[130]. Many studies fail to adequately describe the drying process, and high temperatures may degrade the product and reduce quality. As discussed earlier, blood meal is heat labile, and proteins, peptides, and amino acids can be degraded (e.g., hydroperoxide production and racemisation) which can affect nutrient bioavailability. In practice, the longer heat exposure times during disc and ring drying may lead to heat degradation than observed with spray drying. There is obviously significant variation in blood meal quality and capacity in different fish species for optimum assimilation.

Besides offering a good protein source in aquafeed formulation, blood meal can also provide additional nutritional benefits, such as macro and trace metals, in particular iron. For instance, mammalian blood meal was shown to be an effective dietary source of iron that subsequently reduce the occurrence of cataracts in Atlantic salmon (S*almo salar*)[159]. It was found that iron concentration in the test diet increased to 261 mg kg^{-1} from 78 mg kg^{-1} when there was no blood meal addition. This has subsequently reduced the occurrence of cataracts in the salmon by 12 % with weight gain increasing by 17 % in the blood meal inclusion dietary group. In these investigations, there was a pronounced cataract preventative effect of both the BM fortified and experimental diets compared to the respective reference diets. Another factor is the presence of higher levels of histidine in blood meals and this essential amino acid and especially for salmon entering seawater is now deemed to be critical for mitigating cataractogenesis for Atlantic salmon, (*Salmo salar*)[160]. The histidine is part of the complex polypeptide antioxidant molecule protecting the lens within the salmonid eye and increasing stress and growth demands will necessitate increased dietary levels to meet ocular requirements and/or posterior cortical region and subsequently affect the perinuclear region. It was suggested by these authors that however, that this was most likely due to better vision in the fish, translating to higher feed intake.

However, there is sufficient evidence in the scientific literature and in practice to be very cautious about using appreciable inclusion levels of whole blood meal, with respect to its high iron content. The pro-oxidant characteristics of iron as a transitional metal can cause significant oxidative stress with potential damage to tissues and organs placing demands on antioxidative enzyme systems, and vitamins such as vitamins C and E[162]. Furthermore, high dietary blood meal levels can initiate oxidative induced peroxidation of lipids (oils and fats) producing free radicals and generating undesirable changes in feed quality and risks, e.g., feed rancidness, increase of lipid oxidation products, and reducing feed palatability. Adverse effects of dietary iron overload were also described in rainbow trout by Desjardins *et al.* (1987) using semi-purified test diets with supplemental iron[163]. These authors reported the associated effects of diet rancidity and iron overload (greater than 86 mg kg^{-1}) caused the development of specific histopathological signs, inferior growth and high mortalities in trout. Such studies lead to the recommendation of low to moderate inclusion levels in blood meal (5-15 %) for formulated feeds to avoid iron overloading.

It should also be noted that blood meal provides functional benefits through enhancing feed pellet colouration as well as stability due to binding properties. This is particularly important to farmed shrimp species (e.g., white leg shrimp, *Litopenaeus vannamei*) where colouration is an important parameter in feed attractiveness[163]. Enhanced factors that may affect such organoleptic and gustatory attributes warrant further exploration due to the positive effects of blood meal on taste and palatability for many aquatic species.

4 Future scope for PAPs

The present review has highlighted there is a significant amount of knowledge of using PAPs in aquafeeds. However, there are a number of advancements underway that could enhance their potential in aquafeeds. Advances in the bioavailability of the nutrients for aquatic species can be broadly considered in terms of either being driven by changes in process techniques, by bioprocessing methods or by the synergistic blending of animal proteins.

4.1 Process techniques

The processing of ABPs has evolved over recent decades to become more energy-efficient while at the same time eliminating prion disease risk. Nonetheless, all processes used for the treatment of ABPs have to meet the strict criteria laid down in the EU regulations and this limits the degree of flexibility that might be hoped for when attempting to make technical processing advances. However, there is still scope for technological improvements on PAPs associated with enhancing higher digestibility coefficients for the major nutrients such as protein (and amino acids), energy, and macro-elements, e.g., calcium and phosphorus. For example, Lewis *et al.* (2019) have shown adverse rending temperatures during the production of poultry meal can affect the nutritional value and digestibility[164]. This is evident in the authors' results showing that for every 10 ºC increase after 110 ºC in the poultry meal rendering process, there was a 5 % reduction in protein digestibility. This highlights the importance of controllable rendering techniques is needed to maximise poultry meal potential when used in aquafeeds.

PAPs are currently and may be further enhanced to create functional properties (i.e., benefiting the physicochemical qualities of the feed) or refined to possess new bioactive attributes. These may include bioactive proteins, short-chain peptides, antioxidants, antimicrobials[165-167]. The inclusion of poultry meal in aquafeeds may confer positive benefits in terms of gut health. Hartviksen *et al.* (2014) reported that Atlantic salmon (*Salmo salar*) fed a diet containing a 20 % inclusion of poultry meal displayed a significant increase of autochthonous bacteria (9.61 % of the population)[168]. This primarily consisted of potentially beneficial *Lactobacillaceae, Betaproteobacteria, Enterobacteriaceae*, and Bacilli-like bacteria within the distal intestine when compared to no poultry meal inclusion (basal diet). Notably, a 75 % replacement of fishmeal in a juvenile barramundi (*Lates calcarifer*) diet resulted in higher hindgut microvillus density when compared to the control showing a 7.6 % measurable increase[170]. Although within the same study, a reduction in fish muscle eicosapentaenoic (up to 167 % decrease) and docosahexaenoic (up to 301 % decrease) fatty acids was observed in poultry meal inclusion. This led to an overall lower omega-3/omega−6 fatty acid ratio. This is attributed to the poultry meal possessing a relatively high lipid content of 13.52 % and the fishmeal used in the diet contained a lipid content of 10.76 %. However, poultry fat is notable for its high levels of saturated and omega 6 & 9 fatty acids. Overall, this could influence the end fish fillet product quality and shelf stability, i.e., less unsaturated fatty acids could decrease the rate of lipid peroxidation rate, however, delivering less healthy omega 3 fatty acids to the consumer. Further processing of the poultry meal (e.g., solvent extraction) to remove the lipid fraction could negate these effects.

4.2 Bioprocessing

Another way to remove the oil but also improve nutrient digestibility is the use of hydrolysis (e.g., enzymatic, acid, and alkali) that could be used as a follow-on treatment after the legislative requirement of heat rendering treatment. These processes are often used to produce poultry protein concentrates that have been shown to enhance the effectiveness in the fish diets compared to the standard poultry meal, e.g., growth performance.

Acid hydrolysis can be conducted using a variety of organic food-grade acids such as citric acid and formic acid[171]. Depending on the acid used, this method can be effective at breaking down the complex PAP proteins into smaller oligopeptides and peptides. Furthermore, acid hydrolysis can be used on a wide variety of substrates, even hydrophobic compounds, which normally have low yields, if organic solvents (e.g., ethanol) are also incorporated into the method[172]. By varying one or more of several parameters including, temperature, time, pH, pressure, and specificity or quality of the hydrolytic enzyme, the functionality and processing time of the end product can be optimised[173]. Despite being a versatile method, acid hydrolysis has a few disadvantages, the primary is that the breakdown is often a non-specific process and may not allow for precise extraction of proteins with the desirable bioactivity being produced or amino acid profile.

A more controllable hydrolysis methodology is enzymatic hydrolysis, and it is often used by food industries, e.g., fish protein hydrolysates, plant protein concentrates and isolates. Enzymatic hydrolysis has previously been shown to be an effective method for protein breakdown/refining/extracting across a variety of PAP substrates, including ABP. For example, the use of the enzymes such as Savinase® 16L (Strem Chemicals INC, Newburyport, USA) and various proteases was effective at hydrolysing feather meal. It was also later found that this enzymatic pre-treatment process improved amino acid utilisation by up to 43 % when fed to rainbow trout (*O. mykiss*)[174].

Currently many of the enzymes used for protein hydrolysis are of animal origin as they are designed specifically for protein digestion, pancreatin is a good example of this. Animal-derived enzymes tend to have a particular site of action and cleave specific peptide bonds as a result these enzymes tend to serve a more specific function than enzymes derived from plants and fungi which tend to have a much broader range of functions[175]. More recently, Poolsawat *et al*. (2021) demonstrated the positive effect of replacing fishmeal with enzymatically treated feather meal on the growth and feed utilization of tilapia (*O. niloticus* × *O. aureus*)[176]. This study highlighted that supplemental protease could improve nutrient retention and digestibility of feather meal and enzymatically treated feather meal can replace fishmeal at up to 100 % in diets for tilapia without negative effects on growth and feed utilisation indices. However, in the 9-week experiment using juvenile fish, it should be noted that dietary fishmeal inclusion was 6 % and thus limited exploring higher feather meal levels.

The diversity of enzymes and their specificity at breaking down proteins indicates that there is significant potential for advancement and improvement within the rendering industry. This diversity can potentially give the industry the ability to select and extract specific and desirable peptides from within protein structures. Many peptides produced through enzymatic hydrolysis have displayed positive bioactive properties under *in vitro* examination[167]. This suggests that if these hydrolysed PAPs are incorporated into feeds, the bioactive peptides may confer immune stimulation, better gut health, or stress tolerances in farmed aquatic animals[177].

Another cost-effective approach in improving nutrient bioavailability and bioactivity is to ferment PAPs as a solid-state substrate. De Oliveria *et al*. (2019) demonstrated this as a possible solution, through the use of hydrolytic enzymes produced by *Aspergillus niger* fungi to ensile chicken feather meal under solid-state fermentation conditions, i.e., low moisture and solid substrate bed[178]. The authors found significant amounts of keratinase, protease, and lipase in the feather meal substrate had been produced by the fungi, which could potentially break down the indigestible fibrous keratin protein that makes up the feather meal. While another study found fermenting feather meal with *Bacillus subtilis* was able to replace 20 % of the fishmeal in the silver pompano (*Trachinotus blochii*) diet, which subsequently increased fish weight gain and SGR[179]. In comparison, Dawood *et al*. (2020) study tested the feasibility of fermented poultry meal with Brewer's yeast (*Saccharomyces cerevisiae*)[180]. The authors found that formulating the resulting fermented poultry meal into compound diets and feeding to Nile tilapia (*O. niloticus*) was able to replace 16 % of the fishmeal component (or 40 % fermented poultry meal), without fish growth, palatability, apparent digestibility coefficients of nutrients, haematological indices (e.g., lysozyme & phagocyte activity) being negatively affected. While at a lower dietary of 10 % fermented poultry meal inclusion level, tilapia was observed to have higher growth performance (e.g., final weight, weight gain, and SGR), intestinal enzyme activity (e.g., amylase and protease), and immunological (e.g., lysozyme, phagocytic activity, and bactericidal activity).

4.3 Blending and synergism

The potential of co-feeding PAPs with a solid-state fermentation product feed additive (i.e., fermentation of another substrate product that also contains a natural mixture of digestive enzyme complexes and bioactive peptides and oligopeptides) could offer advantages in enhancing nutrient digestibility and growth performance attributes in farmed fish[181].

Recently, Hong *et al*. (2021) found that a mixture of poultry meal and fermented soybean meal was effective at replacing the fishmeal component in the Asian seabass (*Lates calcifer*) diet[182]. It was determined that with a mixture of 60 % poultry meal and 40 % fermented soybean meal up to 60 % of the FM could be effectively replaced by this blended composite protein without impairing the growth performance indices.

In contrast, the co-feeding of a mixture of PAPs could create a complementary or synergistic meal that could effectively replace fish and plant meals in aquafeeds without the loss of growth performance. For instance, a dietary blend of rendered animal proteins (40:40:20-meat, and bone meal: poultry by-product meal: hydrolysed feather meal) and blood meal at an inclusion of 50 % (replacing 24 % of the fishmeal component) did not negatively influence the growth performance or feed utilisation indices in Siberian sturgeon (*Acipenser baerii*)[183]. To identify the optimal PAP mixtures and harness their synergistic effects, a feed study could be based on the concept of a mixture design in its test feed formulation (e.g., simplex centroid and D optimal matrices) and examined using their associated statistic methods, e.g., response surface analysis and modelling[184]. This can save the need for numerous feed mixture permutations which can be laborious and economically unviable to most feed studies.

Blended complementary protein sources that include poultry meal also offer a strategic potential analogue to fishmeal for aquafeed production. This has been the basis of many investigations to assess the scope of extending the use of avian derived proteins with other terrestrial animal by-products such as porcine blood meals and plasma proteins, this has also been included in bespoke blends with soybean meal and various plant protein concentrates. Such studies have been beneficial to advance the use of rendered materials in aquafeeds. This has been shown by previous work by Fasakin *et al*. (2005)[17] and by Suloma *et al*. (2014)[97] for tilapia as an effective means to enhance the biological value of the substitute protein. Similarly, Li *et al*. (2021) was able to show that 15 % fishmeal crude protein in the diet containing poultry meal and soybean meal was sufficient for the maximum growth and feed efficiency in largemouth bass (*Micropterus salmoides*)[185]. Although the dietary change was inadequate to support intestinal integrity (e.g., increased enteritis), skin structure (e.g., black skin syndrome), eye pathology (e.g., cloudiness, corneal opacity), and liver health (e.g., hepatocyte atrophy). More information on the digestible essential amino acid content of PAPs and requirements for various aquatic species will be necessary to produce viable feed formulations based on a least-cost basis. Species of particular interest would include e.g., Atlantic salmon, rainbow trout, sea bass and sea bream (European and Asian), cobia, mahi mahi, grouper, pompano, yellow tail tuna, and other high-value marine fish of contemporary global interest.

5 Conclusion

The rendering industry provides both a valuable service in safe and sustainable management and recycling of nutrients back into the supply food chain. The nutritional, environmental and economic characteristics of PAPs can be a valuable ingredient for animal feeds such as the expanding aquafeed industry. The BSE epidemic in 1986 has had a significant negative effect on the global rendering and animal feed industries greatly constraining their utilisation and perception. Through the characterisation of rendering processes and the reshaping of EU and other international legislation, the rendering industry played a crucial role in the eradication of BSE from the animal production system. Recent developments in detection and quantification techniques have paved the way for legislative changes allowing the safe re-introduction of non-ruminant PAPs into aquafeeds in 2013. However, there remains scope for further studies to be completed in order to validate PAPs as an effective and safe aquafeed ingredient. New traceability technologies in both information technology and lab verification techniques can potentially dissuade retailers and consumers concerns over PAPs used in animal feeds. The sector has much to offer aquafeed manufacturers in the growing quest for safe, sustainable, and economic feed ingredients in compound diets for both fish and shrimp.

Acknowledgement

The authors would like to acknowledge Ms Ellie-Ann Conneely for her assistance in the editorial review.

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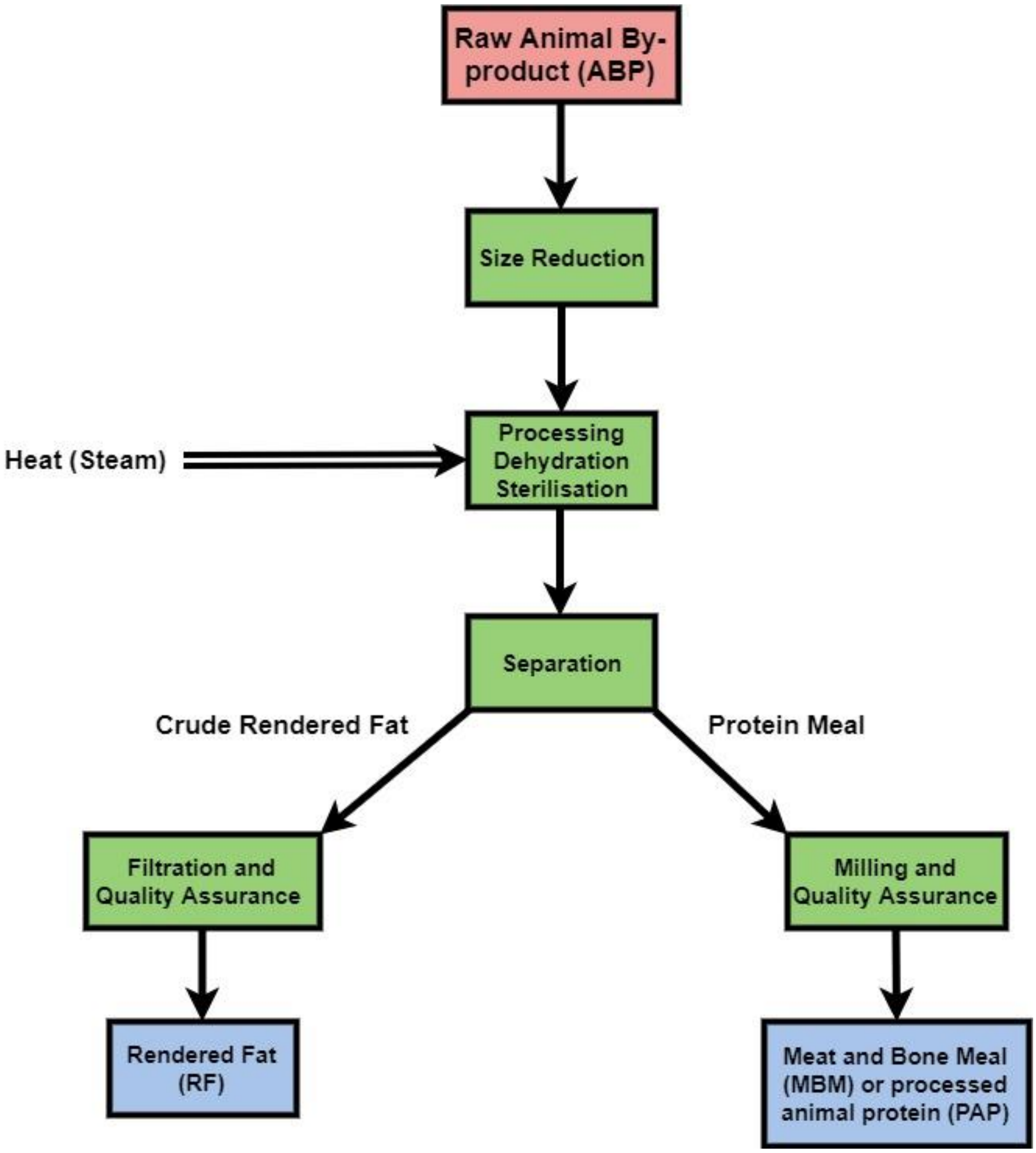
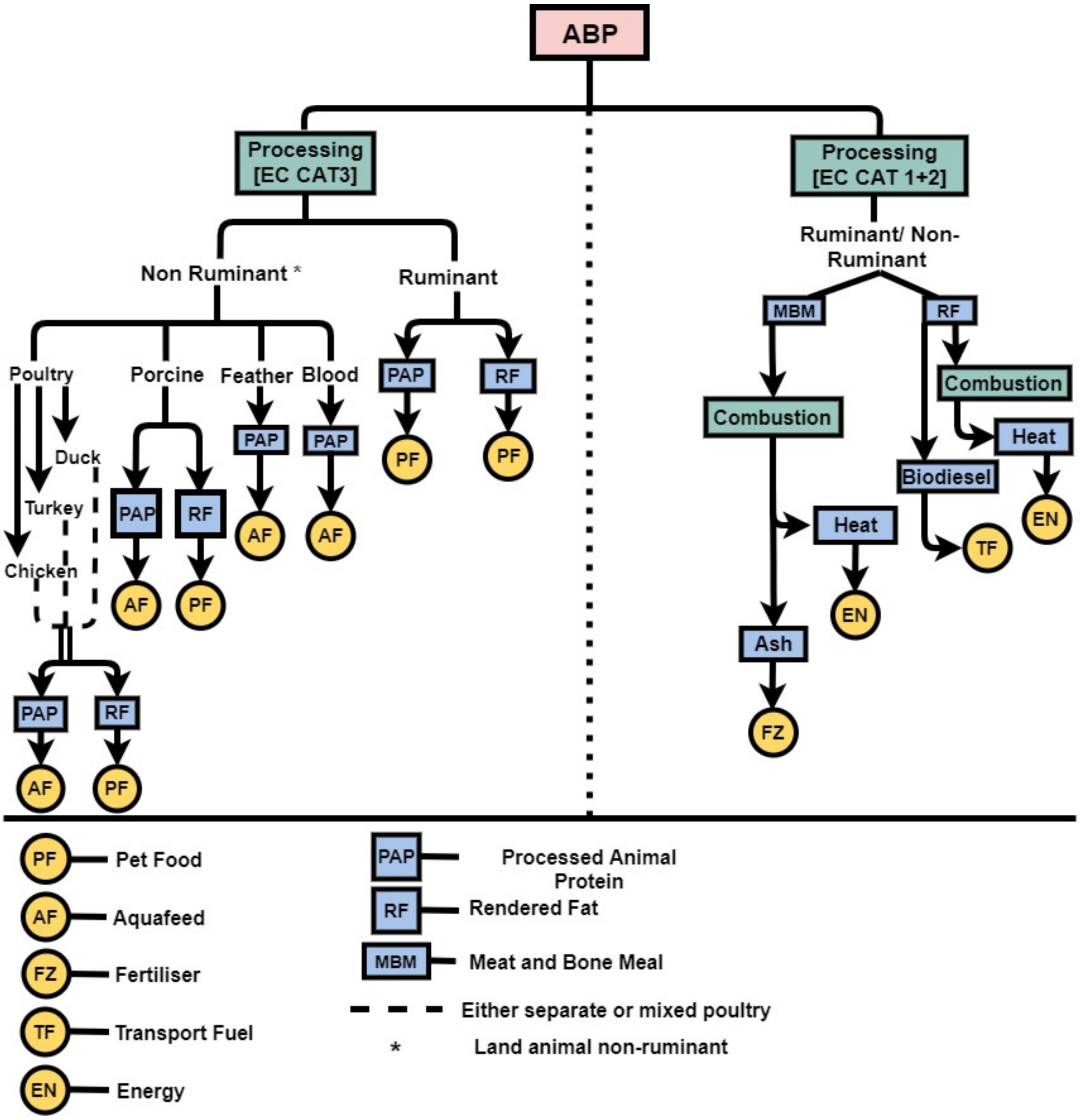
Figure legends

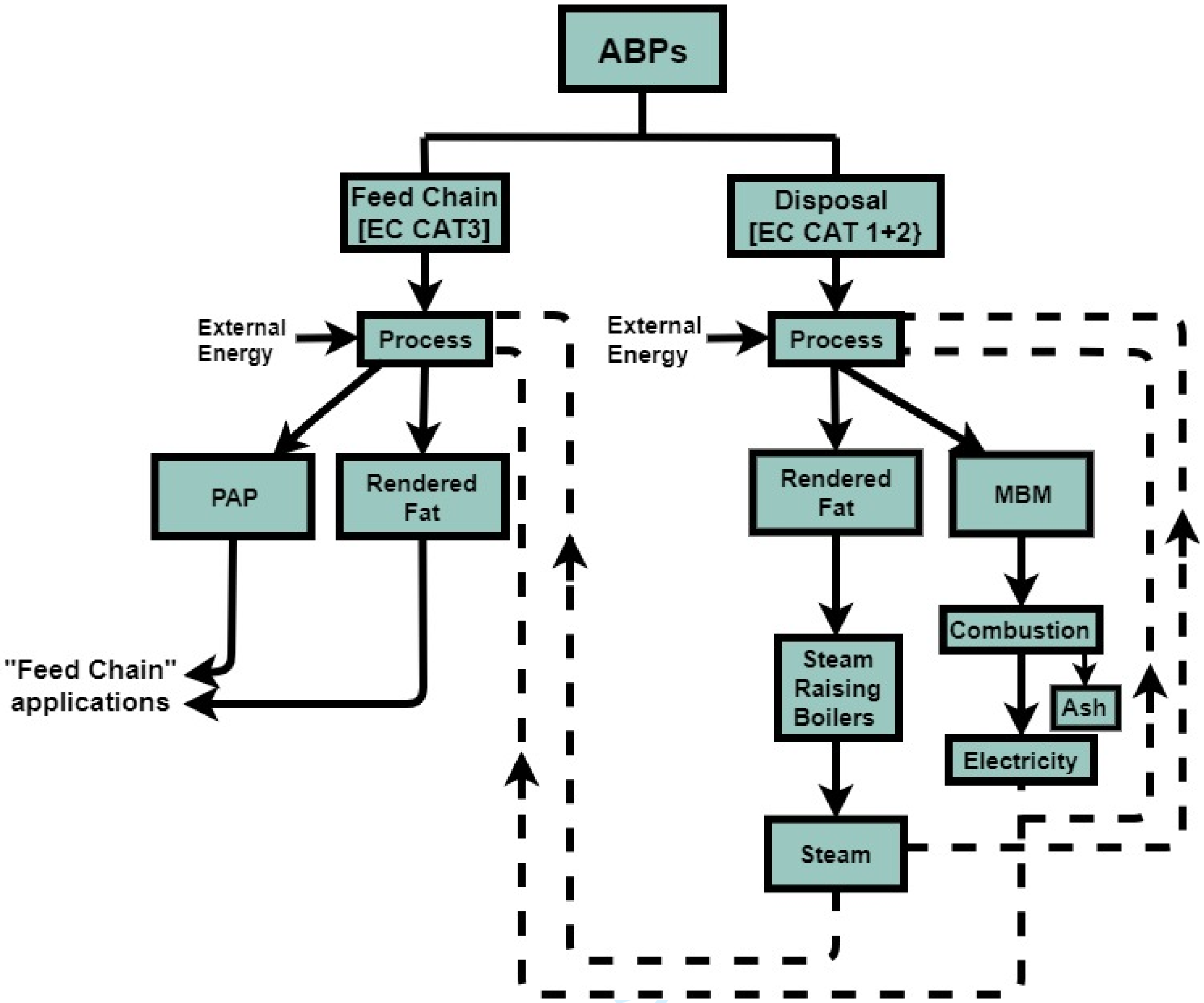
Figure 1: Schematic diagram of the rendering process leading to added value by-products.

Figure 2: Schematic overview of ABP processing, illustrating the biorefinery concept.

Figure 3: Schematic overview of ABP processing, illustrating energy production and utilisation, resulting in low carbon processing and products.


Raw Animal By-product (ABP)
Size Reduction
Heat (Steam)
Processing Dehydration Sterilisation
Separation
Crude Rendered Fat
Protein Meal
Filtration and Quality Assurance
Milling and Quality Assurance
Rendered Fat (RF)
Meat and Bone Meal (MBM) or processed animal protein (PAP)


ABP
Processing [EC CAT3]
Processing [EC CAT 1+2]
Non Ruminant *
Ruminant
Ruminant/ Non-Ruminant
Poultry
Porcine
Feather
Blood
Duck
Turkey
Chicken
PAP
RF
MBM
AF
PF
Combustion
Heat
Biodiesel
Ash
EN
TF
FZ
PF Pet Food
AF Aquafeed
FZ Fertiliser
TF Transport Fuel
EN Energy
PAP Processed Animal Protein
RF Rendered Fat
MBM Meat and Bone Meal
Either separate or mixed poultry
* Land animal non-ruminant


ABPs
Feed Chain [EC CAT3]
Disposal [EC CAT 1+2}
External Energy
Process
External Energy
Process
PAP
Rendered Fat
Rendered Fat
MBM
Combustion
Ash
Electricity
Steam Raising Boilers
Steam
"Feed Chain" applications