It is both possible and practicable to produce feed and fuel from grain. Using the value of grain to produce renewable energy for transport, while using the remaining protein content of the grain as a valuable protein source for livestock and for fish, can be seen as a complimentary and optimal use of all the grain constituents. Consideration must be given to maximise the value of the yeast components, as substantial yeast is generated during the fermentation of the grain starch to produce ethanol. Yeast is a nutritionally rich feed ingredient, with potential for use both as feed protein and as a feed supplement with possible immunity and gut health enhancing properties.

Bioprocessing, with the consequent economies of scale, is a process whereby the value of grain can be optimised in a way that is traditional, natural and sustainable for primarily producing protein and oil for feed with a co-product ethanol as a renewable fuel.

1.1 Introduction

The International Energy Agency (2009) defined biorefining as the sustainable processing of biomass into a spectrum of bio-based products (food, feed, chemicals and minerals) and bioenergy (biofuels, power and/or heat). The controversy in 2008 around the use of grain as a biorefinery feedstock to produce fuel, in particular bio ethanol (Ayre, 2007) suggested lack of public awareness that the process is both sustainable and also an exemplary case of a biorefinery producing both fuel and feed. At present, the issue has not completely abated and there is now an urgent need for greater security and resilience in protein for feed in Europe. The current global approach to sustainable agriculture hinges on balancing supply of the 4Fs: feed, fuel, food and fibre. The incorporation of
biorefinery co-products into animal feeds provides a major conduit for finding balance; excess fibre and feed from production of fuel may be converted into food via animal production. This paper will consider the current situation regarding protein for feed, then specifically address the benefits of an ethanol biorefinery for producing feed protein. Practical aspects of developing an alternative feed material will be discussed, particularly relating to the nutritional composition of the protein. Finally, a dry grind bioethanol refinery will be used as an exemplar in meeting the need for protein production and holistic biorefining.

2.1 The EU Feed Protein Issue

It is well recognised that Europe does not produce sufficient protein of required quality for its manufacture of animal feed (De Visser, Schreuder and Stoddard, 2014) and currently relies on imported protein. A substantial research programme is now being undertaken across Europe (EU, 2013) to identify alternative sources, as innovation in protein crops has been somewhat neglected (Hausling, 2011). Over 66% of the protein used in animal feed in the European Union is imported (Daelemans, 2012), with the remainder sourced from locally produced oilseed and distillers dried grains and solubles DDGS (25%), other protein crops (5%) with 4% from other minor sources. Such heavy reliance on imported material has important implications which raise both economic and environmental concerns. Not only is the energy cost of trans-global transportation set against the product but the growing uncertainty of the international marketplace places significant pressure on the resilience of the European supply chain.

From a nutritional and formulation perspective, a number of factors need to be taken into account when considering protein supply for animal feed. This starts with the concentration of protein in the raw material. There are very few raw materials in which the protein represents one hundred percent of the product, therefore the impact of the non-protein components must be accounted for when formulating a diet. At worst, these components can constitute anti nutritional factors that have a
negative impact on animal health, but more usually, are poorly digested plant carbohydrate fractions that simply dilute the active protein component. However, there are some examples where the associated components have significant positive effects on the protein. This is commonly when the residual component is either an important source of energy such as an oil-seed meal or component with potentially beneficial biological activity, such as the role of yeast in the biorefining example. The amino acid profile of the protein is the second most important consideration. Lysine and methionine tend to be the first limiting amino acids in protein supplements destined for application in animal feed. For nutritionists the final important factor is the availability of the protein in the feed material to the animal itself. The chemical association of protein with plant carbohydrates will influence how the protein is digested and made available for digestion and absorption (Chocht and Annison, 1992) The presence and quantity of anti-nutritional factors must also be quantified to allow for their mitigation (Boye, Zare and Pletch, 2010). Finally, the protein product must be produced in sufficient quantities to make adoption of the product a viable proposition. In order to be considered for incorporation into a commercial feed formulation, any new product not only competes with a basket of alternative protein products on cost, availability and nutritional value, but must also guarantee reliability of supply. A product needs be produced in larger quantities of greater than 50 thousand tonnes per annum to be considered as a commodity protein for feed formulation. It could however have application if the product were to provide a specific, high value, nutritional feed additive as will be exemplified in the bioethanol refinery concept. Table 1 shows the potential avenues available for novel protein sources, spilt into categories based upon their feedstock material.
The EU is a net importer of cereals, and relies heavily on imported soya bean meal (30 million tonnes of soya bean meal per annum) (2010 figures, FEDIOL 2012). Soya bean meal is a good example of a source of protein that contains many of the characteristics defined above as key attributes of a feed protein source. It is also important to reflect that the soya bean crop was first cultivated for its oil content, with the fat extracted protein meal regarded as co-product. It is only relatively recently that soya has been cultivated for its protein yield. There is a need for a balanced approach to cereals/oilseeds and protein crops in the EU in order to begin to solve the protein deficit.

Soya bean meal is the fastest expanding crop in the world and is mainly used in feed for animals in meat and dairy supply chains, but unfortunately this growth has come at considerable environmental and social cost (Minderhoud, 2010). Companies are now being ranked on their commitment to use Responsible Soya (RespSoy, 2015).

### 2.2 Application of proteins in feed for livestock

The animal feed industry may be loosely divided into two sectors: one sector addressing the requirements of ruminant animals (primarily cattle and sheep) and the other sector addressing non-ruminants (primarily fish, pigs and poultry). The ability of ruminants to digest fibre as an energy source and to utilise non-protein nitrogen to meet their amino acid requirements means that the fibrous products are predominantly integrated into ruminant diets, whilst the majority of high protein biorefinery co-products are directed towards non-ruminants. It is important to understand how proteins are employed in feed, with three areas needing distinct consideration, the animal, the feed industry and the consumer.

#### 2.2.1 Animal requirements
The needs of the animal can vary for different segments of the feed industry, particularly in areas such as neonate nutrition. The very high growth rates of commercial strains of fish, pig and poultry render them extremely sensitive to fluctuations in the quality of feed provided and the density of protein and energy in the feed, which limits the inclusion of many co-products. Factors such as protein density which can effect growth and feed conversion and the presence of anti-nutritional factors – for example; lectins, trypsin inhibitors and β conglycin, are important when considering using a protein as a feed material (Gilani, Xiao and Cockell, 2012).

2.2.2 The feed industry

Some reference has already been made to specific needs of the feed industry, but successful adoption of a raw material as a mainstream product requires several criteria to be concurrently met. Reliable supply chains, critical volume of production, consistent quality and the presence of reliable quality control mechanisms are factors specific to the feed industry criteria for mainstream feed ingredients. It is also important that the protein source has the required feed density to allow for flexibility of compound feed production, as feed intake volume (and hence diet nutrient density) frequently becomes a limiting factor, particularly in neonate nutrition. Products must also have little or no fibre contamination, which can effect feed quality and ultimately the acceptance or not of the protein product.

2.2.3 Consumer choice

The opinions of the consumer is a relatively new factor in animal feed production which has gained prominence over the past two decades (Parrot, 2010). The consumer has become increasingly aware and sensitive to the importance of safe feed production and the significance of animal feed as an integral component of the food chain as a result of a significant number of food safety scares which have occurred over the past 30 years (Wall, 2014). Since the mid-1980s, most Western European countries have experienced at least one or more significant food scares (e.g. BSE - considered the cause of Creutzfeldt–Jakob disease, E-Coli, Salmonella, Dioxin residues, Campylobacter). The
consumer now demands a choice in how their food is produced and this includes decisions based on sustainability, animal welfare and use of genetically modified feed ingredients. Public opinion on sustainability of food production includes replacement of fishmeal as a protein source (Hardy, 2010) and less reliance on imported ingredients. Animal welfare considerations have resulted in the continued ban on the use of animal by-products in certain feed formulations and consumers demand a choice as to whether animals have been fed on genetically modified grains or not. From this, it clear that the opinions of consumers now have a highly significant impact on the choice of products that can be used in animal feed.

3.1 The Value of a Biorefinery

Construction of a biorefinery is a significant financial investment requiring a solid business case for the production of the primary product and, more recently, the associated co-product(s). In modern construction of, for example, bioethanol plants, advantages are made of the economies of scale, the use of combined heat and power, and supply chains that are well established for both in bound and outward transport. With scale and high volume comes the added benefit of continuous supply and consistency of product. Additional technology improving co-product value can add significant financial benefit in both existing biorefineries and business plans for future construction.

Biorefining processes are already extensively employed in the production of feed for livestock and in many cases have evolved to accommodate animal, feed industry and consumer requirements for feed protein sources for the different segments of the animal feed industry. The biorefining process can have a significant positive impact on the quality of the protein. Indeed without biorefining processes many proteins would not be suitable for animal feed. When oils seeds were first processed to recover oil, the process was modified to eliminate plant components which were known to be anti-nutritional, such as the inactivation of trypsin inhibitors during soya bean processing. Processing was originally a combination of physical processing, heat and solvent extraction (Clarke and Wiseman, 1999). More recently enzymes have been used to selectively
eliminate plant components which only relatively recently have been identified as being anti-
nutritional, such as addition of phytase to reduce phytate levels in feed materials (Morgan, Walk,
Bedford, Burton, 2015). There are several examples of biorefining in use routinely, including oil
production from which rape meal or sunflower meal can be generated; mono sodium glutamate
production with a rice protein concentrate co-product; and the production of yeast from bioethanol
refining (Burton Scholey, Williams, 2014). The latter will be discussed in more detail later.

It can be as enlightening to examine a case which on the surface appeared viable but fell down
simply because there was no viable cost effective supply chain. It was proposed that green beet tops
would provide an excellent source of plant protein as a co-product to sugar beet production
(Feedipedia, 2015). It was proposed that high protein beet-top meal could be produced, replicating
alfalfa leaf protein. In this case, 8.2 tonnes of fresh leaves at 12% dry matter provided 1 tonne of dry
matter with 15% protein. The process recovered 50% of the protein, to produce a feed material with
52% protein and a residual product for anaerobic digestion. However, this means that 56 tonnes of
leaves were required for 1 tonne food protein. The process was only economical when the leaves
were already in the factory but was totally uneconomical when consideration was given to
transporting the leaves to the factory. Perhaps at some future point consideration will be given to
mechanically pressing the leaf to produce a semi meal product as the tops are removed from the
beet (Van der Poel, Van Krimpen, Viedkamp, Kwakkel, 2013).

3.1.2 Enzymology and the use of endogenous enzymes in animal nutrition:

Anti-nutritional factors are well recognised in the feed industry. Prior to enzymology, processing
technology - often involving heat, was employed to eliminate anti-nutritional factors. This in itself
may be problematic, as lysine is often lost during due to the production of Maillard compounds
(Clarke and Wiseman, 2005). Current processing takes a more targeted approach and has allowed
scientists to identify and eliminate specific anti-nutritional components such as phytate and non-
starch polysaccharides from many non-ruminant feed materials through enzyme treatment and fermentation (Bedford, Partridge, 2010)

3.1.1 European grain production

In Europe significant advances have been made in crop breeding (particularly with wheat, barley and oats) to make grains an important source of biomass, with well-established supply chains and storage facilities. Cultivation practices are widely accepted as best practice and allow for the rotational benefits of cereals with other major EU crops (oil seed rape, corn, sugar beet). These factors have led to the global competitiveness of European growers; grain yields from cereals are highest for the climatic conditions of long cool summers with many hours of daylight for grain filling. The EU also has very knowledgeable and skilled farmers with generations of experience in growing these crops. With these significant benefits it is difficult to find alternative crops that are as productive and financially attractive. Also, alternative crops such as soya bean meal do not traditionally handle climatic irregularities well (i.e. wet harvest, cooler temperatures) and will suffer more from diseases than in the areas they are grown today (US and Latin America) (McFarland and O’Conner, 2014)

3.2 Alternative protein streams from a dry grind ethanol biorefinery

The bioprocessing of starch from cereal, grain or tuber feedstock to produce ethanol is one means of producing renewable fuel alongside a number of co-products that are invariably used in animal feed. The commercial value of ethanol depends heavily on the value of the product it replaces, as a fuel, mineral oil. Therefore when the price of oil falls and the value of ethanol falls there is greater emphasis on the value of the co-product to ensure that the bioprocess remains profitable. Production of ethanol from feedstock occurs via either the “wet grind process” but mainly from the “dry grind ethanol process” (Rausch and Belyea, 2006). The first generation of dry grind ethanol
plants ferment starch to ethanol and in the process produce carbon dioxide and the co-product

distillers dried grains and solubles (DDGS); producing approximately one third of each (Thacker and

Widyaratyne, 2007). After fermentation of the starch, the grain residue consisting of all grain

proteins, the aleuronic layers of the seed, residual fibrous carbohydrates and lipid component are

further processed alongside the yeast generated in the fermentation process. All these residual

components have in the past been combined to produce DDGS which tends to be a one market

commodity that in its current form is mainly used in feed for ruminants (Yang, McAllister, Mckinnon,

Beauchemin, 2012). The fibre to protein ratio of DDGS is approximately 27:33 which is ideal for

ruminant feed, but tends to limit the use of the product in feed for poultry, pigs or in aquaculture

(Thacker and Widyaratyne, 2007; Nyachoti, Haouse, Slominski, Seddon, 2005). Furthermore to assist

in storage and transport the product is dried. Approximately 40% of the energy used in the

bioethanol process is engaged in drying the co-product. It is questionable as the price of energy

increases whether such a high level of energy can be employed in drying feed for ruminants as the

economic model for ruminant production requires low cost feed materials: ruminants require 10kg

of feed for every kg of meat produced, compared with 5kg for pigs and less than 2kg for poultry and

fish (Soil Assoc, 2010).

Bioprocessing relies on economies of scale when plants are constructed and in general, a dry grind

bioethanol plant will, depending on the size of construction of the plant, process between 0.5 and

1.0 million tons of grain per annum. A summary of the production of a 200 Million litre ethanol plant

when either corn or wheat are used as the feedstock is shown in Table 2.

Table 2. Production of a 200ML litre bioethanol plant

The yeast which is both added to the fermentation and then multiplies during fermentation contains

approximately 50% protein in the dry matter and contributes approximately 15% of the protein in

the process. Furthermore the yeast represents 8-10% of the dry matter of the DDGS which has been
commercially recognised as sufficient yeast in the process to develop a new feed protein source, if it can be economically isolated.

Yeast is a valuable protein source, both whole and separated into its constituent parts. Whole yeast is used as a probiotic to modulate gut microbiota, resulting in improved innate immunity, improved disease resistance and improved growth performance (Stone, 1998). Yeast is high in minerals and B vitamins in particular. The B-glucan from the yeast, mannan-oligosaccharides in the yeast cell wall and nucleotides have positive effects on gut microbiota, immunity and disease resistance, enhanced growth performance in both poultry (Hooge, Sims, Sefton, Connolly and Spring, 2003) and pigs (Davis, Maxwell, Erf, Brown, and Wistuba, 2004) and improved gut morphology and mucus production (Santin, Maiorka, Macari, 2001; Moralez-Lopez, Auclair, Garcia, Esteve-Garcia, and Brufau, 2009).

The process for separating yeast protein concentrate (YPC) during bioethanol production is shown in Figure 1. A form of DDGS is still produced via this process, although with a slightly reduced protein content (less than 5% reduction).

A comparison of the nutritional content of DDGS and YPC, both separated from a maize bioethanol distillery is shown in figure 2. Starch and Neutral Detergent Fibre are substantially higher in DDGS, whereas protein and notably lysine are higher in the YPC. This makes this product more appropriate for a monogastric diet than DDGS due to the deleterious effects of excessive dietary fibre content in those species, which lead to reduced nutrient absorption, reduced feed intake and subsequently poorer growth performance (Thacker and Widyaratyne, 2007).
3.2.1 Feeding YPC to animals

YPC is an ideal feed protein for fish and poultry. Digestible amino acid content of yeast protein concentrate has been shown to be comparable with soya for broiler chicks, and higher than the feedstock alone (Burton, Scholey, Williams, 2013), although this is heavily influenced by the drying process used (Scholey, Williams, Burton, 2014a). In feeding studies with broiler chicks, dietary inclusion levels of up to 17.5% bioethanol YPC gave improved performance characteristics (Scholey, Williams, Burton, 2014b). Bioethanol sourced YPC has been fed to several aquaculture species, with 20% dietary inclusion appearing optimal for performance (Omar, Merrifield, Kuhlwein, Williams, Davies, 2012; Gause and Trushenski, 2011a; 2011b). Studies in trout showed an improvement in growth up to 6% higher in trout fed diets with 20% YPC compared with a control diet (see Figure 3). The higher growth rate compared with the controls is probably reflective of the benefits of the yeast and yeast components.

Figure 3: Growth of trout fed diets containing graded levels of YPC

3.3 Protein value

Three factors drive protein value; geographical location, protein composition and presence of additional nutrients. For soya, DDGS and potentially YPC, there is a premium for location from the USA (least expensive) through to the EU and Asia (most costly). Additionally if the protein is a favourable alternative to soya, with less environmental and supply issues, this adds an additional financial premium. Yeast has a further premium applicable as it has additional nutrients and potential health enhancing activity. All these factors add to a substantial increase in value for YPC over soya, particularly in the EU and Asia markets.

Rather than DDGS which is mainly a product for ruminants, biorefineries can produce a diverse valuable protein feed portfolio for livestock, comprised of 58% DDGS for cattle, 10% YPC (for fish/poultry) and remainder a high protein concentrate (for monogastrics).
4.1 Conclusion

The potential to bioprocess plant material to produce both fuel and feed is an exciting prospect. Advanced physical and chemical separation technologies combined with the opportunity to employ enzymes, contributes to the portfolio of techniques to bioprocess material. Protein is an enduring, expanding, high value market, which is expected to increase by 40% by 2050. Currently ethanol biorefineries produce DDGS as a co-product which is a valuable feed ingredient mainly used in cattle feed. However producing high value protein from DDGS is practicable and the technology on offer is appropriate for use in the dry grind ethanol process. Dry grind plants can be adapted downstream to produce valuable protein co-products, which not only add value to the product stream but allow access to growing markets such as pig, poultry and aquaculture and therefore may reduce the reliance of Europe on imported soya bean meal. A rebranding of the dry grind bioethanol process is needed to emphasise that it is a traditional, natural, sustainable, bioprocess producing protein, oil and the co-product ethanol.

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**Table 1: Potential novel protein sources by category**

<table>
<thead>
<tr>
<th>Category</th>
<th>Novel protein sources</th>
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<tbody>
<tr>
<td>Oil seeds</td>
<td>Proteins of defatted soybeans, rapeseed and sunflower seed</td>
</tr>
<tr>
<td>Grain legumes</td>
<td>Peas, Vicia faba, lupines and concentrates, chick peas</td>
</tr>
<tr>
<td>Forage legumes</td>
<td>Lucerne (alfalfa)</td>
</tr>
<tr>
<td>Leaf proteins</td>
<td>Grass, sugar beet leaves</td>
</tr>
<tr>
<td>Aquatic proteins</td>
<td>Algae, both macro- (seaweed) and microalgae, duckweed</td>
</tr>
<tr>
<td>Cereals/pseudo cereals</td>
<td>Proteins from oat and quinoa</td>
</tr>
<tr>
<td>Insects</td>
<td>E.g. mealworm, housefly, house cricket</td>
</tr>
</tbody>
</table>
Table 2. Production of a 200ML litre bioethanol plant

<table>
<thead>
<tr>
<th>Composition of grain</th>
<th>Maize</th>
<th>Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (%)</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Oil (%)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Sugar &amp; starch (%)</td>
<td>65</td>
<td>63</td>
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</table>

<table>
<thead>
<tr>
<th>200 Million litre ethanol plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain K tons</td>
</tr>
<tr>
<td>Ethanol ML litres</td>
</tr>
<tr>
<td>DDGS K tons</td>
</tr>
<tr>
<td>DDGS protein %</td>
</tr>
<tr>
<td>Grain protein K tons</td>
</tr>
<tr>
<td>Yeast protein K tons</td>
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<tr>
<td>Total protein K tons</td>
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<tr>
<td>Total oil K tons</td>
</tr>
</tbody>
</table>
Figure 1: The process for production and separation of YPC from bioethanol processing.
Figure 2: A comparison of the nutrient composition of DDGS and Yeast protein concentrate (YPC)
Figure 3: Growth of trout fed diets containing graded levels of YPC.