1 The Bio Refinery; Producing Feed and Fuel from Grain

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5 Abstract

6 It is both possible and practicable to produce feed and fuel from grain. Using the value of grain to 7 produce renewable energy for transport, while using the remaining protein content of the grain as a 8 valuable protein source for livestock and for fish, can be seen as a complimentary and optimal use of 9 all the grain constituents. Consideration must be given to maximise the value of the yeast 10 components, as substantial yeast is generated during the fermentation of the grain starch to 11 produce ethanol. Yeast is a nutritionally rich feed ingredient, with potential for use both as feed 12 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 13 14 be optimised in a way that is traditional, natural and sustainable for primarily producing protein and 15 oil for feed with a co-product ethanol as a renewable fuel.

16 1.1 Introduction

17 The International Energy Agency (2009) defined biorefining as the sustainable processing of biomass 18 into a spectrum of bio-based products (food, feed, chemicals and minerals) and bioenergy (biofuels, 19 power and/or heat). The controversy in 2008 around the use of grain as a biorefinery feedstock to 20 produce fuel, in particular bio ethanol (Ayre, 2007) suggested lack of public awareness that the 21 process is both sustainable and also an exemplary case of a biorefinery producing both fuel and 22 feed. At present, the issue has not completely abated and there is now an urgent need for greater 23 security and resilience in protein for feed in Europe. The current global approach to sustainable 24 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.

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33 2.1 The EU Feed Protein Issue

34 It is well recognised that Europe does not produce sufficient protein of required quality for its 35 manufacture of animal feed (De Visser, Schreuder and Stoddard, 2014) and currently relies on 36 imported protein. A substantial research programme is now being undertaken across Europe (EU, 37 2013) to identify alternative sources, as innovation in protein crops has been somewhat neglected 38 (Hausling, 2011). Over 66% of the protein used in animal feed in the European Union is imported 39 (Daelemans, 2012), with the remainder sourced from locally produced oilseed and distillers dried 40 grains and solubles DDGS (25%), other protein crops (5%) with 4% from other minor sources. Such 41 heavy reliance on imported material has important implications which raise both economic and 42 environmental concerns. Not only is the energy cost of trans-global transportation set against the 43 product but the growing uncertainty of the international marketplace places significant pressure on 44 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

50 negative impact on animal health, but more usually, are poorly digested plant carbohydrate 51 fractions that simply dilute the active protein component. However, there are some examples where 52 the associated components have significant positive effects on the protein. This is commonly when 53 the residual component is either an important source of energy such as an oil- seed meal or 54 component with potentially beneficial biological activity, such as the role of yeast in the biorefining 55 example. The amino acid profile of the protein is the second most important consideration. Lysine 56 and methionine tend to be the first limiting amino acids in protein supplements destined for 57 application in animal feed. For nutritionists the final important factor is the availability of the protein 58 in the feed material to the animal itself. The chemical association of protein with plant 59 carbohydrates will influence how the protein is digested and made available for digestion and 60 absorption (Choct and Annison, 1992) The presence and quantity of anti-nutritional factors must 61 also be quantified to allow for their mitigation (Boye, Zare and Pletch, 2010). Finally, the protein 62 product must be produced in sufficient quantities to make adoption of the product a viable 63 proposition. In order to be considered for incorporation into a commercial feed formulation, any 64 new product not only competes with a basket of alternative protein products on cost, availability 65 and nutritional value, but must also guarantee reliability of supply. A product needs be produced in 66 larger quantities of greater than 50 thousand tonnes per annum to be considered as a commodity 67 protein for feed formulation. It could however have application if the product were to provide a 68 specific, high value, nutritional feed additive as will be exemplified in the bioethanol refinery 69 concept. Table 1 shows the potential avenues available for novel protein sources, spilt into 70 categories based upon their feedstock material.

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75 Table 1: Potential novel protein sources by category

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77	The EU is a net importer of cereals, and relies heavily on imported soya bean meal (30 million tonnes
78	of soya bean meal per annum) (2010 figures, FEDIOL 2012). Soya bean meal is a good example of a
79	source of protein that contains many of the characteristics defined above as key attributes of a feed
80	protein source. It is also important to reflect that the soya bean crop was first cultivated for its oil
81	content, with the fat extracted protein meal regarded as co-product. It is only relatively recently that
82	soya has been cultivated for its protein yield. There is a need for a balanced approach to
83	cereals/oilseeds and protein crops in the EU in order to begin to solve the protein deficit.
84	Soya bean meal is the fastest expanding crop in the world and is mainly used in feed for animals in
85	meat and dairy supply chains, but unfortunately this growth has come at considerable
86	environmental and social cost (Minderhoud, 2010). Companies are now being ranked on their
87	commitment to use Responsible Soya (RespSoy, 2015).
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97 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).

105 *2.2.2 The feed industry*

106 Some reference has already been made to specific needs of the feed industry, but successful 107 adoption of a raw material as a mainstream product requires several criteria to be concurrently met. 108 Reliable supply chains, critical volume of production, consistent quality and the presence of reliable 109 quality control mechanisms are factors specific to the feed industry criteria for mainstream feed 110 ingredients. It is also important that the protein source has the required feed density to allow for 111 flexibility of compound feed production, as feed intake volume (and hence diet nutrient density) 112 frequently becomes a limiting factor, particularly in neonate nutrition. Products must also have little 113 or no fibre contamination, which can effect feed quality and ultimately the acceptance or not of the 114 protein product.

115 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

123 consumer now demands a choice in how their food is produced and this includes decisions based on 124 sustainability, animal welfare and use of genetically modified feed ingredients. Public opinion on 125 sustainability of food production includes replacement of fishmeal as a protein source (Hardy, 2010) 126 and less reliance on imported ingredients). Animal welfare considerations have resulted in the 127 continued ban on the use of animal by-products in certain feed formulations and consumers 128 demand a choice as to whether animals have been fed on genetically modified grains or not. From 129 this, it clear that the opinions of consumers now have a highly significant impact on the choice of 130 products that can be used in animal feed.

131 **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.

139 Biorefining processes are already extensively employed in the production of feed for livestock and in 140 many cases have evolved to accommodate animal, feed industry and consumer requirements for 141 feed protein sources for the different segments of the animal feed industry. The biorefining process 142 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 143 144 processed to recover oil, the process was modified to eliminate plant components which were 145 known to be anti-nutritional, such as the inactivation of trypsin inhibitors during soya bean 146 processing. Processing was originally a combination of physical processing, heat and solvent 147 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 antinutritional, 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.

154 It can be as enlightening to examine a case which on the surface appeared viable but fell down 155 simply because there was no viable cost effective supply chain. It was proposed that green beet tops 156 would provide an excellent source of plant protein as a co-product to sugar beet production 157 (Feedipedia, 2015). It was proposed that high protein beet-top meal could be produced, replicating 158 alfalfa leaf protein. In this case, 8.2 tonnes of fresh leaves at 12% dry matter provided 1 tonne of dry 159 matter with 15% protein. The process recovered 50% of the protein, to produce a feed material with 160 52% protein and a residual product for anaerobic digestion. However, this means that 56 tonnes of 161 leaves were required for 1 tonne food protein. The process was only economical when the leaves 162 were already in the factory but was totally uneconomical when consideration was given to 163 transporting the leaves to the factory. Perhaps at some future point consideration will be given to 164 mechanically pressing the leaf to produce a semi meal product as the tops are removed from the 165 beet (Van der Poel, Van Krimpen, Viedkamp, Kwakkel, 2013).

166 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 andfermentation (Bedford, Partridge, 2010)

174 3.1.1 European grain production

175 In Europe significant advances have been made in crop breeding (particularly with wheat, barley and 176 oats) to make grains an important source of biomass, with well-established supply chains and 177 storage facilities. Cultivation practices are widely accepted as best practice and allow for the 178 rotational benefits of cereals with other major EU crops (oil seed rape, corn, sugar beet). These 179 factors have led to the global competitiveness of European growers; grain yields from cereals are 180 highest for the climatic conditions of long cool summers with many hours of daylight for grain filling. 181 The EU also has very knowledgeable and skilled farmers with generations of experience in growing 182 these crops. With these significant benefits it is difficult to find alternative crops that are as 183 productive and financially attractive. Also, alternative crops such as soya bean meal do not 184 traditionally handle climatic irregularities well (i.e. wet harvest, cooler temperatures) and will suffer 185 more from diseases than in the areas they are grown today (US and Latin America) (McFarland and 186 O'Conner, 2014)

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188 **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,

- 192 mineral oil. Therefore when the price of oil falls and the value of ethanol falls there is greater
- 193 emphasis on the value of the co-product to ensure that the bioprocess remains profitable.
- 194 Production of ethanol from feedstock occurs via either the "wet grind process" but mainly from the
- "195 "dry grind ethanol process" (Rausch and Belyea, 2006). The first generation of dry grind ethanol

196 plants ferment starch to ethanol and in the process produce carbon dioxide and the co-product 197 distillers dried grains and solubles (DDGS); producing approximately one third of each (Thacker and 198 Widyaratyne, 2007). After fermentation of the starch, the grain residue consisting of all grain 199 proteins, the aleuronic layers of the seed, residual fibrous carbohydrates and lipid component are 200 further processed alongside the yeast generated in the fermentation process. All these residual 201 components have in the past been combined to produce DDGS which tends to be a one market 202 commodity that in its current form is mainly used in feed for ruminants (Yang, McAllister, Mckinnon, 203 Beauchemin, 2012). The fibre to protein ratio of DDGS is approximately 27:33 which is ideal for 204 ruminant feed, but tends to limit the use of the product in feed for poultry, pigs or in aquaculture 205 (Thacker and Widyaratyne, 2007; Nyachoti, Haouse, Slominski, Seddon, 2005). Furthermore to assist 206 in storage and transport the product is dried. Approximately 40% of the energy used in the 207 bioethanol process is engaged in drying the co-product. It is questionable as the price of energy 208 increases whether such a high level of energy can be employed in drying feed for ruminants as the 209 economic model for ruminant production requires low cost feed materials: ruminants require 10kg 210 of feed for every kg of meat produced, compared with 5kg for pigs and less than 2kg for poultry and 211 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.

216 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 itcan be economically isolated.

222 Yeast is a valuable protein source, both whole and separated into its constituent parts. Whole yeast 223 is used as a probiotic to modulate gut microbiota, resulting in improved innate immunity, improved 224 disease resistance and improved growth performance (Stone, 1998). Yeast is high in minerals and B 225 vitamins in particular. The B- glucan from the yeast, mannan-oligosaccharides in the yeast cell wall 226 and nucleotides have positive effects on gut microbiota, immunity and disease resistance), enhanced 227 growth performance in both poultry (Hooge, Sims, Sefton, Connolly and Spring, 2003) and pigs 228 (Davis, Maxwell, Erf, Brown, and Wistuba, 2004) and improved gut morphology and mucus 229 production (Santin, Maiorka, Macari, 2001; Moralez-Lopez , Auclair, Garcia, Esteve-Garcia, and 230 Brufau, 2009). 231 The process for separating yeast protein concentrate (YPC) during bioethanol production is shown in 232 Figure 1. A form of DDGS is still produced via this process, although with a slightly reduced protein 233 content (less than 5% reduction).

234 Figure 1: The process for production and separation of YPC from bioethanol processing

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,

237 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

240 poorer growth performance (Thacker and Widyaratyne, 2007).

241 Figure 2: A comparison of the nutrient composition of DDGS and Yeast protein concentrate (YPC)

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244 3.2.1 Feeding YPC to animals

245 YPC is an ideal feed protein for fish and poultry. Digestible amino acid content of yeast protein 246 concentrate has been shown to be comparable with soya for broiler chicks, and higher than the 247 feedstock alone (Burton, Scholey, Williams, 2013), although this is heavily influenced by the drying 248 process used (Scholey, Williams, Burton, 2014a). In feeding studies with broiler chicks, dietary 249 inclusion levels of up to 17.5% bioethanol YPC gave improved performance characteristics (Scholey, 250 Williams, Burton, 2014b). Bioethanol sourced YPC has been fed to several aquaculture species, with 251 20% dietary inclusion appearing optimal for performance (Omar, Merrifield, Kuhlwein, Williams, 252 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). 253 254 The higher growth rate compared with the controls is probably reflective of the benefits of the yeast 255 and yeast components. 256 Figure 3: Growth of trout fed diets containing graded levels of YPC 257 3.3 Protein value 258 Three factors drive protein value; geographical location, protein composition and presence of 259 additional nutrients. For soya, DDGS and potentially YPC, there is a premium for location from the 260 USA (least expensive) through to the EU and Asia (most costly). Additionally if the protein is a

261 favourable alternative to soya, with less environmental and supply issues, this adds an additional

262 financial premium. Yeast has a further premium applicable as it has additional nutrients and

263 potential health enhancing activity. All these factors add to a substantial increase in value for YPC

264 over soya, particularly in the EU and Asia markets.

265 Rather than DDGS which is mainly a product for ruminants, biorefineries can produce a diverse

266 valuable protein feed portfolio for livestock, comprised of 58% DDGS for cattle, 10% YPC (for

267 fish/poultry) and remainder a high protein concentrate (for monogastrics).

268 **4.1 Conclusion**

269 The potential to bioprocess plant material to produce both fuel and feed is an exciting prospect. 270 Advanced physical and chemical separation technologies combined with the opportunity to employ 271 enzymes, contributes to the portfolio of techniques to bioprocess material. Protein is an enduring, 272 expanding, high value market, which is expected to increase by 40% by 2050. Currently ethanol 273 biorefineries produce DDGS as a co-product which is a valuable feed ingredient mainly used in cattle 274 feed. However producing high value protein from DDGS is practicable and the technology on offer is 275 appropriate for use in the dry grind ethanol process. Dry grind plants can be adapted downstream to 276 produce valuable protein co-products, which not only add value to the product stream but allow 277 access to growing markets such as pig, poultry and aquaculture and therefore may reduce the 278 reliance of Europe on imported soya bean meal. A rebranding of the dry grind bioethanol process is 279 needed to emphasise that it is a traditional, natural, sustainable, bioprocess producing protein, oil 280 and the co-product ethanol. 281 References 282 Ayre, M. (2007). "Will biofuel leave the poor hungry?" BBC News 3-10-17 283 Newshttp://news.bbc.co.uk/1/hi/business/7026105.stm Accessed 30-4-15 284 Bedford, M.R. and Partridge, G.G. (2010) Enzymes in Animal Nutrition, CAB International, London,

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383 Table 1: Potential novel protein sources by category

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

Composition of grainCrude protein (%)811Oil (%)42Sugar & starch (%)6563200 Million litre ethanol plant600524Grain K tons600524Ethanol MI litres200200DDGS K tons240200DDGS protein %2534Grain protein K tons4858Yeast protein K tons1010Total protein K tons2410		Maize	Whea
Crude protein (%)811Oil (%)42Sugar & starch (%)6563200 Million litre ethanol plant600524Grain K tons600524Ethanol MI litres200200DDGS K tons240200DDGS protein %2534Grain protein K tons4858Yeast protein K tons1010Total protein K tons2410	Composition of grain		
Oil (%)42Sugar & starch (%)6563200 Million litre ethanol plant600524Grain K tons600524Ethanol MI litres200200DDGS K tons240200DDGS protein %2534Grain protein K tons4858Yeast protein K tons1010Total protein K tons2410	Crude protein (%)	8	11
Sugar & starch (%)6563200 Million litre ethanol plantGrain K tons600524Ethanol MI litres200200DDGS K tons240200DDGS protein %2534Grain protein K tons4858Yeast protein K tons1010Total protein K tons2410	Oil (%)	4	2
200 Million litre ethanol plantGrain K tons600524Ethanol MI litres200200DDGS K tons240200DDGS protein %2534Grain protein K tons4858Yeast protein K tons1010Total protein K tons5868Total oil K tons2410	Sugar & starch (%)	65	63
Grain K tons600524Ethanol MI litres200200DDGS K tons240200DDGS protein %2534Grain protein K tons4858Yeast protein K tons1010Total protein K tons5868Total oil K tons2410	200 Million litre ethanol plant		
Ethanol MI litres200200DDGS K tons240200DDGS protein %2534Grain protein K tons4858Yeast protein K tons1010Total protein K tons5868Total oil K tons2410	Grain K tons	600	524
DDGS K tons 240 200 DDGS protein % 25 34 Grain protein K tons 48 58 Yeast protein K tons 10 10 Total protein K tons 58 68 Total oil K tons 24 10	Ethanol MI litres	200	200
DDGS protein % 25 34 Grain protein K tons 48 58 Yeast protein K tons 10 10 Total protein K tons 58 68 Total oil K tons 24 10	DDGS K tons	240	200
Grain protein K tons4858Yeast protein K tons1010Total protein K tons5868Total oil K tons2410	DDGS protein %	25	34
Yeast protein K tons 10 10 Total protein K tons 58 68 Total oil K tons 24 10	Grain protein K tons	48	58
Total protein K tons 58 68 Total oil K tons 24 10	Yeast protein K tons	10	10
Total oil K tons 24 10	Total protein K tons	58	68
	Total oil K tons	24	10



406 Figure 1: The process for production and separation of YPC from bioethanol processing



426 Figure 2: A comparison of the nutrient composition of DDGS and Yeast protein concentrate (YPC)







