OPTIMISATION OF DISTILLERY CO-PRODUCTS FOR POULTRY FEED

DAWN VICTORIA SCHOLEY

A thesis submitted in partial fulfilment of the requirements of Nottingham Trent University for the degree of Doctor of Philosophy

AUGUST 2012

ABSTRACT

EU legislation has led to an almost ten-fold increase in bioethanol production between 2003 and 2011. The current distillery co-product, distiller's dried grains with solubles (DDGS), is fed primarily to cattle due to the high fibre content but differentiation of the co-product stream would allow penetration of the large monogastric feed sector. The aim of this project was to evaluate a novel separation process producing a high protein yeast protein concentrate (YPC) from DDGS, and assess the potential of this product as a feed ingredient for poultry.

A pilot plant study modelling the process confirmed the variability of the stillage, but highlighted the viability of the process for ameliorating some of these differences. Inter-batch variability was reduced from 10% to 1.2% in terms of protein content but dry matter content still varied by more than 8%, due to fibre contamination. The batches of yeast cream with increased fibre content had significantly reduced drying rates, from 37.3mg/min to 23.6mg/min, due to the larger particle sizes included. Additionally, the increased range of particle size introduced by fibre contamination would lead to product separation with detrimental implications for transport and handling of the dried product.

It is vital to have measurements of amino acid content and digestibility for any new feed ingredient to ensure accurate feed formulation. Amino acid digestibility was measured *in vivo* in broiler chicks for five YPCs from potable and bioethanol sources produced using three drying technologies. Amino acid digestibility coefficients (COD) were significantly better for bioethanol produced YPC than potable (bioethanol 0.73 and 0.63 compared with 0.58 and 0.52 for potable). This is likely to be due to the addition of exogenous enzymes during the bioethanol process resulting in reduction of some of the detrimental effects of non-starch polysaccharides. Drying methodology affected both total amino acid content and digestible amino acid content, with spray drying being the least damaging method (COD 0.73 and 0.58) and ring drying the most damaging (COD 0.39). Lysine was particularly damaged during the heating process; reducing in total content from approximately 5% of protein to 2.3% of protein

for ring dried material. Freeze dried YPC samples (COD 0.63 and 0.52) may have been negatively affected by the presence of fibre in the YPC due to processing inconsistencies.

In terms of bird performance, bioethanol freeze dried YPC inclusion improved weight gain ($p=0.003$) and feed intake ($p=0.006$) compared with potable, again likely due to the enzyme addition during the bioethanol process. This was confirmed by the measured digesta viscosity increase in birds fed diets with increasing potable YPC inclusion ($p=0.073$). Spray dried YPC did not significantly affect FCR up to 20% inclusion, but both intake and bodyweight gain reduced with rate of inclusion. This may be attributed to spray drying producing a small particle size which increases feeding time and can increase viscosity, as was shown in this project (viscosity of spray dried YPC increased with RoI $p=0.031$). Ring dried YPC was incorporated into pelleted diets and therefore gave the best performance results. FCR for bioethanol YPC was improved from 1.68 for freeze dried material included at 9% of total diet to 1.4 for ring dried material at a higher rate of 12.5% of total diet.

Finally the potential of YPC as a source of available phosphorus was considered, by assessing foot ash content of birds fed diets containing varying YPC levels. Increasing rate of YPC inclusion increased foot ash from 17.3% to 18.5% at 20% inclusion ($p=0.031$). YPC replaced 35% of supplemental phosphorus in chick diets with no significant effects on bone mineralization.

The novel YPC from bioethanol distilleries appears to be a viable protein source for chicks at low inclusion levels (less than 6%). In pelleted diets the inclusion level could rise to 17.5% with no detrimental effects on bird performance. Additionally, YPC provides a source of available phosphorus, reducing the need for supplemental phosphorus in chick diets. The most appropriate drying method appears to be ring drying if care is taken to reduce residence time and heat damage. However there is substantial scope for further improvement of both the process and product as a feed ingredient for poultry.

Acknowledgements

I would like to take the opportunity to thank my Director of Studies, Dr. Emily Burton (nee Clarke) for all her support, guidance, and most of all her unshakable belief in both me and the project. Thank you for taking a chance on an old bird with many other responsibilities!

Thanks also go to my second supervisors, Dr. Anne Coules and Dr. Phillip Bonner. Special thanks must go to my external sponsor, Dr. Peter Williams for his unending enthusiasm for the project.

Funding for this project was gratefully received from the EPSRC and AB Agri, via an industrial CASE studentship.

Other thanks must go to the rest of the chickenopolis team for making the last 3 years fun, specifically, Harriet Lea and Natalie Morgan. For laboratory guidance and discussion, much gratitude goes to Dr. Kirsty Mitchell. For being the saviour in terms of all things mechanical, thanks must go to Robin Drury. Thanks also to Dr Bob Robinson of Nottingham University for friendship and statistics advice.

Finally, this thesis would not have been possible without the total support of my family; my parents especially for childcare and proofreading, my husband for endless support and belief and of course my babies, Bryn and Eden, for being well behaved when mummy was otherwise occupied.

GLOSSARY

AAD – Amino acid digestibility

BRC – Bio-refinery co-product (abbreviation for studies)

BWG – Bodyweight gain

Ca - Calcium

CC – Continuous centrifugation

Centrate – Liquid fraction from separation process

DCP – Di calcium phosphate

DDGS – Distiller's dried grains with solubles

FCR – Feed conversion ratio

FD – Freeze dried

FI – Feed intake

ICP-OES – inductively coupled plasma-optical emission spectrometer

KS - Kolmogorov Smirnoff

MANOVA – multiple analysis of variance

MCP – Mono calcium phosphate

NSP – Non-starch polysaccharide

P - Phosphorus

Paste – Solid fraction from decanter

PVT – Phosphorus validation trial

RD – Ring dried

SD – Spray dried

Stillage – Material from alcohol fermentation post distillation of alcohol

Washing – Adding water to the yeast cream and re-separating through the disk stack

WDDGS – Wheat distiller's dried grains with solubles

Yeast cream – The solids and yeast containing fraction after decanter and disk stack separation

YPC – Yeast protein concentrate; yeast cream dried to a powder

CONTENTS

All procedures undertaken in this thesis have been approved by the Nottingham Trent University College of Arts and Science Ethics committee, and did not require a licence under the Animal Scientific Procedures Act (ASPA, 1986).

CHAPTER 1: REVIEW OF THE LITERATURE

1.1 INTRODUCTION

Demand for renewable fuels is increasing, and growth in production will result in supply of one of the present co-products from bioethanol production (Distillers Dried Grains and Solubles DDGS) far outstripping demand in its current use in animal feed. The aim of this project is to convert a commodity feed co-product stream from a bioethanol production plant, into a high value protein product that could replace plant-sourced feed protein or fishmeal which is currently imported into the EU for feeding pigs, poultry and fish. Unless the existing product can be improved and differentiated, it may revert to a waste product. This would also have major impact on the potable alcohol industry which produces a very similar co-product. The product contains valuable non-GM wheat and yeast proteins plus grain fibre. Limitations to the widespread use of the co-product are that currently, it can only be used in volume for cattle feed due to the high levels of fibre. High proportions of fibre is negative nutritionally for monogastrics as it reduces nutrient absorption in the gut, but the co-product would potentially be a valuable source of protein if separated from the fibre fraction.

Poultry meat is consistently growing in popularity (Magdelaine *et al*., 2008). 800 million broilers are produced annually in the UK, on approximately 3000 broiler units (Defra, 2011a). Profit margins are very small, so co products from other industries are already valuable resources for the feed industry to minimise feed costs. 3.3 million tonnes of poultry feed is used annually in the UK for broilers alone. Globally 48 billion meat birds are produced annually, using 198 million tonnes of feed (FAO, 2010). This huge market could be accessed by making bioethanol co-products more suitable for monogastrics.

A novel, factory scale, continuous-flow process is being developed to separate a yeast-containing, high protein fraction from the distillery stillage (Williams *et al*., 2009; Williams, 2010). This project will initially characterise this novel product, and establish using lab scale

measures whether the product is consistent, before further characterisation of the product *in vivo* as a feed ingredient for poultry.

The technology would be applied in the emerging bioethanol industry, in the existing potable alcohol industry and also in any large scale grain fermentation facility. The differentiation of the co-product could reduce waste from these processes and have positive implications for the industry globally. Information gathered in this project will be fed back into the manufacturing pilot system to optimise the process.

1.2 SUSTAINABLE PROTEIN SOURCES FOR ANIMAL FEED

The increases in consumer demand for meat will require increasing protein supplies for animal feed. The major protein sources in use are soyabean meal, fishmeal and meat and bone meal, with some protein also being sourced from industry co-products and legumes. The majority of protein used in animal feed is from oilseed; Gilbert (2002) quotes an annual figure of 316 million tonnes (Mt) of oilseed protein, 14Mt from animal by-products and 7Mt from fishmeal.

Soyabean meal

Soya bean meal is the by-product of oil extraction and is the most important protein source in the EU, with 40 million tonnes (Mt) imported into the EU per annum and 2.2Mt into the UK, accounting for 80% of animal feed. It is estimated that 60-90% of soya comes from plants that are genetically modified (gmo-compass.org). The EU has only allowed a small amount of non-EU improved GM in feed products (0.1%) since 2011 and this limit makes soya expensive to import. Recently, soya bean meal prices have risen as high as 500 USD per metric tonne (FAO, 2012).

Fishmeal

Fishmeal is a good quality protein source with high total and digestible amino acid content. However fishmeal can be expensive and production has not increased over the past 20 years (Speedy, 2002). Overfishing has led to restrictions being put in place to conserve fish stocks and Hardy (2010) states that the supplies of fishmeal are finite and that further reduction in use of fishmeal will be required in the future. Another issue that limits the inclusion of fishmeal in laying bird diets is a fishy taint which can be detected in the egg yolks (Butler and Fenwick, 1984).

Meat and bone meal

Meat and bone meal (MBM) is derived from the rendering of animal tissues (particularly cattle) and provides a cost effective protein. However the discovery spongiform encephalopathy (BSE) in cows and Creutzfeldt-Jakob disease in human (CJD) led to a total ban on the use of mammalian MBM in the EU in 2001 (TSER 999/2001; ABPR 1774/2002). Although this ban may be lifted in non-ruminant feeds, currently its use is illegal in the UK according to the Transmissible Spongiform Encephalopathies Regulations (2010).

Other protein sources

Co products can provide quality protein, which is inexpensive and sustainable. Important examples include brewery co-products (which will be considered in section 1.4) and maize gluten meal. The latter is rich in protein but deficient in some amino acids including lysine (Chadd *et al*., 2002). Rapeseed meal from biodiesel production is also increasing in volume, particularly in the EU. Other protein sources include legumes which can yield high protein levels, but with poor amino acid profiles. However, worldwide the use of lupins, peas and beans are substantial, particularly in developing countries. Alternatively sources are being

considered including algae from biofuel production (Spolaore, 2006). Lei (2012) estimated that algae could replace a third of soya protein in monogastric diets

1.3 USE OF BIOFUELS

The European commission published the Biofuels directive in 2003 (EU, 2003a) which promotes renewable fuels as a means of reducing carbon emissions. Targets were set for member states and in the UK these were incorporated into the Renewable Transport Fuel Obligation, which specifies that renewable fuels must make up 3.5% of fuel supplied on UK forecourts (Draft RTFO, 2007) increasing up to a 5% inclusion by 2014. In 2010/11, 1507 million litres (Ml) of biofuel were supplied in the UK, of which 41% was bioethanol (618Ml/year), the majority of which was imported from Brazil and the USA (Department for Transport, 2012). In 2011 the EU produced 4400Ml, an increase of 105% on 2007 making it the third largest producer behind Brazil and the US (European bioethanol fuel association, 2009). Table 1.1 compares ethanol production for the five major ethanol producing countries since 2007.

Table 1.1 Global bioethanol production over the last 5 years, by country (RFA, 2012)

	Bioethanol production/ million litres				
Producer	**2011**	**2010**	**2009**	**2008**	**2007**
USA	54,436	51,865	40,068	34,020	24,562
Brazil	21,066	26,161	24,861	24,464	18,972
Europe	4,411	4,566	3,927	2,770	2,155
Canada	1,746	1,346	1,096	900	798
China	2,094	2,045	2,045	1,898	1,837

Ethanol is a high performance fuel, which burns relatively cleanly and emissions are consistently lower than those for petroleum (Lynd, 1996; Grosjean *et al*., 1998), and biodiesel (Patterson *et al*., 2008). The Environmental Protection Agency has confirmed that bioethanol

emits 21% less greenhouse gases than gasoline (Von Blottnitz and Curran, 2007), and studies in general agree that bioethanol has a positive impact on resource depletion (Petrou and Pappis, 2009).

The bioethanol industry is also growing aggressively in the USA, leading to a projected increase of five times the current co-product production, which in turn will lead to a possible tenfold increase in imports into the UK. This coupled with an increased EU production of ethanol (see table 1.2), will mean a 4.2Mt increase in DDGS production by 2012 which is likely to saturate the current markets. By differentiating DDGS, it will add economic value to the ethanol process and reduce the amount of excess DDGS and therefore the amount of potential waste. Most bioethanol production is currently first generation, from feedstock which has the potential for feed use. The use of food as fuel is controversial and has many detractors. Further develpments in technology will allow fibre recovery (second generation) and biomass production of bioethanol.

Table 1.2 Increase in ethanol production in the EU since 2003 (Biofuels platform, 2012)

Year	Production Million litres
2003	446
2004	528
2005	913
2006	1608
2007	1803
2008	2855
2009	3703

1.3.1 Bioethanol from Wheat

The production of bioethanol is a mature technology, which uses enzyme liquefaction and saccharification to produce a relatively clean stream of glucose from the starch component of

the feedstock. The glucose is then fermented by *Saccharomyces cerevisiae* yeast to produce ethanol. The left over mash (stillage) is decanted into a fibrous wet grain and a liquid component, thin stillage, which contains the majority of the yeast protein. Thin stillage has a very high water content, with only around 60-85g/kg dry matter (Mustafa *et al*., 1999), so is evaporated into a syrup and remixed with the wet grain to form Distiller's Dried Grains with Solubles (DDGS). Commonly the starch is supplied by sugarcane (Brazil) or maize (USA), but in the UK the feedstock is wheat.

1.3.2 The Bioethanol Process

A schematic of the bioethanol process is shown in figure 1.1. This shows the production of ethanol from corn which is identical to the process using wheat.

Milling

The initial stage of the bioethanol process involved grinding the grain to increase surface area and allow effective cooking and enzymatic starch breakdown. The whole wheat grain is milled complete, a "dry grind" process. Milling fineness effects alcohol yield, with a more finely ground grain yielding 5-10% more alcohol than coarse (Kelshall and Lyons, 2003). The dry grind method is commonly used in both potable alcohol distilleries and bioethanol plants.

Liquefaction

The milled grain is then mixed with water and heated in a high temperature cooking step. This step gelatinizes the starch and the high temperature and pressure exerts mechanical shear on the structure to disrupt the endosperm. Microbial contamination is also reduced. The temperature needs to be controlled carefully to reduce any possible Maillard's reactions which will reduce alcohol yield (Bringhurst *et al*., 2003).

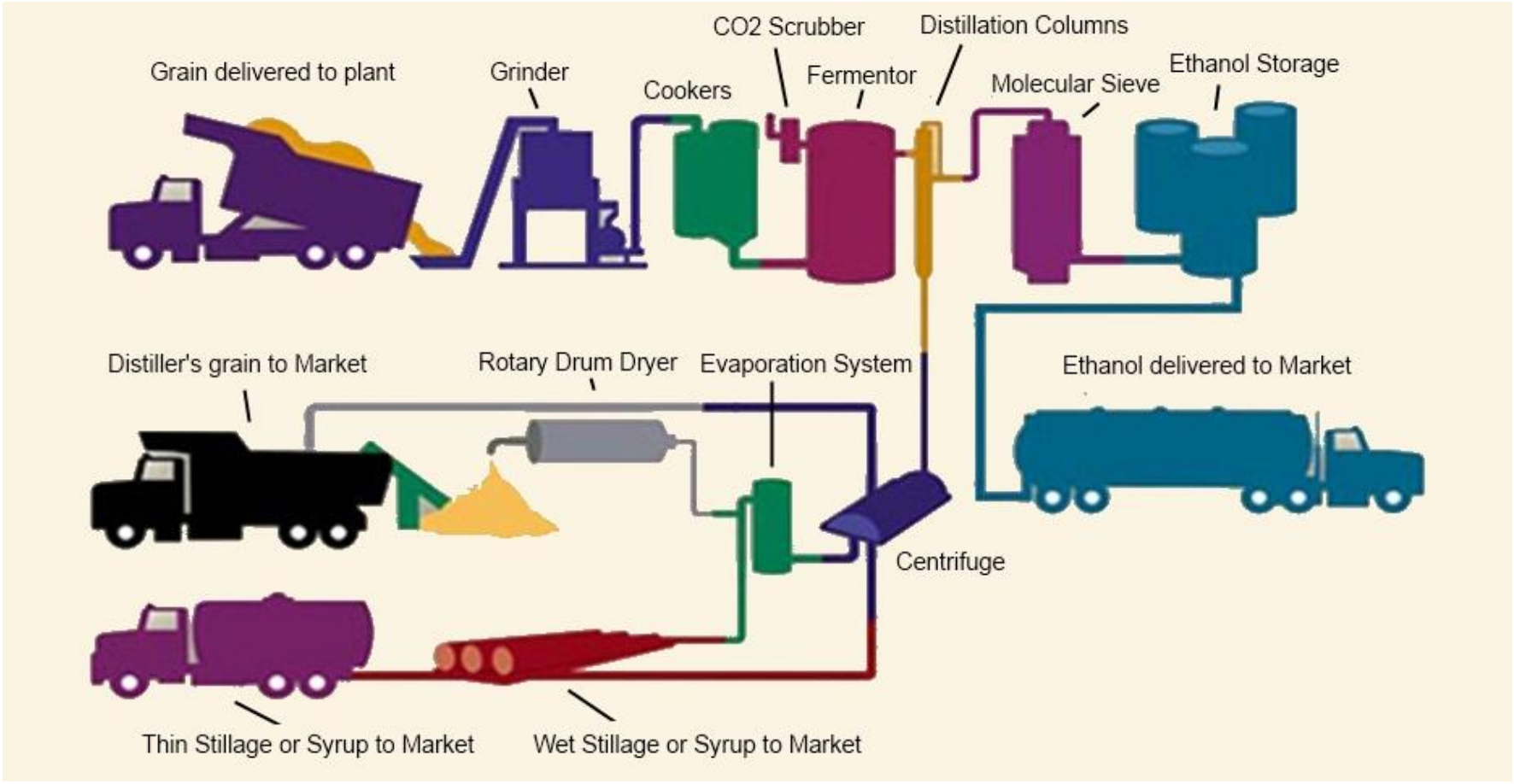

Figure 1.1: The bioethanol process using cereal feedstock (adapted from redtrailenergyllc.com)

Saccharification

After the mash is cooled to 90-100^{o}C, a heat stable amylase is added to breakdown the starch and reduce the viscosity to allow subsequent enzymes to work more efficiently. After further cooling, glucoamylase is added to remove glucose residues from the starch.

Fermentation

The fermentation step is anaerobic, the mash is cooled and the yeast added, typically for between 48 and 72 hours at 30-35^{o}C. An alcohol containing wort is produced with a final alcohol content of 8-12%, depending on the yeast and substrate added. For potable alcohol the efficiency of this step is 84% (Smith *et al*., 2006), with half of this under-recovery due to loss of sugars in yeast growth. In fuel alcohol, the starch conversion efficiency is estimated as 98-99%.

Distillation

Ethanol vaporises at 78^{o}C, alcohol is removed from the wort by heating, to give a 95% ethanol 5% water mixture. This is the end point for potable alcohol production; whereas in fuel alcohol production, molecular sieves are used to adsorb water to give a pure anhydrous alcohol product.

Stillage separation

After fermentation and distillation, the mash left over (stillage) is separated by centrifugation or pressing and extrusion into wet grain and thin stillage. To produce Distillers' Dried Grains with Solubles (DDGS), the thin stillage is dried to a syrup to remove much of the water content and remixed with the grain. In maize alcohol production, 50% of the revenue of the process is derived from the co-products (Wheals *et al*., 1999).

1.3.3 Processing issues with wheat bioethanol

Wheat endosperm contains around 11% non-starch polysaccharides (NSPs), which include beta glucans, arabinoxylans (pentosans) and fructans (Montgomery and Smith, 1956). Arabinoxylans are the major non-starch polysaccharide present in wheat (Choct and Annison, 1990; Chesson, 2001), but content varies with variety and year (Saulnier *et al*., 1995). These NSPs affect processing efficiency and yield, as they have a high water binding capacity and therefore increase mash viscosity, which makes transport difficult during processing. The increase in viscosity makes the DDGS difficult to dry without burning, as the temperature in the drier can be uneven. This increases the likelihood of Maillard's reactions, which produce biologically unavailable lysine derivatives (Plakas *et al*., 1985). This makes the lysine unavailable which reduces the nutritional value of the co-product. Xylanases and other enzymes that break down NSPs are commercially available to reduce viscosity in bioethanol processing (Sorenson, 2006; Novozymes, 2009) and are used routinely in bioethanol processing (Bamforth, 2009).

1.3.4 Potable alcohol production

The broad process for potable alcohol production is the same as fuel alcohol. Starch is degraded to glucose and then fermented to alcohol by yeast, and the alcohol is separated by distillation from the residual material, which is usually dried to form DDGS.

Barley is the feedstock for the whisky making process. The main difference with potable alcohol is that producers are confined by the Scotch Whisky Order (1990), which only allows the use of grain, water and yeast in production. The emphasis is on a high quality, traditional product, throughput being less important. The processing time is around 100 hours for potable alcohol production compared with 60 hours for fuel alcohol. No enzymatic treatment is allowable in potable alcohol production, whereas fuel alcohol production has no such constraints; exogenous enzymes and chemicals are added to improve processing efficiency and

throughput, and to reduce energy costs. The malting process of potable alcohol production synthesises enzymes, specifically amylases and glucanases which break down starch and glucans.

Barley is malted and then milled to a fine flour which is then mixed with water in a mash tun and heated. This promotes the enzymatic hydrolysis of the constituents of the malted barley, and in this process the starch is converted to fermentable and non-fermentable sugars and proteins are partially degraded. A mash tun is a large drum with rotating blades which initially separates the liquid "wort" and the waste draff via perforations which allow the watery wort to flow out to the fermentation stage. Yeast is added to ferment the sugars to alcohol and this is then distilled off by heating the still with steam vapour. Stills in Scotland are typically copper. Scotch whisky is double distilled, with the initial distillation occurring in a large wash still to producing an alcohol content of around 21%. This is then re-distilled in a smaller spirit still to produce a product containing 63-72% alcohol pre-aging. The waste from the first distillation is called "pot ale" and this is frequently mixed with draff to produce a distillers dark grains which is similar to DDGS. A schematic of the potable alcohol process is shown in figure 1.2. Significant differences in DDGS composition have been seen between different distilleries, particularly in residual starch and total sugars, suggesting different alcohol conversion efficiencies (Cottrill *et al.*, 2007). There is also the potential to market the DDGS product as organic due to the restricted ingredient list.

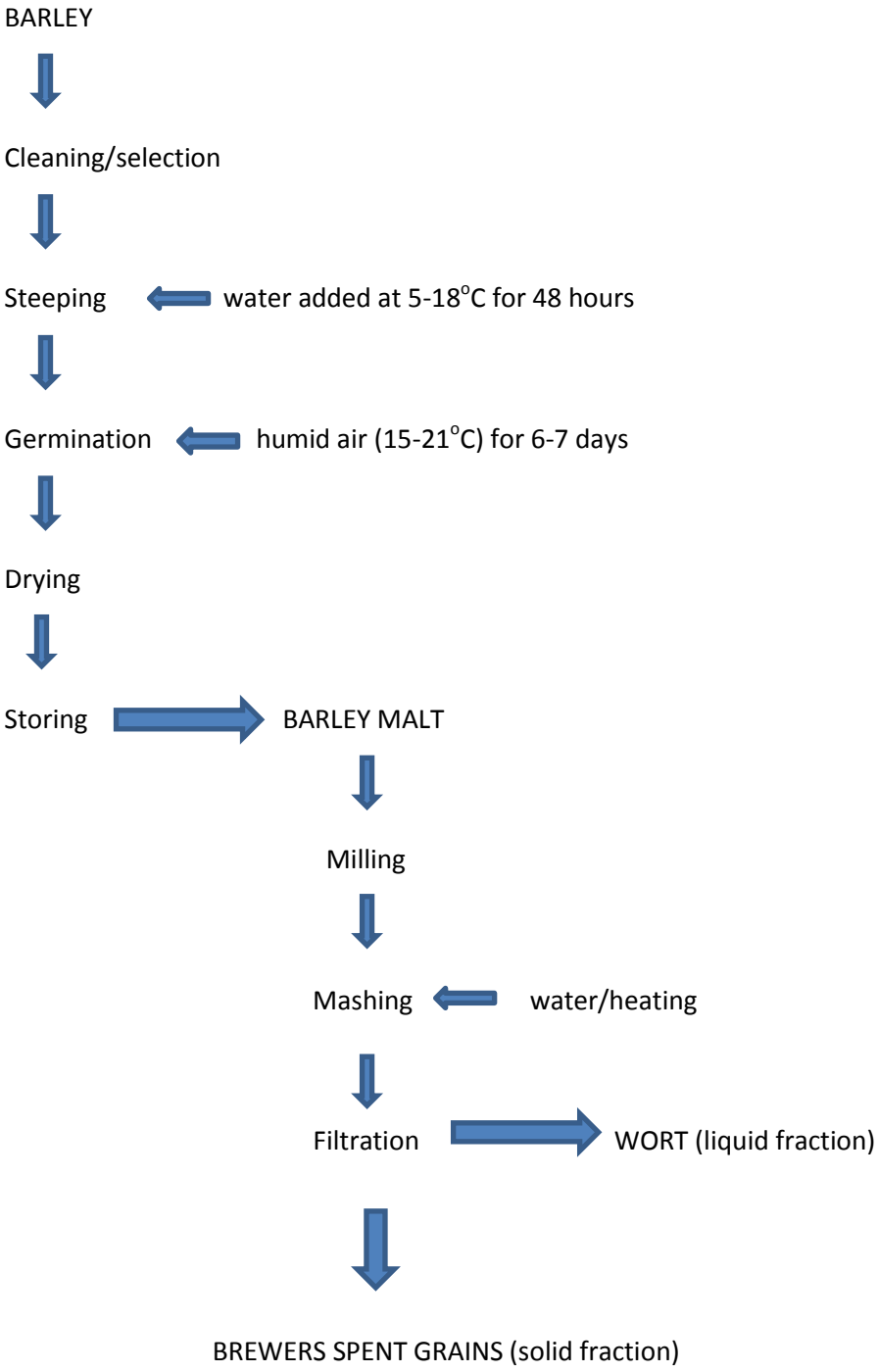

Figure 1.2 Schematic of the potable alcohol process.

1.4 DDGS

Distillers dried grains with solubles are produced from de-alcoholised fermentation residues, after the yeast fermentation of grains to convert starch to alcohol (Weigel *et al*., 1997). Most of the work to date on DDGS as a feed ingredient has been using maize DDGS, which is

produced in the USA in large quantities. In the UK and most of Europe the feedstock of choice will be wheat, which brings different challenges. The three main challenges for wheat as a feedstock are the high fibre content, variability and increased anti-nutritional factors (NSPs).

1.4.1 Variability in DDGS production

Maize DDGS has been found to be very variable in nutritional value both within and between plants, especially for amino acids (Cromwell *et al*., 1993; Spiehs *et al*., 2002). With wheat, varietal differences and processing have been shown to affect alcohol yield (Swanston *et al*., 2007; Schingoethe, 2006) and co product composition (Lan *et al*., 2008; Azafar *et al*., 2012). Significant differences in DDGS composition have been seen between different distilleries, particularly in residual starch and total sugars, suggesting different alcohol conversion efficiencies (Cottrill *et al*., 2007). Belyea *et al*. (2010) assessed DDGS from a number of bioethanol plants at different times over the course of a year. Fermentation batches were found to be the most influential source of variation. However starch composition of the parent grain can also affect ethanol yields. Particle size distribution has been measured for ground maize at the first stage of the bioethanol process (Rausch *et al*., 2005) and considerable variation was found between batches and plants. In the fermentation step, there are many processing factors which effect the composition of the final product, including temperature, concentration of solids and water quality (Rausch and Belyea, 2006). After fermentation, the stillage is pressed and the separation can be imperfect, allowing some fibre to escape the sides of the press, so that the grains and stillage will vary considerably in composition. The stillage and grains are re-combined to form DDGS and this process is difficult to control and the proportions of each may not be consistent. Noll *et al*. (2007) investigated the effect of adding varying amount of solubles back to the fibre and found that increased soluble addition increased fat, energy and mineral content and decreased amino acid digestibility.

Finally the DDGS is dried using processing conditions that can vary widely which may impact protein quality of the DDGS (Swietkiewicz and Koreleski, 2008). During drying, reducing sugars

may bind to the ε-NH_2 group of lysine via Maillard reactions, which makes the lysine nutritionally unavailable. Maillard reactions increase the darkness of the product which has been correlated to chemical composition (Cozannet et al., 2010).

1.4.2 Nutritional value of DDGS as a feed ingredient

Removal of the starch from the wheat source through fermentation triples the concentration of valuable nutrients such as protein in the DDGS product (Thacker and Widyaratyne, 2007). DDGS nutrient content varies between feedstock types (i.e. wheat and maize) and also between batches of the same feedstock (Azarfar *et al*., 2012).This nutritional variability found in DDGS can affect feed manufacturing as nutrients contributed by DDGS can vary so widely that the final feed can be out of tolerance for final product specification (Behnke, 2007). For example, maize DDGS has been found to vary in calcium and sodium levels in particular (Batal and Dale, 2003) and wheat DDGS has increased protein and decreased fat when compared to maize DDGS, as shown in the comparison table 1.3.

Table 1.3 Comparison of nutritional content of wheat, wheat DDGS and maize DDGS (from Thacker and Widyaratyne, 2007)

Variable	Wheat	Wheat DDGS	Maize DDGS
Moisture	11.8	8.1	11.8
Crude protein	19.8	44.5	30.3
Non-protein N	4.6	10.2	5.4
Fat	1.8	2.9	12.8
Ash	2.1	5.3	4.8
ADF	2.7	21.1	14.6
NDF	9.4	30.3	31.2

Amino acid content of DDGS does not necessarily correspond to the increased protein content, as some amino acids (such as proline and alanine) concentrate more rapidly than others (such

as histidine and leucine) during fermentation (Liu, 2011). Due to the high possibility of Maillard reactions during DDGS drying, lysine digestibility is a major concern for the use of DDGS as a feed ingredient. Maillard reactions occur when reducing sugars and amino acids, proteins or other nitrogen containing compounds are heated together. The reaction is a complex series of stages, beginning with a condensation between a reducing sugar and most commonly the ε-amino group of lysine (Purlis, 2010). The condensation product is rearranged to form an Amadori product which is then degraded to by-products dependant on the reaction pH. When pentoses and hexoses are involved, HMF (hydroxymethylfurfural) is formed which reacts to form brown polymers (Martins *et al.*, 2001). Maillards impair the nutritional content and the bioavailability of amino acids and proteins (Moralez *et al*., 2007). Colour has been correlated to amino acid digestibility by a number of researchers (Batal and Dale, 2006; Fastinger *et al*., 2006), with lysine digestibility reducing from 80% to 60% with darker DDGS (Ergul *et al*., 2003). Very dark samples have recently been reported to give very low ileal digestibility values in pigs (Cozannet *et al*., 2010) and in cockerels (Cozannet *et al*., 2011a). This heat processing damage also negatively affect the digestible energy content of both corn (Fastinger *et al*., 2006) and wheat DDGS (Cozannet *et al*., 2011b). In wheat DDGS, amino acid digestibility was found to be lower in pigs when compared to wheat and some amino acids (including lysine) were significantly reduced by the drying process (Pederson and Lindberg, 2010).

Unfortunately the fermentation of starch means that DDGS also contains increased levels of fibre and the anti-nutritional factors present in wheat. Currently wheat DDGS is successfully used in ruminant nutrition at inclusion levels of between 25 and 35% without effecting nutrient digestibility or growth characteristics (Li *et al*., 2011; Yang *et al*., 2012), but the high fibre content decreases feed intake and limits nutrient utilisation in both pigs (Nyachoti *et al*., 2005) and chicks (Thacker and Widyaratyne, 2007), as the fibre increases dietary bulk. Wheat DDGS contains varying amounts of non-starch polysaccharides (NSPs) (Saulnier *et al.*, 1995).

These increase viscosity, which causes issues for mixing and transport during processing (Smith *et al.*, 2006), and can cause uneven drying which reduces the amino acid content of the DDGS, particularly lysine (Cromwell *et al.*, 1993; Waldroup, 2007). The increase in digesta viscosity is also a problem for a feed constituent, increasing digestive transit and reducing nutrient absorption in poultry (Choct and Annison, 1992a; 1992b). In pigs, supplementing DDGS with a carbohydrase enzyme has increased nutrient digestibility and growth in some studies (Emiola, 2009; Yoon *et al*., 2010) but not all (Jones e*t al*., 2010; Yanez *et al*., 2011). Agyekum *et al*. (2012) found a trend towards decreased villus height and villus crypt ratio with 30% DDGS inclusion (wheat/corn blend) in pigs, and a decreased dressing percentage which was removed by addition of a multi- carbohydrase enzyme.

For maize DDGS, suggested inclusion rates for broilers are 24% when fully balanced for amino acids (Shim *et al*., 2011) and 6% when not fully supplemented (Lumpkins *et al*., 2004). Wang *et al*. (2007) did suggest that there may be a possible loss of breast meat yield at 20% maize DDGS inclusion. Inclusion rates for layers are suggested at 10% (Lumpkins *et al*., 2005), and Masa'deh *et al*. (2011) found that egg weight was reduced when more than 15% DDGS was included in the diet.

Wheat DDGS has been used successfully in pig feed at 30% with no effect on performance traits (McDonnell *et al*., 2011) and in broiler feeds at 15% inclusion (Thacker and Widyaratyne, 2007, Youssef *et al*., 2008). Loar *et al*. (2010) suggested that feeding DDGS levels of 15% or higher may adversely affect young chicks less than 28 days of age, but that feed intake in older chicks is reduced if chicks are not exposed to DDGS in starter diets. Although levels up to 12% have no effect on meat quality or consumer acceptance, above these levels there may be a negative effect on thigh meat as increased fatty acids may increase oxidation (Corzo *et al*., 2009; Schilling *et al*., 2010).

DDGS has also been considered in aquaculture diets; in tilapia, Schaeffer *et al*., (2010) found that higher levels of DDGS (over 20%) reduced feed conversion ratio and bodyweight gain and hypothesised that replacing fishmeal with DDGS would require supplementation at higher inclusion levels. In diets supplemented with lysine p to a 40% DDGS in tilapia has been shown to be acceptable in terms of performance (Li *et al*., 2011).

If DDGS could be differentiated and made more suitable for monogastrics, it would enable the co-product to be more easily utilised via the large pig and poultry feed markets. Some work has been carried out to produce a higher protein DDGS, including the Elusieve process, which uses a combination of sieving and air flow to produce a higher protein enhanced DDGS (Svinivasan *et al*., 2008; 2009). This process has been used with maize DDGS and the high protein fraction fed to chickens with a small increase in final bodyweight (Loar *et al*., 2009) but no differences in energy or amino acid (Kim *et al.*, 2010). Sieving wheat DDGS showed increased energy digestibility in rainbow trout (Randall and Drew, 2010). The improvements to DDGS using this process appear to be marginal at present.

1.4.3 Processing issues with DDGS

DDGS has been found to deleteriously effect pellet quality; increasing DDGS content has been negatively correlated with pellet durability (Shim *et al*., 2011) and has been shown to increase the quantity of fines (Loar *et al*., 2010). The most common reactions which affect pelleting are protein denaturation and starch gelatinisation. Behnke and Beyer (2002) found that starch is usually involved in the bonding between particles which helps to produce a durable pellet. In DDGS production, the majority of the starch has been fermented to alcohol, and this couples with the relatively high oil content. The bonds formed are mostly hydrophilic in nature and oil in the DDGS can produce a hydrophobic coating which inhibits binding, thereby producing a poor quality pellet (Behnke, 2007). Loar and Corzo (2011) also discussed the effect of oil

addition on the pelleting of DDGS, concluding that the oil addition required to add energy to DDGS containing diets would form a more viscous diet, which required more energy to pellet. Loar *et al*. (2010) also found that higher DDGS inclusion increased energy use in the condenser due to the viscosity of the mash. Production rate was shown to decrease with 30% DDGS inclusion, which may be due to reduced supplemental rock phosphate, which has a scrubbing effect in the die (Loar *et al*., 2010). However the same study also showed a decrease in energy through the pellet mill to be due to the fat content, which will lubricate the product through the die. The energy considerations could be mitigated by post pelleting fat addition if required.

1.5 SEPARATION OF YEAST PROTEIN FROM STILLAGE

The yeast component of the DDGS has been estimated to make up a minimum of 5.3% of the DDGS protein content (Ingledew, 1999). Han and Liu (2010) found that yeast contributes 20% towards DDGS proteins in maize bioethanol production. This yeast is a valuable source of protein if it can be economically separated from DDGS, leaving a high fibre fraction suitable for ruminant feeding. A novel, continuous centrifugation (CC) process to separate a yeast protein concentrate (YPC) from stillage is under development by the industrial sponsor. This separation will also leave a fibre fraction, which is still nutritionally appropriate for ruminant feed (with a less than 2% reduction in protein) and a watery, sugar-rich fraction. Once separated, the YPC is then dried to produce a powder. This YPC powder may be suitable as either a protein source or a feed additive for monogastric feeds. However, as with DDGS, batch variability and product drying remain key issues, and the product is likely to still contain some of the anti-nutritional factors present in wheat.

The separation undertaken in this project has three distinct stages; decanting, liquid removal and drying.

1.5.1 Decanter separation

Decanters are solid walled, horizontal centrifuges used to separate suspensions with a high concentration of solids. In the case of ethanol stillage, the decanter is the first stage of separation after distillation. A decanter houses a rotating horizontal bowl with a cylindrical and a conical section, and a scroll integrated in the bowl (see Figure 1.3). The stillage enters the separation chamber through a centrally arranged feed pipe, and due to centrifugal forces, the solid particles are flung towards the wall of the chamber. The rotating screw in the separating bowl conveys the solids to the cone end of the bowl where they are then discharged. Regulating tubes allow the level of liquid to be altered within the bowl. The liquid phase, containing the yeast flows in the opposite direction to the solid discharge through the cylindrical part of the bowl to discharge under gravity. This separated liquid will contain varying amounts of solid matter depending on the size of the regulating tube and the speed of flow of the material. This liquid fraction is then further clarified using a centrifugal separator or disk stack.

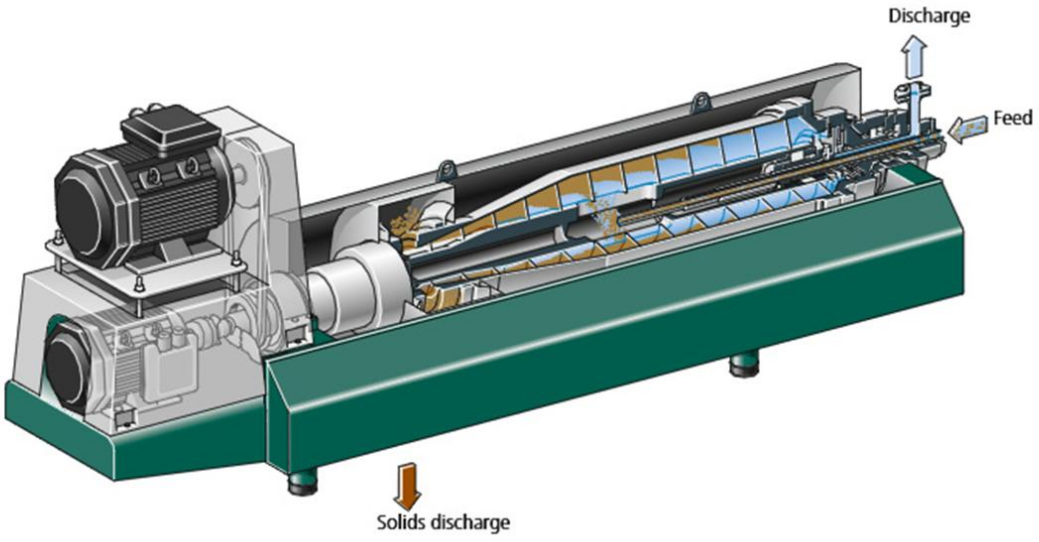

Figure 1.3 Cross sectional diagram of a decanter (GEA Westfalia, 2011)

1.5.2 Disk stack centrifuge

The disk stack is a continuously operating nozzle centrifuge which is specially designed for liquid separation. Using a centrifuge to remove water is more cost effective than drying (V'ant Land, 1991).The disk stack comprises a rotating bowl equipped with a large number of inserts; conically arranged discs which are stacked into the bowl with small interspaces (see Figure 1.4). The spacers are between 0.3 and 2mm, which creates a large separation area in a small space. The bowl is able to rotate up to 15,000rpm.

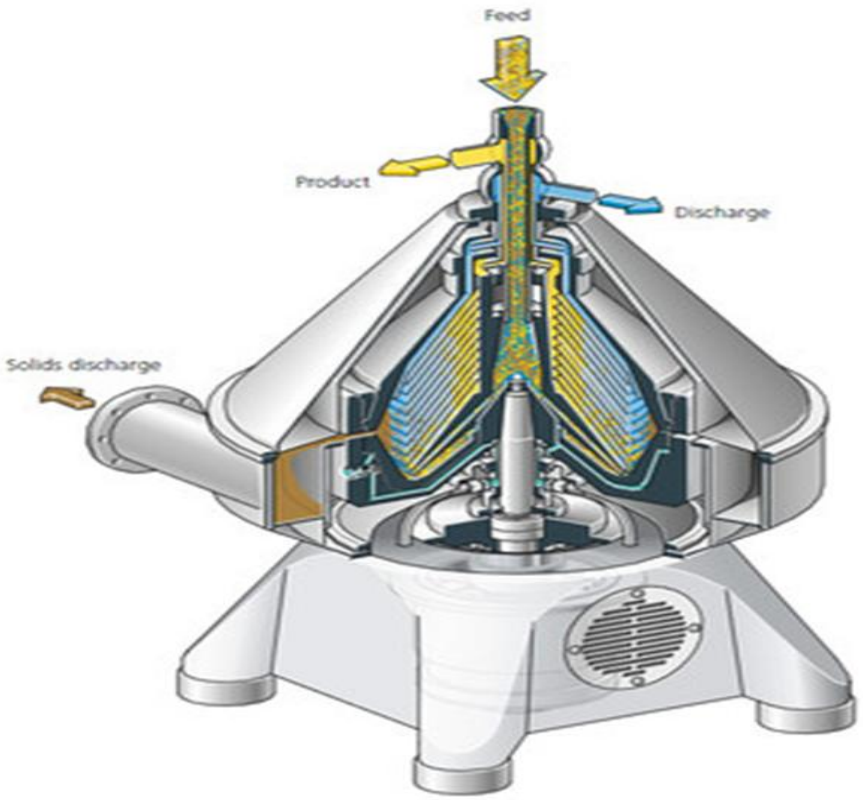

Figure 1.4 Cross section of a disk stack centrifuge (GEA Westfalia, 2011)

The liquid fraction from the decanter enters the bowl through a central feed tube and the product is accelerated and conveyed into the disc portion of the separator. Solids are flung

against the underside of the disk above due to their higher density and then they flow down the disc. The separated solids are continuously discharged though nozzles at the bowl periphery (see figure 1.5 for close detail).

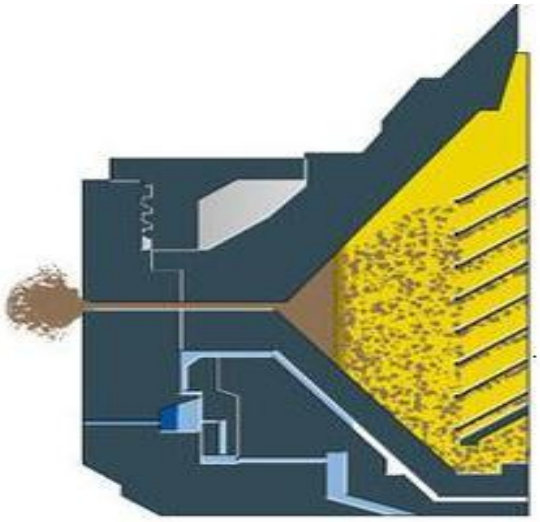

Figure 1.5 Cross section of the discharge nozzle of a disk stack centrifuge (GEA Westfalia, 2011)

The liquid in the bowl is then picked up by a centripetal pump which discharges the liquid. In the case of the alcohol stillage separation, the solid fraction from the disk stack is referred to as a yeast cream and the liquid fraction as a centrate. A yeast cream separated from the disk stack is shown in figure 1.6.

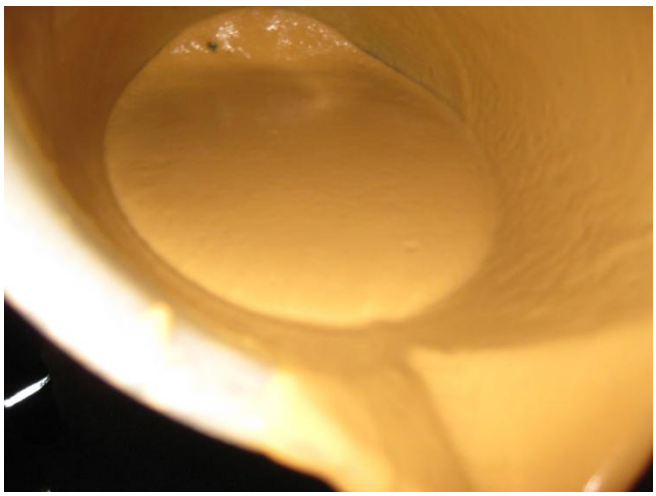

Figure 1.6: Yeast cream separated by the disk stack process.

1.5.3 Drying process

After much of the water is removed via centrifugation, it is necessary for the yeast cream (about 20% dry matter) from the disk stack process to be dried to a powder for shelf life considerations and ease of transport and handling. A powder can also be easily and thoroughly mixed with other feed stuffs prior to pelleting of diets. The type of drying can affect nutritional content of the finished product, although product deterioration is usually due to the application of excess heat, rather than the moisture removal (Morris *et al*., 2004). The effect of the drying on the structure of the yeast cell wall may also be an issue. Drying types can be classified by either the mechanism of heating or mechanism of vapour transport. Air drying requires a high temperature air which supplies the heat and removed the water vapour whereas vacuum drying uses a reduction in pressure to remove the vapour (Chen *et al*., 2008). The specific drying methods used in this study will be described in more detail in the following sections. When choosing a drying technology, consideration needs to be made of required capacity, moisture content, particle size and drying characteristics of the material and maximum allowable temperature.

Freeze drying

Using a freeze drier to remove water from the yeast cream is the least aggressive form of drying. Freeze drying is a third generation drying technology, which has 4 distinct stages, freezing, vacuum, sublimation and condensing. There is no application of heat, instead the material is frozen to -50^{o}C and a vacuum removes liquid and which condenses in the chamber of the drier. The material is unchanged by the process, making this method of drying a good control method for assessing the effect of other drying regimens. It has been shown to overcome issues of structural damage to the end product (Karel, 1975, Dalgleish, 1990) and the absence of air prevents oxidative deterioration, and there is no possibility of heat damage. Freeze drying technology is more commonly used for smaller scale applications due to cost implications as it is 4-8 times more costly than air drying (Ratti, 2001). Lin Hsu *et al*. (2003)

compared hot air, drum and freeze drying of Yam flours and found anti-oxidants were more preserved in freeze dried samples, but otherwise little difference was observed between the drying technologies.

Ring drying

Ring drying is a first generation drying technology where hot air flows over an extensive area to remove water from the surface of the product. A heated air stream moves the material to be dried through a vertical column and as the particles lose moisture they are transported to the top of the column. The bulk of the evaporation occurs in this initial period when the wet product comes into contact with the hot airstream. The particles are then further dried by moving them through one or more rings attached to the column. The ring dryer is differentiated from a flash drier by the presence of an internal classifier, which uses the centrifugal effect of an airstream passing round a curve to concentrate the product into a moving layer. Adjustable splitter blades are used to convey the heavier semi-dried material back into the dryer for another pass through the system. Any lighter, dryer product is removed on the first pass. This selective extension of residence time allows the ring dryer to process traditionally hard to dry materials. However there is more possibility of burning and overheating occurring, and consistency of product may be an issue as the material is not all dried for the same length of time. Ring drying is a more cost effective method of large scale drying when compared to spray drying and freeze drying.

Spray drying

Spray drying is a second generation drying technology, which is defined by the formation of a spray of droplets, which are produced for optimum evaporation and contact with air. A spray dryer converts a suspension to a powder in a single processing step. A nozzle or atomiser is used to convert the liquid input into a fine spray, usually with droplet sizes of 100-200um (Niessen, 2002). The liquid fraction is sprayed into a hot vapour stream which vaporises the liquid. This is a very rapid method of drying which produces a consistent particle size and often

a fine, free flowing, powder end product. It also offers an easy and continuous drying process. However spray drying is not appropriate for viscous liquids as they are difficult to atomise into a consistent, fine spray. Clogging of the nozzles can occur and the material can clump to the side of the spray dryer, leading to overheating and uneven burning of the product. Spray drying has been shown to be a suitable method for preserving viable yeast (Luna-Solano *et al*., 2003), but it is a high energy demand process, which needs to be carefully managed to minimise resource use (Luna-Solano *et al*., 2005). A comparison of ring and spray drying processes is shown in table 1.3

Table 1.3 Comparison between ring and spray dried product (adapted from Vega Mercado *et al*., 2001)

Measure	**Ring Dried**	**Spray Dried**
Particle size	10 - 3000µm	10 - 500µm
Residence time	1 - 300 min	5 - 100 sec
Feed characteristics	Solid	Fluid

1.6 YEAST

Yeast (*Saccharomyces cerevisiae*) is produced in the bioethanol process, with 0.071g yeast being produced for every gram of starch (Spencer Martins and Van Uden, 1977). Ingeldew (1999) calculated that yeast makes up at least 3.9% of the DDGS biomass, and 5.3% of the DDGS protein content. More recent work by Han and Lui (2010) found that yeast contributes as much as 20% towards the protein in maize DDGS.

1.6.1 Yeast as a protein source

Yeast contains valuable proteins, B vitamins, nucleotides and high inositol and glutamic acid levels (Silva *et al*., 2009). Composition of yeast is affected by the growth substrate during

fermentation (Ingledew, 1999), especially the ratio of Carbon to Nitrogen (Vananuvat, 1977). The temperature of the process will also affect the composition as high temperatures may denature some nutrients (Stone, 1998).

Yeast has been considered as a protein source in animal feed for many years. In 1945, Klose and Fevold fed yeast as a sole protein source but found it deficient in essential amino acids. This has also been substantiated by more recent research (Caballero and Sgarbieri, 2000). Sell *et al*. (1981) fed methanol grown yeast as a replacement for soya bean meal, and found it could replace 75% if issues of palatability and texture are addressed. Baker's yeast has been fed successfully to broilers and layers at inclusion up to 10% (Yalcin *et al*., 1993; Onol and Yalcin, 1995), but some studies found higher levels of yeast (15% and higher) decreased growth and performance (Daghir and Abdul-Baki, 1977; Waldroup, 1971). Shannon and McNab (1972) fed paraffin grown yeast to broilers with a 10% inclusion resulting in decreased FCR and a 20% inclusion also depressing growth. Poo and Millan (1990) replaced soya with a protein supplement made from beer yeast (*Saccharomyces carlbergensis*) and suggested 50% as a maximum substitution level, above which bodyweight gain decreased although there was no reduction in intake.

In other species, yeast has been fed as a sole protein source (22.5% inclusion in diet) to foals with no adverse effects except a depression of intake with increasing inclusion (Winkler *et al*., 2011). In fish, yeast has been used to replace 50% fishmeal, with no significant differences in growth and improved protein conversion (Oliva-Teles and Goncalves, 2001). Omar *et al*. (2012) investigated bioethanol YPC in carp diets and found 15-20% inclusion was optimal in terms of performance. Gause and Trushenski (2011a; 2011b) also replaced fish meal with ethanol derived yeast in sunshine bass, and found no adverse effects on performance with inclusion levels of up to 22.5% of the diet, but that palatability and production performance was impaired with full replacement of fish meal with yeast (30% dietary inclusion). Half of dietary

fishmeal has been replaced with brewer's yeast in tilapia, with no adverse effects (Zerai *et al*., 2008).

Lower inclusion levels of yeast have been fed to pigs with positive results. 5% inclusion has been shown to improve ADG and intake in nursery pigs (Carlson *et al*., 2005), and whey yeast inclusion was shown to improve pig performance (Spark *et al*., 2005). In fish Essa *et al*. (2011) supplemented catfish diets with low levels of brewer's yeast (2% and less) with improved performance.

In broiler chicks an additive level of 0.3% inclusion showed an improvement in weight gain of 11% and the birds accumulated less abdominal fat (Miazzo *et al*., 2005), whereas Ciurescu (2001) found that 6% inclusion did not affect bird performance. Yalcin *et al*. (2008) fed 4.5% brewer's yeast to quail with no adverse effects on performance or serum uric acid. Low levels of baker's yeast (less than 2%) also improved performance characteristics in broiler chicks with no effect of serum uric acid (Shareef and Al-Dabbagh, 2009).

Recent work using distillery sludge showed a decrease in weight gain in meat birds with increasing inclusion levels (Rameshwari and Karthikeyan, 2005), which could be due to anti-nutritional factors which may also have been present in the sludge. Although there are some conflicting findings, the evidence discussed above suggests that yeast could have a positive effect on performance, especially at lower inclusion levels. This would support the possible use of the novel YPC at low levels in poultry feed.

Knott and Shurson (2004) fed a separated yeast cream from maize DDGS. The separation process used was not described, but the fractions appear to be similar to those produced by the CC process. An increase in gain:feed was shown in late grower pigs with 7.5% inclusion. However any improvements in gut health were seen only with the separated residual soluble fraction, which questions whether some of the beneficial yeast components have been removed by the separation stage. This residual soluble fraction could be equated to the sugary liquid produced during the novel CC process, so the possibility that important nutrients are

being removed during processing needs to be considered. However, Sharif *et al*. (2012) fed a washed yeast sludge from potable alcohol production at 4,8,12 % inclusion to broilers, with increased weight gain and feed intake. The nutritional content of washed and unwashed yeast was also compared and they found that washing reduced the mineral content but increased protein, energy and amino acids.

It has been suggested that yeast may have a positive effect on palatability due to the glutamic acid content (Rose, 1987). Vananuvat (1977) found that inclusion of *Saccharomyces cerevisiae* in poultry diets increased palatability and food intake. However several authors have pointed to a negative palatability factor and reduced intake in poultry fed yeast containing diets (Daghir and Sell, 1982; Succi *et al*., 1980.; Vananuvat and Chiraratananon, 1977). Feeding pelleted or crumbed yeast containing feeds has been shown to increase intake, and if intake is equal, performance is similar to soya (Daghir and Sell, 1982). This palatability issue has implications for the feeding of the powder YPC as chick diets for *in vivo* studies are usually mash based in research scale facilities. However measuring feed conversion efficiency gives a measure of any positive performance regardless of any lowered intake.

Another issue may be that the bioethanol yeast may be more exhausted of cellular components, due to the cell cycling process and the exposure to high levels of ethanol (higher than during potable production). The protein content has been found to be lower for ethanol yeast than traditional brewer's yeast, as the yeast is only used once in the potable process (Yamada *et al*., 2005).

1.6.2 Yeast fractions as dietary additives

Perez *et al*. (2011) suggested that up to 10% maize DDGS inclusion in diets may have a beneficial effect on chick intestinal health, due to the increased microbial diversity in the caeca, which could be interpreted as an improved stability of the microbiota. This effect may be due to the yeast component of DDGS particularly the sugars in the yeast cell walls, which make up 20% of the yeast dry weight (Ingledew, 1999). These sugars include mannanoligosaccharides

(MOS) which are considered to be a prebiotic with three modes of action: improved pathogen adsorption (Spring, 2000), improved gut health (Baurhoo *et al*., 2009; Brummer *et al*., 2010) and immune modulation by binding to specific immune receptors (Kogan and Kocher, 2007). MOS inclusion in diets has also been shown to improve bodyweight gain and FCR (Feed Conversion Ratio) in poultry (Parks *et al*., 2001; Hooge *et al*., 2003) and pigs (Davis *et al*., 2004). MOS supplementation may shift intestinal microflora towards beneficial organisms (Geier *et al*., 2009; Baurhoo *et al*, 2009). Spring *et al*., (2000) showed an inclusion of MOS decreased Salmonella concentrations in the caeca of birds, and Elmusharaf *et al*. (2007) found an improvement in some coccidiosis lesions with MOS supplementation.

Yeast cell walls may also contain other biologically important fractions and the inclusion of whole yeast cell walls in diets has been shown to have a positive effect on gut morphology in broiler chicks (Moralez-Lopez *et al*., 2009). Yeast cell wall from lysed cell culture increased bodyweight gain and reduced intestinal coliform population (Stanley *et al*., 2004), and improved goblet cell numbers in broilers (Reisinger *et al*., 2012). Yeast cell wall components have also been shown to improve intestinal morphology and mucosa along with performance in chicks (Zhang *et al*., 2005; Santin *et al*., 2001).

Due to the high ethanol exposure during the process, the bioethanol yeast may have a thicker, toughened cell wall, which is more resistant to enzyme proteolysis (Caballero and Sgarbieri, 2000). Rumsey *et al*. (1991a) showed that salmonids fed disrupted yeast had an increased nitrogen absorption compared with whole yeast. This improvement in nutrient utilisation in homogenised yeast has also been shown in poultry (Vananuvat, 1977; Vananuvat and Chiraratananon, 1977) and shrimp (Coutteau *et al*., 1990).

Recent studies have assessed extracted yeast protein as an additive, in which the yeast cell wall was enzymatically hydrolysed. These can be compared to the YPC investigated in this thesis, as it has been mechanically disrupted, particularly by the shear forces exerted during the disk stack processing step. Silva *et al*., 2009 fed diets with 2% yeast extract to broiler chicks

and found improved FCR at 21 days of age, but did not find an effect on immune response in broilers. In a similar experiment, yeast derived protein was fed at 0.1% to broilers with improved performance and a humoral response against Newcastle disease was also observed (Haldar *et al*., 2011). The latter study also showed an increase in villus height which may suggest an improved absorptive capacity of the gastrointestinal tract.

1.6.3 Uric Acid production from dietary yeast inclusion

Yeast has 7 times more nucleic acids than traditional protein sources (Giec and Skupin, 1988). Levels are high enough that Ingledew (1999) suggested that yeast should use a conversion factor of 5.5 to calculate percentage protein from nitrogen, as opposed to 6.25 for other protein sources such as soya bean meal. Excretion of nucleic acids is limited in man and monogastrics, which can lead to a build-up of uric acid in the lower extremities, leading to gout (Stone, 1998); however high levels of uric acid are needed to cause gout in chicks (Peterson *et al*., 1971), due to its conversion into a soluble compound by binding to a protein in the kidney nephron (Braun and Dantzler, 1997). Nucleic acid content is even less problematic for fish as they have a very active liver uricase enzyme (Rumsey *et al*., 1991b).

In humans, low concentrations of uric acid act as an important anti-oxidant, which accounts for two thirds of the total plasma antioxidant capacity. Free radicals are produced in the mitochondria during respiration or by the immune system, and can damage tissues and lead to various pathologies and cancer (Graff *et al*., 1999). Antioxidants have multiple modes of action to quench free radicals (McGraw and Ardia, 2004). Hellstein *et al*. (1997) proposed uric acid as a potent combatant of oxidants as it scavenges free radicals from oxygen and stabilises ascorbate through iron chelation (Verdejo, *et al*., 2008). Uric acid is more important still in birds as they produce more oxidants than mammals due to their increased basal metabolic rate. Simoyi *et al*. (2002) found that reducing these oxidants improves meat quality and improves bird health, and found a correlation between oxidant activity and plasma uric acid

levels. Cohen *et al*. (2007) also found that uric acid was highly correlated with antioxidant capacity in chicks.

Hevia and Clifford (1977) found that uric acid in excreta increased with increased protein intake, and that protein efficiency ratio decreased as uric acid increased up to a level of 3000mg uric acid per day, therefore uric acid content of excreta can be used as a method of estimating dietary protein quality (Miles and Featherston, 1976).

High levels of uric acid in excreta are broken down into ammonia by microbial uricase so uric acid content of excreta is also important environmentally. Some minerals inhibit microbial uricase activity, especially zinc and copper by up to 90% (Kim and Patterson, 2003).

1.7 THE BROILER CHICKEN AS A NUTRITIONAL MODEL

1.7.1 Poultry production and consumption

Poultry meat is consistently growing in popularity (Magdelaine *et al*.,2008), due to low price points, no cultural or religious obstacles, and the dietary and nutritional advantages of poultry meat above other meats. UK consumption is greater than the EU average, at around 30kg/capita/year and UK consumption of poultry has doubled over the last 20 years (Food ethics council, 2007). Table 1.4 shows the consumption of poultry per capita worldwide (2006 figures).

However poultry meat prices fell 45% between 1994 and 2002 (Magdelaine et al, 2008), so competitive production is needed to ensure profitability.

Table 1.4 : Poultry consumption for 2006 (Magdelaine *et al*., 2008)

Country	Poultry consumption kg/capita for 2006
United States	54
Brazil	33.3
Mexico	26.1
EU	22.2
China	26.1

Value of the UK poultry industry exceeds £2 billion in 2010 (Crane *et al*., 2011) and 74 million broiler chickens were produced in the UK in 2011, a drop in production of 5.5% compared with the previous year (Defra, 2011a). This reduction is due to an increase in input costs, particularly feed prices, which have increased from £149 per tonne in October 2006 to £278 per tonne in October 2011 (Defra, 2011b). 1,223 thousand tonnes of broiler feed is produced annually in the UK (2011 figures). In the US, the poultry industry is worth over $830 billion per annum (Dunham, 2009). The poultry sector also extends to laying birds, which uses 160 thousand tonnes of feed per annum and produces around 26 thousand cases of eggs per year in the UK (Defra, 2012). Other poultry in production such as turkeys, ducks and geese make up a smaller, but still significant proportion of the poultry sector. Poultry meat production in the UK for August 2012 stood at 102 thousand tonnes of broiler meat, 14.8 thousand tonnes of turkey and 2.4 thousand tonnes of duck (all carcass weight) (Defra, 2012).

In terms of raw materials, 356 thousand tonnes of distillery co-products were used to produce animal feed in 2011, which is an increase of 23.7% on the previous 12 month period (Defra, 2011b). The majority of these co-products are used in ruminant feed but a huge market could be accessed by making alcohol co-products more suitable for monogastrics.

1.7.2 Digestive tract of the chick

Mechanical, chemical and enzymatic processes occur throughout the gastrointestinal tract, but absorption only occurs in the small intestine (Larbier and Leclercq, 1994). Digestion and absorption in the chicken are very efficient processes; digesta may take as little as 2-4 hours to transit the tract.

The alimentary tract of the chick may be up to 6 times longer in total than the length of the bird. In the chick the gastrointestinal tract is precocious and can make up 25% of bird live weight at hatching. By 8 weeks this has reduced to less than 5% (Larbier and Leclercq, 1994). The length of the individual component sections of the digestive tract varies with size of bird,

feed type and other factors. For example, more fibrous feedstuffs are associated with large digestive tracts (Sturkie *et al.*, 1965).

Mechanical, chemical and enzymatic processes occur throughout the gastrointestinal tract, but absorption only occurs in the small intestine (Larbier and Leclercq, 1994). Digestion and absorption in the chicken are very efficient processes, digesta may take 10 hours to transit the tract or as little as 2-4 hours.

Buccal cavity

Most foods are ingested whole, with only a small amount of breakdown occurring in the beak. Feed is passed down the pharynx by a forward and upward movement of the head (Hill, 1976). When drinking water the beak closes and the water flows passively into the oesophagus as the head is raised. This is coordinated by the tongue and other parts of the buccal cavity.

The tongue is narrow, pointed and triangular in cross section, and situated in the lower beak. It is rigid with few intrinsic muscles, and is attached to the hyoid bone which confers mobility to move food and water into the oesophagus. Birds have no soft palate and no distinction between the mouth and the pharynx (Duke, 1986a). There are few taste buds, therefore taste may have a minor role in feed consumption (Hill, 1976; Scott *et al*., 1976). The few taste buds that are present vary in location and number between species. Food consumption appears to relate to energy levels of the diet (Duke, 1986a).

Salivary glands are numerous and secret a salivary fluid which is rich in mucous. This moistens and lubricates the food bolus, assisting its passage into the oesophagus. The volume of saliva produced increases during feeding to 7-30ml per day, dependant on feeding conditions and parasympathetic stimulation (Duke, 1986a; Chodnik, 1948). Saliva is alkaline, contains amylase and is rich in bicarbonate ions (Hill, 1976; Larbier and Leclercq, 1994). The amylase has little effect on digestion as food passes through the mouth too rapidly for digestion to occur (Duke, 1986a).

Oesophagus and Crop

These organs produce mucous for lubrication in response to feeding. The oesophagus is chicks is relatively long, 11-12cm in a 20 day old bird, (Calhoun, 1954), growing to 20cm in a mature bird (Sturkie, 1965; Hill, 1976). The oesophagus is situated between the proventriculus and the pharynx and has two parts; upper (cervical/cranial) and lower (caudal/intra-thoracic) and the crop is situated between these two regions. The crop and oesophagus are similar in microstructures, except the mucous glands are situated only at the juncture of the oesophagus (Sturkie, 1965; Hill 1976)

In the chick the crop is well developed and acts as a temporary food store and reservoir. This allows the bird to regulate the transit of digesta and its digestive efficiency, and enables it to eat large quantities of food at one time. In the crop, food is moistened and mixed with saliva and water to aid subsequent digestion. Food is mixed by three different types of muscle and passed into the proventriculus as required. When the gizzard is empty, the food bypasses the crop and moves directly into the proventriculus. Empty crops contract at 1 to1.5 minute intervals, less frequently than this when containing food. Emptying is determined by capacity, fullness of gizzard, food particle size and moisture content (Hill, 1976; Larbier and Leclercq, 1994). If digesta moves rapidly on from the gizzard, then crop emptying is also accelerated, for example if the feed is wet or finely ground.

Proventriculus and Gizzard

The proventriculus and gizzard are two stomachs with complementary roles. The proventriculus secretes hydrochloric acid, pepsin and mucous, whereas the gizzard has a mechanical function.

The proventriculus is ovoid, has a thickened wall and is located between the lower oesophagus and the gizzard. It contains gastric glands which secrete hydrochloric acid and pepsinogen. Entry into the proventriculus is regulated by the gizzard and depends on the crop and lower oesophagus. Contractions one minute apart move the ingesta through. If the feeding is ad lib,

nervous and chemical stimulation causes the proventriculus to secrete gastric juice, so the proventriculus and gizzard are mainly acidic (Hill, 1976; Larbier and Leclercq, 1994). No digestion occurs until the gizzard, where the food is held and gastric secretions act (Duke, 1986a). Food moves through the proventriculus too quickly for digestion to occur.

The gizzard is a thickened biconvex organ with a tough lining and extensive muscularity, due to its purpose as a grinding organ and site of initial proteolysis (Duke 1986a). The bird needs to have insoluble mineral grit present for successful digestion as it improves food grinding through abrasive contact, and improves motility by increasing the size of the contractions.

The proventriculus produces hydrochloric acid (pH 2 – 3.5), which solubilises mineral salts, ionises electrolytes and disrupts tertiary structures of dietary proteins in the digesta while in the gizzard. Food is moistened to make a paste (chyme) which enters the intestine after peptic digestion commences.

Small Intestine

The small intestine is in 3 parts, the duodenum, jejunum and ileum. In adult birds, the whole small intestine is 120-140cm long, whereas in young birds (20 days) the duodenum is 12cm and the jejunum and ileum combined is 49cm (Calhoun, 1954). The small intestine is the principal site of digestion involving enzymes of both intestinal and pancreatic origin. Hormones are secreted which principally regulate gastric and intestinal actions (Duke, 1986a).

The duodenum is a U shaped loop around the pancreas (Hill, 1976; Larbier and Leclercq, 1994). There are two components of pancreatic secretion, aqueous and enzymatic. The aqueous secretions contain a high concentration of bicarbonate ions to neutralise the chyme and give optimum pancreatic enzymic activity. The enzymatic secretions contain peptidases, amylases, saccharidases and lipases to digest protein, carbohydates and lipids. Pancreatic juice has a pH of between 6.4 and 6.8 in chickens (Hulan *et al*., 1972).

Enzymes are secreted as proenzymes into the intestinal lumen; the presence of ingesta converts them to active status and the peptide hormone secretin, influences pancreatic juice

secretion. Secretin in formed in the intestinal walls in addition to cholecystokinin-pancreozyme (CCK-PZ). Release of secretin is stimulated by gastric hydrochloric acid and food reaching the duodenum. CCK-PZ is released by stimulus from amino acids, peptides and fats from the feed. Secretin causes an initial secretion of aqueous and enzymatic components (Duke, 1986b). Intestinal juice contains pancreatic and bile secretions and enzymes which are secreted by the brush border of the small intestine, which are involved in the hydrolysis of oligosaccharides. The gizzard-duodenal junction acts as a filter to prevent grit and large particles of food from entering the duodenum. The goblet cells (Crypts of Lieberkuhn) secret mucous as Brunner's glands do not occur in birds.

Bile from the liver at pH6 enters the duodenum through two ducts. Lipids are emulsified by this bile and this facilitates the action of pancreatic lipase and lipid absorption. Bile synthesis and secretion progress with age, which means that older birds can digest lipids adequately. (Larbier and Leclercq, 1994). Bile also contains amylase so it has an involvement in carbohydrate digestion (Duke, 1986b). Amylase first appears in chicken bile ay 4-8 weeks of age, it has greater activity when secreted from the gall bladder as opposed to the liver (Farner, 1943). The bile and pancreatic ducts enter the caudal end of the ascending limb of the duodenum; this marks the commencement of the jejunum.

Pancreatic trypsin is detected in poultry embryos at 18 days incubation (Sell *et al.*, 1991). Specific trypsin activity in the pancreas decreases after hatching until 4 days of age and then increased up to 14 days. There is then no change in activity until 20 days of age.

Meckel's diverticulum marks the beginning of the ileum. The jejunum and ileum are similar in length, structure and function; they are the major absorptive regions of the digestive tract. The ileum terminates with a ringed valve before branching into two caeca. The digesta is propelled through the ileum by peristalsis and segmentation movement. The small intestine is filled with villi, which increase the surface area of the small intestine to improve nutrient absorption. Villi are projections of epithelial and sub epithelial tissue and are covered by delicate absorptive

tissue. Bird villi contain capillary bed and lymph vessels. Absorptive cells are large cylindrical structures with basal nuclei and an apical membrane covered with microvilli (Hill, 1976). Villi are tubular and contain secretory cells and mucous secreting goblet cells and are associated with the Crypts of Leiberkuhn. Villi also contain undifferentiated cells which may undergo mitotic division to become the precursors of absorptive cells which then develop further and migrate either up the villus or down the crypts.

Large Intestine: Caeca and Colon

The large intestine is 20cm long in an adult bird and 5cm in a young bird (Calhoun, 1954). Caeca are paired, blind ended sacs at the junction of the small and large intestine. They are thought to produce a limited amount of B vitamins and have involvement in water absorption (Feltwell and Fox, 1978). Caeca contain microorganisms which degrade undigested nutrients, but few are absorbed. However products of fermentation may be absorbed from the caeca (Hill, 1976).

The ileo-caecal-colonic junction (sphincter) controls the flow of chyme between the colon and the caeca. When the ileum distends the sphincter relaxes to allow colonic movement and when the colon distends it contracts. As the colon contracts, digesta moves towards the caeca or cloaca depending upon the peristaltic direction. If the birds are fed *ad libitum*, the caeca replenishes regularly and evacuation results from a strong distal end contraction. Emptying frequency of the caeca varies with caecal distension, pH, electrolyte activity and diet. There is minimal food digestion in the large intestine. Microbes decompose some undigested food particles and form excreta, which passes into the cloaca (Sturkie, 1965; Larbier and Leclercq, 1994).

Broiler chickens are often used as a model for investigating feed ingredients due to their rapid growth rate, particularly in the starter and grower phases (day 1 to 28). This speed of growth makes them particularly sensitive to dietary changes. Broilers are genetically programmed for

fast tissue deposition, which makes them physiologically vulnerable and any nutritional deficiencies will have a clear effect on performance. There are also distinct changes in the digestive tract of the broiler as it grows which makes quantification of negative dietary impact easier.

1.7.3 AME measurement

The energy content of poultry feed is crucial to balance growth and feed efficiency with profitability. Feed can represent 65% of the production cost in broilers, with energy making up the largest proportion of this cost (Dozier *et al*., 2007). A reduction of 0.5MJ/kg can be the difference between profitability and loss for poultry (Austin *et al.*, 1999). If other nutrient ratios are maintained, increasing energy input increases FCR and bodyweight gain (Waldroup, 1981). Energy use in poultry diets is measured as apparent metabolisable energy, which is a measure of gross energy in the feed minus the gross energy in the excreta (NRC, 1994). This is commonly measured quantitatively by collecting feed intake and excreta output or by relating dietary energy throughput to that of an inert marker. The use of inert markers will be discussed further in the subsequent section (1.7.4).

1.7.4 The use of inert markers in poultry nutrition

Inert markers are regularly used in digestibility studies as they make it possible to calculate the digestibility of a nutrient without measuring quantitatively feed intake and excreta output. The inert markers are vital when investigating nutrient uptake of the gastro-intestinal tract at a particular point (Short *et al*., 1996). An inert marker needs to be easily analyzed; indigestible and non-absorbed by the animal while maintaining digestive transit at the same speed as other dietary nutrients; physiologically inactive; non-toxic and easily mixed into the diet to produce a homogenous concentration (Jagger *et al*., 1992; Sales and Janssens, 2003). Some of the markers used include chromic oxide, titanium dioxide and acid insoluble ash. Although chromic oxide is widely used as a marker, there have been difficulties reproducing results and mixing

uniformly in feed (Vohra, 1972). It is also potentially hazardous (Peddie *et al*., 1982). Acid insoluble ash is another suggested marker, but the intestinal flow may not reflect feed passage accurately (Cheng and Coon 1990). Titanium dioxide has been evaluated for use in ruminants (Titgemeyer *et al*., 2001; Meyers *et al*., 2003a) and poultry (Short *et al*., 1996) and Peddie *et al*. (1982) found TiO_2 measurement gave less variation than total excreta collection. Meyers *et al*. (2003b) could not obtain consistent results with the Short *et al*. (1996) method, but the method has been used with success by other laboratories. Although fewer studies have been conducted to evaluate TiO_2 as a dietary marker in poultry in comparison to other species (Sales and Janssens, 2003), it has been used successfully to measure amino acid digestibility (Rodehutscord *et al*., 2004), energy (Ventura *et al*., 1994) and calcium and phosphorus concentrations (Perez-Vendrell *et al*., 2001).

1.8 AMINO ACID PROFILES IN POULTRY NUTRITION

The protein requirement of chickens is actually a requirement for amino acids, and digestibility of these can vary widely dependent on the feed material. There are 22 amino acids, all of which can be considered to be essential physiologically, but 11 cannot be synthesised by poultry and therefore must be supplemented in the diet. These 11 essential amino acids are lysine, methionine, tryptophan, threonine, arginine, isoleucine, leucine, histidine, phenylalanine, valine and glycine. Glycine can be synthesised *in vivo* by poultry, but at a rate which is inadequate for growth in modern broiler chickens. Tyrosine and cysteine are classed as semi essential as they can be synthesised from phenylalanine and methionine respectively. Several of these essential amino acids can be defined as more limiting than others, where limiting is defined as meaning that a deficiency will produce measurable signs, such as retarded growth, which would be reversible by supplementation. Practically speaking, the two most limiting amino acids for growth in poultry are methionine and lysine (Lemme *et al*., 2004),

with the most limiting being related to feedstuff as opposed to dietary need. For poultry the requirements are stated in the NRC (1994) recommendations, which are used in the industry for diet formulation.

Digestibility of amino acids should not be confused with availability. Availability can be defined as "an amino acid in a form suitable for digestion, absorption and utilisation" (Lemme *et al*., 2004). Digestibility is the greatest single contributor to availability therefore it has become the favoured technique for estimating amino acid availability, as measuring the latter directly is expensive and time consuming.

1.8.1 Measuring Amino acid digestibility

Protein quality depends on both amino acid content and digestibility, which can be reported as apparent, standardised or true digestibility, the latter accounting for endogenous losses as well as the amino acids disappearing from the intestine. *In vivo* digestibility is the favoured method for assessing amino acid digestibility due to the validity of in animal measurement and the ability to measure all amino acids concurrently. Measurements can be ileal or excreta based.

1.8.2 Excreta versus ileal amino acid digestibility measurement

Excreta was commonly used from the 1970s onwards, but has several drawbacks. The excreta contains a urine component, which is thought to contain negligible amino acids (Terpstra, 1978), but this may not always be true in every case (McNab, 1995). Also excreta use ignores the contribution of hindgut microorganisms to the amino acid profile and their effect on protein utilisation. The caecal component can affect amino acid estimates by as much as 25% as the caecal microflora can modify the amino acid content (Parsons *et al*., 1982; Ravindran and Bryden, 1999). To overcome this, commonly cockerels are caecetomised (Parsons, 1986), and tube fed directly into the crop, but this uses mature birds, so does not take into account age differences, plus it is not a normal *ad libitum* feeding situation. Amino acid digestibility has been shown to increase with bird age (Ten Doeschate *et al*., 2003; Huang *et al*., 2005) and alter depending on strain and bird sex (Kim and Corzo, 2012). Excreta measures may also be

misleading as heat damaged proteins may be voided in excreta as metabolites (McNab, 1995; Fernandez and Parsons, 1996).

Payne *et al*. (1968) first suggested ileal digestibility of amino acids as a more appropriate measure than excreta but this method is more time consuming and labour intensive. The amino acids digested in the terminal ileum have been found to be significantly higher than those in excreta, as the undigested carbohydrates may increase fermentation and therefore microbial proliferation in the hind gut, leading to microbial protein synthesis and a net gain of amino acids in the excreta (Hew *et al*., 1998). This suggests that ileal is a more accurate measure of AAD than excreta. There are distinct differences in digestibility quantified by the 2 systems, so any comparisons of feed ingredients need to be using the same methodology (Ravindran *et al*., 1999; Garcia *et al*., 2007). However recent work by Kim *et al*. (2012) compared the use of precision fed assays with a standardised method with a sole protein source and found all methods were comparable.

1.8.3 Methods of Ileal digestibility measurement

To measure ileal, or pre-caecal digestibility, samples can be taken either directly post mortem from the ileal section of the gastrointestinal tract, or through an in-dwelling fistula. The latter is labour and time intensive and the birds need to be mature for the surgery, which makes the results less applicable to growing birds. A regression method was developed by Short *et al*. (1999), in which graded levels of protein are fed as a sole source of dietary nitrogen. A linear extrapolation of the protein to 1000g/kg allows an estimate of digestible amino acids to be calculated. Whatever method is used, quantitative collection is not possible so an inert marker needs to be used, which allows *ad libitum* feeding of the test diet. Drawbacks to the use of ileal digestibility are the number of bird replicates required and the complexity of the technique, particularly with the different inclusion levels of the regression method (Lemme *et al*., 2004). Rodehutscord *et al*. (2004) established that 3 levels of inclusion was sufficient for regression analysis. The test diet needs to be formulated with added energy in the form of carbohydrates

and vitamin and minerals, to reduce the possibility of dietary refusal or deficiencies. The effect of the diet is also reduced by only pre-feeding for three days prior to sample collection (Kluth and Rodehutscord, 2010).

Kluth *et al*. (2005) found that the length of the ileal section used for sampling significantly effects the amino acid digestibility and therefore the proximal third of the ileum should not be considered.

1.8.4 True and Apparent amino acid digestibility

Apparent digestibility does not account for endogenous amino acids losses, as opposed to true digestibility which corrects for endogenous losses. When the diet contains a low protein level, the endogenous amino acids make up a greater proportion of the amino acids in the digesta, whereas as the dietary protein content increases, the endogenous contribution will be comparatively reduced, and therefore more representative of the true digestibility (McNab, 1989). McNab (1989) suggested that these losses were constant across protein intakes, whereas Ravindran and Bryden (1999) found that the levels varied dependent on protein source and level. The latter also found effects of fibre and anti-nutritional factor content on endogenous amino acids.

1.8.5 Measurement of endogenous amino acid losses

Endogenous amino acid losses can be influenced by dietary protein concentration and feed intake (Li *et al*., 1993). There are three common methods for measuring endogenous losses; using a nitrogen free diet, using fasted birds and the regression method. The first two of these have been criticised as they put the body into negative nitrogen balance and therefore the birds may not be considered normal physiologically (Low, 1990), and several authors have claimed a lack of reproducibility of the measurements (Donkoh and Moughan, 1999; Rutherford *et al*., 2004).

In the regression method (as detailed in section 1.7.3) the intercept can be considered as a measure of endogenous amino acid losses, which allows a measure of true and apparent

digestibility to be ascertained in the same experiment. This method does not allow for changes in ileal flow (Fuller, 1988), and there will be errors due to the inaccuracy of a trendline fitting to 3 points of a curve. Differences in the slope of the extrapolation curve will result in errors at both the intercept and the extrapolated 1000g/kg value. Rodehutscord *et al*. (2004) suggested that regression should be adopted as a standard method of quantifying amino acid digestibility, and that if the slope of the regression was used a measure of digestibility then no correction was needed for endogenous losses as they are already accounted for in the intercept.

The issue with the protein-free method issue is that proteolytic enzymes are not stimulated (Ravindran and Bryden, 1999), so studies have assessed using highly digestible protein sources which are assumed to be fully digested, but this may not be the case (Lemme *et al*., 2001). Lemme *et al*., 2004 detailed a method for estimating endogenous amino acid secretions using enzymatically treated casein, but this method still requires endogenous secretions to be quantified separately to digestibility so requires extra time and labour.

1.8.6 Effects of feed ingredients on amino acid digestibility

The variability of amino acid digestibility of DDGS has been considered in Section 1.4.2, but there are also differences in the parent grain, with different wheat cultivars having varying amino acid digestibilities (Ravindran *et al.*, 1999). Non starch polysaccharides have been shown to be highly variable in wheat (Choct *et al*., 1999a; Selle *et al*., 2002) and they may physically encapsulate the protein from wheat and thereby reduce amino acid digestibility (Selle *et al.*, 2009). Wheat non starch polysaccharides have been shown to increase endogenous amino acid secretions via increased digesta viscosity increasing the peristaltic resistance, leading to increased endogenous protein losses (Angkanaporn *et al*., 1994). Varying amino acid digestibilities in cereal varieties have been shown to be positively affected by xylanase supplementation, which can equalise some of these differences (Kluth *et al*., 2009). Xylanase acts by counteracting the viscosity increases induced by soluble NSPs and reducing the effect where cell wall components may encapsulate nutrients (Cowieson, *et al*., 2006).

Xylanase supplementation has been shown to increase amino acid digestibility in wheat diets fed to broilers (Hew *et al*., 1998; Selle *et al*., 2009) and turkeys (Boguhn and Rodehutscord, 2010). The fibre content of the diet can also increase endogenous amino acid losses through reduced digestive efficiency (Adedokum *et al*., 2012).

1.9 NON-STARCH POLYSACCHARIDES IN BIRD NUTRITION

Polysaccharides in the wheat endosperm are composed of glucosans and arabinoxylans (pentosans) (Montgomery and Smith, 1956) with the latter being the major polysaccharide present in non-starch polysaccharides (Choct and Annison 1990; Chesson, 2001). Wheat arabinoxylans consist of a (β1-4) linked D-xylose chain to which L-arabinose moieties are attached (Delcour *et al*., 1999) and these can make up 5% of wheat (Pettersson and Aman, 1988) but this is highly variable. Delacour *et al*. (1999) measured pentosan content in milled wheat fractions and found they varied between 1.4 and 30% of dry matter content. Insoluble NSPs can absorb large quantities of water without effecting gut motility. In wheat and barley there are about 6 times as many insoluble NSPs to soluble (Choct, 2002), whereas maize contains almost solely insoluble NSPs (Huyghebaert *et al*., 2011).

Soluble fractions of the β glucan and arabinoxylan solubilise post ingestion and influence nutritional value by several mechanisms. High levels of soluble arabinoxylans can turn the gut contents into a highly viscous gel, which reduces the availability of nutrients to the bird (Choct and Annison, 1992a; 1992b). The solubilised pentosans can form a network that reduces diffusion rates for nutrients, with particular effect on larger molecules, such as fat (shown in rye by Fengler and Marquardt, 1988). Viscosity increases can be detrimental due to decreased rates of endogenous enzyme diffusion and a negative effect has been shown on pancreatic enzymes (Classen, 1996). Increased feed passage time decreases feed intake and the reduced digesta flow rate causes overgrowth of the microbial population of the small intestine. Increased fermentation occurs in the small intestine when a large amount of viscous NSPs are

present in the diet (Choct and Kocher, 2000) and the proliferation of fermentative organisms are detrimental for digestive efficiency and bird health (Choct *et al*., 1996). This was previously implied by Antoniou and Marquadt (1982), who showed that antibiotic treatment improved fat absorption. Indirectly the proliferation of fermentative microflora leads to volatile fatty acid production in small intestine which competes for digestible nutrients (Choct *et al*., 1996; 1999b).

NSPs can also increase the depth of the unstirred water layer in the epithelial lining of the small intestine (Hedemann, 2009) which is rate limiting for absorption and impedes passage of digestive enzymes into the gut (Austin *et al*., 1999). This thicker unstirred layer at the mucosal surface slows the uptake of released sugars and amino acids impairing digestibility. Other effects of NSPs include detrimental effects on gut morphology (Gutierrez-Alamo *et al*., 2008) and increase in apoptosis of epithelial cells (Teirlynck *et al*., 2009). Annison (1991) found a significant correlation between AME and NSP content, which was suggested to be due to the water-soluble NSP component which has been suggested to be the main factor negatively effecting wheat AME and poultry performance (Gutierrez-Alamo *et al*., 2008). Sensitivity to pentosans is reduced with age (Pettersson and Aman 1988) which suggests that a gut based microflora capable of degrading NSPs develops with time.

The xylose and arabinose moieties are increased in WDDGS, and the arabinoxylan content is twice that of the parent grain (Zijlstra *et al*., 2007). It is therefore possible that the process used in this project may also be separating high levels of non-starch polysaccharides, which may be detrimental to the nutritional value of the YPC.

1.9.1 The use of carbohydrase enzymes in poultry nutrition

All broiler feed routinely contains NSP hydrolysing enzymes (Huyghebaert *et al*., 2011) which reduce viscosity and increase nutrient digestion and digestive passage rate so there is less substrate for fermentative organisms . Enzymes can ameliorate negative effects on

performance (Bedford and Classen, 1992); reduce pathogenic bacterial proliferation (Jackson *et al*., 2003) and small intestinal fermentation (Choct *et al*., 1996).

Xylanase has been shown to improve performance in wheat diets (Yang *et al*., 2008), specifically those that induced high viscosity (Adeola and Bedford, 2004). Multi- enzyme supplementation has been shown to improve nutrient digestibility and bird performance in barley diets (Garcia *et al.,* 2008) and reduce viscosity (Lavinia, *et al*., 2010). Starch digestibility has been improved by enzyme use in both wheat and barley diets, by xylanase and β glucanase enzymes respectively (Juanpere *et al*., 2005). The impairment of fat digestion can also be eliminated by the use of multicarbohydrase enzymes (Meng *et al*., 2004; Rodriguez *et al*., 2011). Improvements with enzyme addition occur up to 28 days and after this viscosity reduction seems to play a lesser role (Olukosi *et al*., 2007), which may be due to increased consumption, maturation of the digestive system or increased indigenous microbial population (Campbell and Bedford, 1992).

Xylanase has been shown to not have a major effect on performance in diets including brewers DDGS (Pettersson *et al*., 1987) and Denstadli *et al*. (2010) suggested that the brewing process has reduced the NSP component present in barley. This agrees with previous studies which reported a decrease of β glucans of 80% during the malting process (Wang *et al*., 2004). However it is possible that addition of enzymes will retain positive effects through reducing variation in batches of fermentation products. Although Gutierrez-Alamo *et al*. (2008) found that differences in wheat cultivar were not eliminated by enzyme addition, Svihus and Gullord, (2002) found that enzyme addition decreased batch to batch variations. Enzyme addition has also been shown to decrease variability in barley (Campbell *et al*., 1989; Classen *et al*., 1988).

1.10 PHOSPHORUS

Phosphorus is one of the more expensive components of animal feed and oversupply can lead to environmental issues. Increased excretion of water soluble phosphorus in manure leads to an increased run off from the soil (Sharpley and Moyer, 2000), which is turn can lead to

eutrophication of water sources. Calcium and phosphorus deficiency in growing birds lead to a lack of skeletal calcification and abnormal bone development. It is important to consider available phosphorus, as from some sources, phosphorus is bound in phytate. This bound phosphorus is unavailable for use nutritionally by pigs and poultry as they lack the enzyme phytase (Jacques, 2003).

1.10.1 Normal bone development in poultry

Bone composition

Collagen makes up around 85% of the organic bone matrix, and as the major organic component of bone it provides support to the mineral component. Collagen is cross linked and this fibre arrangement can influence bone strength (Albright, 1987). Other organic components of bone include lipids and non-collagenous proteins, the latter of which contributes to the stabilisation of the collagen/mineral matrix (Termine and Gehron-Robey, 1996). There is also a proportion of water present, which may have visco-elastic properties. Crosslink content has been correlated with bone strength in older birds, but this is also dependent on relative mineral content (Rath *et al.* 1999). Various minerals are required to facilitate collagen synthesis and crosslinking, including Vitamins B6, C and K (Weber, 1999).

Mineralisation of bone

The major minerals in bone are calcium and phosphorus, which form around 65% of the bone weight and 95% of the bone mineral matrix. They take the form of hydroxyapatite crystals which deposit in holes between collagen fibres. These crystals provide stiffness and bind to collagen to improve the shear strength of the bone. Low calcium leads to a release of bone minerals via homeostatic mechanisms, so adequate dietary calcium is required to reduce bone turnover and maintain bone strength, so confounding factors which influence calcium absorption, such as phytate, need to be monitored (Rath *et al*., 2000). Vitamin D is required for intestinal calcium absorption and there is a complex relationship between this vitamin, other

calcitropic hormones and calcium and phosphorus. Dietary formulation can adversely affect bone growth, with high protein detrimentally effecting calcium balance (Heany, 1998) and saturated fats reducing bone mineralisation (Wohl *et al*., 1998). As the main component of the bone is minerals, the density of these is used as a measure of bone strength.

Bone maturity in Poultry

It has been suggested that bone development cannot keep up with growth in genetically fast growing poultry leading to increasing load on the bones. Bone mass increases with age (Seeman, 1999) and this contributes to bone strength. Bone maturity appears to take longer than the growth process in the bird, as small increases in bone ash have been recorded between 25 and 35 weeks of age (Rath *et al*, 2000). In younger birds (5 weeks and less), bone breaking strength is lower which may be due to an increase proportion of minerals to collagen making the bone brittle. Rath *et al.* (2000) assessed bone ash up to 43 days of age and found a peak between week 3 and week 5, after which ash was consistent, but strength decreased. Changes in collagen cross links may translate to a stiffer bone in younger birds which has less tensile strength. Tibia continue to grow but bone density was not found to increase past 4 weeks of age (Talaty *et al*., 2009)

1.10.2 Phosphorus in DDGS

Wheat DDGS (WDDGS) has more available phosphorus than wheat (Nyachoti *et al*., 2005). The increase in available phosphorus is due to the degradation of IP6 to IP5, 4, 3, and 2 during the process so less of the phosphorus is bound in phytate (Zijlstra *et al*., 2007). Liu and Han (2011) found that in maize DDGS, inorganic and phytate phosphorus increased 10.37 and 2.54 times compared with maize suggesting that phytate is degraded to inorganic phosphorus during the process. The authors suggested that this was due to yeast phytase activity.

Cottril *et al*. (2007) suggested that the process of bioethanol production may affect the available phosphorus, as heating may destroy phytic acid. Several authors have shown this increase in heating phosphorus availability in maize DDGS (Martinez-Amezcua *et al*., 2004; Parsons *et al*., 2006). Maize DDGS has more non-phytate P (73.2%) and there is no relation between phytate P and total P in DDGS (Tahir *et al*., 2012).

The phosphorus content of maize DDGS has been shown to be extremely variable, possibly due to differences in processing (Spiehs *et al*., 2002; Waldroup, 2007). Wheat DDGS has also been shown to contain more total phosphorus and calcium than maize DDGS (Ortin and Yu, 2009). However, Thacker and Widyaratne (2007) found a decrease in available phosphorus with WDDGS inclusion in chickens, although previously this phosphorus was found to be highly available in pigs (Stein and Shurson, 2009). This contrasts with findings for maize DDGS where increased phosphorus retention has been recorded in laying hens (Masa'deh *et al*., 2011) and pigs (McDonnell *et al*., 2011). This suggests that digestible phosphorus may be less available in wheat DDGS compared with maize DDGS. WDDGS also appears to contain more water soluble phosphorus, which is a larger environmental management issue. Excretion of water soluble phosphorus has been shown to increase with WDDGS inclusion in both pigs (Widyaratne and Zijlstra, 2007) and poultry (Leytem *et al*., 2008).

It has previously been noted that yeast can contain substantial phosphorus (Sell *et al*., 1981), and this combined with the high phosphorus content in wheat DDGS suggests that the separated YPC may be a good source of phosphorus. Yeast culture fed at 0.25% inclusion in broiler diets increased phosphorus digestibility and Gao *et al*. (2008) postulated that this was due to the action of yeast phytase.

1.10.3 Eutrophication

Phosphorus oversupply can lead to environmental issues such as eutrophication. Over application of phosphorus on agricultural land can result in elevated levels of soil phosphorus (Daniel *et al*., 1998) which then leads to an increased run off from the soil (Sharpley and Moyer,

2000). When receiving waters are over-enriched with minerals like phosphorus, algae is over produced, leading to low oxygen levels with a detrimental effect on other aquatic life (Correll, 1998).

1.10.4 Bone mineralisation methodologies

It is important to measure bone mineralisation in chicks, to ensure the balance between optimal delivery of expensive mineral sources and bone health.

Tibia ash is currently recommended for evaluation of Vitamin D activity by the Association of Official Analytical Chemists (AOAC, 2000). As the tibia is the fastest growing bone in the body, it is considered sensitive to dietary mineral changes. This makes the tibia the most popular selection as a bone mineralisation marker (Skinner and Waldroup, 1995).

However, tibia ash can be labour intensive and time consuming, and methodology differences can affect the results (Orban *et al*., 1993). Several authors have compared results from fresh, dried and fat free tibias. Kim *et al*. (2004) found no differences in ash content with different preparative procedures, whereas Hall *et al*. (2003) found that the use of fat free tibias provided more resilient results which required less replications to show treatment differences. Zhang and Coon (1997) found that bone ash was a good indicator of bone status, but was improved by correction for bone volume.

Alternative methods have been suggested to reduce time and labour required for tibia ash measurement. Toe ash has been used routinely as an alternative to tibia ash for over 70 years (Baird and McMillan, 1942; Evans and Carver, 1944), and is recognised by the AOAC (2000) as an alternative to tibia ash in the measurement of vitamin D activity. This method reduces labour and time required, by direct ashing of the toe bones without a flesh removal step, and has been found to have a linear relationship with tibia ash (Yoshida and Hoshi, 1983). Ravindran *et al*. (1995) found that toe ash was more sensitive to phosphorus bioavailability when used in conjunction with bodyweight. Drawbacks to this method include the small weight of the toe ash, which can lead to greater variation (Mendez and Dale, 1998), and this

method may not always reflect the bone mineralisation in cases of compromised fat metabolism (Huff, 1980).

Foot ash was suggested as an alternative to toe ash for bone mineralisation evaluation by Dale and Garcia (2004), due to the larger sample weight and simplicity and speed of collection. Preliminary results by the same research group had found a good correlation between both toe and tibia ash, and tibia and foot ash (Mendez and Dale, 1998; Mendez *et al*., 1998). Further work by Yan *et al*., 2005 found a strong relationship between both extracted tibias and foot ash (R^2 = 0.92), as well as between extracted and unextracted tibias (R^2 = 0.95). Garcia and Dale (2006) evaluated foot ash over a series of experiments and found that dietary phosphorus was accurately reflected in the foot ash measurements. They also investigated the effect of fat extraction of the foot or tibia, and found no effect on the sensitivity of the measures.

To date all the evaluation of the foot ash method has been using young chicks (up to 2 weeks old). It needs to be investigated whether foot ash will be suitable for measurement of bone mineralisation in older birds (Yan *et al*., 2005).

Femur bone strength could be a greater issue for processing related skeletal concerns than tibia strength. This could be due in part, to a higher external torsion on the femur when compared to the tibia in larger birds (Thorp, 1992). Early studies have reported that femur ash is more reflective of total skeletal mineralisation than other bones studies (Itoh and Hatano, 1964; Dilworth and Day, 1965). These studies also determined that when studying the ratios of calcium to phosphorus, the femur bone was more sensitive to dietary changes than the tibia. This finding was supported by Moran and Todd (1994), who reported that when broilers are fed a low phosphorus diet, the femurs were the most sensitive bone to dietary changes. From these findings it is clear that there is a lack of consensus in terms of the most appropriate way to measure bone mineralisation.

The level of phosphorus in the YPC needs to be carefully quantified to reduce an over-supply and reduce any possible environmental impact. This is particularly the case as much of the

phosphorus in WDDGS is water soluble therefore washing may remove much of it. Commonly in poultry feed, phytase is added to improve phosphorus digestibility, but may increase the water soluble phosphorus in the excreta, which in turn increases run off with deleterious environmental effect (Powers and Angel, 2008).

1.11 CONCLUSION

The novel separating process investigated in this project is producing a possible new feed ingredient for the monogastric market. This ingredient, YPC may have some nutritional qualities in common with the previous bioethanol co-product, DDGS, and also with yeast alone. However the actual nutritional make-up of the product is currently unknown, and may be markedly different to DDGS or yeast. Based on the previous research on DDGS and yeast, YPC may be suitable as a feedstuff for monogastrics, but extensive research is needed as YPC is currently unstudied.

Initial characterisation of this product *in vivo*, using broilers as a sensitive model, is needed to prove the product is a viable feed ingredient. Further characterisation will be needed to address the other possible issues, including available phosphorus contribution and presence of any anti-nutritional factors. If the YPC could be fully characterised as a novel feedstuff suitable for feeding to poultry, waste from the bioethanol process may be reduced, and this would in turn have a positive effect on the potable alcohol industry. In addition the YPC may show positive nutritional qualities which would be advantageous to the large monogastric industry.

1.12 AIMS AND OBJECTIVES

The overall aim of this project was to evaluate yeast protein concentrate (YPC) as a feed ingredient for use in poultry feed. Specific objectives were:

- Assess YPC variability and effect of processing.
- Quantify amino acid content and digestibility in broiler chicks.
- Evaluate performance of chicks fed diets incorporating YPC.
- Analyse the effect of non-starch polysaccharides present in the YPC.
- Assess the available phosphorus content of YPC

CHAPTER 2: MATERIALS AND METHODS

2.1 INTRODUCTION

This chapter covers the general materials and methods used throughout the both the poultry trials and the laboratory analysis. Eight bird trials were completed as detailed below (table 2.1), and an initial pilot scale processing trial.

Table 2.1: Description of individual trials in the study

Trial	Description	Chapters
Oelde	Variation of samples of alcohol stillage and effect of separation machinery settings on proximate content of YPC and viscosity of supernatants.	3
BRC01	Measurement of the amino acid digestibility of YPC from potable and bioethanol sources.	4
BRC02	The effect of feeding different inclusion levels of YPC from potable and bioethanol sources on performance parameters and digesta viscosity in chicks.	5
BRC03	The effect of feeding different inclusion levels of washed and unwashed YPC on performance parameters and digesta viscosity in chicks.	5 and 6
BRC04	The effect of washing on the amino acid digestibility of bioethanol YPC.	4
BRC06	Measurement of the amino acid digestibility of ring dried YPC from a potable source.	4
BRC07	The effect of feeding different inclusion levels of ring dried YPC from a potable sources in pelleted diets on performance parameters in chicks.	5 and 6
PVT01	Comparison of different leg bones for measurement of bone mineralisation.	6

2.2 ANIMALS, HOUSING AND MANAGEMENT

All procedures carried out were approved in advance by the College of Science ethics committee. All animals were euthanized using a Schedule one method as determined by the Animal Scientific Procedures Act (ASPA, 1986).

All animal trials used Ross 308, male broiler chicks, supplied within 24 hours of hatching by PD Hook, Cote Hatchery, Oxon. The chicks were grouped in preheated 0.64m^2 pens in a purpose built, insulated poultry house. Any unusually sized birds were not allocated to trial pens. Temperatures were maintained at 35^{o}C for the first 24 hours by heating fans with supplementary heat lamps and reduced to 21^{o}C by day 21. Birds were checked at least twice daily to ensure environmental conditions were adequate, and heating and ventilation were adjusted accordingly. The chicks were bedded on 5cm washed, fine sand which was chosen for its inert properties (Bilgili *et al*., 1999) for all trials except BRC07. In study BRC07, chicks were bedded on 3cm of wood shavings, which were topped up as necessary throughout the duration of the trial. Lighting was provided for 23 hours a day with one hour of complete darkness for all trials except BRC07. For this study, in line with commercial standards, lighting was increased by an hour a day from day 1 until 6 hours of darkness was achieved. Feed and water were always available *ad libitum*. Dates and weights of dead birds and reasons if culled were recorded.

2.3 DIET FORMULATION

Birds were fed commercially available chick starter crumb for the amino acid digestibility trials (chapter 4), prior to trial diets. The composition and analysis of all the trial diets are detailed in the appropriate chapter. Diets were either manufactured by a commercial diet manufacturer or mixed onsite. For the latter the ingredients were individually weighed and mixed dry for 5 minutes in a ribbon mixer (Rigal Bennett, Goole, UK) before oil addition. The diets were then mixed for a further 5 minutes with an intermediary brush down within the mixer to remove oil

clumps. Titanium dioxide was carefully incorporated into the diets as an inert marker by mixing with other minor ingredients prior to inclusion in the dry mix to ensure homogeneity. Titanium dioxide content (5g/kg) was selected to ensure the digesta samples would contain sufficient titanium for laboratory measurement.

Diets for all studies were allocated randomly to pens within the study room to reduce any effect of room position.

2.4 TRIAL PERIOD

2.4.1 Feed intake

Each pen of chicks was fed solely from an individual experimental bag of diet (pre-weighed). Troughs were positioned to minimise spillage. At the end of the trial, any remaining feed in the bag and trough was weighed, and where practicable, any spilled food collected and weighed.

2.4.2 Bird weights

Chicks were weighed on Day 1 for all performance trials and any unusually sized birds were excluded. For performance trials, birds were weighed on a weekly basis by pen using a top pan balance (Mettler Toledo, Leicester, UK).

2.4.3 Excreta Collection

Excreta samples were collected by placement of a foil-lined, wire-topped tray into the pen for 2 to 3 days. Trays were placed under the feeding troughs to ensure frequent use by the chicks. At the end of the collection period, the trays were removed and any contaminating feed and sand removed. Foils were wrapped allowing a small gap for air circulation and dried at 80^{o}C for 5 days in a forced air oven.

2.4.4 Post Mortem Sample Collection

On the final day of trials requiring digesta collection, feed was removed for an hour before re-feeding for an hour to ensure gutfill. The birds were then euthanised in a separate room by cervical dislocation. Only one pen of birds was removed to the culling room at any time, to reduce stress to the birds. Only trained and competent persons culled birds to ensure death was rapid.

Serum collection

Immediately after euthanasia, the abdominal cavity was opened and blood was collected from the heart using a 20G needle and syringe. One bird per pen was used for blood collection. Blood was collected into a 15ml tube and allowed to clot at room temperature for up to 3 hours before centrifuging at 3000rpm for 10 minutes to separate out red blood cells. The serum was poured into tubes and frozen at -20^{o}C until analysis.

Digesta collection

The gastrointestinal tract from the duodenal loop to the ileal-caecal junction was gently removed and the digesta collected from the small intestine by gentle digital pressure, taking care not to disturb the mucosal lining. The small intestine was separated into jejunum and ileum for purposes of digesta collection, with Meckel's Diverticulum taken as the separation point between the two. Digesta from the proximal small intestine (jejunum) and distal small intestine (ileum) were pooled into separate pots per pen. The ileal samples were collected for digestibility analysis as the majority of dietary protein digestion and absorption would be expected to be completed by this point in the gastrointestinal tract. The jejunal samples were collected to allow determination of the viscosity of the digesta supernatant.

Bone collection

Feet were removed at the tibial-tarsal junction, taking care to ensure consistency of removal. Feet were placed into one bag per pen and left and right feet were collected separately. In the validation study, PVT01, feet were stored individually by bird. Feet were either immediately dried or stored at -20^{o}C for analysis. For PVT01, toes were removed at the joint between the second and third toe bones of the middle toe of each foot. Toes were kept with the respective foot for later comparison purposes, and stored individually by bird at -20^{o}C for analysis. Tibia bones were separated at the tibial-tarsal junction and the tibial-femural junction and placed into one bag per bird. Femurs were removed from the tibial-femoral junction to the hip socket and placed into one bag per bird. Both femurs and tibias were stored at -20^{o}C for analysis. See section 2.5.6 for detail of the bone analysis.

2.4.5 Viscosity measurement

Jejunal samples were pooled into one pot per pen and mixed before aliquoting into eppendorf tubes for centrifugation at 13,000rpm for 10 minutes in a microcentrifuge (SLS, Goole, UK). The viscosity of the supernatant collected was then measured using a Brookfield DVII+ spinning disc viscometer with a cone and plate attachment (Model LVTDV-II, Brookfield Engineering Laboratories Inc., Stoughton, MA., USA). The viscometer was maintained at 41^{o}C to mimic chick body temperature and the measurements were taken as soon as practicable after centrifugation. The viscometer was calibrated with water prior to duplicate measurements using 0.5ml of supernatant from each pen.

2.5 ANALYTICAL METHODS

2.5.1 Dry Matter Determination

Diets and excreta were analysed for dry matter content as follows. 2 to 3g of finely ground sample were accurately weighed into pre-weighed crucibles and dried for a minimum of 72

hours at 105^{o}C until constant weight. The samples were cooled in a desiccator on removal from the oven before reweighing.

2.5.2 Crude Protein Determination

Samples of diets and raw materials were analysed for nitrogen content using the Kjeldhal method (AOAC official method 2001.11). 1g of sample was accurately weighed into a boiling tube and both copper and selenium catalyst tablets added (BDH). 12.5ml of concentrated nitrogen free Sulphuric acid was added to each tube prior to heating in a Kjeltec digestor unit (Foss Tecator, Cheshire, UK) at 450^{o}C for 45 minutes. Tubes were then allowed to cool before 75ml of distilled water was added to each tube and the tubes distilled in a 2100 Kjeltec distillation unit (Foss Tecator, Cheshire, UK). This unit adds 50ml of 10M sodium hydroxide before distilling for 3 minutes. The resulting ammonia is expelled into a conical flask containing 25ml 4% boric acid with indicator. The boric acid was then titrated back to its original colour using either 0.1M or 1M hydrochloric acid, depending on the expected protein content of the sample. Protein was calculated from nitrogen content by multiplication by a conversion factor of 6.25. Although N*6.25 typically overestimates the true protein content (Sriperm *et al*., 2011), for consistency all diets and YPC analysis have been calculated using this value.

2.5.3 Extractable Fat Determination

Samples of diets and raw materials were analysed for extractable fat content by the Soxhlet method as outlined by the Association of Official Analytical Chemists (AOAC official method 2003.05). Up to 5g of sample was accurately weighed into an extraction thimble (Fisher), which was then refluxed for 18 hours with 150ml 40-60^{o}C petroleum ether and the extracted fat was weighed after evaporation of the ether.

2.5.4 Ash Determination

Dry samples of feed and raw materials were weighed accurately into pre-weighed ceramic crucibles, before ashing for 13 hours in a muffle furnace at 650^{O}C. Crucibles were cooled in a desiccator prior to reweighing.

2.5.5 Energy Determination

Diets and excreta were analysed for gross energy content using a bomb calorimeter (Instrument 1261, Parr Instruments, Illinois, USA). Diets were mixed with a small amount of water and mixed before pelleting with a pellet press (Parr Instruments, USA). Pellets were then dried overnight at 105^{o}C in a drying oven prior to analysis to improve combustion. Pellets were weighed into a tin crucible and placed in the bomb (Parr Instruments, USA). 10cm of fuse wire was cut and threaded through the holes ensuring that the wire was in contact with the pellet. The bomb was then assembled and filled with oxygen. The filled bomb was carefully placed into the calorimeter bucket containing 2 litres of water and the wires connected. The calorimeter explodes the pellet in the pure oxygen environment and measures the energy produced in kJ/kg.

2.5.6 Foot and Bone ash measurement

Tibia and femur bones were wrapped individually in foil and autoclaved for 15 minutes at 121^{o}C. The bones were then stripped free of flesh and connective tissue and dried in an oven set to 105^{o}C for 3 days. The dried bones were weighed into pre-weighed ceramic crucibles and ashed for 13 hours at 650^{o}C, then cooled in a desiccator before re-weighing. Bone ash is expressed a bone ash weight as a percentage of dry bone weight.

Toes and feet were dried in an oven at 105^{o}C for 5 days, then weighed into pre-weighed crucibles and ashed at 650^{o}C for 13 hours. Larger feet were broken into pieces when dry before ashing and ashed in 2 separate crucibles. Ash weight was recorded after cooling in a desiccator. Foot and toe ash are expressed as foot/toe ash weight as a percentage of dry

foot/toe weight. Ashed feet were then ground with a pestle and mortar to a powder and stored in a sealed pot for further analysis for phosphorus and calcium content.

2.5.7 Uric acid measurement

Uric acid was measured using an Amplex Red uric acid kit (Invitrogen, Paisley, UK). Standards were prepared using the 5mM uric acid provided in the kit, to produce a range of standards with final concentrations in the assay of between 125µM to 10µM. 50µl of each standard was pipetted onto a 96 well plate in duplicate. 5µl of each sample was pipetted into the designated wells on the plate in duplicate and made up to 50µl with reaction buffer. An example of a plate layout is shown in Figure 2.1.

well ID	1	2	3	4	5	6	7	8	9	10	11	12
A	0	S60	1	5	9	13	17	21	25	29	33	37
B	0	S60	1	5	9	13	17	21	25	29	33	37
C	S10*	S80	2	6	10	14	18	22	26	30	34	38
D	S10	S80	2	6	10	14	18	22	26	30	34	38
E	S20	S100	3	7	11	15	19	23	27	31	35	39
F	S20	S100	3	7	11	15	19	23	27	31	35	39
G	S40	S125	4	8	12	16	20	24	28	32	36	40
H	S40	S125	4	8	12	16	20	24	28	32	36	40

*S denotes standards

Figure 2.1: Plate layout for uric acid assay kit.

To start the reaction, 50µl of 100µM Amplex red reagent was added containing 0.4units/ml horse radish peroxidase (HRP) and 0.4units/ml uricase. Plates were then incubated at 37^{o}C for 30 minutes protected from light, before reading at 560nm on a microplate reader (Multiskan FC, Thermo Scientific, Welwyn Garden City, UK). A standard curve was plotted of absorbance at 560nm against concentration of uric acid in µM, and the sample concentrations were calculated using this. An example standard curve for the assay is shown in Figure 2.2. Finally sample concentrations were corrected to mg/dl.

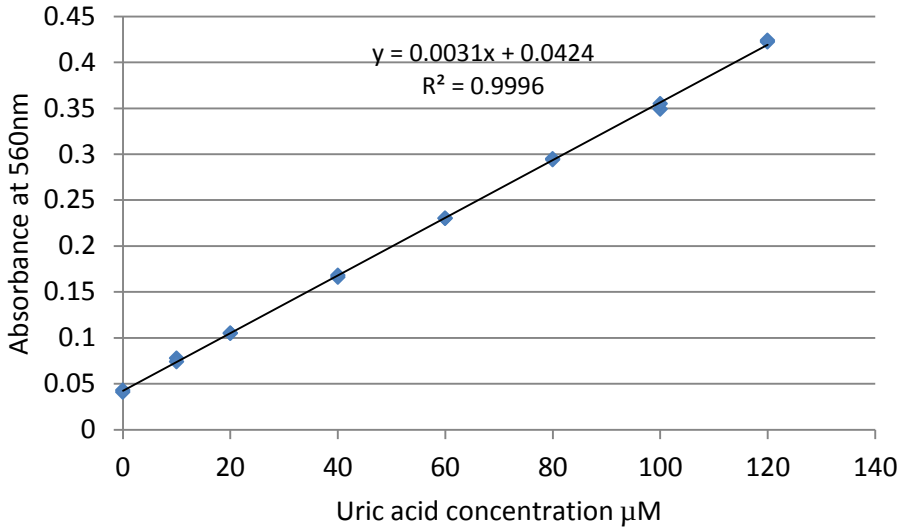

Figure 2.2: Example standard curve for the Amplex red uric acid kit.

2.6 DIGESTIBLITY RELATED MEASURES

Digesta samples were frozen immediately upon collection and then freeze dried to a constant weight in a Lyotrap freeze drier (LTE Scientific, Oldham, UK). Samples were then finely ground using a pestle and mortar and stored in sealed pots until analysis. Digesta samples were analysed for titanium dioxide and amino acid content for the amino acid trials BRC01, BRC04 and BRC06.

Excreta samples were collected as previously described and dried at 80^{o}C for 5 days. Samples were then ground to pass through a 1mm sieve using a coffee grinder (Whittards, Chelsea, UK) and stored in sealed pots until analysis. Excreta samples were analysed for dry matter, titanium dioxide and gross energy content.

2.6.1 Titanium Dioxide Determination

Titanium dioxide was incorporated into all diets as an inert marker at an inclusion rate of 5g/kg. Titanium dioxide (TiO_2) was quantified in diets, excreta and digesta by the method of Short *et al.*, (1996). Samples of 0.3-0.4g of digesta were accurately weighed into ceramic crucibles and

ashed at 650^{o}C for 13 hours and allowed to cool. The crucibles were placed on a hotplate and 15ml of 7.4M sulphuric acid was pipetted into each. The crucibles were then heated to simmering until the sample was completely dissolved (approximately 2-3 hours). Crucibles were allowed to cool before the contents were qualitatively transferred using distilled water into 100ml volumetric flasks, via a Whatman 541, hardened, ashless filter paper (Fisher Scientific). 10ml of hydrogen peroxide (30 volumes) was added to each flask, before making to volume with distilled water. Flasks were stoppered and mixed and absorbance measured on a UV spectrophotometer (Cecil CE3410, Cecil instruments, Cambridge, UK) at a wavelength of 410nm. A series of standards were prepared from 5ug/ml titanium dioxide and these were used to produce a standard curve. All samples and standards were read against a zero standard as a blank. The amount of titanium in the sample was calculated using the following equation:

Mg Ti / mg sample = absorbance * 100 / coefficient * mass of sample (mg)
where the coefficient is obtained from the regression analysis of the standard curve.

2.6.2 Amino acid analysis

Amino acid analysis was carried out using a Biochrom 30 amino acid analyser (Biochrom, Milton Road, Cambridge, UK) based on ion exchange chromatography with post column derivatisation with Ninhydrin using lithium citrate buffers. The analyser was managed using BioSys control software and EZChrom Elite Data handling software.

Sample preparation

Samples were weighed into 100ml glass bottles, 500mg for raw materials and 1g for digesta. The bottles were cooled to 4^{o}C prior to oxidation. Oxidation solution was prepared fresh by mixing 1ml hydrogen peroxide (30 volumes) with 9ml formic acid (73.5%) with 0.05M phenol. The oxidation solution was then incubated at 25^{o}C for an hour and then cooled to 4^{o}C for an hour prior to use. 10ml of cooled oxidation solution was added to each sample bottle to

oxidise the cysteine and methionine to cysteic acid and methionine sulphate respectively, in order to prevent their destruction during hydrolysis. Bottles containing the samples and oxidation solution were incubated for 16 to 18 hours at 4^{o}C.

Excess oxidation reagent was decomposed by addition of 0.84g of sodium metabisulphate to each sample bottle. 50ml of hydrolysis reagent (6N hydrochloric acid with 0.01M phenol) was then added to each bottle and the samples incubated at 110^{o}C for 24 hours.

Bottles were cooled in the freezer before quantitatively transferring the contents into wide neck conical flasks, rinsing with tri-sodium citrate buffer (pH 2.2). The flasks were then partly neutralised by addition of 35ml of 7.5N sodium hydroxide, with the flasks kept on ice to reduce overheating. The pH of each flask was adjusted to 2.2 using 7.5N sodium hydroxide, 1N sodium hydroxide and hydrolysis reagent as required.

Flask contents were transferred to a series of 200ml volumetric flasks, containing 4ml of 10µmol/ml norleucine as an internal standard. The volumetric flasks were then made to volume with tri-sodium citrate buffer (pH 2.2), stoppered and mixed. 10ml from each flask was centrifuged at 3000rpm for 5 minutes and the supernatant filtered through a 0.2µm filter into an analytical glass vial and stored at 4^{o}C until analysis.

The sample vials were loaded onto the Biochrom 30 amino acid analyser using an autosampler and 20µl of each injected. Cycle time was around 180 minutes per sample.

Calculation of amino acid content

Standards were prepared containing 200nmol/ml of each of 18 amino acids, 100nmol/ml each of cysteic acid and methionine sulfone and 200nmol/ml norleucine. These were used to prepare calibration graphs of peak area against amino acid concentration for each amino acid. After correction of peak areas by comparison with the internal standard, the calibration graphs were used to quantify the amino acid content of the samples, and this result was corrected for sample weight.

Determination of coefficient of amino acid digestibility

Using the titanium dioxide measurements the amino acid results for the chick digesta samples were used to calculate apparent amino acid digestibility using the following equation:

$$1-(aa_{dig} * marker_{feed}) / (aa_{feed} * marker_{dig})$$

Where: aa_{dig} = amino acid concentration in the digesta

$marker_{feed}$ = titanium dioxide concentration in the diet

aa_{feed} = amino acid concentration in the diet

$marker_{dig}$ = titanium dioxide concentration in the digesta

Apparent digestible amino acid content of the YPC

The determined apparent digestible amino acid content of the diets was regressed against rate of inclusion of the protein source. The linear regression was then extrapolated to a rate of inclusion of 100% (or 1000g/kg) protein. This method gives a figure for apparent digestible content of the protein source for each amino acid measured. Dividing this figure by the total content of the specific amino acid in the protein gave a coefficient of apparent amino acid digestibility.

2.6.3 Phosphorus and Calcium Determination

Phosphorus and Calcium content was measured in feed (PVT01) and foot ash (BRC03) using Inductively Coupled Plasma mass spectroscopy with Optical Emission Spectrometry (ICP-OES)(ICP-MS model PQ Excell, VG Elemental, Waltham, MA, USA). 0.5g samples of foot ash were weighed in duplicate into 50ml conical flasks and incubated overnight with 10ml of aqua regia (1 part nitric acid to 3 parts hydrochloric acid) before heating to boiling for 90 minutes. One blank flask with just aqua regia was prepared for each 5 samples measured. Samples were allowed to cool before a further addition of 5ml aqua regia to each flask. Samples were again

heated for 30 minutes to reconstitute before cooling. The flask contents were each filtered into a 50ml volumetric flask, and the flask rinsed three times with ultrapure water. Volumetric flasks were made to volume with ultrapure water and mixed before aliquots were poured into 15ml conical tubes and stored for analysis at 4^{o}C. Standards were prepared with differing levels of calcium and phosphorus (dependant on the sample to be measured)using 1000ppm ICP grade standards (Fisher Scientific, Loughborough, UK) diluted in ultrapure water. All tubes were run on the ICP_OES set to analyse calcium at a wavelength of 317.933 and phosphorus at a wavelength of 213.617.

CHAPTER 3: CONSISTENCY OF YPC COMPOSITION THROUGH THE SEPARATION PROCESS AND THE EFFECT OF YPC INCORPORATION INTO DIETS ON PELLET QUALITY

3.1 INTRODUCTION

This chapter investigates the effect of processing conditions on the composition and drying properties of the YPC. The effect on pellet quality of incorporation of the YPC into poultry diets is also examined.

Variation is one of the main issues with incorporation of DDGS into animal feed as it makes accurate formulation impossible. Both maize and wheat DDGS have been found to vary widely in nutritional composition (Spiehs *et al*., 2002; Lan *et al*., 2008) with fermentation batch being the most important source of variation (Belyea *et al.*, 2010). Within the fermentation there are many factors which can affect the co-product composition including temperature and water quality (Rausch and Belyea, 2006) and particle size (Rausch *et al*., 2005). The drying of DDGS also affects protein quality and digestibility (Swietkiewicz and Koreleski, 2008).

A pilot plant trial was carried out at the facilities of GEA Westfalia in Oelde, Germany, to obtain samples from different settings of the separation machinery. These samples were then analysed to quantify any compositional effect of the equipment changes. The machinery used were a decanter and disk stack which are specialised separation devices with complimentary roles. The decanter provides an initial separation of the solid and liquid fraction of the alcohol stillage stream. This separation is controlled by the speed of delivery of the stillage into the decanter and by the speed of the rotation of the drum. The disk stack acts as a dewatering stage to produce a higher dry matter final product, in this case, a yeast cream. The separation through the disk stack is also determined by the speed of the rotation of the drum and the speed of the input stream. Using the two pieces of machinery in series allows more control of the separation process, with the decanter removing the majority of the fibre fraction from the stillage and the disk stack separating a yeast cream from the liquid fraction (centrate).

Next to nutritional content, pellet quality can be considered the most important factor in a finished feed. DDGS has been found to adversely affect the quality of pellets (Shim *et al*., 2011), and therefore it is important to quantify the effect of YPC inclusion on the durability of the pelleted feed. The most reliable method of determining pellet quality is a pellet durability index (Beyer *et al*., 2000), but other methods are also widely used. This chapter shows a model of the process using a stillage which typifies the type of material used, but is not definitive as it derives from an alternative feedstock to those used in later chapters.

3.2 METHODS

3.2.1. Pilot plant trial

The raw material used for the pilot plant trial was post-distillation stillage from a maize based, potable alcohol distillery. Six Intermediate bulk containers (IBCs) of material from the same fermentation batch were transported to Germany the week prior to the trial. All the IBCs were sampled to assess variability within the fermentation batch. The IBC material was centrifuged to see the yeast and fibre split in the material and this can be seen for two of the IBCs in figure 3.1.

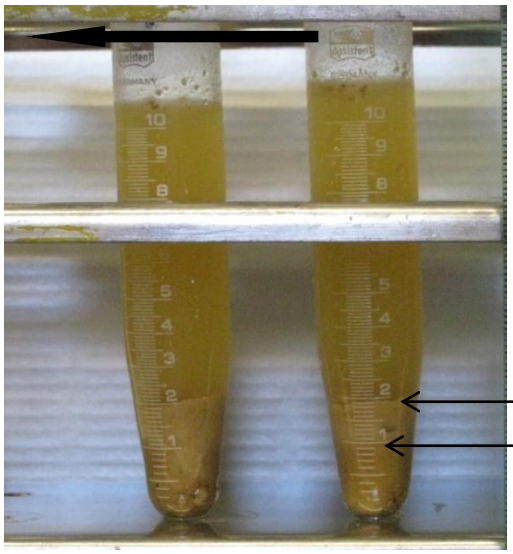

Figure 3.1: Spun sample of the IBC raw material. The fibre fraction can be seen as a layer below 10%.

The first IBC was used to optimise the running of the separating machinery. The remaining IBCs of stillage were separated through the decanter to produce specific spun volumes in the liquid fraction, where spun volume is a factory measure involving centrifugation of a sample in a graduated tube as shown in figure 3.2. These spun volumes gave an indication of the level of solid material present in the separated, liquid fraction. The yeast content of the stillage was estimated at around 5% (Ingeldew, 1999), so the machinery settings were aligned to produce liquid fractions with spun volumes on either side of this 5% figure. Individual runs are detailed in table 3.1 and the spun volumes for two of the runs are shown in figure 3.2.

Table 3.1: IBC treatments during Oelde pilot plant trials

IBC number	Spun volume achieved (%)	Disk stack run number
II	1.5	XI
III	2.5	X
IV	7.5	IX
V	6	VIII
VI	5	VII

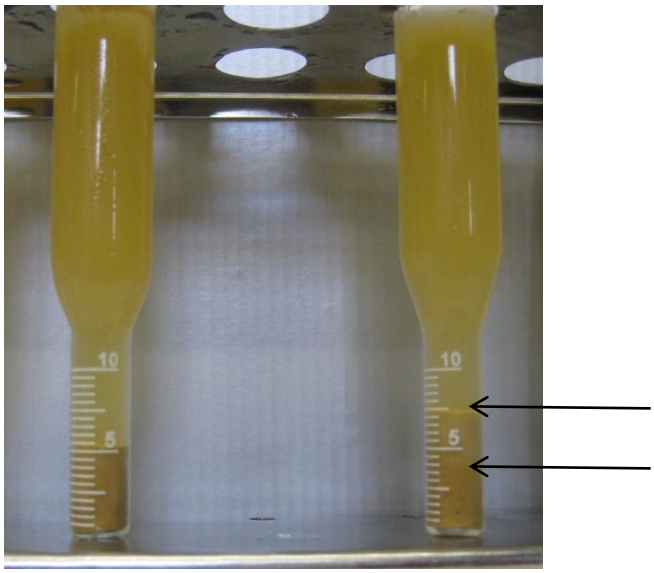

Figure 3.2: Spun volumes of 5% and 7.5% corressponding to decanter runs VI and IV

Each decanter run was then processed through the disk stack, in a de-watering step, to give a higher dry matter yeast cream and a liquid fraction (centrate) of less than 0.2% spun volume. Examples of the spun volume of both these fractions is shown in figure 3.3. It can be seen that

the centrate has very little solid matter, whereas the yeast cream is approximately 40% solid matter.

Figure 3.3: Spun volumes of centrate and the yeast cream from the disk stack process

Half litre samples were collected from each run of the decanter of the fibre fraction (paste) and liquid fraction (centrate). Samples were taken from the middle of the decanter run for consistency. 0.5 litre samples were also collected from the disk stack de-watering runs, again from both liquid (centrate) and yeast cream fractions (YC). Samples were shipped to Nottingham Trent University within a week and then sub samples were taken and sent immediately to a commercial laboratory for analysis for dry matter and protein content. A further subsample was stored at -20^{o}C for laboratory analysis at NTU for all samples except IBC II and yeast cream from run VII where insufficient sample was sent from Oelde to allow for subsampling.

3.2.2 Laboratory analysis of pilot plant samples

In addition to the external laboratory analysis, the pilot plant samples were analysed in house at NTU. Samples were removed from -20^{o}C storage and allowed to defrost overnight at room temperature. Raw material from the IBCs and the final centrates from the disk stack were

analysed for spun weight and the viscosity of the supernatant was measured. The yeast creams were also analysed for spun weight and drying time was quantified for the pelleted fraction.

Spun weight measurement

Samples of IBC, centrate and yeast cream were defrosted overnight and mixed before aliquoting into pre-weighed eppendorf tubes. Samples were then weighed before centrifugation for 10 minutes at 14,000rpm in a microfuge. Supernatant was carefully decanted from each tube and the pellet and tube re-weighed. Spun weight is calculated as the pellet weight as a proportion of the whole sample weight pre-centrifugation. Six replicates of each sample were analysed.

Viscosity of supernatants

After the centrifugation during the spun volume measurement, supernatant from the IBCs and centrates were decanted, with care taken not to disturb the pellets. Supernatants from each sample were pooled and viscosity measured using a Brookfield DVII+ spinning disc viscometer with a cone and plate attachment (Model LVTDV-II, Brookfield Engineering Laboratories Inc., Stoughton, MA., USA). The viscometer was maintained at 41^{o}C and was calibrated with water prior to duplicate measurements using 0.5ml of supernatant from each sample.

Drying time measurement

Each yeast cream sample was mixed and aliquoted into a 50ml tube and centrifuged at 4200 x g in a bench top centrifuge (Universal 32R, Hettich Centrifuges, Newport Pagnell, UK). The supernatant was decanted to waste from each tube and a proportion of the pellet weighed into a pre-weighed tin crucible and placed in a drying oven at 80^{o}C. Crucibles were removed from the oven at five minute intervals and cooled in a desiccator before re-weighing and returning to the oven. Drying was assessed over a period of 90 minutes and rate of drying in mg/minute calculated.

3.2.3 Pellet Quality tests on diets from study BRC07

The four pelleted diets manufactured for study BRC07 were assessed for pellet quality using three measures. These diets contained graded levels of a ring dried, potable sourced, yeast protein concentrate, at dietary inclusion levels of 0, 7.5, 12.5 and 17.5%.

Percentage fines measurement

Percentage fines were measured by sieving a weighed quantity of pellets through a 2mm square hole sieve with any fines collected weighed. 5 replicate samples were analysed for each diet. Results are expressed as fine weight as a percentage of sample weight, referred to as percentage fines.

Pellet durability Index (PDI)

Pellet durability index was measured by the method of Beyer *et al*. (2010). Briefly, pellets were sieved though a 2mm square holed sieve and then the pellets remaining on the sieve were weighed and tumbled on a roller mixer for 10 minutes before re-sieving. The pellet durability index was calculated as the ratio of the mass of pellets remaining on a sieve after tumbling to the initial mass before tumbling. Each diet was subsampled and measured in duplicate.

Pellet drop test

A pellet drop test was carried out using the method described by Tumuluru *et al*. (2010). Single pellets were dropped from a 1.85 metre height onto a metal plate. Mass retained in the pellet is expressed as a percentage of initial weight. Measurements were repeated 12 times for each diet.

3.2.4 Statistical analysis

After KS testing of the data to confirm normality, one way ANOVAs were used to investigate variation of spun weight of the different samples taken from the pilot plant. A one way

ANOVA was also used to assess the difference in drying rate between different separation runs. Finally one way ANOVAs were used to assess the effect of graded levels on YPC inclusion in diets on pellet quality. Where appropriate Duncan post hoc testing was used to separate significances at $p>0.05$. The statistical package used was SPSS v.19 (IBM statistics, 2012).

3.3 RESULTS

The analysis of the samples from Oelde will be considered in several sections for clarity. The initial raw material samples will be considered as an estimate of batch to batch variation from the fermentation process. Subsequently, the fibre and centrate (high liquid) fractions will be considered to quantify the reproducibility of the separation process. Finally the yeast cream fraction analysis will be reported to compare consistency of the final product. Results will be compared to the spun volume measures made in the pilot plant and referred to as spun volume to differentiate from spun weight measurements.

3.3.1 Intermediate bulk container analysis

Analysis for dry matter and protein (commercially analysed) are shown in table 3.2. The IBCs of stillage were very variable even though they were from the same fermentation batch from the same maize based, potable alcohol plant. However this is likely due to the low dry matter content of the stillage, which would make taking a homogenous, representative sample difficult. This can also be seen with the protein content which is also highly variable. This can also be seen in table 3.3, which shows the spun weights. These are less variable than the commercially analysed samples as care was taken to get a representative sample and this was confirmed by analysis of 6 replicate samples for each IBC. However, there is still significant variation between the IBCs.

Table 3.2: Analysed differences in Bulk IBCs (Intermediate bulk containers)[1]

Raw Material	Protein (%)	DM (%)	Protein on a DM basis (%)
IBC II	2.2	1.7	129.4
IBC III	1.2	3.6	33.3
IBC IV	1.8	3.5	51.4
IBC V	1.1	1.2	91.7
IBC VI	2.1	2.8	75.0

[1]Analaysis performed by commercial laboratory

Table 3.3: Spun weights of the Bulk IBCs

Raw Material	% spun weight (S.E.)
IBC 3	18.34 (0.974)[c]
IBC 4	28.84 (0.848)[a]
IBC 5	17.71 (0.774)[c]
IBC 6	22.13 (0.099)[b]
p value	p<0.001

Table 3.4 shows the measured supernatant viscosity of the IBCs. All the measured viscosities were extremely low and very similar between containers. They are also in the range expected for water, between 0.65 and 1 at between 40^{o}C and 20^{o}C respectively.

Table 3.4: Viscosity of IBC supernatant

Raw Material	Viscosity of supernatant / cP (S.E.)
IBC 3	0.835 (0.015)
IBC 4	0.850 (0.010)
IBC 5	0.800 (0.010)
IBC 6	0.825 (0.005)

3.3.2 Fibre fraction and centrate analysis from Oelde separation

Table 3.5 shows the dry matter measurements (commercial analysis) for the centrate and solid samples (paste) from the decanter separator. The dry matter content of the disk stack centrate is also shown.

Table 3.5: Dry Matter (DM) of samples taken from liquid and paste fractions from decanter and disk stack runs[1]

IBC No.	Spun solids (%)	DM centrate (%)	DM paste (%)	DM Disk stack centrate (%)
II	1.5	1.8	18.2	1.7
III	2.5	1.7	17.0	1.6
VI	5.0	2.3	25.9	1.8
V	6.0	2.6	21.1	1.7
IV	7.5	2.8	25.8	1.7

[1]Analysis performed by commercial laboratory

Table 3.6 shows the NTU analysis of spun weight for the disk stack centrate samples. Although all the spun weights are below 4% there is significant variation between the separation runs.

Table 3.6: Centrate spun weights for the disk stack separation runs.

Disk Stack run	% spun weight (S.E.)
VII	2.62 (0.122)^{bc}
VIII	3.02 (0.416)^{b}
IX	3.16 (0.057)^{ab}
X	3.66 (0.151)^{a}
XI	2.34 (0.051)^{c}
p value	p=0.001

Table 3.7 shows the viscosity of the supernatant from the disk stack centrate. Again the samples all had extremely low viscosities comparable with water. Due to the similarity of the measures from these and the IBC samples, no further viscosity measures were recorded.

Table 3.7: Viscosity of the centrate supernatant

Disk stack run	Viscosity of supernatant cP (S.E.)
7	0.830 (0.000)
8	0.785 (0.005)
9	0.810 (0.010)
10	0.810 (0.010)
11	0.855 (0.025)

3.3.3 Yeast cream analysis from Oelde separation trial.

Table 3.8 shows the dry matter and protein content of the yeast creams separated from each IBC as measured by the commercial laboratory. In terms of protein on a dry matter basis (which approximates to the dry YPC measure) the yeast creams varied from 41.5% to 47.2%. A sample of the bulk yeast cream was also taken after the separation was complete and analysis for these are shown in table 3.9.

Table 3.8: Yeast cream (YC) DM and protein from each run

IBC number	Spun solids	YC DM (%)	YC protein (%)	YC Protein on a DM basis (%)
II	1.5	18.6	7.9	42.5
III	2.5	14.7	6.1	41.5
VI	5	17.6	8.5	48.3
V	6	16.6	7.2	43.4
IV	7.5	12.3	5.8	47.2

Table 3.9: Protein and dry matter content of bulk yeast creams (YC) from runs VI through VI

IBC Number	DM Bulk YC (%)	Protein bulk YC (%)	YC Protein on a DM basis (%)
VI	10.1	4.9	48.5
V	16.7	8.1	48.5
IV	18.3	9.1	49.7

These analyses show the bulk yeast creams produced were of a similar protein content on a dry matter basis, with only around 1% difference in protein. The percentage dry matter increased with the increased spun solids in the decanter fraction. The higher spun solids runs contain an inclusion of fibre as well as yeast which is also be separated through the decanter. However the total protein on a DM basis indicates that the samples yield consistent amounts of yeast protein.

Table 3.10 shows the spun weights of the yeast creams analysed at NTU. There are significant differences in the solids content of these creams, which correspond to the spun volume of the material achieved through the decanter. These results show that the disk stack removes a proportion of liquid from the feed and therefore the solid content from the decanter is the determining factor for the solid content of the yeast cream.

Table 3.10: Spun weights of yeast creams from the disk stack

Yeast cream from run	**Spun volume from decanter %**	**% spun weight (S.E.)**
VIII	6	69.21 (0.663)[b]
IX	7.5	74.76 (1.143)[a]
X	2.5	58.07 (1.068)[c]
XI	1.5	55.70 (0.416)[c]
p value		P<0.001

3.3.4 Drying time of yeast cream from disk stack separation trial

The drying rates of the pelleted yeast cream samples from different runs through the separation process are shown in table 3.11. There are significant differences between the different yeast creams with the higher spun solid fractions drying at a higher rate, and the lowest spun volume fraction drying at a significantly lower rate. The drying rates are shown in figure 3.4, where the strong linearity of the drying is clear with R^2 values for all batches greater than 0.99.

Table 3.11: Drying rates of yeast cream from the pilot plant trial

Yeast cream from run	Drying rate mg/min (S.E.)
VIII	37.31 (0.671)[a]
IX	37.99 (1.0972)[a]
X	28.89 (1.602)[b]
XI	23.66 (1.028)[c]
p value	p<0.001

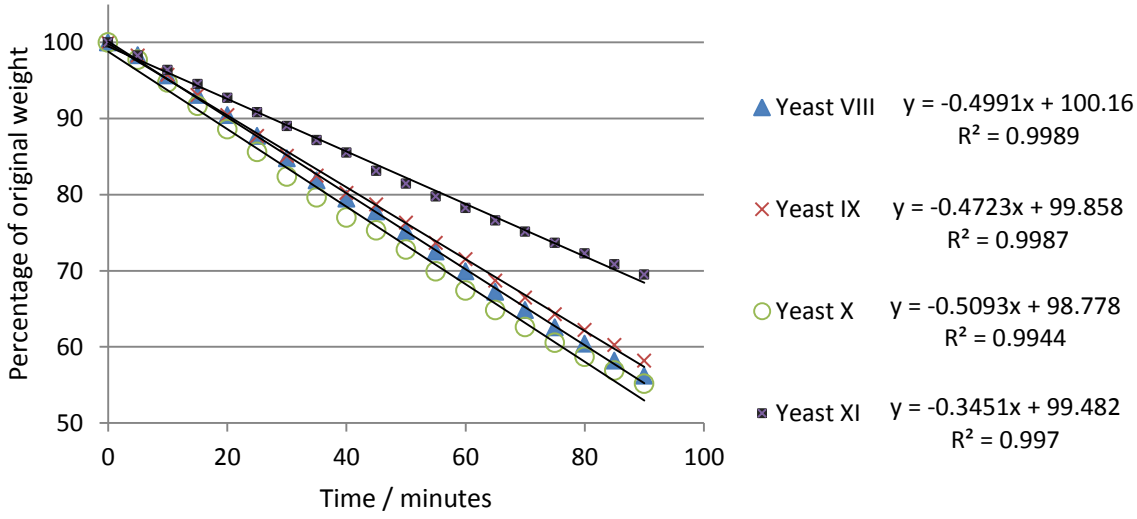

Figure 3.4: Drying rates of the yeast creams from the pilot plant study

3.3.5 Pellet quality of diets containing graded levels of ring dried yeast protein concentrate

The pellet quality measures are all detailed in table 3.12. The values for pellet durability and pellet drop durability are all very high; near to 100%, although there was a significant lower pellet durability in the 17.5% YPC inclusion diet. There are significantly lower fines in the higher two YPC inclusion levels, but all diets had a very low level of fines.

Table 3.12: Pellet Quality tests for the diets containing graded levels of ring dried potable YPC.

Diet	Treatment	Fines (%)	Pellet drop durability (%)	Pellet durability (%)
AA	0% YPC	2.96[a]	99.48	99.79[a]
BB	7.5% YPC	2.38[ab]	99.58	99.83[a]
CC	12.5% YPC	1.02[c]	99.74	99.78[a]
DD	17.5% YPC	1.56[bc]	99.73	99.38[b]
p value		0.001	0.179	0.001

3.4 DISCUSSION

Variation in raw material and separation

The results in this chapter are indicative of the type of variation expected with the process, but are not definitive as the material is not identical to that used in later chapters. There are significant differences in the physical properties of the raw material. The results for protein and dry matter from the commercial laboratory are highly variable, and the dry matter results appear very low when compared with the spun weight results. It is possible that the commercial analysis was carried out on the sample supernatant as the raw material rapidly settled out, and if efforts were not made to ensure thorough mixing, these results would not be representative of the whole material.

With the spun weight measurements, care was taken to ensure the sample was well mixed, but it was noted in the pilot plant that it may have been difficult for the factory operators to have taken a representative sample from the IBCs. This was due in part to the high liquid content and the nature of the suspension, which contained a large variation in particle sizes. The IBCs are delivered as one tonne containers and as such are difficult to mix, although a factory scale agitator was used in an attempt to achieve homogeneity. The spun weights of the different IBCs of raw material are significantly different from one another, with greater than 10% difference in the extremes for the containers. Large differences have been observed between batches of DDGS (Spiehs *et al*., 2002; Cromwell *et al*., 1993) and brewer's grains (Santos *et al*.,

2003) which was suggested to be due in part to differences in cultivar, harvest time and brewing technology. In this study all IBCs were taken from the same fermentation batch. Belyea *et al*. (2010) found that fermentation batch was the largest contributor to variation in DDGS and this pilot plant study shows that even within one fermentation there is considerable variation. Yeast is subject to many stressors during the process the most important of which are ethanol inhibition and increased osmotic pressure (Zhao and Bai, 2009) which will vary during the process as more ethanol is produced. Other fermentative conditions such as temperature will vary during the process and these factors will contribute towards the inconsistency of the IBCs of raw material.

Viscosity of both the IBC and centrate supernatant was extremely low, on par with levels expected for water. Maize contains almost solely insoluble NSPs (Huyghebaert *et al*., 2011) but it is the soluble NSPs which form viscous gels and increase viscosity (Choct and Annison, 1992a; 1992b). The stillage in this trial was from a maize based potable distillery so it would be expected that viscosity would be low. This would not be expected to be the case in distilleries using a wheat or barley feedstock, both of which contain substantial soluble NSPs (Choct, 2002).

Through the decanter separation step the dry matter of the liquid fraction can be seen to increase with the machinery settings to increase spun volume, confirming that more solid matter was separated. The solid fraction (paste) from the decanter varied widely in terms of dry matter, but this corresponds to the variation in the dry matter seen in the raw materials. The liquid fraction (centrate) from the disk stack has a very low dry matter but there is still significant variation between IBCs. This variation could be in part due to the difficulty in thoroughly mixing the low DM material, but it this variation needs to be investigated further if the centrate is to be used for a further purpose. It has been suggested that this centrate could be used as a liquid feed stream for anaerobic digesters, but this requires a very low solid

content, less than 3% (Gunaseelan, 1997) so any variation may have a detrimental effect on the equipment.

The yeast cream separated through the disk stack is the most comparable to the yeast protein concentrates used in the rest of this project, prior to the drying step. The commercial analysis shows that the protein content of the yeast cream (on a dry matter basis) ranges from 41.5 to 48.3%, which is similar in range to the YPCs evaluated in later chapters. It is notable that the bulk yeast cream samples, which are taken from a mixed container of the whole separation run, are much more consistent than the samples taken midway through the separation run. The dry matter of the yeast creams increases with the increasing spun volumes through the decanter, but the protein content (on a dry matter basis) are very similar in the 3 batches collected. This is likely to be due to the fibre contamination of the higher spun volume runs, as can be seen in figure 3.2, which would increase the dry matter content without contributing any protein. The yeast protein component appears to be consistent regardless of the decanter settings. This is confirmed by the spun weight measures, which were significantly different between batches, with the 7.5% spun volume decanter run having the greatest spun weight. All spun weights for the yeast creams were greater than 55% which illustrates the dewatering action of the disk stack process. The disk stack appears to remove a consistent amount of water from the feed stream, so a higher input of solids will give a higher DM yeast cream. The operators at Oelde are experts in the use of the equipment and therefore it may be that poorer consistency of final product will be the case at an industrial scale.

Filling transporters with consistent amounts of material is a major cost implication of co-product production (Clementson and Ileleji, 2010). Bulk density effects how much material can be transported so the proportion of fibre and yeast in the YPC product will greatly influence transport costs (Ileleji and Rosentrater, 2008). Large variations have been reported in the physical characteristics of DDGS (Shurson, 2005; Rosentrater, 2006; Ilileji *et al*., 2007) and the present study shows similar variation when differing levels of spun solids are produced in

the yeast cream. Handling DDGS separates out particle sizes and alters bulk density (Clementson and Ileleji, 2010) as a decrease in particle size increases bulk density (Zhou *et al*., 2008). Particle size distribution can affect flowability and cause powders to segregate when handled (Barbosa-Canovas *et al*., 2005) and this particle separation has been observed with DDGS during discharge (Ilileji *et al*., 2007). DDGS is also hygroscopic which can lead to caking and causing poor flowability making it difficult to transport (Saragoni *et al*., 2007). Particle size must also be considered during drying of the yeast cream to ensure caking does not occur.

Drying tests

With the exception of browning reactions during heating, unless temperatures are high (exceeding 120^{o}C) there has been shown to be no negative effect of drying on the composition of DDGS (Lopez and Pascual, 1981), so a lower temperature was chosen to minimise compositional changes in the yeast creams. The yeast creams varied in drying rates which has implications for YPC production as it may be difficult to dry batches with any consistency leading to an increased possibility of Maillard reactions. Although all the materials had been centrifuged to remove excess water prior to drying, the lowest spun weight material was the slowest to dry. This suggests that the material which has some fibre content is quicker to dry, possibly due to the larger particle size of the fibre compared to the yeast cells. This can be seen clearly in figure 3.5 which shows a correlation between spun weight and drying rate. Fibre particles have a flatter shape and lower mass than non-fibre particles in DDGS (Srinivasan and Singh, 2008). Drying differences can result from physical and chemical interactions between particles including particle size and shape (Ganesan *et al*., 2008). It is possible that residual sugars act as binders which form fine particles into denser pellets which are slower to dry (Kingsly *et al*., 2010). A high proportion of residual sugars in the separated YPC may also increase browning reactions (Labuza and Baisier, 1992).

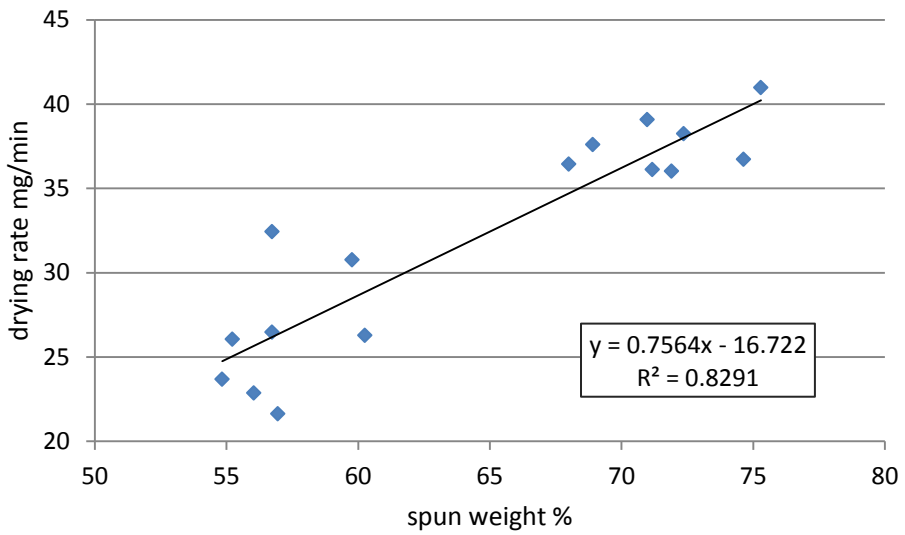

Figure 3.5: Correlation between spun weight and drying rates of yeast cream samples

Pellet Quality

Pellet quality is very important for poultry production as pelleted feed with a higher PDI (pellet durability index) is more likely to remain intact during handling prior to feeding (Beyer *et al.*, 2000). Pellets are produced by a series of reactions, the most common of which are protein denaturation and starch gelatinisation. Several factors effect pellet quality, including the amount of starch and oil content. DDGS has been found to deleteriously affect pellet quality; Shim *et al*. (2011) showed that increasing DDGS content was negatively correlated with pellet durability and Denstadli *et al*. (2010b) found that higher inclusion levels of brewer's grains reduced the pellet durability index from 85% in control diets to 68% at 40% inclusion. It has also been suggested that the oil in the DDGS can produce a hydrophobic coating which inhibits binding, thereby producing a poor quality pellet (Behnke, 2007). Although there are some significant differences in the pellet durability in this study, these are unlikely to have practical implications as all values derived are relatively high (pellet durability was near 100% for all inclusion levels) and the number of fines were extremely low. It appears from the results in this study that the inclusion of up to 17.5% of ring dried potable YPC has no negative effect on the durability of the pellet which agrees with the findings of Srinivasan *et al*. (2009) who found

that separating a proportion of the fibre from DDGS improved pellet durability. These diets were manufactured in a small scale facility which may not be indicative of the pellet quality form a higher throughput process. The pellets were also noted to be very hard and durable and it may be that they were over-conditioned. The pellets were also handled with comparative care and had less handling in terms of storage and transport than commercial feed, which may also contribute to their durability.

3.5 CONCLUSION

The variability seen in DDGS could translate to variability of YPC and this was confirmed by variation between one tonne containers within the same fermentation batch. However much of this variation appeared to be due to insufficient mixing of the material as one each batch was fully separated the yeast cream was consistent on a dry matter basis. The material used was also from a different feedstock to other YPC material used in this project, but this separation can be used as a model for the process. Fibre contamination only increased dry matter and did not affect protein content. However fibre inclusion increased drying rates which has implications for consistency of drying batches. Fibre can also be an issue for transport and handling of the final product as the particle size differences would lead to segregation of the product on handling. Viscosity of the material was extremely low due to low soluble NSP content of the maize feedstock.

DDGS deleteriously effects pellet quality, however, at a pilot plant scale there was no detrimental effect of YPC inclusion on pellet quality. This finding would need to be confirmed at a commercial scale.

CHAPTER 4: EFFECT OF FEEDING YPC ON APPARENT ILEAL DIGESTIBILITY IN BROILER CHICKS

4.1 INTRODUCTION

This chapter investigates the amino acid content and apparent ileal amino acid digestibility of yeast protein concentrates (YPC) from different sources, prepared using different drying methodologies. It is important to quantify amino acid content and digestiblities to ensure the requirements of the bird are met, and they are routinely used to formulate diets in a commercial setting. These YPCs were all separated using the decanter and disk stack process as detailed in chapters 1 and 3, from both bioethanol and potable alcohol distilleries. The separated yeast cream was then dried to a powder using three different drying technologies. The total amino acid content of the YPCs was measured and then the YPCs were incorporated into diets as a sole protein source to allow *in vivo* measurement of amino acid digestibility.

The current co-product from alcohol production is distiller's dried grains with solubles (DDGS). DDGS has been found to be particularly variable in amino acid content (Spiehs *et al*., 2002), with lysine digestibility a particular concern due to the high potential for the formation of Maillard's reaction during the drying process (Swietkiewicz and Koreleski, 2008). Yeast has been shown to be deficient in certain amino acids, specifically methionine and arginine (Klose and Fevold, 1945; Daghir and Sell, 1982). It is therefore important to quantify the amino acid availability of the YPC to assess any deficiency issues which may be suggested by previous studies.

Processing conditions may affect amino acid digestibility, particularly the drying stage as heat damage can cause the formation of indigestible complexes from some amino acids. The drying process will affect the chemical structure of the product and this is particularly dependant on the residence time in the dryer. The effect of a washing step during processing the YPC will

also be considered in this chapter as this will potentially remove non-starch polysaccharides and alter the drying characteristics. Sharif *et al*. (2012) found that washing a distillery sludge (similar to a yeast cream used in this project) increased amino acid content.

4.2 METHODS

There are several methods for measuring amino acid digestibility and in this chapter the regression method of Short *et al*. (1999) has been used. For regression amino acid measurement, graded levels of the protein to be assessed are fed as a sole nitrogen source and a linear extrapolation to 1000g/kg gives an estimate of the digestible amino acid content. Although this method has not gained widespread acceptance due to the complexity and large numbers of birds required (Lemme *et al*., 2004), Rodehutscord *et al*. (2004) suggested that regression should be adopted as a standard method of quantifying amino acid digestibility. The latter also found that the linear correlation was highly significant therefore three inclusion levels of protein were sufficient.

Three trials will be described in this chapter, and the yeast protein concentrates (YPCs) used in each trial are detailed in Table 4.1.

Table 4.1 Amino acid digestibility studies on yeast protein concentrates (YPCs)

Trial number	**YPC Source**	**Drying process**	**Other**
BRC01	Potable	Freeze dried (FD)	-
	Bioethanol	Freeze dried (FD)	-
BRC04	Potable	Spray dried (SD)	Washed (W)
	Bioethanol	Spray dried (SD)	-
BRC06	Potable	Ring dried (RD)	-

4.2.1 Yeast protein concentrates

All yeast protein concentrates used in the amino acid digestibility studies were separated using the decanter and disk stack process previously detailed in chapter 1 and 3.

In BRC01, two yeast protein concentrates were evaluated. The potable YPC used was separated from a barley based distillery and the bioethanol YPC was separated from a wheat based bioethanol plant, both in the UK. Both YPCs used in this trial were freeze dried (FD) and vacuum packed to remove any effect of drying process on the amino acid content. This trial also included soya as a protein control at the three different inclusion levels.

In BRC04, two yeast protein concentrates were evaluated. One yeast protein concentrate used was separated from a barley-based potable alcohol distillery, and this YPC underwent a washing process twice before drying. The washing process involved mixing the separated yeast cream from the disk stack separation process with water before re separation through the disk stack. The second YPC evaluated was separated from a wheat-based bioethanol plant and did not go through the washing process. Both YPC products were spray dried (SD) post separation.

In BRC07, one yeast protein concentrate was evaluated. A potable YPC from a barley distillery was separated using the disk stack separator and dried using a commercial scale ring drier (RD).

4.2.2 Study diets

Diets were either produced by a commercial feed manufacturer (BRC01) or made in house (BRC04 and BRC06). The potable (P) and bioethanol (B) yeast protein concentrates (YPC) and soya bean meal were incorporated into mash diets at 200, 400 and 600g/kg inclusion, as a sole protein source with added vitamins, minerals and oil. An inert marker was added at 5g/kg and the remainder of the diet made up with an equal mix of dextrose and wheat starch. Diet formulation is shown in table 4.2.

Table 4.2 Diet formulation for amino acid digestibility studies (g/kg)

Ingredient	200g/kg inclusion	400g/kg inclusion	600g/kg inclusion
Yeast protein concentrate	200	400	600
Soya oil	50	50	50
Vitamin/mineral premix*	50	50	50
Titanium dioxide	5	5	5
Dextrose	347.5	247.5	147.5
Wheat starch	347.5	247.5	147.5

*see Appendix 1

In house diets were mixed using a ribbon mixer, with the titanium dioxide incorporated into a portion of the dextrose prior to adding to the mixer to ensure homogenous mixing. Dry ingredients were mixed for 5 minutes before addition of oil and then mixed for a further 5 minutes.

4.2.3 Study Protocol

Birds were obtained from a commercial hatchery on day of hatch and housed as previously described in Chapter 2. All birds were fed a commercial chick starter crumb from day 1 to day 23, then split into groups of 4 birds per pen and fed one of the trial diets for a further 3 days. Feed and water were available *ad libitum.* Each protein source (YPC) was fed at 3 inclusion levels to between 4 and 6 pens per inclusion level (depending on trial). Specifics for each trial are detailed in table 4.3.

Table 4.3 Number of replicates and pens per trial

Trial identifier	Protein sources	Drying	No. of reps per inclusion level
BRC01	Soya/potable/bioethanol YPC	Freeze dried	4
BRC04	Potable (washed)/bioethanol YPC	Spray dried	5
BRC06	Potable YPC	Ring dried	6

After three days of feeding the test diets, the birds were culled by cervical dislocation and digesta collected from the distal end of the small intestine (ileum) as identified as the portion between Meckel's diverticulum and the ileal-caecal-colonic junction. Care was taken to use gentle digital pressure when removing digesta to minimise disruption of the mucosal lining of the intestine. Digesta samples were pooled into one pot per pen and frozen at -20^{o}C, prior to freeze drying and grinding.

In study BRC06, birds were also weighed by pen as they were put onto trial diets and again at the end of the study. Trial diets in this study were fed for 5 days for logistical purposes only and birds were culled on day 26 as per the previous studies.

4.2.4 Laboratory analysis

Samples of digesta and protein sources (YPCs and soya) were analysed for titanium dioxide content by the method of Short *et al*., 1996. Amino acid content of digesta and protein sources was carried out using a Biochrom 30 amino acid analyser based on ion exchange chromatography as detailed in chapter 2. Briefly, samples oxidised with performic acid prior to acid hydrolysis with norleucine added as an internal standard, and then analysed against prepared standards as described in full in chapter 2. In trial BRC06, the recorded peaks for proline and methionine could not be fully separated and therefore could not be quantified.

The determined apparent digestible amino acid content of each protein source was regressed against rate of inclusion of the yeast protein concentrate and extrapolated to 1000g/kg to give the apparent digestible content for each amino acid measured. Extrapolations were carried out for each replicate sequentially; for example, pens 1, 2 and 3 in trial BRC01 were extrapolated and so forth. A coefficient of apparent amino acid digestibility (COD) was then calculated using the total content of the respective protein source.

Complete trial diets were also analysed for protein, moisture, fat and ash as described previously in chapter 2.

4.2.5 Data Analysis

After KS testing to confirm normality, statistical analysis was carried out using one way ANOVA to compare the digestible amino acid content and coefficients of digestiblity of the different protein sources. Duncan post hoc tests were used where appropriate to elucidate differences between sources. In BRC06, the effect of YPC inclusion on bodyweight gain and digesta viscosity were also analysed using one way ANOVA with Duncan post hoc tests. The statistical package used was SPSS v.19 (IBM statistics, 2012).

4.3 RESULTS

Analysis for each of the protein sources studied in this chapter is detailed in table 4.4. The washing process appears to have removed fat and had a concentrating effect on the protein of the YPC. It is also notable that the protein content of the YPC has increased in the final study, which is likely to be due to the improvements in processing as the operators become familiar with the equipment.

Table 4.4: Proximate analysis of protein sources for amino acid digestibility trials

Trial number	Protein source	Dry matter g/kg	Protein g/kg	Fat g/kg	Ash g/kg
BRC01	Potable FD	912.56	365.81	73.03	27.73
	Bioethanol FD	864.76	347.43	78.41	36.07
	Soya	874.15	443.95	17.83	35.26
BRC04	Potable SD W	911.44	676.09	2.15	37.59
	Bioethanol SD	914.05	380.99	90.99	17.38
BRC06	Potable RD	805.33	510.51	3.36	25.68

4.3.1 Dietary analysis for amino acid studies BRC01, 04 and 06

Tables 4.5, 4.6 and 4.7 show the diet analyses for the trials BRC01, BRC04 and BRC06 respectively. The protein levels in the graded dietary treatments increase with protein source

inclusion as would be expected from the formulation. Fat content was higher in the bioethanol YPC containing diets as the fat level was particularly high in the raw material.

Table 4.5: Proximate analysis of amino acid diets from BRC01

Treatment	Dry matter g/kg	Protein g/kg	Fat g/kg	Ash g/kg
200g/kg Soya	906.91	84.11	114.27	64.94
400g/kg Soya	898.16	183.54	51.18	74.30
600g/kg Soya	890.89	289.25	53.70	86.40
200g/kg Potable FD	917.88	83.56	188.60	61.79
400g/kg Potable FD	914.61	152.40	276.94	58.78
600g/kg Potable FD	916.88	228.47	182.92	68.57
200g/kg Bioethanol FD	907.32	79.89	270.70	49.03
400g/kg Bioethanol FD	895.72	150.33	277.02	66.25
600g/kg Bioethanol FD	877.32	215.09	263.82	65.78

Table 4.6: Proximate analysis of amino acid diets from BRC04

Treatment	Dry matter g/kg	Protein g/kg	Fat g/kg	Ash g/kg
200g/kg Potable SD W	909.25	97.02	50.63	45.21
400g/kg Potable SD W	909.89	192.32	28.89	57.59
600g/kg Potable SD W	928.62	257.97	29.79	67.04
200g/kg Bioethanol SD	914.47	124.36	67.52	53.02
400g/kg Bioethanol SD	907.76	234.47	84.48	49.96
600g/kg Bioethanol SD	910.50	336.40	106.49	56.08

Table 4.7: Proximate analysis of amino acid diets from BRC06

Treatment	Dry matter g/kg	Protein g/kg	Fat g/kg	Ash g/kg
200g/kg Potable RD	888.11	122.08	21.25	46.37
400g/kg Potable RD	876.68	176.25	22.86	50.01
600g/kg Potable RD	864.84	235.99	23.71	57.94

4.3.2 Total amino acid content of yeast protein concentrates (YPCs) from different sources with different drying conditions

The total amino acid contents of the soya and the YPC protein sources used in the amino acid digestibility trials are shown in table 4.8. Soya was numerically higher in amino acid content than the YPCs, with the exception of the bioethanol SD YPC which was similar. Lysine content was much lower in all the YPCs when compared with soya except the potable washed SD YPC. Ring dried potable YPC had lower total amino acid content than the other YPCs and soya for almost all amino acids measured.

Table 4.8 Total amino acid content of YPCs and soya (g/kg)

Amino acid	Soya	Potable FD	Bioethanol FD	Bioethanol SD	Potable SD washed	Potable ring dried
cysteine	13.9	7.4	13.2	12.0	5.1	8.9
aspartic acid	54.4	29.4	22.0	31.7	48.4	16.4
methionine	7.7	8.2	6.5	11.6	9.5	--
threonine	19.9	15.8	12.8	19.6	23.4	8.3
serine	24.1	17.6	16.9	26.8	21.7	8.8
glutamate	78.6	71.8	83.6	193.4	55.7	23.0
glycine	19.3	15.8	14.8	21.4	19.4	8.3
alanine	21.4	20.5	15.4	22.8	30.5	11.2
valine	24.2	21.7	17.6	33.4	29.6	10.8
isoleucine	23.3	17.8	14.7	27.2	24.4	8.9
leucine	39.2	30.6	26.6	50.0	37.1	14.8
tyrosine	19.6	14.5	13.7	20.0	16.7	4.5
phenylalanine	25.9	18.9	17.6	33.7	22.4	8.9
lysine	30.0	17.9	12.9	18.3	34.2	11.8
histidine	14.2	9.8	9.2	15.4	10.8	4.1
arginine	37.9	15.7	17.7	28.0	21.7	9.6
proline	26.2	28.4	33.5	64.7	19.4	--

4.3.3 Digestible amino acid content of YPC and soya

The digestible amino acid content of the different YPCs and soya are shown in table 4.9. Statistical differences at $p<0.05$ are denoted by superscript letters. Soya was highest in digestible amino acid content in all but 7 amino acids with bioethanol SD YPC being equal to or higher than soya for all amino acids apart from cysteine, aspartic acid and lysine. Potable SD then Bioethanol FD are the next highest in terms of digestible amino acid content, followed by potable FD. Potable RD YPC had the lowest digestible amino acids across almost all amino acids.

Table 4.9: Digestible amino acid content of soya and YPCs from potable and bioethanol sources (g/kg)

Amino acid	Soya	Potable FD	Bioethanol FD	Potable SD	Bioethanol SD	Potable RD	p value
cysteine	8.92 (0.550)^{a}	1.47 (0.510)^{d}	6.68 (0.563)^{b}	1.40 (0.436)^{d}	7.22 (0.827)^{b}	4.65 (0.209)^{c}	p<0.001
aspartic acid	41.94 (1.755)^{a}	10.43 (1.407)^{d}	10.28 (1.250)^{d}	28.55 (2.238)^{b}	18.86 (1.981)^{c}	4.13 (0.520)^{e}	p<0.001
methionine	6.37 (0.225)^{b}	4.60 (0.560)^{c}	4.20 (0.298)^{c}	6.81 (0.646)^{b}	8.95 (0.710)^{a}	--	p<0.001
threonine	14.86 (0.752)^{a}	6.91 (1.126)^{b}	7.14 (0.709)^{b}	13.29 (1.048)^{a}	12.67 (0.941)^{a}	2.58 (0.279)^{c}	p<0.001
serine	18.69 (0.684)^{a}	8.41 (0.341)^{b}	10.38 (0.745)^{b}	9.68 (0.613)^{b}	18.60 (1.462)^{a}	2.46 (0.292)^{c}	p<0.001
glutamate	60.93 (3.037)^{b}	43.57 (1.868)^{c}	58.40 (1.873)^{b}	30.95 (1.151)^{d}	158.39 (7.258)^{a}	13.81 (3.423)^{e}	p<0.001
glycine	14.29 (0.919)^{a}	6.96 (0.356)^{c}	9.03 (0.593)^{b}	8.87 (0.630)^{bc}	13.26 (0.981)^{a}	2.58 (0.212)^{d}	p<0.001
alanine	16.37 (0.780)^{ab}	10.07 (1.241)^{c}	9.25 (0.702)^{c}	17.53 (0.929)^{a}	14.85 (0.827)^{b}	4.36 (0.304)^{d}	p<0.001
valine	18.43 (0.943)^{b}	12.31 (1.448)^{c}	11.41 (0.627)^{c}	17.46 (0.722)^{b}	24.87 (1.864)^{a}	4.09 (0.325)^{d}	p<0.001
isoleucine	18.01 (0.960)^{b}	10.12 (1.103)^{c}	8.59 (0.658)^{c}	15.66 (0.932)^{b}	18.79 (1.373)^{a}	3.74 (0.301)^{d}	p<0.001
leucine	29.94 (1.400)^{b}	18.19 (0.859)^{d}	17.55 (1.153)^{d}	24.91 (1.070)^{c}	37.82 (1.837)^{a}	6.90 (0.546)^{e}	p<0.001
tyrosine	16.00 (0.479)^{a}	8.03 (0.345)^{c}	8.84 (0.599)^{bc}	9.89 (0.712)^{b}	15.63 (0.869)^{a}	1.63 (0.174)^{d}	p<0.001
phenylalanine	20.14 (0.960)^{b}	11.80 (0.537)^{c}	12.60 (0.695)^{c}	12.69 (0.645)^{c}	27.46 (1.037)^{a}	4.23 (0.325)^{d}	p<0.001
lysine	24.20 (0.817)^{a}	8.68 (0.763)^{d}	7.59 (0.896)^{d}	21.81 (0.534)^{b}	12.29 (0.700)^{c}	5.42 (0.559)^{e}	p<0.001
histidine	11.48 (0.332)^{a}	4.60 (0.285)^{c}	6.29 (0.362)^{b}	7.22 (0.060)^{b}	10.82 (0.766)^{a}	1.65 (0.177)^{d}	p<0.001
arginine	31.90 (0.733)^{a}	9.60 (0.727)^{e}	12.73 (0.722)^{d}	16.62 (1.086)^{c}	23.98 (0.965)^{b}	5.05 (0.349)^{f}	p<0.001
proline	20.75 (0.867)^{c}	18.36 (1.260)^{c}	25.92 (0.519)^{b}	12.24 (0.509)^{d}	55.01 (2.750)^{a}	--	p<0.001

highest statistically
lowest statistically

4.3.4 Coefficients of digestibility of YPCs from different sources with different drying conditions

Coefficients of digestibility (COD) are shown in table 4.10, letters denote statistical differences at $P<0.05$ level. There were no significant differences in COD for glutamate and methionine, although in the latter there was a trend ($P=0.055$) towards spray dried YPC content being greater than freeze dried YPC. Soya had the highest or equal highest COD for all amino acids, followed by bioethanol spray dried YPC, which was equal to soya for 11 out of 17 amino acids. Bioethanol freeze dried YPC CODs were equal to soya for 6 amino acids and potable spray dried CODs were equal for 2 amino acids. Ring dried potable YPC had the lowest COD for all amino acids measured except cysteine, for which the other potable YPCs were worse.

Table 4.10: Coefficients of digestibility for YPCs and soya

Amino acid	Soya	Potable FD	Bioethanol FD	Potable SD	Bioethanol SD	Potable RD	p value
cysteine	0.64 (0.039)^{a}	0.20 (0.069)^{b}	0.50 (0.043)^{a}	0.28 (0.085)^{b}	0.60 (0.069)^{a}	0.52 (0.023)^{a}	p<0.001
aspartic acid	0.77 (0.032)^{a}	0.36 (0.048)^{cd}	0.47 (0.057)^{bc}	0.59 (0.046)^{b}	0.59 (0.062)^{b}	0.25 (0.032)^{d}	p<0.001
methionine	0.82 (0.029)	0.56 (0.068)	0.65 (0.046)	0.72 (0.068)	0.77 (0.061)	--	p=0.055
threonine	0.74 (0.038)^{a}	0.44 (0.071)^{cd}	0.56 (0.055)^{bc}	0.57 (0.045)^{bc}	0.65 (0.048)^{ab}	0.31 (0.033)^{d}	p<0.001
serine	0.78 (0.028)^{a}	0.48 (0.019)^{c}	0.61 (0.044) ^{b}	0.45 (0.028)^{c}	0.69 (0.055)^{ab}	0.28 (0.033)^{d}	p<0.001
glutamate	0.77(0.038)	0.61 (0.026)	0.70 (0.022)	0.56 (0.021)	0.82 (0.038)	0.60 (0.149)	p=0.198
glycine	0.74 (0.048)^{a}	0.44 (0.023)^{c}	0.61 (0.04)^{b}	0.46 (0.032)^{c}	0.62 (0.046)^{b}	0.31 (0.025)^{d}	p<0.001
alanine	0.77 (0.037)^{a}	0.49 (0.061)^{cd}	0.60 (0.045)^{bc}	0.57 (0.030)^{bc}	0.65 (0.036)^{b}	0.39 (0.027)^{d}	p<0.001
valine	0.76 (0.039)^{a}	0.57 (0.067)^{b}	0.65 (0.036)^{ab}	0.59 (0.024)^{b}	0.74 (0.056)^{a}	0.38 (0.030)^{c}	p<0.001
isoleucine	0.77 (0.041)^{a}	0.57 (0.062)^{b}	0.58 (0.045)^{ab}	0.64 (0.038)^{b}	0.69 (0.050)^{a}	0.42 (0.034)^{c}	p<0.001
leucine	0.76 (0.036)^{a}	0.59 (0.028)^{b}	0.66 (0.043)^{ab}	0.67 (0.029)^{ab}	0.76 (0.037)^{a}	0.47 (0.037)^{c}	p<0.001
tyrosine	0.82 (0.024)^{a}	0.55 (0.024)^{b}	0.65 (0.044)^{b}	0.59 (0.043)^{b}	0.78 (0.043)^{a}	0.36 (0.038)^{c}	p<0.001
phenylalanine	0.78 (0.037)^{a}	0.63 (0.028)^{bc}	0.71 (0.039)^{ab}	0.57 (0.029)^{cd}	0.81 (0.031)^{a}	0.47 (0.036)^{d}	p<0.001
lysine	0.81 (0.027)^{a}	0.48 (0.043)^{c}	0.59 (0.069)^{bc}	0.64 (0.016)^{b}	0.67 (0.038)^{b}	0.46 (0.047)^{c}	p<0.001
histidine	0.81 (0.023)^{a}	0.47 (0.029)^{c}	0.69 (0.040)^{b}	0.67 (0.006)^{b}	0.70 (0.050)^{ab}	0.40 (0.043)^{c}	p<0.001
arginine	0.84 (0.019)^{a}	0.61 (0.046)^{cd}	0.72 (0.041)^{bc}	0.77 (0.050)^{ab}	0.86 (0.034)^{a}	0.53 (0.036)^{d}	p<0.001
proline	0.79 (0.033)^{a}	0.65 (0.044)^{b}	0.77 (0.016)^{a}	0.63 (0.026)^{b}	0.85 (0.043)^{a}	--	p<0.001

highest statistically

lowest statistically

4.3.5 Bodyweight gain and viscosity measures from amino acid study BRC06

Bodyweight gain

The total bodyweight gain of the birds fed the different test diets can be seen in table 4.12. Bodyweight gain was significantly higher for the 60% inclusion diet (p=0.015), but there was no significant diffence in the bodyweight gain of the other diets.

Table 4.12: Bodyweight gain for each diet over the final 5 days of the study period in birds fed graded levels of ring dried potable YPC

Diet	Treatment	Bodyweight gain per bird (g) (S.E.)
A	20% Potable RD YPC	56.7 (9.61)
B	40% Potable RD YPC	71.9 (11.74)
C	60% Potable RD YPC	105.9 (10.53)
p value		0.015

Viscosity of digesta supernatant

The viscosity of the digesta supernatent of the birds fed the ring dried YPC are shown in table 4.13. Readings were taken from pooled digesta from each pen. There were no significant differences in diegsta supernatant viscosity between the different inclusion levels of YPC.

Table 4.13: Digesta supernatant viscosity of chicks fed graded levels of ring dried YPC

Diet	Viscosity Cp (S.E.)
20% YPC	2.43 (0.200)
40% YPC	2.48 (0.243)
60% YPC	2.23 (0.247)
p value	0.711

4.4 DISCUSSION

Total amino acid content of the yeast protein concentrates and soya

Figure 4.1 shows a graphical comparison of all total amino acid content for the protein sources measured in this chapter. Spray dried YPC from both potable and bioethanol sources appears to be comparable with soya in terms of total amino acid content. However freeze dried YPC had a lower total amino acid content than spray dried YPC for both potable and bioethanol sources, which was unexpected as freeze drying reduces structural damage (Dalgleish, 1990) as removes excess heat which is usually the cause of product deterioration (Morris *et al*., 2004). This could be due in part to differences in feedstock composition or fermentative parameters. Different varieties of wheat have been shown to effect DDGS composition (Lan *et al*., 2008; Azafar *et al*., 2012) and this may translate to differences in the YPC composition. Processing conditions including temperature, concentration of solids and water quality can also effect co-product composition (Rausch and Belyea, 2006) with fermentation batches found to be the most influential source of variation (Belyea *et al*., 2010). However both the bioethanol and potable YPCs were similarly affected, which suggests that the differences may be due to post distillery processing. The freeze dried YPCs were the first products to be separated via the disk stack process, it is possible that these YPCs were contaminated with a substantial fibre fraction which would increase the dry matter of the material without increasing the protein content as described in chapter 3. Increased operator familiarity with the equipment as the project continued is likely to have improved separation of the spray dried material and therefore improved the yeast content. Both SD YPCs contained higher protein than the FD YPCs which suggests less fibre contamination.

Table 4.14 shows a comparison of the total content of the indispensable amino acids of the bioethanol feedstock (wheat) and the traditional bioethanol co-product (wheat DDGS) with the bioethanol sourced YPCs. Both YPCs have an increased amino acid content when compared with

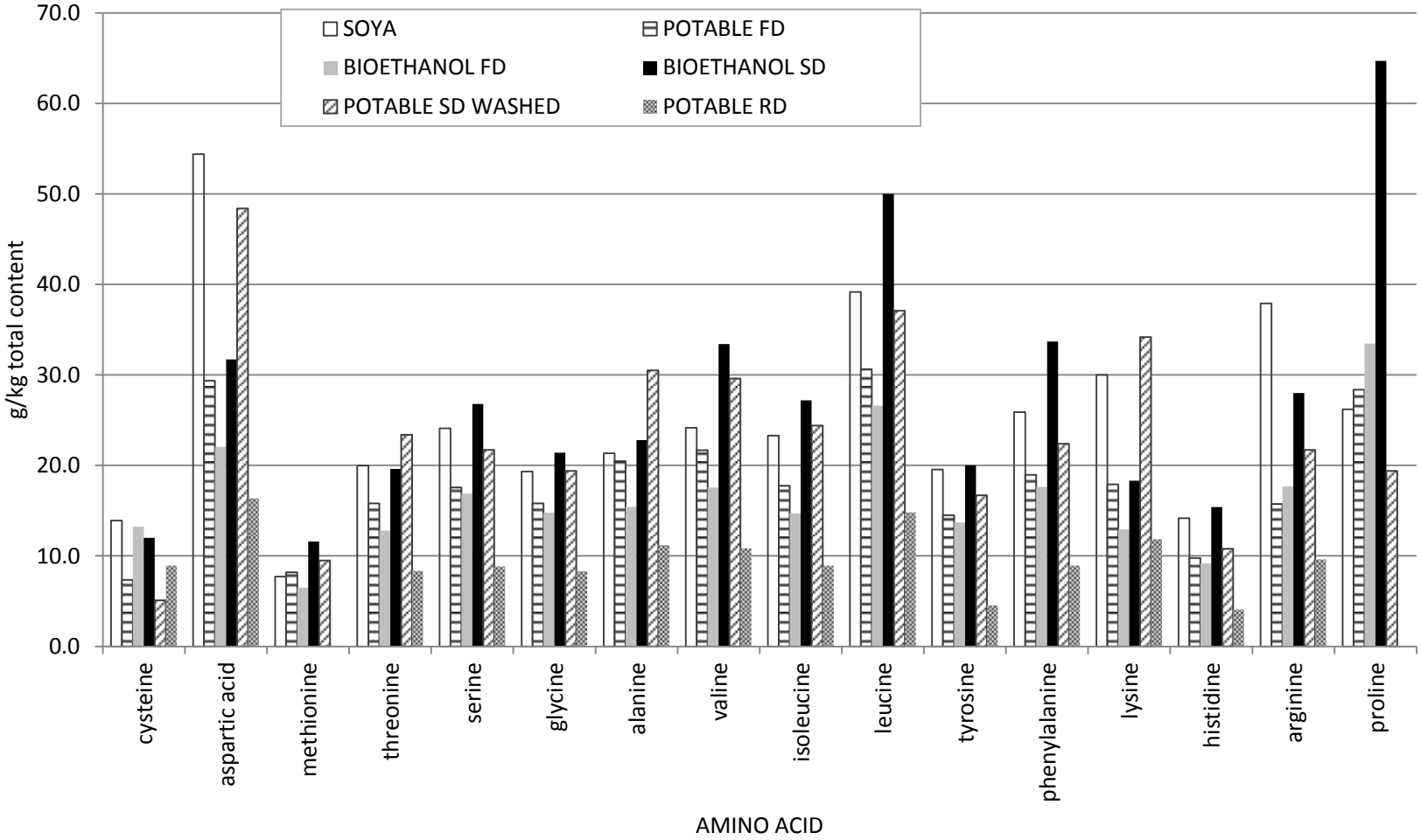

Figure 4.1 Total amino acid content of soya and the different YPCs assessed

wheat DDGS, which is due in part to the increased protein, 35-38% compared with an average (based on 5 samples) measured protein of 21% for WDDGS (Bandegan *et al*., 2009). The spray dried bioethanol YPC is 80% higher in protein than WDDGS, but an average of 93% higher for indispensable amino acids.

Table 4.14: Comparison of total amino acid content (g/kg) of YPCs from wheat feedstocks with wheat and wheat DDGS

Amino acid	Wheat[1]	Wheat DDGS[1]	Bioethanol YPC FD	Bioethanol YPC SD
Arginine	7.1	16.1	17.7	28
Histidine	3.6	8.2	9.17	15.4
Isoleucine	5.4	13.7	14.69	27.2
Leucine	10.3	26.3	26.6	50
Lysine	4.2	7.4	12.9	18.3
Methionine	2.6	6.1	6.5	11.6
Phenylalanine	7.3	18.1	17.6	33.7
Threonine	4.4	11.8	12.8	19.6
Valine	6.7	17	17.6	33.4

[1]Bandegan *et al*., 2009

It is particularly notable that lysine is 147% higher in the spray dried YPC. Lysine is the first limiting amino acid in DDGS, but in brewer's yeast historically methionine, arginine and leucine are limiting (Klose and Fevold, 1945; Daghir and Sell, 1982). This is due to yeast utilizing free amino acids for growth during fermentation, with different amino acids being taken up at specific points in the fermentation process (Jiranek *et al*., 1995). For example proline is assimilated slowest by yeast, but in DDGS proline content is relatively higher as it has not been utilized as a nitrogen source by the yeast (Han and Lui, 2010).

Table 4.15 compares the total amino acid content of the bioethanol spray dried YPC with a previously measured bioethanol yeast (Han and Lui, 2010) and a commercial purified yeast protein extract (NuPro; Wang *et al*., 2009). The bioethanol YPC is higher in amino acids than the whole yeast analysed previously and is comparable to the purified yeast protein. This may be due in part to the mechanical disruption of the disk stack process, which shears the cell

walls of the yeast, making the cell contents more available. The high ethanol content in bioethanol production has been shown to toughen yeast cell walls making them more resistant to proteolysis (Caballero and Sgarbieri, 2000). This could explain the lower amino acid content found in the bioethanol yeast (Han and Lui, 2010) as this has not had the mechanical shear forces exerted on it from the disk stack.

Table 4.15: Comparison of total amino acid content (g/kg)of yeast and bioethanol YPC

Amino acid	**NuPro**[1]	**Bioethanol Yeast**[2]	**Bioethanol YPC SD**
Cysteic acid	5	4	12
Aspartic acid	38	34	32
Threonine	22	18	20
Serine	19	17	27
Glutamic acid	85	63	193
Glycine	19	14	21
Alanine	29	18	23
Valine	25	17	33
Isoleucine	18	14	27
Leucine	36	24	50
Tyrosine	17	8	20
Phenylalanine	19	14	34
Lysine	28	26	18
Histidine	10	9	15
Arginine	19	16	28
methionine	11	7	12

1.Wang *et al*., 2009; 2. Han and Lui, 2010

Although the potable spray dried YPC had the highest protein content, this has not translated to higher amino acid content, which is similar to the bioethanol SD YPC and soya. It has been shown in DDGS that increased protein content does not necessarily correspond to increased amino acid content, as some amino acids (such as proline and alanine) concentrate more rapidly than others (such as histidine and leucine) during fermentation (Liu, 2011). Table 4.16 compares the barley based potable YPCs with the feedstock and a distillery sludge in terms of

essential amino acid content. Distillery sludge is a co-product of potable alcohol production similar to DDGS, but without drying. This table also compares the effect of washing on amino acid content of distillery sludge and YPC.

Table 4.16: Comparison of essential amino acid content of barley, washed and unwashed distillery sludge (DS) and washed and unwashed potable YPC, all from barley feedstock

Essential amino acids	**Barley[1]**	**DS washed[2]**	**DS unwashed[2]**	**Potable YPC**	**Potable washed YPC**	**Percent increase from washing**
Threonine	3.2	10.5	8.3	15.8	23.4	48.1
Valine	5	12.5	9.9	21.7	29.6	36.4
Methionine	1.8	6.9	5.4	8.2	9.5	15.9
isoleucine	3.5	12.9	10.2	17.8	24.4	37.1
Leucine	7.1	15	11.8	30.6	37.1	21.2
Phenylalanine	5	11.6	9.1	19	22.4	17.9
Histidine	2.4	6.4	5	9.8	10.8	16.1
Lysine	3.7	5.5	4.4	17.9	34.2	91.1
Arginine	5.1	18.6	14.7	15.7	21.7	38.2

1.Ravindran *et al*., 2005; 2. Sharif *et al*., 2012

YPC from potable sources has a greater content of indispensable amino acids than either barley alone or distillery sludge. The protein content of the distillery sludge was 27.4% for unwashed, rising to 34.8% for washed (Sharif *et al*., 2012) which is comparable with the unwashed potable YPC at 36.5% protein. This suggests that protein alone is not the reason for the improved amino acid profile of the YPC. Differences in cultivar and fermentation will contribute, but it is likely due to the improved amino acid content of yeast combined with the mechanical disruption of the cell walls improving the accessibility of the yeast cell components compared to the mixed distillery sludge (which will have a fibre component).

Average increase of amino acid content by washing was 35.8% with the YPC compared with an increase of 27% found by Sharif *et al*. (2012). The latter also found a consistent increase across amino acids, whereas there is a great deal of variability in the increases in amino acid content

in the washed potable YPC, with differences after washing ranging from 16% to over 90%. Although some of these differences may be due fermentation and batch variations between the two products, most is likely to be attributed to the drying process, especially considering the reduced stickiness observed during the spray drying of the washed material (Masons Chemicals, personal communication). It is notable that lysine in particular was increased in the washed material, which suggests that the washing process has reduced the propensity for Maillard reactions during the drying process. The washing process used by Sharif *et al*. (2012) was a much simplified process which did not use a mechanical separation and this may have had some effect on the amino acid content, particularly as there would have been less disruption of the yeast cell walls compared to the mechanical shear from the disk stack process. The ring dried potable YPC has much lower total amino acid content when compared with all other protein sources measured. This YPC was extremely dark, as can be seen in figure 4.2, which would suggest that it has been burnt during the ring drying process. During ring drying, selective extension of residence time increases the possibility of burning and overheating occurring (Vega Mercado *et al*., 2001). The consistency of ring dried product can also be an issue as some of the material recycles through the dryer and therefore the material is not all dried for the same length of time. Maillard reactions impair the nutritional content and the bioavailability of amino acids and proteins (Moralez *et al*., 2007) and have been shown to occur during the drying of DDGS. Pederson and Lindberg (2010) found that some amino acids (including lysine) were significantly reduced by the drying process, which also seems to be the case with the ring dried YPC.

Figure 4.2: Comparison of yeast protein concentrates tested; from left to right (top row), potable freeze dried, bioethanol freeze dried, potable washed spray dried, (bottom row) potable ring dried and bioethanol spray dried

Colour has been used previously as a measure of protein quality in DDGS (Cromwell *et al*., 1993; Shurson, *et al*., 2005) with darkness of the product correlating with chemical composition (Cozannet *et al*., 2010). This is due to the formation of brown polymers at the end of the series of reactions that take place when nitrogen compounds and reducing sugars are heated together (Martins *et al*., 2001). This browning reaction can be clearly seen in figure 4.2, so much of the reduction in amino acid content of the ring dried YPC is likely to be due to heat damage.

Lysine is the amino acid which is most effected by Maillard reactions. Soya contains 30g/kg lysine, and most of the investigated YPCs contained around half this amount, with the exception of the spray dried washed potable YPC, which contained 34g/kg total lysine. During spray drying of the bioethanol YPC, it was observed that the material was adhering to the sides of the dryer which could have led to uneven drying and patches of burning which goes some

way towards explaining the lower lysine content of this product. However the freeze dried YPCs also contained lower levels of lysine, which cannot be explained by heat damage. These YPCs were the pilot runs through the separation facilities and it is possible that the poor separation is the cause of the reduced amino acid content.

Cozannet *et al*., (2011b) found that lysine ranged from 0.83 to 3.0g per 100g crude protein (CP) in wheat DDGS. For comparison, the lysine content of the protein sources measured in this chapter are shown as a proportion of crude protein in table 4.17, which shows the lysine varies from 2.3 to 5g/100 CP, with the spray dried YPCs and FD potable YPC all being similar. These YPCs contain substantially more lysine on a proportion of total protein basis than WDDGS, and this implies that Maillard reactions, although present, may have a less deleterious effect on the lysine content of YPC than DDGS.

Table 4.17: Lysine In the YPCs as a proportion of crude protein

YPC	Crude protein	Lysine %	Lysine g/100g CP
Bioethanol FD	34.74	1.29	3.713
Bioethanol SD	38.1	1.83	4.803
Potable FD	36.58	1.79	4.896
Potable SD	67.61	3.42	5.058
Potable RD	51.05	1.19	2.321

Digestible amino acid content and coefficients of digestibility

The digestible amino acid contents of the protein sources investigated in this chapter are shown in figure 4.3 and the coefficients of digestibility are shown in figure 4.4. All values refer to apparent ileal digestible amino acids, and the coefficients of digestibility are calculated from these and the total amino acid content. The coefficients of digestibility for each amino acid for each protein source have been detailed in table 4.10, and for simplicity, the average COD and digestible amino acid content for each protein source is shown in table 4.18 to show a comparative ranking and a feedstock comparison. Ravindran *et al*. (2005) quote the COD of soya as 0.82 which is a little higher than measured in this study, but it is difficult to make direct

comparisons with published data due to methodology differences such as bird age and site of sample collection.

Table 4.18: Mean coefficients of digestibility and digestible amino acid content of YPCs, soya and parent grains

Protein source	**Coefficients of Digestibility**	**Digestible amino acid content**
Soya	0.777	21.9
Bioethanol Spray dried	0.727	28.2
Bioethanol Freeze dried	0.632	12.6
Potable Spray dried	0.581	15.0
Potable freeze dried	0.516	11.4
Potable Ring dried	0.394	4.2
Wheat[1]	0.830	12.12
Barley[1]	0.670	9.78

[1]Ravindran *et al*., 2005

Both the barley and wheat based YPCs have lower CODs than the base feedstock cereals and soya, however the feedstocks have considerably lower protein content so the ileal digestible amino acid content is higher for all the YPCs compared with the respective parent grain, with the exception of the ring dried YPC. Of the YPCs investigated, the bioethanol SD YPC was the most digestible and the ring dried potable YPC the least digestible. To compare the YPCs directly to a yeast product, the true amino acid digestibility coefficient of Nupro yeast extract has been estimated as 0.87 (Nanney *et al*., 2007), which is also higher than those recorded here. However this is estimated using a different methodology (caecetomised cockerel assay) so is not directly comparable. It would also be expected that yeast extract would be more digestible as they removal of the cell wall allows quicker access to the free amino acids within the yeast cell.

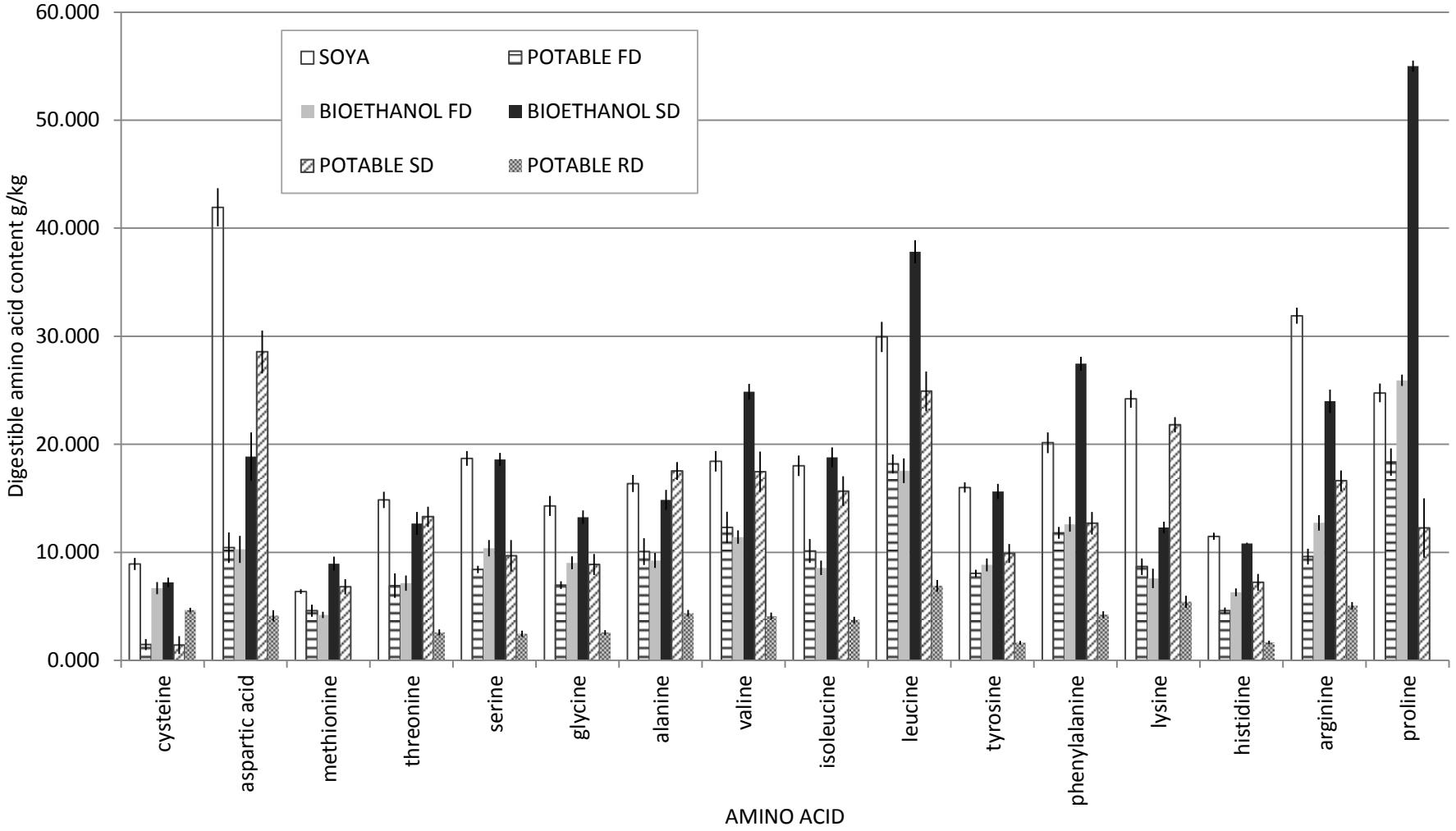

Figure 4.3: Digestible amino acid content of the protein sources tested in BRC01.04 and 06

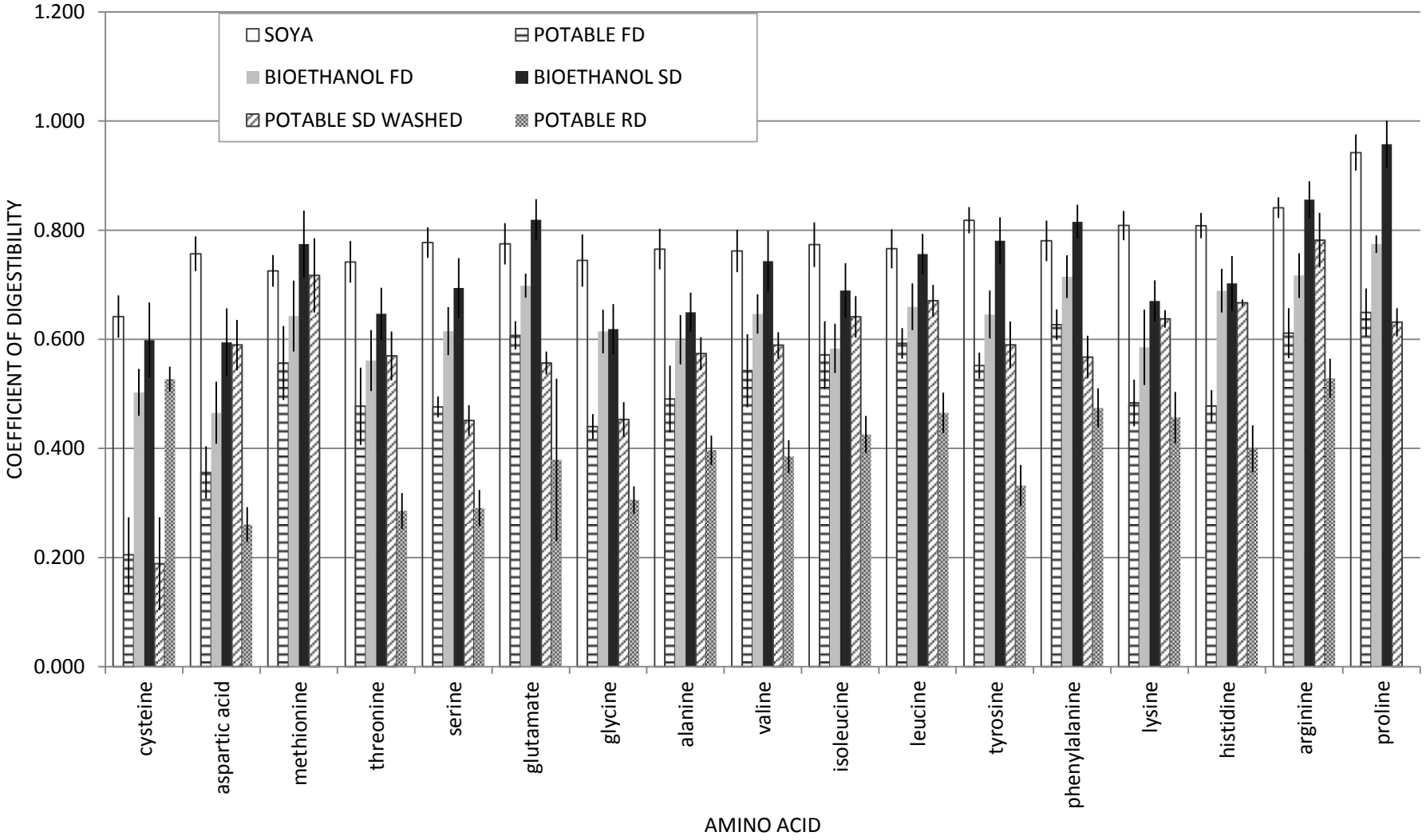

Figure 4.4 Coefficients of digestibility of soya and each YPC assessed in studies BRC01, 04 and 06

The differences in amino acid digestibility measures between groups can also be seen clearly in table 4.19, which is a comparison of coefficients of digestibility for wheat sourced YPC, wheat and the traditional bioethanol co-product DDGS. It is clear from this table that there are significant differences between measurements of the same material from different research groups. Some of this will be due to differences in methodology as there is no consensus as to the most appropriate way to measure amino acid digestibility. Differences in cultivar, growth conditions and time of harvest have been shown to effect amino acid digestibility (Ravindran *et al*., 2005) and WDDGS has also been shown to be extremely variable for amino acid digestibility (Bandegan *et al*., 2009) with fermentation parameters playing a major role (Belyea *et al*., 2010).

Table 4.19: Comparison of essential amino acid coefficients of digestibility for wheat, wheat DDGS and YPCs from wheat feedstock.

Essential amino acids	**Wheat** [1]	**Wheat**[2]	**YPC Bio FD**	**YPC Bio SD**	**WDDGS**[3]	**WDDGS**[4]
Threonine	0.73	0.59	0.56	0.65	0.62	0.56
Valine	0.82	0.74	0.65	0.74	0.68	0.64
Methionine	0.91	0.75	0.65	0.77	0.76	0.68
isoleucine	0.84	0.78	0.58	0.69	0.72	0.61
Leucine	0.86	0.81	0.66	0.76	0.76	0.64
Phenylalanine	0.86	0.82	0.71	0.81	0.86	0.69
Histidine	0.82	0.7	0.69	0.7	0.66	--
Lysine	0.79	0.64	0.59	0.67	0.4	0.69
Arginine	0.82	0.74	0.72	0.86	0.71	0.74

[1]Ravindran *et al*., 2005; [2]Huang *et al*., 2007; [3]Bandegan *et al*., 2009; [4]Kluth and Rodehutscord, 2010

A comparison of the potable YPCs with the cereal feedstock (barley) and a traditional co-product from potable distilleries (brewer's grains) is shown in table 4.20. It can be seen that

both potable YPCs are less digestible than barley, and brewer's grains. However the brewer's grains described in this study (Donkoh and Attoh-Kotoku, 2007) were wet grains and therefore did not have any drying or processing damage. It is also notable that threonine has poor digestibility throughout due to it being the major amino acid in endogenous amino acid secretions (Ravindran, 2004).

Table 4.19: Comparison of coefficients of digestibility for potable YPCs, barley and brewers grains.

Essential amino acids	Barley[1]	Brewers grains[2]	Potable FD	Potable SD W
Threonine	0.6	0.72	0.44	0.57
Valine	0.71	0.8	0.57	0.59
Methionine	0.77	0.82	0.56	0.72
isoleucine	0.7	0.81	0.57	0.64
Leucine	0.73	0.82	0.59	0.67
Phenylalanine	0.73	0.85	0.63	0.57
Histidine	0.63	0.65	0.47	0.67
Lysine	0.65	0.76	0.48	0.64
Arginine	0.7	0.83	0.61	0.77

[1]Ravindran *et al*., 2005; [2]Donkoh and Attoh-Kotoku, 2007

Interestingly, bioethanol YPC coefficients of digestibility are better than for potable YPC even though it has been suggested that bioethanol yeast may be more exhausted of cellular components when compared to potable yeast, as the latter is only used once in the potable process (Yamada *et al*., 2005). The feedstock will play a part in the differences as the potable feedstock was barley and the bioethanol was wheat based. Barley has poorer COD than wheat (Ravidran *et al*., 2005) but the differences can also be found within cereal type with different wheat cultivars found to have varying amino acid digestibilities (Ravindran *et al,* 1999; Kluth *et al*., 2009).

Difference in potable and bioethanol COD may be due to the presence of non-starch polysaccharides in the potable YPC. Non starch polysaccharides may physically encapsulate the

protein and thereby reduce amino acid digestibility (Selle *et al.*, 2009). Wheat non starch polysaccharides have been shown to increase endogenous amino acid secretions via increased digesta viscosity increasing the peristaltic resistance, leading to increased endogenous protein losses (Angkanaporn *et al*., 1994). The potable YPCs tested are all from distillaries that use a barley feedstock. Potable distilleries do not use exogenous enzymes during the fermentation process as they are constrained by law (Scotch Whisky Order, 1990). As barley contains substantial non-starch polysaccharides, it may be that these will pass through the disk stack seperator and be present in the YPC.

In chapter 5, digesta supernatent viscosity was assessed in chicks fed YPC containing diets, and increasing dietary levels of potable YPC led to a significant increase in digesta supernatant viscosity. However the viscosity of the chick digesta supernatent measured in BRC06 were much lower previously seen (even in the control diets), despite the higher YPC inclusion levels. This could be due to the short time period the digestiblity diets are fed for, although Kluth and Rodehutscord (2010) showed that 3 days feeding were sufficient for accurate amino acid measurement, it may be that it is insufficient to see a viscosity effect via changes in gut microflora and intestinal fermentation rates. Also sensitivity to pentosans decreases as the birds age (Pettersson and Aman 1988), especially after 3 weeks of age, which may be due to the maturation of the digestive system or increased indigenous microbial population (Campbell and Bedford, 1992); so it may be that in these studies the birds were too old to record a viscosity increase.

The freeze dried YPC had a significantly lower COD when compared with spray dried YPC from the same source. This may be due to the increased levels of fibre which may be present in the freeze dried samples due to poor separation. The freeze dried YPCs were the first products to be separated through the pilot plant and it is likely that the operators were less familiar with the equipment and were overcautious to ensure full capture of the yeast, which may have led to fibre contamination of the YPC. If this is the case, the fibre could be having a deleterious

effect on the amino acid digestibility, as Ravindran and Bryden (1999) found negative effects of fibre on endogenous amino acids. Dietary fibre also decreases feed intake due to increase in dietary bulk and has been shown to limit nutrient utilization in chicks (Thacker and Widyaratyne, 2007). Fibre content of the diet can increase endogenous amino acid losses (Adedokum *et al*., 2012)

The darker colour of the ring dried YPC seen in figure 4.2 is reflected in its low coefficients of digestibility. Heat processing damage and Maillard reactions have been shown to negatively affect the digestible energy content in both maize (Fastinger *et al*., 2006) and wheat DDGS (Cozannet *et al*., 2011a). Very dark samples were also reported to give very low ileal amino acid digestibility values in pigs (Cozannet *et al*., 2010) and in cockerels (Cozannet *et al*., 2011b). In wheat DDGS fed to pigs, digestibility of some amino acids (including lysine) were significantly reduced by the drying process (Pederson and Lindberg, 2010).

Lysine availability is one of the major concerns due to the possibility of Maillard reactions during the drying process. Colour has been correlated to amino acid digestibility by a number of researchers (Batal and Dale, 2006; Fastinger *et al*., 2006), with lysine digestibility reducing from 80% to 60% with darker DDGS (Ergul *et al*., 2003). Lysine digestibility in the ring dried YPC was measured at 47% but this is not significantly different to the digestibility of the freeze dried potable YPC, implying that heat damage may not be the only cause of low lysine digestibility. Papadopoulos (1989) states that the processing time is the most important factor in heat damage of amino acids therefore the controlled pilot plant conditions may not be indicative of the damage which may occur in a commercial setting where the YPC may be dried for longer.

Lemme *et al*. (2004) suggest a dietary lysine inclusion of 1.25% ileal digestible lysine for broilers, and only potable spray dried YPC resulted in greater than this level of lysine in the ileal digesta (2.18%), due to the high total content of lysine in this YPC. The bioethanol spray dried lysine was approaching the suggested digestible lysine, which is unexpected as the spray

dried unwashed material was observed adhering to the spray drier which may have led to formation of unavailable lysine derivatives. Although the washed material appears to have increased available lysine, it does not appear that spray drying is deleterious to amino acid quality.

Bodyweight gain

Bodyweight gain was only recorded for the period of test diet feeding in study BRC06 where bodyweight gain increased significantly with YPC inclusion levels. This suggests that palatability was not an issue with the ring dried potable YPC assessed in this study. The spray and freeze dried YPC products were very fine in terms of particle size and it may be that the ring drying process has reduced the dustiness of the material. Certainly, spray and freeze drying are known to produce a free flowing product with small particle size (Niessen, 2002).

4.5 CONCLUSION

In conclusion, spray dried YPC is comparable to soya in terms of amino acid content, but with lower digestibility coefficients. Bioethanol YPC is has greater digestible amino acids than potable, and this is likely to be due to exogenous enzyme use in bioethanol production. These enzymes have been shown to reduce non starch polysaccharide content, counteracting the viscosity increases and increasing amino acid digestibility in diets fed to broilers (Hew *et al*., 1998; Selle *et al*., 2009).

Drying method can have a detrimental effect on amino acid content and coefficients of digestibility, with ring drying proving very damaging in terms of amino acid availability.

The separation technique needs to be carefully monitored to ensure consistency and to reduce any fibrous contamination of the YPC. The fibre content of the diet can increase endogenous amino acid losses (Adedokum *et al*., 2012) and deleteriously affect the amino acid digestibility.

CHAPTER 5: EFFECT OF FEEDING YEAST PROTEIN CONCENTRATE (YPC) SEPARATED FROM DIFFERENT SOURCES USING A RANGE OF PROCESSING CONDITIONS ON BIRD PERFORMANCE AND DIGESTA VISCOSITY

5.1 INTRODUCTION

This chapter investigates the performance of broiler chicks fed diets containing varying levels of yeast protein concentrate (YPC) from different sources, with different processing treatments. The possible effect of some anti-nutritional factors in the YPC will also be investigated, specifically uric acid and non-starch polysaccharides content.

The current co-product from both bioethanol and potable alcohol production is distiller's dried grains with solubles (DDGS). The use of DDGS in chick diets is limited by the high fibre content, but when supplemented with amino acids has been fed successfully to broilers at 15% inclusion (Thacker and Widyaratyne, 2007; Youssef *et al*., 2008). DDGS contains nutritionally valuable yeast protein, which if separated successfully could be potentially fed at higher dietary inclusion levels to monogastrics. This project uses a novel separation technology combined with a dewatering process to produce a high yeast content powder. Yeast has been fed as animal feed for a number of years and although there is some conflicting research, at lower levels there have been positive effects on performance reported (Miazzo *et al*., 2005; Silva *et al*., 2009).

One of the issues with feeding DDGS is it can contain non-starch polysaccharides (NSP) which are present in cereals in differing quantities. These are deleterious to poultry as they can increase digesta supernatant viscosity and reduce nutrient digestibility (White *et al*., 1981; Choct and Annison, 1992b), as well as increasing small intestinal fermentation (Choct *et al*., 1996). It is entirely possible that the separation procedure used to obtain the yeast protein

concentrate may also concentrate these NSPs, and with the fibre removal during the process, this would comparatively increase the final proportion of the NSPs in the YPC.

It may be possible to reduce the concentration of NSP by washing (mixing with water post separation and re-separating) the YPC prior to drying. This could also make the material more consistent to dry, reducing the drying time needed. This could be a substantial saving to the process when it is estimated that drying can account for 40% of energy costs (Stock and Klopfenstein, 1982). If the material cannot be dried evenly the nutritional content could be compromised and consistency would be an issue. Over -heating can lead to the formation of Maillard's complexes, binding lysine in a biologically unavailable form (Plakas *et al*., 1985). It may also be possible to negate the effects of these deleterious carbohydrates by addition of exogenous enzymes during the process. Although the use of exogenous enzymes is prohibited in the production of whisky (Scotch Whisky order, 1990), they are used routinely during bioethanol processing to increase extract volume (Bamforth, 2009). Therefore a comparison of bioethanol YPC with potable YPC would indicate whether enzymes added post separation either to the YPC directly or to the mixed feed would be beneficial.

Yeast contains a high proportion of nucleic acid nitrogen, around 7 times the amount present in other protein sources (Ingledew, 1999). Due to the lack of an active liver uricase in poultry, excretion of these nucleic acids is limited, leading to a build-up of uric acid in the lower extremities (Stone, 1998) with negative consequences for bird health. Measuring uric acid in serum would deliver a quantifiable measure of the effect of YPC inclusion, and determine whether higher levels have negative implications for bird health. However, Hellstein *et al*. (1997) proposed uric acid as a potent scavenger of oxidants at lower level and Simoyi *et al*. (2002) found that reducing these oxidants improves meat quality and improves bird health, and found a correlation between oxidant activity and plasma uric acid levels. It may be that a lower level of serum uric acid has a positive antioxidant effect, whereas higher levels are detrimental to bird health.

5.2 METHODS

This chapter details the results for three separate bird studies as shown in table 5.1. Husbandry was carried out as detailed in chapter 2. In BRC02 and BRC03, yeast protein concentrates from bioethanol and potable alcohol sources were included in mash broiler chick diets to assess any effect on bird performance. The YPCs were dried using different technologies in each trial, to further assess any effect of drying on bird performance. Digesta supernatant viscosity was also quantified in these studies as a measure of the presence of deleterious carbohydrates in the YPC. In BRC03, the potable sourced YPC was washed prior to drying to investigate whether this would reduce the increase in digesta viscosity associated with non-starch polysaccharides. The effect of YPC incorporation on the apparent metabolisable energy of the diets was also investigated in this study. The final study (BRC07) was designed to assess the effect of feeding pelleted diets containing graded levels of YPC on bird performance. Serum uric acid and litter dry matter (as a measure of potential NSP effect) were also measured in this trial. All YPCs and diets used in this chapter were analysed for moisture, protein, fat and ash content using the methods detailed in Chapter 2. Other laboratory methods are also described in detail in chapter 2.

Table 5.1 Performance studies with Yeast protein concentrate (YPC) inclusion

Trial number	**YPC source used**	**Drying process**	**Other treatments**	**Data collected**
BRC02	Potable	Freeze dried	-	Performance
	Bioethanol	Freeze dried	-	Viscosity
BRC03	Potable	Spray dried	Washed	Performance & AME
	Bioethanol	Spray dried	-	Viscosity
BRC07	Potable	Ring dried	Pelleted	Performance
				Serum uric acid

5.2.1 BRC02: Performance of birds fed diets with graded levels of potable and bioethanol yeast protein concentrates

This study investigated the effect of dietary inclusion of graded levels of yeast protein concentrates separated from different sources on the performance of broiler chicks from day 1 to day 15 post hatch. This study also investigated whether the presence of any deleterious carbohydrates in the YPC was having a negative effect on the viscosity of the chick digesta supernatant.

Yeast protein concentrates

In this study, 2 different yeast protein concentrates were evaluated. The potable YPC used was separated from a barley based distillery and the bioethanol YPC was separated from a wheat based bioethanol plant, both in the UK. The decanter and disk stack processes previously detailed in chapter 1 and 3 were used in both cases. Both YPCs used in this trial were freeze dried and vacuum packed prior to transferal to the diet manufacturer.

Study diets

Diets were formulated and produced by a commercial feed manufacturer using a basal diet formulation shown in table 5.2, to NRC (1994) recommendations. This basal diet was then mixed with soya bean meal or YPC as shown in table 5.3. The potable (P) and bioethanol (B) yeast protein concentrates (YPC) were incorporated into mash diets at 3, 6 and 9% inclusion, as a direct replacement for soya bean meal in the diets.

Table 5.2 Basal diet formulation for BRC02

Raw Material	**Proportion of diet (g/kg)**
Wheat – standard[#]	842.5
Full fat soya extruded [#]	66.9
L Lysine HCl	4
DL Methionine	3.3
Soya Oil	26.8
Limestone	20.1
Monocalcium phosphate	20.1
Salt	3.3
Sodium bicarbonate	2
Vitamin E: Biotin (75:125)	1.3
Vitamin/mineral premix*	3.3
Choline chloride 50%	0.6

*see Appendix 1 [#]see Appendix 2

Table 5.3 Dietary treatments in BRC02

Diet	**Treatment**	**Basal mix/kg**	**Soya[#] /kg**	**Potable YPC /kg**	**Bioethanol YPC /kg**
A	Control	15	5.0	---	---
B	3% Potable	15	4.4	0.6	---
C	6% Potable	15	3.8	1.2	---
D	9% Potable	15	3.2	1.8	---
E	3% Bioethanol	15	4.4	---	0.6
F	6% Bioethanol	15	3.8	---	1.2
G	9% Bioethanol	15	3.2	---	1.8

[#]see appendix 2 for analysis

Study Protocol

140 male Ross 308 chicks (PD Hook, Cote, Oxon.) were fed one of seven wheat/soya bean meal-based mash diets from 1 to 15 days of age. Each experimental diet was fed to 5 pens, each containing 4 birds. One pen was considered to be one replicate.

Bird performance was evaluated by measuring feed intake, bird weight gain and (subsequently) feed conversion ratio on a weekly basis. The birds were weighed on Day 1, Day 8 and Day 15. Dates and weights of dead birds and reasons if culled were recorded. On Day 15 the feed troughs were removed for an hour and returned for an hour prior to euthanasia to ensure gut fill. Post euthanasia digesta was removed from the section of the gastrointestinal tract between the termination of the duodenal loop and Meckel's diverticulum (designated as jejunum) and pooled into one pot per pen. The sample was homogenised prior to centrifugation at 5000 rpm for ten minutes. A Brookfield DV2 digital cone and plate viscometer maintained at 41˚C was used to measure viscosity of the digesta supernatant in duplicate.

Data Analysis

After Kolmogorov Smirnoff (KS) testing to confirm normality, statistical analysis was carried out using one way ANOVA in the first instance, with Duncan post hoc testing to compare the effect of dietary treatment on feed intake, bodyweight gain, FCR and viscosity. Further analysis was carried out using MANOVA, again with Duncan post hoc tests (where required) to examine the effects of yeast source and rate of dietary inclusion on FCR, feed intake, bodyweight gain and viscosity. The statistical package used was SPSS v.19 (IBM statistics, 2012).

5.2.2 BRC03: washed and unwashed bioethanol yeast protein concentrate

This study investigated the effect of washing the yeast protein concentrate (YPC) prior to drying on the performance of broiler chicks, when included at graded levels in starter diets.

The effect of washing on removal of any non-starch polysaccharides (NSPs) present in the YPC was evaluated by measuring the viscosity of the digesta supernatant.

Yeast protein concentrates

In this study, 2 yeast protein concentrates were evaluated. One yeast protein concentrate used was separated from a barley-based potable alcohol distillery, and this YPC underwent a washing process twice before drying. The washing process involved mixing the separated yeast cream from the disk stack separation process with water before re separation through the disk stack. The second YPC evaluated was separated from a bioethanol plant and did not go through the washing process. Both YPC products were spray dried post separation.

Study diets

Diets were formulated by a commercial feed producer (Target Feeds, Cheshire, UK) to NRC (1994) recommendations with the YPC directly replacing solvent extracted ('hi pro') soya bean meal. Diets were manufactured in house, using the formulations in Table 5.4. The diets contained 0, 5, 10 or 20% of the two yeast protein concentrates as previously described; a washed, potable alcohol derived YPC (P) and an unwashed, bioethanol derived YPC (B). Dietary treatments are shown in Table 5.5.

Table 5.4 Basal diet formulation for BRC03

Raw Material	Proportion of diet (g/kg)
Wheat – standard[#]	636.5
HiPro Soya[#]	0
Full fat soya extruded[#]	50.0
L Lysine HCl	3.0
DL Methionine	2.5
Soya Oil	20.0
Limestone	15.0
Mono Calcium Phosphate	15.0
Salt	2.5
Sodium bicarbonate	1.5
Vitamin E: Biotin (75:125)	1.0
Vitamin/mineral premix*	2.5
Choline choride 50%	0.5

*see Appendix 1[#]see Appendix 2

Table 5.5 Dietary treatments in BRC03

Diet	Treatment	Basal mix /kg	Soya /kg	Bioethanol YPC /kg	Potable YPC /kg
A	0% YPC	26.25	8.75	---	---
B	5% YPC B	26.25	7.00	1.75	---
C	10% YPC B	26.25	5.25	3.50	---
D	20% YPC B	26.25	1.75	7.00	---
E	5% YPC P (washed)	26.25	7.00	---	1.75
F	10% YPC P (washed)	26.25	5.25	---	3.50
G	20% YPC P (washed)	26.25	1.75	---	7.00

Study protocol

168 male Ross 308 chicks were fed one of seven wheat/soya bean meal-based mash diets from 0 to 21 days of age. Birds were sourced from a parent flock ages between 30 and 50 weeks (PD

Hook, Cote, Oxon.). The diets contained 0, 5, 10 or 20% of one of two yeast protein concentrates, one which had been washed with water twice prior to spray drying. Each experimental diet was fed to 6 pens, each containing 4 birds. One pen was considered to be one replicate.

Bird performance was evaluated by measuring feed intake, bird weight gain and (subsequently) feed conversion ratio on a weekly basis. The birds were weighed on day 1, day 8, day 15 and day 21. Dates and weights of dead birds and reasons if culled were recorded. Excreta was collected from each pen between day 19 and day 21, by use of wire collection trays as detailed in chapter 2. On day 21 the feed troughs were taken away for an hour and returned for an hour prior to euthanasia to ensure gut fill. Post euthanasia digesta was removed from the section of the gastrointestinal tract between the termination of the duodenal loop and Meckel's diverticulum and pooled into one pot per pen. The sample was homogenised prior to centrifugation at 5000 rpm for ten minutes. A Brookfield DV2 digital cone and plate viscometer at 41˚C was used to measure viscosity of the digesta supernatant in duplicate. Excreta was dried and ground prior to analysis of gross energy by bomb calorimetry.

Data Analysis

After KS testing to confirm normality, statistical analysis was carried out using one way ANOVA with Duncan post hoc testing to compare the effect of dietary treatment on feed intake, bodyweight gain, FCR, apparent metabolizable energy (AME) and viscosity. Further analysis was carried out using MANOVA, again with Duncan post hoc tests (if appropriate) to examine the effects of yeast source and rate of dietary inclusion on FCR, feed intake, bodyweight gain, AME and viscosity. The statistical package used was SPSS v.19 (IBM statistics, 2012).

5.2.3 BRC07 – Performance of birds fed ring dried potable YPC containing diets and effect on serum uric acid content

This study investigated the effect of pelleting diets containing graded levels of YPC from a potable source on bird performance and litter moisture content. The effect of the high levels of nucleic acid nitrogen in yeast was also assessed by measuring serum uric acid content.

Yeast protein concentrates

In BRC07, one yeast protein concentrate was evaluated. A potable YPC from a barley distillery was separated using the disk stack separator and dried using a commercial scale ring drier.

Study diets

Diets were formulated by a commercial diet manufacturer (Target Feeds, Cheshire, UK) to be as iso-nitrogenous, using predicted as opposed to actual protein levels of the raw materials, and otherwise to NRC (1994) recommendations. Total dietary phosphorus was also reduced with increasing YPC inclusion to account for the estimated available phosphorus present in the YPC. Diet formulations are shown in Table 5.6. Diets were also manufactured and pelleted at a commercial diet producer to produce 2-3mm diameter pellets. A proportion of the diets was passed through a grinder with a 1mm screen to produce a crumb diet for the first week of the trial.

Table 5.6: Diet formulation for BRC07 diets g/kg inclusion of materials

Raw Material	**0% YPC**	**7.50% YPC**	**12.50% YPC**	**17.50% YPC**
Wheat[#]	576.5	581.5	588.5	588.5
Yeast protein	0	75.0	125.0	175.0
Hipro Soya[#]	200.0	120.0	65.0	15.0
Full fat soya extruded[#]	180.0	180.0	180.0	180.0
L lysine HCl	1.0	1.0	1.0	1.0
DL methionine	2.0	2.0	2.0	2.0
Soya oil	5.0	5.0	5.0	5.0
Limestone	13.0	15.0	15.0	16.0
Di-calcium Phosphate	14.0	12.0	10.0	9.0
Salt	3.0	3.0	3.0	3.0
Sodium bicarbonate	1.0	1.0	1.0	1.0
Vitamin/mineral premix*	4.0	4.0	4.0	4.0
Commercial coccidiostat	0.5	0.5	0.5	0.5

*see Appendix 1 [#]see Appendix 2

Study protocol

Birds were sourced from PD Hook (Cote hatchery, Oxford), from a flock aged 34 weeks. 192 day old male Ross 308 chicks were weighed before random allocation to one of 48 pens. Pens contained 4 birds and each pen was considered one replicate. 12 pens were randomly allocated to each dietary treatment. Diets contained 0, 7.5, 12.5 or 17.5% of a potable derived YPC which had been ring dried.

Birds were weighed by pen on arrival, on D8, D15 and at the end of the trial on day 22 and feed intake recorded weekly. Birds were observed twice daily during the trial and any observations related to health recorded in a trial diary. Any dead birds were weighed.

Birds were culled on day 22 by cervical dislocation and blood samples collected by post mortem cardiac puncture, from one bird per pen. Blood samples were allowed to stand for 2 hours to allow clotting to occur before centrifugation at 3000rpm for 5 minutes. Serum was

aliquoted into tubes and stored at -20^{o}C for analysis. Serum uric acid content was measured in serum using an Amplex Red kit (Invitrogen) as detailed in Chapter 2.

Samples of litter were collected on D21 of the study to assess litter dry matter content. A representative sample was collected from at least 3 points in the pen. The dry weight as a proportion of wet weight of litter is expressed as percentage litter dry matter.

Data Analysis

KS tests were carried out to confirm normality of the data before further analysis. Statistical analysis was carried out using one way ANOVA with Duncan post hoc testing to investigate the effect of increasing dietary YPC inclusion on FCR, feed intake, bodyweight gain, serum uric acid content and litter dry matter. The statistical package used was SPSS v.19 (IBM statistics, 2012)

5.3 RESULTS

The nutritional analyses for each YPC investigated in this chapter are shown in table 5.7. The freeze dried YPCs were similar in terms of nutritional content, whereas the washed YPC has a much higher protein content. The ring dried YPC contained intermediate protein levels and lower fat content. Fat content varied depending on processing of the YPC, with the washed yeast and the ring dried yeast containing very low fat levels in comparison to the other YPC.

Table 5.7 Analysis of Yeast protein concentrates used in the 3 performance trials.

Trial number	YPC	Dry Matter g/kg	Protein g/kg	Fat g/kg	Ash g/kg	Gross Energy MJ/kg
BRC02	potable freeze dried	912.56	365.81	73.03	27.73	20.74
	bioethanol freeze dried	864.76	347.43	78.41	36.07	20.71
BRC03	potable spray dried (washed)	911.44	676.09	2.15	37.59	22.05
	bioethanol spray dried	914.05	380.99	90.99	17.38	24.36
BRC07	potable ring dried	805.33	510.51	3.36	25.68	21.28

5.3.1 BRC02: Performance of birds fed graded levels of YPC from potable or bioethanol sources.

Diet analysis

Table 5.8 shows the nutritional analysis of the diets fed in BRC02. Although these diets were commercially formulated to be iso-nitrogenous, the protein content varied from 14 to 19%. The most marked differences were in ash content, which varied from around 5% to nearly 30% ash. This had a subsequent effect on the energy content as the higher mineral content of these diets (particularly diet B) reduced the gross energy of the diets by a considerable margin.

Table 5.8: Analysis of potable and bioethanol Yeast Protein Concentrate (YPC) diets

Diet	Treatment	Dry matter g/kg	Ash g/kg	Protein g/kg	Fat g/kg	Energy (MJ/kg)
A	0% YPC inclusion	866.6	55.09	175.47	17.61	19.54
B	3% Potable YPC	910.91	290.9	152.97	35.52	13.44
C	6% Potable YPC	906.46	214.8	175.94	37.45	17.95
D	9% Potable YPC	901.89	140.49	177.51	44.64	18.42
E	3% Bioethanol YPC	869.76	48.96	178.93	32.84	19.92
F	6% Bioethanol YPC	859.19	58.13	187.58	27.48	19.93
G	9% Bioethanol YPC	838.93	118.84	191.4	5.87	20.47

Performance

Table 5.9 shows that both bodyweight gain and feed intake were significantly increased in birds fed the bioethanol-derived yeast protein concentrate (YPC) compared to the potable alcohol-derived YPC and the control (0% YPC) diet. No significant difference in FCR was observed between the two YPC sources, although numerically the FCR increased with rate of YPC inclusion.

Table 5.9: Effect of YPC source and rate of inclusion (RoI) on mean bird performance from 1-15d post hatch

Treatment	**Feed intake per bird (g) (S.E.)**	**Bodyweight gain per bird (g) (S.E.)**	**FCR (S.E.)**
0% YPC inclusion	240.0 (44.15)[ab]	380.7 (51.70)[ab]	1.64 (0.124)
3% Potable YPC	286.5 (26.96)[ab]	417.2 (34.94)[ab]	1.46 (0.023)
6% Potable YPC	223.5 (19.45)[a]	346.8 (23.89)[a]	1.57 (0.126)
9% Potable YPC	202.5 (18.51)[a]	326.9 (23.56)[a]	1.63 (0.070)
3% Bioethanol YPC	328.4 (33.55)[b]	467.3 (33.35)[b]	1.45 (0.083)
6% Bioethanol YPC	330.4 (8.68)[b]	457.0 (6.06)[b]	1.39 (0.041)
9% Bioethanol YPC	265.2 (35.30)[ab]	427.8 (32.66)[ab]	1.68 (0.165)
p value	0.03	0.022	0.315

Variation Source	**p value**		
Yeast source	0.003	0.006	0.556
RoI (L)	0.144	0.053	0.126
Yeast source*RoI	0.608	0.525	0.469

(Letters denote statistical significance at p>0.05)

Table 5.9 shows that both bodyweight gain and feed intake were significantly increased in birds fed the bioethanol-derived yeast protein concentrate (YPC) compared to the potable alcohol-derived YPC and the control (0% YPC) diet. No significant difference in FCR was observed between the two YPC sources, although numerically the FCR increased with rate of YPC inclusion.

Viscosity

Table 5.10 shows that, whilst overall there was no significant effect (P=0.308) of dietary treatment on viscosity of the supernatant digesta, there is a trend suggesting that the potable YPC produces higher digesta viscosity than the potable derived YPC (p=0.073). Due to the increasing numerical viscosity with the higher inclusion levels, the data was considered for each YPC separately, but there was no significant effect of rate of rate of YPC inclusion with either YPC, due to the high individual bird variation.

Table 5.10: Viscosity of digesta supernatant of birds fed graded levels of potable and bioethanol YPC

Treatment	**Viscosity /cP (S.E.)**
0% YPC inclusion	4.84 (0.855)
3% Potable YPC	5.59 (1.262)
6% Potable YPC	7.01 (1.008)
9% Potable YPC	7.39 (0.977)
3% Bioethanol YPC	5.25 (0.615)
6% Bioethanol YPC	5.53 (0.458)
9% Bioethanol YPC	5.42 (0.750)
p value	0.308

Variation Source	**P value**
Yeast source	0.073
RoI (L)	0.451
Yeast source*RoI	0.61

5.3.2 BRC03: Performance of birds fed spray dried YPC with and without a washing step in the process

Dietary analysis

Table 5.11 shows the dietary analysis of the diets fed in BRC03. These diets were made in house to be iso-calorific. There were slight differences in diet protein content, relating to the differing protein level of the washed and unwashed YPC (see table 5.8)

Table 5.11: Dietary analysis of washed and unwashed YPC containing diets.

Diet	Treatment	DM g/kg	Ash g/kg	Protein g/kg	Fat g/kg	Energy g/kg
A	Control	867.94	58.29	208.68	23.70	18.25
B	5% P washed	902.38	57.11	195.50	43.24	18.90
C	10% P washed	896.02	54.05	184.85	43.15	18.89
D	20% P washed	896.81	52.25	171.80	33.38	18.96
E	5% B	889.40	60.64	199.11	42.41	19.05
F	10% B	899.79	51.06	205.04	36.09	19.14
G	20% B	898.12	49.93	213.59	48.87	19.15

Performance

Table 5.12 shows that the FCR significantly increased with rate of inclusion for both YPCs investigated ($p=0.044$). There were no significant differences between the washed and unwashed YPC for feed intake, bodyweight gain or FCR. However rate of inclusion of both YPCs significantly reduced bodyweight gain and feed intake. For both feed intake and bodyweight gain, there were no significant differences between the control (0% inclusion) diet and the 5% inclusion level of either YPC, whereas the 20% inclusion level significantly decreased FI and BWG compared with the control. For FCR, this translates into a significant higher FCR for the 10 and 20% inclusion levels of the washed YPC when compared to the control diet.

Table 5.12 Effect of rate of washed and unwashed YPC inclusion on mean bird performance from 0-21d post hatch

Treatment	Bodyweight gain per bird (g) (S.E.)	Feed intake per bird (g) (S.E.)	FCR (S.E.)
0% YPC	572.5 (23.85)[b]	955.2 (41.53)[c]	1.67 (0.057)[a]
5% YPC B	505.0 (26.76)[b]	903.83 (35.31)[c]	1.80 (0.054)[ab]
10% YPC B	507.8 (12.9)[b]	908.9 (20.09)[c]	1.79 (0.064)[ab]
20% YPC B	344.7 (10.12)[a]	679.4 (7.61)[a]	1.98 (0.074)[ab]
5% YPC P (washed)	549.8 (13.85)[b]	933.4 (12.78)[c]	1.70 (0.038)[a]
10% YPC P (washed)	444.4 (34.70)[b]	888.3 (51.36)[b]	2.06 (0.221)[b]
20% YPC P (washed)	311.8 (8.30)[a]	693.7 (52.32)[a]	2.22 (0.163)[b]
P value	<0.001	<0.001	0.034

Variation source	P value		
Yeast	0.615	0.577	0.188
RoI	<0.001	<0.001	0.044
Yeast*RoI	0.069	0.679	0.209

(Letters denote statistical significance at p>0.05)

The apparent metabolisable energy (AME) for each of the dietary treatments are detailed in Table 5.13.

Table 5.13: Apparent metabolisable energy content of diets with different inclusion levels of washed and unwashed Yeast Protein Concentrate.

Treatment	**Apparent Metabolisable Energy (MJ/kg) (S.E.)**
0% YPC	12.36 (0.306)
5% YPC B	13.51 (0.323)
10% YPC B	12.36 (0.447)
20% YPC B	11.71 (0.270)
5% YPC P (washed)	13.65 (0.646)
10% YPC P (washed)	12.55 (0.722)
20% YPC P (washed)	11.12 (0.854)
P value	0.288
Variation source	
Yeast	0.641
RoI	0.143
Yeast*RoI	0.219

The gross energy of the diets was consistent across all treatments in this study. There was a numerical decrease in AME with increasing inclusion of both washed and unwashed YPC, but this was not significant.

Viscosity

The viscosity of the digesta supernatant for each treatment is shown in Table 5.14. The control diet with 0% YPC produced digesta supernatant with significantly lower viscosity (p=0.034) compared with the 20% inclusion of the washed YPC. The washed bioethanol sourced YPC had the highest digesta supernatant viscosity of all the dietary treatments, but this was only significantly higher than the control diet and the 5% inclusion diets. Further analysis of the data by MANOVA, showed a significant increase in viscosity with rate of YPC inclusion (p=0.031), but showed no significant differences between YPC sources and the control diet.

Table 5.14 Viscosity of digesta supernatant of birds fed graded levels of washed and unwashed bioethanol sourced YPC

Treatment	**Viscosity / cP (S.E.)**
0% YPC	4.48 (0.389)[a]
5% YPC B	4.71 (0.237)[ab]
10% YPC B	6.21 (0.453)[bc]
20% YPC B	6.01 (0.671)[abc]
5% YPC P (washed)	4.69 (0.399)[ab]
10% YPC P (washed)	5.71 (0.827)[abc]
20% YPC P (washed)	6.938 (0.977)[c]
P value	0.034
Variation source	
Yeast	0.485
RoI	0.031
Yeast*RoI	0.141

5.3.3 BRC07: Performance of birds fed pelleted diets with ring dried potable YPC inclusion

Dietary analysis

Table 5.15 shows the dietary analysis of the diets fed in BRC07. These diets were made formulated to be as similar as possible in terms of nutrition, and were made and pelleted in a commercial facility. The control (0% inclusion) diet has less protein (1.7% less than the average of the other diets) and lower manganese levels compared to the test diets, which suggests that less vitamin/mineral premix was added. Other analyses were consistent across diets.

Table 5.15: Dietary analysis for BRC07 diets

Diet	DM g/kg	Protein content g/kg	Fat g/kg	Energy MJ/kg	Fibre g/kg	Manganese (mg/kg)	Ash (g/kg)
0% YPC	883	192	55.2	16.916	29	107	41.5
7.5% YPC	871	210	51.5	16.683	32	130	40.5
12.5% YPC	873	211	51.1	16.767	24	125	40.1
17.5% YPC	877	206	51.0	16.990	26	126	40.8

Performance

Feed intake was significantly lower for the 0% inclusion diet over both the initial 2 week trial period ($p=0.033$) and the full 3 week period, when compared with the other dietary treatments ($p>0.001$), as shown in table 5.16. There were no significant diffences in the intake of any of the YPC containing diets at any point in the trial. There was no significant difference in feed intake in week 1 between any of the diets.

Table 5.16: BRC07 Cumulative Feed intake for each diet weekly over the study period

Diet	Intake/bird D1 to D8 /g (S.E.)	Intake/bird D1 to D15 /g (S.E.)	Intake/bird D1 to D21 /g (S.E.)
0% YPC	157.9 (5.96)	479.2 (25.66) [a]	933.9 (44.16) [a]
7.5% YPC	143.8 (11.58)	550.1 (24.14) [b]	1164.1 (54.88) [b]
12.5% YPC	153.7 (11.16)	554.5 (10.62) [b]	1196.2 (36.54) [b]
17.5% YPC	149.0 (8.03)	538.2 (12.07) [b]	1155.2 (24.7) [b]
P value	0.744	0.033	<0.001

Table 5.17 shoes the bodyweight gains per bird for each week of the study for the 4 diets. Bodyweight gain (BWG) was significantly lower ($p<0.001$) in birds fed the 0% YPC diet compared with the YPC containing diets over the whole 3 week period of the trial. There was a numerical trend towards reduced BWG with increasing YPC inclusion, but this was not significant ($p=0.094$).

The bodyweight gain for the first week was significantly lower for the 0% inclusion diet. Over the initial 2 week period of the trial, the bodyweight gain for the 7.5% inclusion diet was also significantly greater than for the 17.5% diet ($p>0.001$).

Table 5.17: BRC07: Cumulative Body weight gains per bird weekly over the study period

Diet	BWG/bird D1 to D8 /g (S.E.)	BWG/bird D1 to D15 /g (S.E.)	BWG/bird D1 to D21 /g (S.E.)
0% YPC	79.1 (2.25) [a]	261.1 (9.91) [a]	629.9 (32.71) [a]
7.5% YPC	111.4 (5.09) [b]	389.4 (13.93) [c]	890.9 (23.73) [b]
12.5% YPC	104.9 (11.16) [b]	368.1 (10.96) [bc]	856.3 (21.18) [b]
17.5% YPC	102.9 (8.03) [b]	352.3 (6.36) [b]	829.7 (14.98) [b]
P value	<0.001	<0.001	<0.001

Feed conversion ratios are shown for the trial period, for each diet in table 5.18. All three YPC diets have significantly lower feed conversion ratios (FCR) over the full 3 week trial period, when compared to the 0% YPC diet ($p=0.002$). The diet containing 7.5% YPC inclusion had the lowest FCR numerically which was approaching significance when compared to the other

inclusion levels (p=0.053). This reduction in FCR was also recorded in the first week and the first 2 weeks of the study.

Table 5.18: BRC07: Feed conversion ratios for each diet weekly over the study period

Diet	FCR D1 to D8 /g (S.E.)	FCR D1 to D15 /g (S.E.)	FCR D1 to D21 /g (S.E.)
0% YPC	2.01 (0.097) [a]	1.84 (0.086) [a]	1.49 (0.042) [a]
7.5% YPC	1.28 (0.06) [b]	1.41 (0.034) [b]	1.30 (0.039) [b]
12.5% YPC	1.46 (0.084) [b]	1.51 (0.035) [b]	1.40 (0.019)[b]
17.5% YPC	1.45 (0.079) [b]	1.53 (0.039) [b]	1.39 (0.022) [b]
P value	<0.001	<0.001	0.002

Litter Dry Matter

Litter dry matter content at the end of the study is shown in Table 5.19. The 7.5% inclusion diet have significantly wetter litter than both the 0% YPC and the 17.5% YPC diets (p=0.016). Photographs were also taken of the litter quality at the end of week 1 and a comparison between the extremes of the dietary treatments (0% and 17.5%) is shown in Figure 5.1. It can be seen that the highest YPC inclusion diet led to more excreta production and a wetter, darker appearance to the pen litter.

Table 5.19: BRC07 litter dry matter

Treatment	Litter DM % (S.E.)
0% YPC	66.9 (2.87)[a]
7.5% YPC	46.7 (3.86)[b]
12.5% YPC	55.1 (5.28)[ab]
17.5% YPC	60.9 (4.02)[a]
p value	0.016

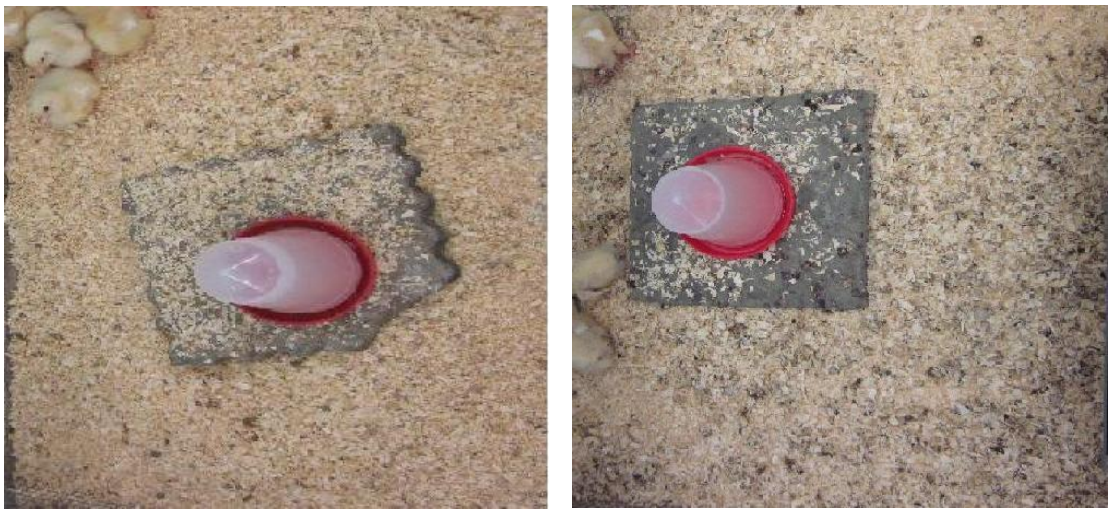

Figure 5.1: Litter quality in week 1. 0% YPC diet is shown on the left, 17.5% YPC diet on the right.

Uric acid was measured in the serum collected at the end of the study, and the levels are shown in Table 5.20. There were no significant differences in serum uric acid concentrations between birds fed any of the diets.

Table 5.20: BRC07 serum uric acid content

Treatment	**Uric acid mg/dl (S.E.)**
0% YPC	7.72 (0.463)
7.5% YPC	7.58 (0.362)
12.5% YPC	8.29 (0.587)
17.5% YPC	8.37 (0.482)
p value	0.569

5.4 DISCUSSION

There is a marked variation between nutritional content of YPCs produced from different sources, and particularly related to processing variables. The composition of the YPC is dependent on many variables; feedstock composition, processing conditions and yeast strain

for example. Additionally, the novel separation process adds a further level of variability as discussed in Chapter 3. It appears from the analysis of the YPCs used in this chapter that the washing process may reduce fat levels and have a concentrating effect on the protein, as found by Sharif *et al*. (2012). The increased fat content of the washed bioethanol YPC translates to a higher energy content, compared with all the other YPCs which were similarly calorific. The ring dried YPC also had a higher protein and lower fat content, which may be due to improved separation in the secondary stage as the operators became more familiar with the equipment and process. Differences in feedstock or fermentation process may account for some of the differences. The bioethanol sourced YPCs are likely to also have viscosity reducing enzymes as they are routinely added during processing, which will affect the drying of the YPC (Bamforth, 2009). The potable yeast used in BRC03 (washed) produced a blue coloured ash (see figure 5.2) suggesting a copper contamination, which is likely to be from the potable copper stills used for whisky production.This is important to note for future studies using potable product as copper is toxic to poultry in larger concentrations. An overall mineral analysis of the ash needs to be considered before use as a feed ingredient or additive.

Figure 5.2: Ashed potable spray dried yeast as fed in BRC03

There was also a distinct difference in texture of the different YPCs, with the spray dried YPCs being particularly fine and powdery.

5.4.1 Performance of broiler chicks fed graded levels of potable or bioethanol sourced YPC (BRC02)

The data collected from this study was compromised by the variation in nutritional content of the diets, particularly with respect to apparent metabolisable energy and mineral content. This appears to have been due to an error at the commercial manufacturing plant.

The significant improvement in feed intake and bodyweight gain of bioethanol YPC when compared with both the potable alcohol YPC and the control (0% inclusion) diet suggests that the bioethanol YPC may be improving the palatability of the diet, possibly due to the glutamic acid content of the yeast, which may have a flavour enhancing effect (Rose, 1987). Some studies have shown a negative palatability effect of yeasts fed in mash diets (Daghir and Sell, 1982; Succi *et al*., 1980), and at the highest inclusion level in this study there is a numerical reduction in intake, although this is not significant. The YPCs used in this study were freeze dried and so were free flowing powders which made the diets slightly dusty, and more difficult to manipulate into the beak, therefore increasing feeding time for the same quantity of diet (Amerah *et al*., 2007a). Schiffman (1968) reported that birds also showed a preference for larger particles. For rate of inclusion (RoI) there was a trend towards reduced bodyweight gain with increasing inclusion level of YPC ($p=0.053$) which suggests that there will be a limit to inclusion level, after which there will be a detrimental effect on bird performance. Previous studies have shown contrasting results for bird performance with some studies showing an improved performance at inclusion levels less than 6% (Miazzo *et al*., 2005; Ciurescu *et al*., 2001) and some showing a reduction in performance at increasing inclusion levels (Rameshwari and Karthikeyan, 2005).

It may be that at lower inclusion levels (below 6%) the yeast enhances performance, but this is negated at higher inclusion levels (9% and above) by the negative effects of anti-nutritional factors, either nucleic acids or NSPs. This suggests that the YPC may be more suitable for use at lower inclusion levels of less than 5% as opposed to as a sole protein source. With DDGS, a

maximum inclusion level of 15% has been suggested (Thacker and Widyaratne, 2007, Youssef *et al*., 2008) but this particular study indicates that a lower inclusion level for YPC may be appropriate.

Viscosity inducing components of the diet do not appear to be an issue in the bioethanol YPC in this study. This is likely to be due to the use of exogenous enzymes to reduce viscosity (Bamworth, 2009) which are widely used in the bioethanol industry to increase extract volume and improve handling during processing. However there was an increase in digesta supernatant viscosity with increased inclusion level of potable alcohol produced YPC, although this is not significant, due to high individual bird variation. Barley contains NSPs, particularly β glucans (Holtekjølen *et al*., 2006) which have a negative effect on digesta viscosity due to fractions solubilising post ingestion and cross linking forming a highly viscous gel (Chesson, 2001). The increased viscosity slows digestive passage time and provides a supportive environment for microbial fermentation (Choct and Kocher, 2000). Antoniou and Marquadt (1982) showed that antibiotic treatment improved fat absorption, further suggesting that intestinal microbial overgrowth is an issue in broilers.

Whisky distilleries, such as the one from which the potable YPC in this trial was derived, are constrained by legislation (Scotch Whisky Order, 1990) which prohibits the use of exogenous enzymes, or any other additives to improve the process. The observed increase in digesta supernatant viscosity may go some way to explain the reduced feed intake of the potable YPC compared with the bioethanol YPC, as the increase in gut transit time means the bird is physically incapable of eating larger quantities of feed (Fengler and Marquardt, 1988; Gutierrez-Alamo *et al*., 2008). However there seems to be no strong correlation (R^2 = 0.0703) between FCR and viscosity (see figure 5.3)

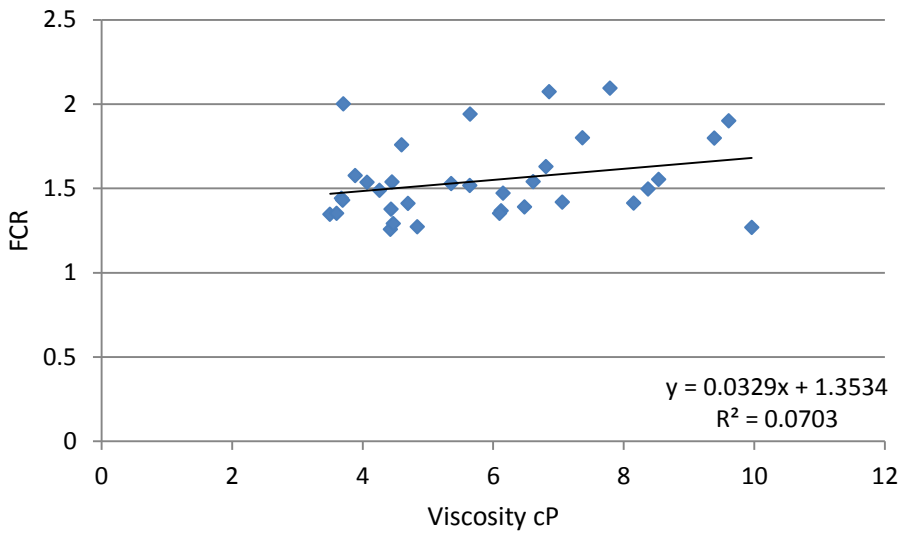

Figure 5.3 Correlation between FCR and Viscosity for BRC02

There is an increase in FCR at higher inclusion levels which may be due in part to the negative nutritional effects of nucleic acids in the yeast (Stone, 1998). Yeast has been shown to contain a high level of nucleic acids compared to other protein sources, and ingestion of these can lead to metabolic problems in poultry (Ciurescu *et al*., 2001). However the FCR is not significantly different in the YPC diets when compared with the control (0% inclusion).

YPC derived from bio-ethanol production appears to have a positive effect on feed intake and bodyweight gain at low inclusion levels (less than 6% total dietary content), during the first 2 weeks post hatch, when compared with potable alcohol produced YPC and a control diet. This could be due to the mannanoligosaccharide content of the yeast cell walls, which have been shown to have a positive effect on bird performance (Parks *et al*., 2001) and gut morphology (Morales-Lopez *et al*., 2009).Overall, this trial suggests there is a limit to inclusion level of YPC, beyond which performance would be adversely effected.

5.4.2 Performance of chicks fed graded levels of spray dried YPC, with and without a washing stage during processing

The YPCs used in this study were all spray dried, which may affect palatability as spray drying produces a fine, free flowing powder of less than 200µM (Niessen, 2002) which can be difficult for the bird to manipulate, thus increasing feeding time. Waldroup (1997) recommended that particles of less than 600µm should be avoided in poultry feed. Very fine mash diets can also cause hypertrophy of the small intestine (Nir *et al.*, 1994) and smaller gizzard size as the gizzard reverts to a transit organ due to lack of grinding required (Taylor and Jones, 2004; Nir and Ptichi, 2001). Advantages to spray drying are that the specification of the powder is constant, and it offers an easy, continuous drying which should reduce structural damage (Vega Mercado *et al*., 2001). Mash diets are not usually fed to broilers commercially, as it is generally accepted that high quality pellets give better performance than lower quality pellets or mash (Amerah *et al*., 2011). Mash diets also increase feed wastage and spillage, especially with very fine diet, where the particles are easily displaced by the birds. This is particularly an issue in small birds which may dust bathe in the feed troughs and produce clouds of fine particles which cannot be recorded as feed spillage.

Washing the YPCs with water may reduce the anti-nutritional factors present and improve processing, as the soluble NSPs can dissolve in the water and therefore be removed by the second separation step. Indeed it was observed during the drying process that the washed yeast cream was easier to dry, and resulted in less blocking of the machinery, suggesting viscous compounds may have been removed. Additionally, the unwashed yeast cream had a tendency to adhere to the sides of the spray dryer and needed to be manually cleared repeatedly (Mason's Chemicals, personal communication). This may have led to parts of the YPC burning so the material produced may lack consistency and the nutritional value may be compromised. However historically, work on cereals found washing did not remove all anti-nutritional factors (Antoniou and Marquadt, 1982).

Despite the improvements seen in processing, overall FCR significantly deteriorated with rate of inclusion, for both YPCs. However the FCRs for the unwashed bioethanol YPC diets were not significantly different to the control diets at any inclusion level, but the FCR was significantly worsened in the washed diet at inclusion levels of 10% and above. Univariate analysis showed no significant differences between the YPC sources for any performance measure. Both feed intake and bodyweight gain were reduced with increasing YPC inclusion ($p<0.001$), with the 20% inclusion level significantly lower than both the control diet and the lower inclusion levels for both YPCs. This suggests a limitation to the inclusion level of this product. Recent studies on washed distillery sludge found that lower inclusion levels (8%) had a positive effect on feed intake and bodyweight gain which was not seen here (Sharif *et al*., 2012).This could be due to a number of factors. Knott and Shurson (2004) showed that the residual solubles fraction from the separation method they used had a positive effect on gut morphology, so it may be that some nutrients are being removed during the CC process. It may also be due in part to the higher proportion of nucleic acids present in yeast (Stone, 1998) which may cause an increase in uric acid at a level that has negative metabolic effects (Baker and Molitoris, 1974). Palatability could also be an issue (Daghir and Sell, 1982; Succi *et al*., 1980), which may be due in part to the small particle size of the spray dried YPC. The small particle size may make the diet difficult to handle and increase feed wastage: several studies have shown a reduction on feed intake and bodyweight gain with fine textured feed at 21 days of age (Nir *et al*., 1994; Amerah *et al*., 2007a). This may be because the finer particles reduce gizzard development and therefore digestive transit control, decreasing gut transit and feed intake (Amerah *et al*., 2007b).

The gross energy values of all the diets (manufactured in house and designed to be iso-energetic) were consistent in this study (mean 18.9 +/- 0.117). Bioethanol YPC had higher gross energy than the washed potable YPC, which may be due to feedstock composition or processing differences, but this did not affect the overall dietary energy content. There was no

significant effect of RoI or YPC source on AME, indicating no adverse effect of either YPC on energy utilisation. Figure 5.4 shows a correlation between AME and viscosity, which shows no specific relationship (R^2 = 0.0716), however AME does decrease slightly with increasing viscosity.

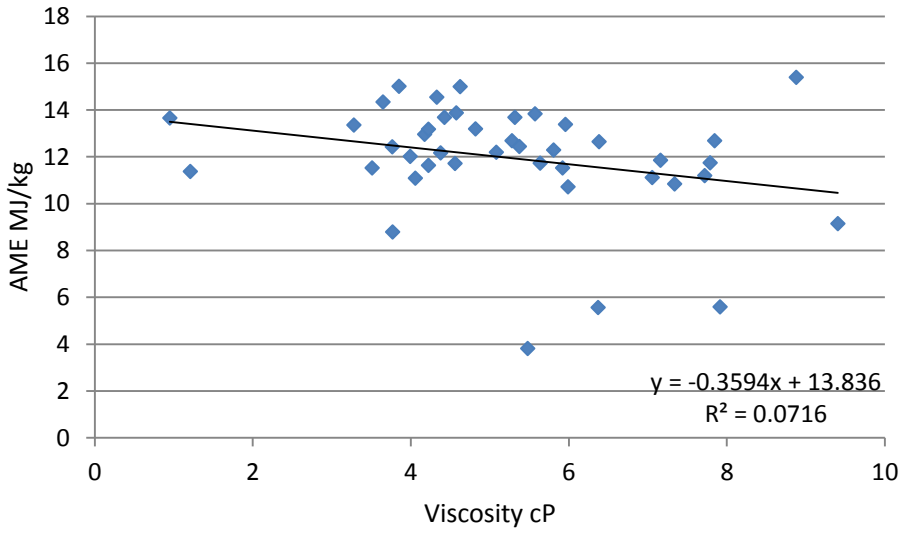

Figure 5.4: Correlation between AME and viscosity for BRC03

Despite the lack of effect on AME, viscosity was elevated at higher inclusion levels of YPC (p=0.031), with the highest viscosity measured in the washed 20% potable YPC diet. This higher viscosity suggests the presence of deleterious non-starch polysaccharides in the YPC. It is notable that the 20% potable diet has still produced a lower viscosity than that seen with the 9% diet in BRC02 which suggests that washing is having some effect on non-starch polysaccharide concentration. Interestingly, there was no significant difference between YPC sources, as it would be expected that bioethanol YPC would have had viscosity reducing enzymes added during the mashing stage. However, although there is an effect of RoI, the viscosity of the bioethanol YPC reduced at the 20% inclusion levels, which does not support the theory that the viscosity increase is due to non-starch polysaccharide content in the YPC. This effect could be due to the particle size of the material. Small particle size (less than 0.5mm) can increase digesta viscosity, which may be due to increased NSP release or greater

degradation of the small particles (Yasar, 2003). Spray dried material produces a very fine material between 500µm and 10µM (Vega Mercado *et al.*, 2001) which is certainly fine enough to produce this increase in viscosity. Figure 5.5 shows that as viscosity increases, so does FCR, but there is a lot of bird to bird variation so the relationship is not

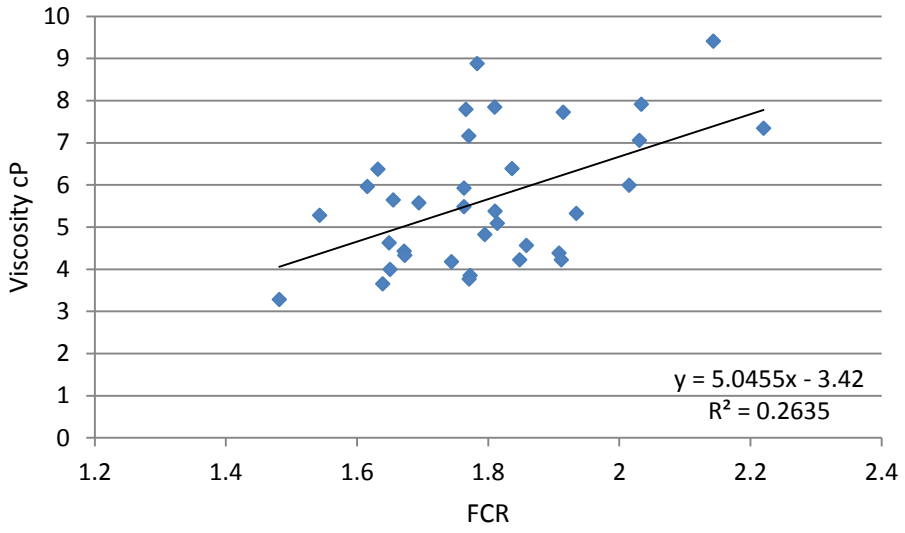

Figure 5.5: Correlation between FCR and viscosity for BRC03

Washing the YPC twice with water is wasteful in terms of both water and energy usage. With performance limitations and the ready availability of exogenous enzymes to control the effect of NSPs, this study has not shown washing to be a productive addition to the process.

5.4.3 Performance of chicks fed pelleted diets containing graded levels of ring dried potable YPC

The YPC used in this study was derived from a potable source and ring dried. The diets were pelleted to remove any issues with particle size and the negative palatability issue of yeast products sometimes seen in mash diets (Daghir and Sell, 1982; Succi and Pialorsi, 1980). Pelleting feed with a yeast component has been shown to increase intake (Daghir and Sell, 1982).

Throughout the study the 0% YPC diet (control) was noted to be less palatable than the other diets. In house comparisons show that normal control mash diets at NTU can produce an FCR at 28 days between 1.39 and 1.59, and the control diet in this study falls within this range. However it is generally accepted that high quality pellets give better performance than mash diets (Loar and Corzo, 2011). This is due to reduced time spent feeding, and ease of manipulation into the beak by the bird, it would be expected that the pelleted control diet would produce a lower FCR than those measured in mash diets. The FCR is also lower than the Aviagen performance objectives for male Ross 308 growth, which suggest an FCR of 1.328 at 22 days of age (Aviagen, 2007). This implies that the control diet in this trial did not optimally meet the nutritional requirements of the birds. The diet was designed in line with curretn commercial guidelines suggesting that it may have been manufactured incorrectly. However initial commercial dietary analysis shows no major issues apart from a small protein reduction (20.6% to- 21.1% in the test diets compared with 19.6% in the control diet) and lower manganese levels in the control diet compared to the test diets, which suggests that less vitamin/mineral premix was added. The small protein reduction is unlikely to be sufficient to have caused the reduction in performance but it may be that the control diet was sub-clinically deficient for some vitamins or minerals and this has effected the feed intake and growth. It was also noted that when the diets were ashed, the 0% YPC diet ash had a blue tinge (see figure 5.3) which suggests a higher than usual copper inclusion in the diet. As feed mills used to produce poultry diets also produce pig diets, this may be a carry over from a batch of copper supplemented diet, which could potentially be toxic to the birds.

Figure 5.3 Ashed control (0%) diet from BRC07 showing slight blue colour.

Intake was lower for the control diet ($p<0.001$), but there was no significant diffence in the intake of the YPC containing diets. This suggests that there is no negative palatability effect from including up to 17.5% of ring dried, potable YPC in pelleted broiler diets. However the possible problems with the incorrect manufacture of the control diet limit the veritability of the performance data of this study.

Similarly, bodyweight gain (BWG) was significantly lower ($p<0.001$) in birds fed the 0% YPC (control) diet compared with the YPC containing diets. There was a numerical trend towards reduced BWG with increasing YPC inclusion, but this was not significant ($p=0.094$). This indication of reduced BWG with higher yeast inclusion could be due to the high proportion of nucleic acid nitrogen present in yeast, which may be causing sub-clinical uric acid buildup. Alternatively it could be due to the presence of deleterious carbohydrates, which increase digesta viscosity and subsequent risk of sticky droppings and wet litter. Viscosity was not measured specifically in this study, but litter moisture was quantified as a estimate of the effect of non-starch polysaccharides. The presence of NSPs are well documented to reduce nutrient digestilbility by increasing the unstirred layer in the digesta and increasing gut transit time (Austin *et al*., 1999; Chesson, 2001)

All three YPC diets have significantly improved feed conversion ratios (FCR) when compared to the 0% YPC diet ($p=0.002$), once again probably due to issues with the control diet. The diet containing 7.5% YPC inclusion had the lowest FCR numerically which was approaching

significance when compared to the other inclusion levels (p=0.053). Other authors have also reported positive effects on performance at low yeast inclusion levels, less than 2% (Miazzo *et al*., 2005; Silva *et al*., 2009). This may be due in part to the performance enhancing effects of yeast cell wall components (Parks *et al*., 2001; Moralez-Lopez *et al*., 2009). However it is impossible to pronounce a performance enhancing effect of low level (7.5%) YPC inclusion from this study, due to the questionable control diet data. All the performance data suggests a trend towards lower performance with the highest inclusion level of YPC. Although this is not significant at 17.5%, there is evidence to suggest that higher levels may have a detrimental effect on performance.

Yeast contains a high proportion of nucleic acid nitrogen, around seven times the amount present in other protein sources (Stone, 1998). The nucleic acids increase production of uric acid, which can build up in the joints of the birds with negative consequences for bird health (Peterson *et al*., 1971). However, birds are able to tolerate quite high levels (up to 25mg/dl) as the uric acid is converted into a soluble compound by binding to a protein in the kidney nephron. (Braun and Dantzler, 1997). No significant differences were seen in the serum uric acid concentration between any of the diets. This suggests that uric acid build up is not detrimental for diets containing up to 17.5% ring dried potable YPC as a protein source. Numerically the increasing levels of YPC inclusion showed an increase in serum uric acid content, which again suggests a possible limit to the inclusion level. However the levels from the 17.5% inclusion diet were similar to those measured by Mcfarland *et al*. (1979), who found a range of serum uric acid of 4.94-9.69mg/dl compared this study where the levels reached 8.37, which suggests that at all YPC inclusion levels, uric acid in serum was not elevated. Hellstein *et al*. (1997) proposed uric acid as a potent scavenger of oxidants. Uric acid is more important in birds as they produce more oxidants than mammals and uric acid has been found to be highly correlated with antioxidant capacity in chicks (Cohen *et al*., 2007). Simoyi *et al*. (2002) found that reducing these oxidants improves meat quality and improves bird health,

and found a correlation between oxidant activity and plasma uric acid levels (up to 16.8mg/dl). It may be that the increase in uric acid from the YPC ingestion may decrease oxidant damage therefore improving bird health.

The litter dry matter was measured as there was noticably wet litter in the pens of the birds fed the YPC containing diets at the beginning of the study. These loose droppings suggest the presence of deleterious carbohydrates and would potentially be a welfare and production issue, causing pododermititis and hock burn (Martland, 1984). Litter dry matter was significantly lower for the diet with 7.5% YPC, when compared to both the control diet and the 17.5% inclusion level diet ($p=0.016$). However this increase in litter moisture is unlikely to be due solely to the inclusion of the YPC as then it would be expected that the moisture would only be increased in the higher inclusion diets and not in the control diet. It is probable that the increased loose droppings were an initial effect as the gut adapted to the presence of the YPC protein, and also in part due to the increased feed intake in the pens fed the 7.5% inclusion diet leading to increased excreta output. It has been shown that age of the bird has implications for the effect of non-starch polysaccharides, with the sensitivity to pentosans reducing with increasing bird age (Pettersson and Aman, 1988). It may be that the observed wet litter at the start of the study was due to NSPs in the YPC, but by the time the litter was sampled on day 22, the birds had adapted to the diet. This may be due to maturation of the digestive system or increased indigenous microbial population (Campbell and Bedford, 1992).

5.5 CONCLUSION

There are some conflicting performance results from the different YPCs characterised in these studies. Bioethanol produced YPC appears to be preferable in terms of performance compared to potable YPC, which is likely due to the deleterious presence of non-starch polysaccharides, which have been treated in the former with exogenous enzymes. In terms of production parameters, washing does not appear to have any positive effect on digesta viscosity. Therefore, with cost implications of the longer process and water consumption, washing does not appear to be a valuable addition to the process. Drying methods had a large effect on particle size of the final product, with finer particles appearing to have a negative effect on palatability and digesta viscosity. Uric acid content was not shown to be significantly different at levels up to 17.5% inclusion.

These results imply that YPC can be used with no detrimental effect on performance at levels up to 12.5% inclusion in pelleted diets. Higher levels have a trend towards lower FCR (feed intake and BWG), which suggests that there is a ceiling on inclusion level for optimum bird performance.

CHAPTER 6: CAN YEAST PROTEIN CONCENTRATE (YPC) BE USED AS AN AVAILABLE SOURCE OF PHOSPHORUS?

6.1 INTRODUCTION

This chapter investigates whether yeast protein concentrate (YPC) separated from either potable or bioethanol refineries contains substantial amounts of phosphorus, and whether this phosphorus is bio-available and therefore improves bone mineralisation in broiler chicks. As foot ash was used to quantify the bone mineralisation, a study was also carried out to validate this technique in birds up to 6 weeks of age.

The genetic selection of broilers for rapid growth has contributed in part to an increase in bone abnormalities. The most significant form of loss to the poultry industry in the EU is from leg abnormalities in the birds. These disorders and lameness can lead to mobility problems and then to starvation and dehydration as the birds are unable to move around to access feed and water. Leg problems can cause high rates of mortality, morbidity and condemnation at processing plants and these can reach 30% (Pines *et al*., 2005) leading to heavy economic losses. Data from 1996 suggests that losses could be more than €112 million per annum (Bennett *et al*., 1999). A recent study in Denmark, using meat birds of a type widespread in Europe, reported that 30% of birds sampled have impaired walking abilities (Sanotra *et al*., 2002).Typically broiler diets are supplemented with calcium and phosphorus to reduce problems with skeletal calcification and abnormal bone development. These two minerals are the major contributor to the mineral matrix in bone and improve bone strength by creating a matrix with the collagen fibres. However phosphorus is expensive and in limited supply globally and therefore the available phosphorus in diets needs to be quantified. Some phosphorus is bound as phytate and therefore unavailable to monogastrics as they lack endogenous phytase (Jacques, 2003). Unavailable phosphorus in feed can lead to increased

concentration of phosphorus in excreta, increasing soil phosphorus levels (Daniel *et al*., 1998). The leaching of phosphorus from soil is an environmental problem as it is known to cause eutrophication of water sources (Correll, 1998).

Wheat DDGS has been shown to contain more phosphorus than both maize DDGS (Ortin and Yu, 2009) and wheat (Nyachoti *et al*., 2005), which may be in part due to the yeast component, as yeast contains substantial phosphorus (Sell *et al*., 1981). It may also be due to the destruction of phytate during the bioethanol liquefaction process by endogenous yeast phytase (Cottril *et al*., 2007; Liu and Han, 2011). It has been suggested that the phosphorus is less biologically available from wheat DDGS compared with maize DDGS (Thacker and Widyaratne, 2007; McDonnell *et al*., 2011), and that it may contain large water soluble component (Widyaratyne and Zijlstra, 2007; Leytem *et al*., 2008).

Available phosphorus content is often measured indirectly using bone mineralisation. There are several methods which are routinely used to quantify bone mineralisation based around the comparison of ashed bone to dry bone weight. Commonly, toe or tibia ash are used, with tibia ash the most commonly used criteria for assessment of commercial calcium and phosphorus content (Skinner and Waldroup, 1995). Tibia ash is time consuming, both in terms of collection of material and preparation (Orban *et al*., 1993) and differences in methodology can affect results (Hall *et al*., 2003). Toe ash has been shown to have a linear relationship with tibia ash (Yoshida and Hoshi, 1983) and may be more sensitive to dietary changes (Ravindran *et al*.,1995). However toe ash allows a certain amount of subjectivity when collecting the material, and the sample size can be so small that any errors are disproportionately large (Mendez and Dale, 1998).

Foot ash has been shown to give comparable results to toe and tibia ash up to two weeks of age (Mendez and Dale, 1998; Yan *et al*., 2005), and has been shown to reflect dietary phosphorus levels (Garcia and Dale, 2006). The advantages of foot ash as a measure of bone mineralisation are speed of sample collection and reproducibility due to larger sample weight

(Dale and Garcia, 2004), but this method has not been validated for use in birds over 14 days old.

As birds age, more of the small bones may become fully mineralised and larger bones, such as femurs, may allow measures to be more representative at older bird ages. Several studies have suggested that the femur is more representative of total skeletal mineralisation (Itoh and Hatano, 1964; Dilworth and Day, 1965), and Moran and Todd (1994) suggested that it may be more sensitive to dietary changes. Femur strength may also be important for processing related issues (Thorp, 1992).

In this validation study (PVT01), tibia, toe, foot and femur ash were compared in low and high phosphorus and calcium diets over a six week period to establish the most consistent and appropriate methodology for measuring bone mineralisation in birds of different ages.

Findings from three separate studies are detailed in this chapter. In addition to the validation study there are two studies to consider YPC as a source of available phosphorus. These other studies have been reported in detail in chapter 5; this chapter describes only the bone mineralisation data from those studies in order to relate the findings to available phosphorus content of the YPC.

6.2 METHODS

Three studies are discussed in this chapter. The first is the validation study PVT01, which compares methodology for the measurement of bone mineralisation. The other studies have previously been described in detail in Chapter 5 but additional samples were taken to quantify bone mineralisation. BRC03 compared spray dried potable and bioethanol YPC products with and without a washing step in the process. BRC07 compared graded levels of ring dried potable YPC in pelleted diets, where the YPC was considered to contribute to the available phosphorus and the supplemental phosphorus reduced accordingly. All husbandry and lab techniques are described in detail in chapter 2.

6.2.1 Bone mineralisation methodology validation study PVT01

This study compares the bone mineralisation of broiler chicks up to 42 days of age, fed 2 diets with differing phosphorus and calcium content. Bone mineralisation was quantified using four different leg bones; the more commonly used toe and tibia compared with foot and femur ash.

Study diets

Diets were formulated and produced by a commercial feed manufacturer using the diet formulations shown in table 6.1. Diets were formulated to be as nutritionally similar as possible, with the exception of phosphorus and calcium content. The Low diet was formulated at a low but adequate level of phosphorus and calcium, whereas the High diet contained double those inclusion levels, but both are within normal ranges. These levels were selected in an attempt to produce a measureable difference in bone mineralisation. Both feeds had common ingredients and levels of other nutrients were formulated to conform to NRC (1984) recommendations (Table 6.1)

Table 6.1 Composition of low and high phosphorus and calcium broiler feeds for PVT01

Ingredient	High Diet (g/kg)	Low Diet (g/kg)
Wheat[#]	663.5	677.5
Hipro Soya[#]	250	250
Lysine	3	3
Methionine	2.5	2.5
Soya oil	40	40
Limestone	20	10
Monocalcium Phosphate	8	4
Sodium Chloride	2.5	2.5
Sodium Bicarbonate	1.5	1.5
Vitamin E:Biotin (75:125)	1	1
Vitamin Mineral Premix*	2.5	2.5
Choline chloride	0.5	0.5
Titanium dioxide	5	5

*see appendix 1[#]see appendix 2

Study Protocol

72 male Ross 308 chicks (PD Hook, Cote, Oxon) were fed one of two wheat/soya based mash diets from day 1 to day 42. The experimental diets were each fed *ad libitum* to 6 pens containing 6 birds. The lighting regimen used was 24 hours light on day 1, with darkness increasing by an hour a day until 6 hours of darkness was reached, and which was maintained throughout the remainder of the study.

One bird per pen was euthanized weekly by cervical dislocation (6 birds per dietary treatment per week). Both feet were removed from each bird at the tibial-tarsal joint, prior to dissection of tibia and femur bones from both legs. The middle toe of each foot was removed at the joint between the second and third toe bones. All bones were immediately frozen individually at -20^{o}C until analysis. The bones removed are all shown in figure 6.1.

Toes and feet were dried at 105^{o}C for 5 days (until constant weight) in a forced air oven prior to ashing at 650^{o}C for 13 hours. Weights of dried and ashed toe were added back to the corresponding foot to calculate whole foot ash for each foot.

Tibia and femur bones were prepared using the method used by Bolin-Frankenbach *et al*. (2001). Individual legs were foil wrapped and autoclaved at 121^{o}C for 15 minutes, and the flesh, including cartilage caps, carefully removed by hand. The stripped bones were then oven dried for a minimum of 3 days (until constant weight) at 105^{o}C prior to ashing at 650^{o}C for 13 hours. The ash weight of each bone (or foot/toe) was expressed as a percentage of dry weight, referred to throughout as percentage ash.

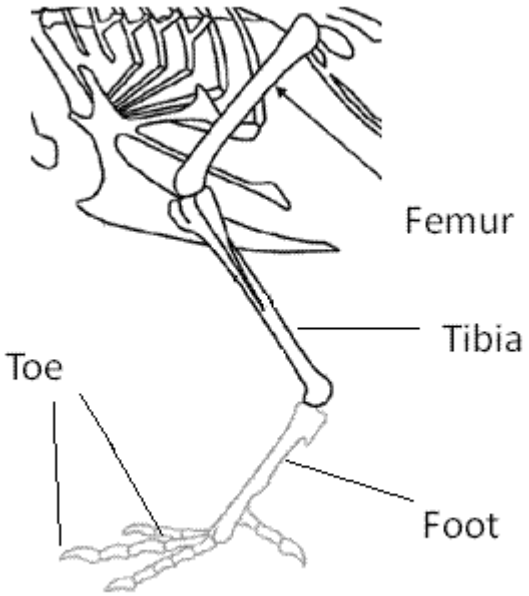

Figure 6.1 Diagram of a chicken leg, showing detail of the collected bones (Wikimedia.com).

Data Analysis

After Kolmogorov Smirnoff (KS) testing to confirm normality, statistical analysis was carried out using paired t tests to compare high and low dietary treatments for each bone type at each age. The statistical package used was SPSS v.19 (IBM statistics, 2012). Coefficients of variation for each method were calculated to give a measure of precision and reproducability.

6.2.2 Bone mineralisation in chicks fed graded levels of washed and unwashed yeast protein concentrates (BRC03)

As part of study BRC03, previously described in chapter 5, bone mineralisation data was collected to assess the effect of dietary YPC inclusion. Details of the study carried out, including diet formulations are recorded in full in Chapter 5. Briefly, 168 male Ross 308 chicks were fed one of seven wheat/soya bean meal-based mash diets from 0 to 21 days of age. The diets contained 0, 5, 10 or 20% of two yeast protein concentrates, one which had been washed

with water twice prior to spray drying. YPC details are shown in table 6.2. Each experimental diet was fed to 6 pens, each containing 4 birds. One pen was considered to be one replicate.

Table 6.2: Yeast protein concentrates assessed in BRC03

YPC source	Parent grain	Drying procedure	Other treatment
Potable	Barley	spray dried	washed
Bioethanol	Wheat	spray dried	--

On Day 21, the percentage foot ash was determined using the method described by Garcia and Dale (2006). Briefly, the total foot was removed at the tibial-tarsal joint and dried for 5 days at 105°C, before re-weighing and ashing at 650°C for 14 hours. Percentage ash was calculated as the ash weight as a percentage of dry weight. All 4 chicks in a pen were analysed individually using the left foot of each chick.

Bone ash was finely ground after weighing and pooled into one pot per pen. The phosphorus and calcium content of the bone was then measured using ICP-OES following an aqua regia digestion step, as detailed in Chapter 2.

Statistical analysis was carried out after KS testing to confirm normality, using one way ANOVA with Duncan post hoc testing to compare the effect of dietary treatment on percent foot ash, and calcium and phosphorus content of the bone ash. Further analysis if appropriate, was carried out using MANOVA, again with Duncan post hoc tests to examine the effects of yeast source and rate of dietary inclusion on percentage foot ash. The statistical package used was SPSS v.19 (IBM statistics, 2012).

6.2.3 Bone mineralisation of chicks fed yeast protein concentrate as a source of phosphorus (BRC07)

Full details of the study BRC07, including diet formulations are included in chapter 5. Briefly, 192 day old male Ross 308 chicks were fed one of four wheat/soya based diets containing 0,

7.5, 12.5 or 17.5% of a potable derived YPC which had been ring dried. Diets were presented as 2-3mm pellets. 12 pens of 4 birds were randomly allocated to each dietary treatment, with one pen regarded as one replicate. Supplemental phosphorus was reduced with increasing YPC inclusion to allow for the estimated available phosphorus present in the YPC based on existing knowledge of yeast. Table 6.3 shows the di-calcium phosphate and YPC inclusion levels of each dietary treatment. Limestone was increased slightly with YPC inclusion level to account for the effect of reducing di-calcium phosphate on the available calcium.

Table 6.3: Dietary yeast protein concentrate (YPC) and di-calcium phosphate(DCP) inclusion levels in study BRC07

Diet	YPC inclusion g/kg	DCP inclusion g/kg	% DCP reduction
0% YPC	0	14	-
7.5% YPC	75	12	-14.3
12.5% YPC	125	10	-28.6
17.5% YPC	175	9	-35.7

On Day 22, the left foot of each bird was removed at the tibia-tarsal joint for measurement of percentage foot ash. The feet for all 4 chicks in a pen were analysed individually. Statistical analysis was carried after KS testing to confirm normality, using one way ANOVA with Duncan post hoc testing to compare the effect of dietary treatment on percent foot ash. The statistical package used was SPSS v.19 (IBM statistics, 2012).

6.3 RESULTS

6.3.1 Bone mineralisation methodology validation study (PVT01) results

Table 6.4 shows the comparison between the formulated values for the test diets and the measured values analysed in house. The analysed values closely reflect those the diets were formulated to, with some variation in both phosphorus and calcium content. However the

diets provide substantial differences in mineral content to enable differences in bone mineralisation to be measured.

Table 6.4: Dietary analysis of high and low diets from the validation study (PVT01)

	HIGH Formulated values	**HIGH Analysed values**	**LOW Formulated values**	**LOW Analysed values**
Gross Energy Content (MJ/kg)	19.5	19.3	19.5	19.9
Phosphorus Content (g/kg)	8	7.77	4	4.44
Calcium Content (g/kg)	20	22.68	10	13.06
Dry Matter Content (g/kg)	--	872.53	--	872.73
Protein Content (g/kg)	200	208	200	204.4
Ca:P ratio	2.50	2.92	2.50	2.94

Table 6.5 shows the percentage bone ash per week for each of the six weeks of the study for both diets. The results are separated into toe, foot, tibia and femur ash results for each diet on each week of the study. The foot and toe ash have substantially lower percentage ash values, due to the inclusion of skin and tissue in the dry weight.

Table 6.5: Percentage bone ash for each method for both dietary treatments, for weeks 1 through 6 of study PVT01

		Toe		Foot		Tibia		Femur	
Week	**Diet**	**Ash (%)**	**S.E.**	**Ash (%)**	**S.E.**	**Ash (%)**	**S.E.**	**Ash (%)**	**S.E.**
1	**Low**	10.04	0.851	12.93	0.161	35.97	0.525	36.32	0.868
1	**High**	10.08	0.805	12.99	0.295	37.26	0.620	37.12	0.499
2	**Low**	9.66	0.327	12.30	0.155	38.89	0.453	39.03	0.857
2	**High**	10.82	0.163	13.50	0.184	41.63	0.589	40.68	0.771
3	**Low**	10.01	0.273	13.22	0.142	42.95	0.469	42.93	0.991
3	**High**	11.10	0.322	14.97	0.252	45.42	0.489	45.34	1.377
4	**Low**	11.46	0.339	13.76	0.179	41.07	0.596	41.70	0.587
4	**High**	12.22	0.360	15.40	0.319	44.22	0.295	44.56	0.645
5	**Low**	11.59	0.337	13.84	0.281	40.58	0.666	40.33	0.424
5	**High**	12.09	0.363	15.09	0.218	43.62	0.367	44.43	0.980
6	**Low**	10.59	0.310	14.47	0.317	43.57	0.741	41.50	0.649
6	**High**	10.88	0.232	15.48	0.251	47.83	0.985	45.06	0.355

Table 6.6 shows the results of the statistical tests to compare the high and low diets. Each bone type (toe, foot, tibia and femur) were considered individually for each week of the study, to monitor which bones were showing a statistical difference between the two diets. Different levels of significance are denoted by the shaded boxes.

Table 6.6: Paired t test p values from PVT01 comparing low and high dietary treatments, for each methodology for weeks 1 through 6.

Week	Toe	Foot	Tibia	Femur
1	0.954	0.889	0.169	0.542
2	0.012	<0.001	0.003	0.271
3	0.039	<0.001	0.001	0.175
4	0.203	0.001	0.002	0.021
5	0.257	0.002	0.005	0.001
6	0.449	0.05	0.017	<0.001

p<0.05
p<0.01
p<0.001

Coefficients of variation for each week, for each diet are shown in table 6.7 for toe, foot, tibia and femur ash. These show that the toe ash measurements are highly variable, particularly in young chicks. This is likely due to variation in harvesting technique as the low weight of the toe makes small variations in precision more apparent. Foot ash appears to be comparable with tibia and femur ash for reproducibility.

Table 6.7: Coefficients of variation for each bone ash measure for PVT01

Week	Diet	Toe	Foot	Tibia	Femur
1	Low	29.37	4.29	5.06	8.28
1	High	27.65	7.87	5.77	4.60
2	Low	11.73	4.37	4.03	7.61
2	High	5.21	4.72	4.90	6.56
3	Low	9.45	3.71	3.78	8.00
3	High	10.05	5.82	3.73	10.52
4	Low	10.25	5.51	5.02	4.87
4	High	10.22	7.17	2.31	5.01
5	Low	10.07	7.04	5.68	3.64
5	High	10.40	5.00	2.92	7.64
6	Low	10.15	7.6	5.88	5.42
6	High	7.40	5.62	7.13	2.73
Mean		12.66	5.73	4.68	6.24

6.3.2 Bone mineralisation of chicks fed graded levels of washed and unwashed yeast protein concentrates (BRC03)

Table 6.8 shows the percentage foot ash for each dietary inclusion level of the washed (potable)and unwashed (bioethanol) yeast protein concentrates (YPCs). Foot ash is significantly higher for the 20% inclusion level of both YPCs, compared to the lower inclusion levels. This was confirmed by further analysis by MANOVA (with Duncan post hoc tests) which showed a significant effect of rate of YPC inclusion (RoI; p=0.001), but no significant effect of YPC source.

Table 6.8: Percentage foot ash for each yeast protein concentrate (YPC) inclusion level in study BRC03

Diet	**Foot ash / % (S.E.)**
0% YPC	17.36 (0.135)[ab]
5% YPC B (Bioethanol)	17.55 (0.509)[abc]
10% YPC B (Bioethanol)	17.17 (0.397)[a]
20% YPC B (Bioethanol)	18.47 (0.643)[c]
5% YPC P (Potable washed)	16.87 (0.287)[a]
10% YPC P (Potable washed)	17.52 (.343)[abc]
20% YPC P (Potable washed)	18.066 (0.572)[bc]
p value	0.003
Variation source	
YPC	0.485
RoI	0.031
YPC*RoI	0.141

Table 6.9 shows the phosphorus and calcium content as a percentage of the foot ash for each dietary inclusion level.

Table 6.9: BRC03 - Bone ash calcium and phosphorus content for each YPC inclusion level as a percentage of foot ash weight.

Treatment	% Bone Calcium (S.E.)	% Bone Phosphorus (S.E.)
0% YPC	40.37 (0.991)	17.91 (0.493)
5% YPC B (Bioethanol)	39.69 (1.470)	17.65 (0.779)
10% YPC B (Bioethanol)	41.60 (0.854)	18.60 (0.523)
20% YPC B (Bioethanol)	39.29 (1.186)	17.85 (0.948)
5% YPC P (Potable washed)	41.07 (0.702)	18.30 (0.321)
10% YPC P (Potable washed)	40.05 (1.316)	17.54 (0.577)
20% YPC P (Potable washed)	39.97 (1.060)	17.58 (0.559)
p value	0.788	0.876

Table 6.10 shows the percentage phosphorus and calcium content as a proportion of the dry foot weight and the respective phosphorus to calcium ratio in the foot for each dietary treatment.

Table 6.10: BRC03 - Phosphorus and calcium content as a percentage of foot dry weight (wt.) and Ca:P ratio of the bone for all diets

Treatment	% dry wt. foot Ca (S.E.)	% dry wt. foot P (S.E.)	dry wt. Ca:P (S.E.)
0% YPC	7.01 (0.172)	3.11 (0.086)	2.26 (0.014)
5% YPC B	6.81 (0.252)	3.03 (0.134)	2.25 (0.022)
10% YPC B	7.14 (0.147)	3.19 (0.089)	2.24 (0.027)
20% YPC B	7.26 (0.219)	3.29 (0.175)	2.21 (0.047)
5% YPC P (washed)	6.93 (0.118)	3.09 (0.054)	2.24 (0.018)
10% YPC P (washed)	7.01 (0.231)	3.07 (0.101)	2.28 (0.018)
20% YPC P (washed)	7.22 (0.191)	3.18 (0.101)	2.28 (0.018)
p value	0.660	0.680	0.550

No significant differences were seen between any of the dietary treatments for bone phosphorus or calcium content, whether as a proportion of ash weight or dry foot weight. There were also no significant differences in the bone calcium to phosphorus ratio.

6.3.3 Bone mineralisation of chicks fed yeast protein concentrate as a source of phosphorus

The percentage foot ash for each diet is shown in table 6.11. There were no significant differences in foot ash between any of the YPC inclusion levels and the control diet (p=0.195).

Table 6.11: BRC07 – percentage foot ash for birds fed pelleted diets with increasing ring dried potable YPC inclusion levels and reducing di-calcium phosphate.

Diet	Foot ash / % (S.E.)
0% YPC	15.41 (0.141)
7.5% YPC	15.63 (0.119)
12.5% YPC	15.78 (0.115)
17.5% YPC	15.70 (0.152)
p value	0.195

6.4 DISCUSSION

The initial section of this chapter focuses on a validation study which was originally conceived to validate the use of foot ash as a method of measuring bone mineralisation in chicks up to 6 weeks old. Tibia and toe ash are both recognised methods of quantifying bone mineralisation, with tibia ash being the most commonly used (Skinner and Waldroup, 1995). The validation study was designed to compare these established methods with foot and femur ash measurements using diets formulated to produce differences in bone mineralisation.

6.4.1 Dietary suitability in study PVT01

The validation study successfully produced diets with measurable differences in analysed dietary calcium and phosphorus. This translated into measureable differences in bone

mineralisation between the high and low diets using some methods. Not all weeks and methods showed statistically significant differences between the dietary treatments, which shows that the dietary differences are not so extreme as to produce differences regardless of methodology. It is important to consider the ratio of calcium to phosphorus in the diets. The NRC (1984) recommends 10g/kg Ca and 4.5g/kg P which is a ratio of 2.2:1, whereas other authors have suggested 2:1 as optimum (Shafey, 1993; Skinner and Waldroup, 1992). Excess calcium can bind to phosphorus and make phytate phosphorus less soluble and therefore less available to the bird. Although the ratios in PVT01 are higher than formulated, the ratios for both the high and low diets are very similar in this study. The high calcium of 22.68g/kg may have had a supressing effect on growth as performance can be reduced due to the increased pH in the crop and ileum (Shafey *et al*., 1991). During this study, pH levels were measured as part of a different project but no difference between diets was observed. Mean pH across all diets was found to be 2.57 in the proventriculus and 7.02 in the ileum at 6 weeks of age (Morgan *et al*., 2012). Wien and Schartz (1985) found that calcium solubility decreased when the ileal pH increased from 6 to 7, and this may affect bioavailability of other minerals from the gastrointestinal tract (Shafey, 1993), so this may have occurred in this study but growth was not monitored in PVT01 as the aim of the study was to compare mineralisation methodology. However Shafey and McDonald (1990) did not report any effect on performance up to 30g/kg calcium, as long as phosphorus was also relatively high (12g/kg).

6.4.2 Suitability of mineralisation methods for different ages of bird

In week one of the study, no differences in mineralisation were found between high and low diets using any leg bone. This could be due to the small size of the birds which would make any inter sample differences appear much larger, however with the exception of toe ash measurements, the coefficients of variation are not large for the other methods when compared to other study weeks. Week 1 may be too early in the lifetime of the bird for dietary

treatments to have had any measurable effect on bone mineralisation. Itoh and Hatano (1964) also found no significant differences when measuring bone minerals in chicks at 1 week of age. Toe ash only showed a significant difference between diets when the birds were 2 and 3 weeks old. Ravindran *et al*. (1995) found toe ash was a sensitive measure of phosphorus availability in birds aged 21 days. The lack of differences at week 1 is likely to be due to the small weight of the sample, which means that a small difference in technique when removing the toes can make a large difference in relative standard deviation. The average dry toe weight was 59mg and the average weight of ash was less than 6mg, so any small errors in weighing were magnified. Mendez and Dale (1998) suggested that the small ash weight would lead to greater variation, and in this study, toe ash had higher coefficients of variation throughout compared with the other bones measured. At the start of the study, these differences may also be due to the large individual variation of newly hatched chicks as observed by Yoshida and Hoshii (1982). It is possible that the relative inexperience of the collectors of the toe bones in this study has led to a larger standard error, but attempts were made to reduce this by using the same collector and same technique throughout. It could be expected that the small bones of the toe would be fully mineralised early in the life of the bird due to their small size, so this may be one of the reasons why in later weeks the differences in diet are not apparent in the toe ash. The results of this study suggest that toe ash may be an inappropriate measure of bone mineralisation in older chicks (greater than 21 days old).

Foot and tibia ash were significantly ($p<0.05$) higher in the high diet for weeks 2 through 5. P values were consistently lower for foot ash for this period. By week 6, the foot ash is only approaching significance ($p=0.05$) whereas tibia ash is still showing significant differences. This could suggest that the foot bones are approaching full mineralisation by 6 weeks of age, and therefore less effective at showing differences between dietary treatments. It appears that foot ash is comparable with tibia ash at showing dietary mineral differences up to 5 weeks of age.

Foot ash has comparatively low standard errors, due to the simplicity of removal of the required bones, and the relatively large sample size compared with toe ash. Dale and Garcia (2004) suggested that foot ash improved replication due to relatively large sample weight compared with toe ash whilst maintaining the simplicity and speed of collection. In this study, the coefficients of variation for the foot ash samples were less than 8% throughout the study, even with the smaller birds, which is comparable to the coefficients for both tibia and femur ash.

Foot ash has been shown to reflect dietary P up to 2 weeks of age (Garcia and Dale, 2006), and the foot ash levels found in the aforementioned study are compared with the findings from PVT01 for the same age of chicks (14 days) in table 6.12.

Table 6.12: Foot ash levels of 2 week old chicks comparing PVT01 with Garcia and Dale (2006)

Garcia and Dale, 2006		**PVT01**	
Phosphorus content %	**Foot ash %**	**Phosphorus content %**	**Foot ash %**
0.53	10.93	0.44	12.3
0.91	13.46	0.77	13.5
1.29	15.45		

Table 6.4.1 shows that the foot ash percentages for PVT01 are higher than was found for slightly higher supplemental phosphorus levels by Garcia and Dale (2006). The Garcia and Dale diets are the equivalent to 0.24,0.32 and 0.4% available phosphorus, but this was not measured in PVT01. In PVT01, the diets were formulated to a consistent calcium to phosphorus ratio, whereas the data above is based on a calcium level of 0.95% throughout, which translates to ratios between 2.38 and 3.95. These high Ca:P ratios could contribute to the lower percentage foot ash measured by Garcia and Dale. Other differences could be due to differences in technique, or different fat composition of the birds, which affects foot ash values, as highlighted by Huff (1980).

Tibia ash is recommended by the AOAC (2000) for the measurement of Vitamin D activity. As a bone mineralisation method, it is the most commonly used bone for measuring phosphorus availability (Skinner and Waldroup, 1995). In PVT01, tibia ash showed significant differences between the dietary treatments from week 2 onwards although the magnitude of the significance reduced from week 3 (p=0.001) to week 6 (p=0.017). This may suggest that the tibia bones are almost fully mineralised by week 6 and therefore are becoming less sensitive for showing dietary phosphorus and calcium differences. This finding suggests that tibia ash may not be an appropriate measure in studies which assess bone ash of birds older than 6 weeks but this requires further confirmation. Talaty *et al*. (2009) also recorded that although tibia bones continued to increase in size, the bone density did not significantly increase past 4 weeks, and Rath *et al*. (2000) found that the bone ash was consistent after week 5.

Average coefficients of variation for tibia ash were lower than for any other leg bone type, which evidences the precision of the method. Skinner and Waldroup (1995) assessed percentage tibia ash over the same time period as PVT01 and a comparison of results from the studies is shown in table 6.13. The phosphorus levels of the high diet in PVT01 are comparable to this study, but the calcium levels and hence the Ca:P ratio were much higher in PVT01 than this paper. The results show the same trend to increasing tibia ash content as the birds age, but the results from PVT01 are generally higher. This is likely to be due to the use of extracted tibias in the Skinner and Waldroup paper, which has been shown to reduce percentage bone ash (Hall *et al.,* 2003). Interestingly, the standard errors are no higher using unextracted tibias, which contrasts with the findings of Hall *et al*. (2003) which found that extraction improved the power of the tibia ash method.

Table 6.13: Comparison of tibia ash from PVT01 and Skinner and Waldroup (1995) over 6 weeks

	Skinner & Waldroup 1995		**PVT01**
Age /days	**Tibia ash %**	**Diet**	**Tibia ash %**
7	36.52 (0.45)	Low	35.97 (0.53)
		High	37.26 (0.62)
14	39.30 (0.70)	Low	38.89 (0.45)
		High	41.63 (0.59)
21	41.86 (0.80)	Low	42.95 (0.47)
		High	45.42 (0.49)
28	41.93 (0.45)	Low	41.07 (0.60)
		High	44.22 (0.30)
35	42.43 (0.34)	Low	40.58 (0.67)
		High	43.62 (0.37)
42	42.86 (0.78)	Low	43.57 (0.74)
		High	47.83 (0.99)

Femur ash has been reported to be the most similar bone in terms of mineralisation to whole skeletal values (Itoh and Hatano, 1964), and that it is more responsive to dietary changes than the tibia (Dilworth and Day, 1965). It may also be important when considering processing related injuries as there is high external torsion on the femur in birds at processing age (Thorp, 1992). In this study, the femur ash showed no differences between the high and low diet in birds up to 3 weeks of age. The percentage femur ash was significantly greater for the high diet compared with the low diet in weeks 4 to 6. At week 6 the differences were highly significant ($p>0.001$) for femur ash, and this contrasts with the results for foot and tibia ash, which appeared to become less significant with older birds. This suggests that the femur bone is still mineralising at 6 weeks of age and may be a more appropriate bone to use when making dietary comparisons in older chicks (over 6 weeks of age).

Tibia ash has been previously found to be consistently greater than femur ash, therefore femur integrity cannot be implied from tibia measurements (Applegate and Lilburn, 2002). This is

confirmed in this study, where it can be seen that tibia and femur ash do not always show the same differences between dietary treatments dependent upon the age of the birds.

6.4.3 Correlations between different methodologies for measuring bone mineralisation

Ravindran *et al*. (1995) found that toe ash was a sensitive measure of phosphorus availability in chicks of 21 days old and found a strong correlation between toe and tibia ash which was not reflected in this study. A correlation plot of toe and tibia ash is shown in figure 6.1 and there is no relationship apparent (R^2 = 0.0174). The relationship between toe and tibia ash has also been shown by Yoshida and Hoshii, (1982) and Yan *et al*. (2005) who reported an R^2 of 0.8776. Some of these differences can be explained by the differences in methodology as the previous papers all used a greater range of dietary Ca and P values and in some cases pooled all toes per bird to increase sample weight. This lack of correlation is repeated in PVT01 between toe and femur ash, as shown in figure 6.2 (R^2=0.0992). It seems likely that the variation of toe ash, combined with the small sample weight has disguised any relationships between toe ash and other bone ash.

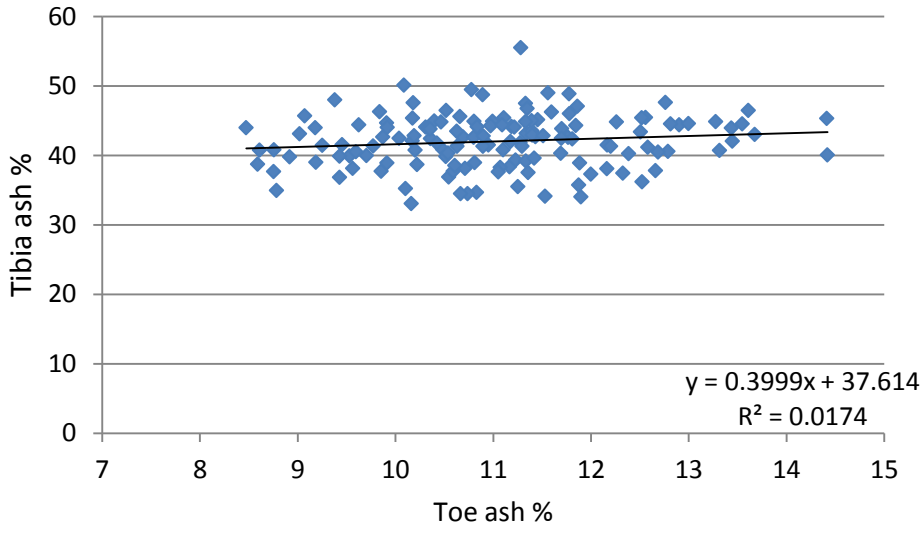

Figure 6.1 Correlation between toe and tibia ash percentages for PVT01

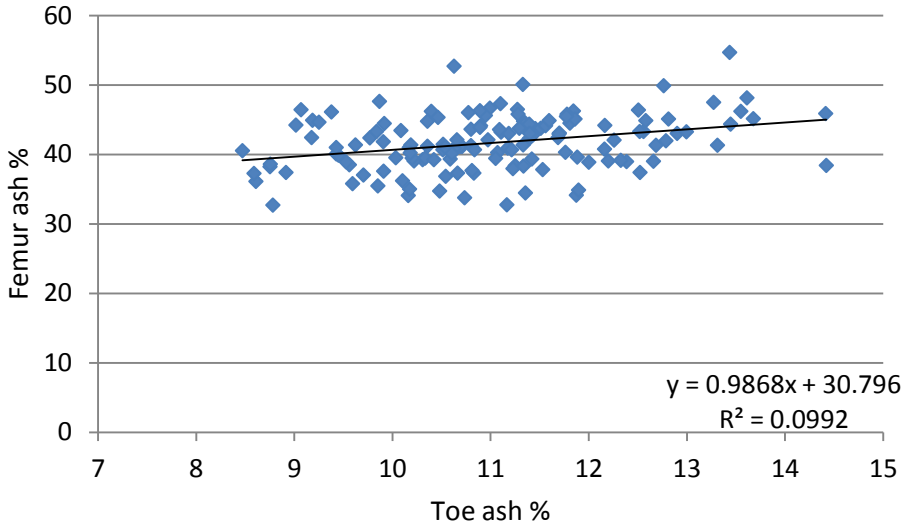

Figure 6.2: Correlation between femur and toe ash for PVT01

Foot ash was suggested originally by Mendez and colleagues (Mendez and Dale, 1998; Mendes *et al*., 1998) who found a correlation between tibia and foot ash (R^2 = 0.85) as well as a relationship between toe and tibia ash (R^2= 0.82). Yan *et al*. (2005) found a strong correlation between extracted tibias and foot ash (R^2=0.92) which was also not as clearly shown in this trial. Although it is clear from figure 6.3 that there is some relationship between the two methods, there is not a strong correlation (R^2 =0.455). This may be due to the small number of replicates in this study (6 birds per diet per week) and the restricted range of dietary phosphorus levels. Also, unlike the Yan *et al*. paper, which only studied birds aged 21 days, the study PVT01 assessed a range of ages up to 42 days, which will increase variation. This is especially the case for foot and toe ash, where differences in lipid content due to nutrition, disease or other factors, can have an effect on the ash values (Huff, 1980). In PVT01, unextracted tibias were used whereas Yan used extracted tibias in the correlation with foot ash, although they did find a correlation between extracted and unextracted tibias (R^2=0.95). Figure 6.4 shows a relationship between toe and foot ash (R^2 = 0.3991) which, although not described in other papers, could be implied from the correlations between tibia and toe and tibia and foot ash. This correlation between toe and foot ash is weaker than between foot and tibia due to the large variation found in the toe ash measurements.

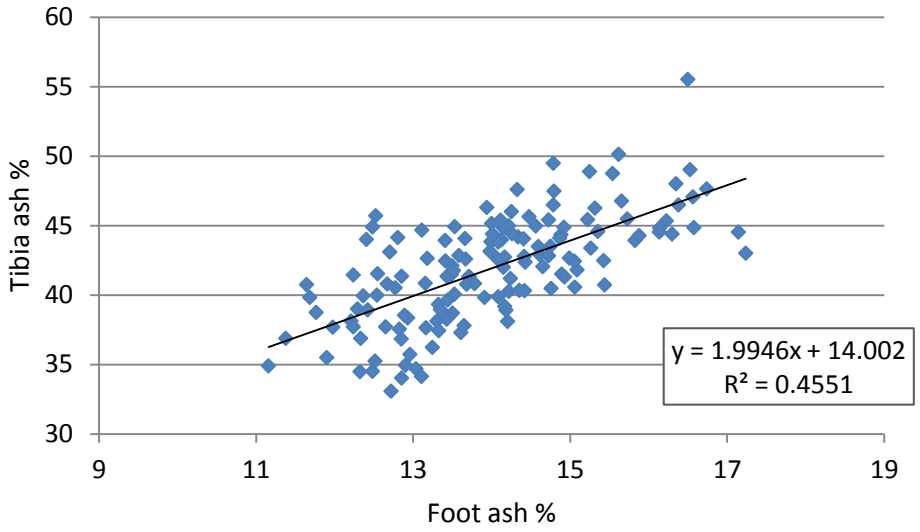

Figure 6.3: Correlation between foot and tibia ash in study PVT01

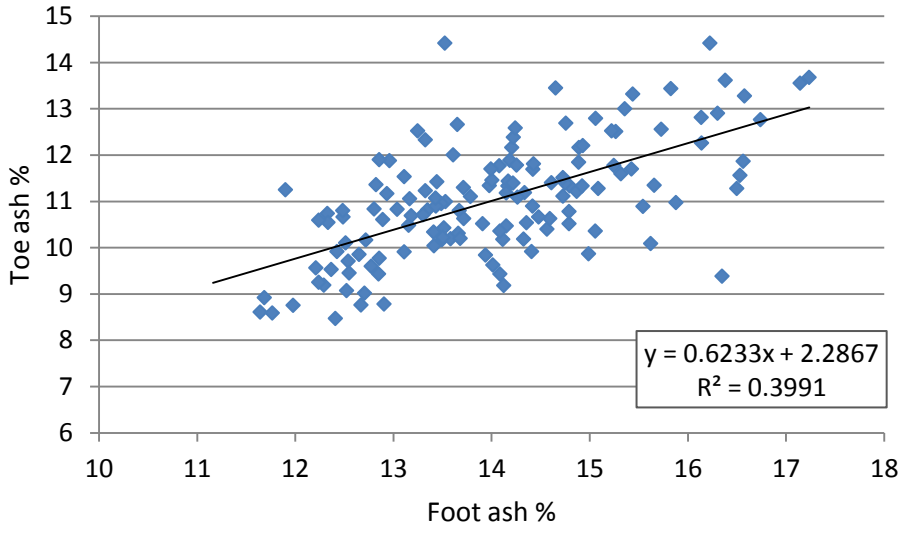

Figure 6.4: Correlation between foot ash and toe ash for PVT01

Femur ash was found to correlate with tibia ash (figure 6.5) and, to a lesser extent, with tibia ash (figure 6.6). The regression coefficients are modest at R^2=0.4723 and R^2= 0.4152 respectively, which is likely to be due to the small replicate size and narrow range of ash values

achieved with only two phosphorus levels. Femur bones are reported to be more sensitive to differing ratios of Ca:P in older birds (Moran and Todd, 1994), and this may have had some effect on the results.

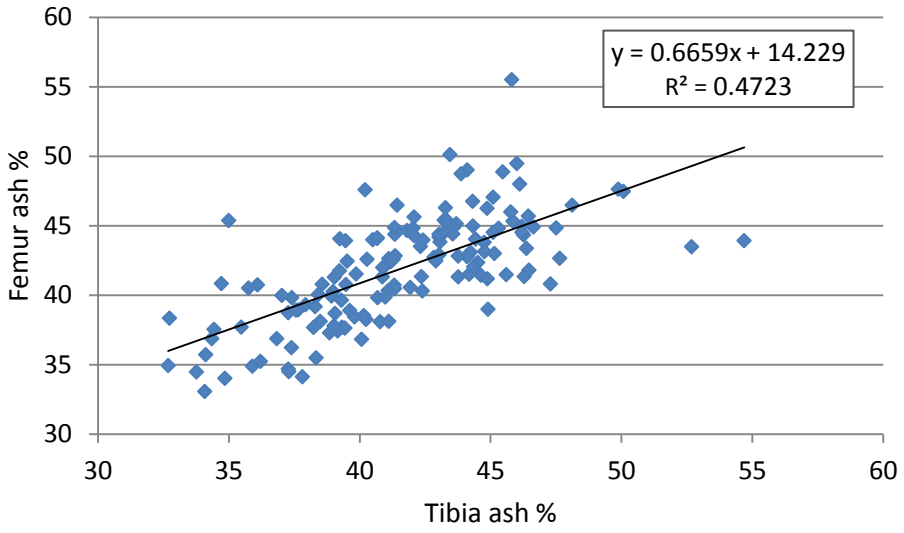

Figure 6.5: Correlation between tibia and femur ash for PVT01

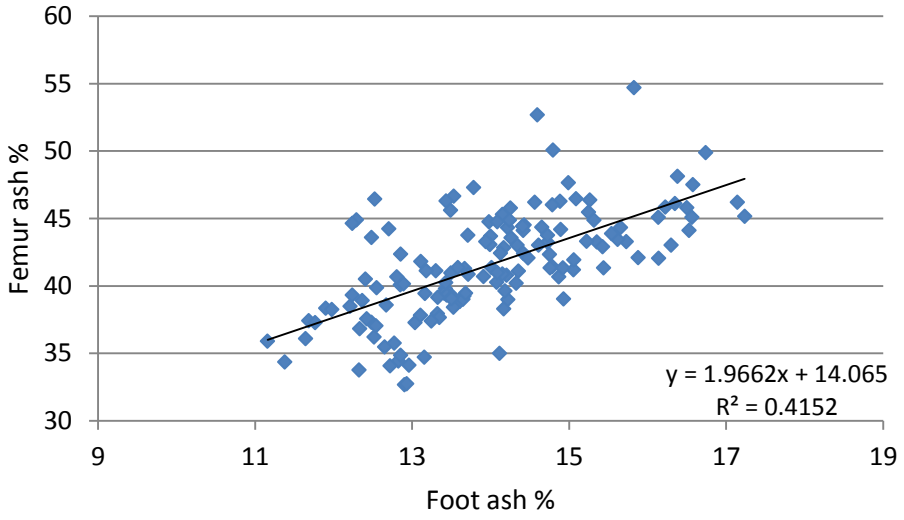

Figure 6.6: Comparison between femur and foot ash for PVT01

6.4.4 Limitations of methodology

Kim *et al*. (2004) compared different preparatory procedures (fresh, dried and fat free) for tibia ash and found no differences in bone ash content. However it has been suggested that extracted tibias are a more resilient measure requiring less replications to show dietary differences (Hall *et al*., 2003), so it may be possible that fat free tibias would have provided a more robust correlation in this study. However, findings from PVT01 showed un-extracted tibia ash coefficients averaged less than 5% compared to Hall's findings where un-extracted, autoclaved tibias had high coefficients of variation (21.53%). This may be due to differences in technique, in PVT01, great care was taken to remove all flesh from the bones in a consistent way. Also Hall only dried the bones for 24 hours, which may have been insufficient to remove all moisture. It also needs to be considered that the extraction method for tibias is labour intensive and uses harmful chemicals (ethanol and ether). Bones were frozen to allow more care and time to be taken with the bone preparation, and this has been shown to have no effect on bone ash (Park *et al*., 2003).

It is likely that the high and variable organic matter content and lipid content of the older chicks has reduced the sensitivity of foot ash as a measure by 6 weeks. Fat extraction has been used to reduce variation in bone ash measurement since first noted by Bethke *et al*., (1923), and it may be that using extracted feet would more consistent results in older birds as suggested by Garcia and Dale (2006). However the latter paper found no difference in the sensitivity of foot ash as a measure of available phosphorus in birds aged up to two weeks when the foot was extracted prior to ashing, so it is unlikely to be necessary in younger birds. In older birds, extracted foot ash may be a more suitable method, as the larger feet cause issues when ashing, due to the high organic matter and fat content and can set off laboratory alarms (Yan *et al*., 2005).

Average coefficients of variation over the whole study show that tibia, femur and foot ash are comparable in terms of precision, but toe ash had greater variation. Much of this variation

comes from the very high standard errors in week 1, without these, the toe ash variation reduces to 9.49%, but this is still twice as high as recorded for tibia ash. Toe ash in this study did not appear to give reproducible results, which may be due to technical inconsistencies removing the toes. Also some of the studies on toe ash have pooled all the toes on each foot in order to increase the sample weight and thereby reduce variation (Yan *et al*., 2005). However this method would increase the time required for the collection of the toes, especially when compared with foot ash collection.

6.4.5 YPC as a source of available phosphorus

Phosphorus inclusion in diets is vital to reduce bone abnormalities as in conjunction with calcium it is the major mineral component of bone. The organic matrix of bone is essentially collagen based and the collagen fibres create holes which the combination of P and Ca (as hydroxyapatite crystals) fill, thereby improving the strength of the overall bone. Phosphorus also plays an important role in cellular metabolism in regulatory mechanisms, as part of the cellular energy potential.

Wheat Distiller's dried grains with solubles (WDDGS) has been shown to contain more phosphorus than wheat (Nyachoti *et al*., 2005) and this may come from the yeast fraction, which makes up at least 5.9% dry weight of DDGS (Ingledew, 1999) or 20% of the protein (Liu, 2011).

Available phosphorus has been shown to decrease with increasing dietary inclusion of WDDGS (Thacker and Widyaratne, 2007), which suggests that WDDGS would not be a viable source of available phosphorus in poultry feeds. However laying hens were shown to have increased P retention in diets with 25% maize DDGS and reduced P excretion (Masa'deh *et al*., 2011), which contrasts to the findings in pigs where increased maize DDGS reduced phosphorus uptake (McDonnell et al., 2011). Wheat DDGS has also been shown to increase excretion of

water soluble phosphorus in poultry (Leytem *et al*., 2008) which is detrimental to the environment.

Trial BRC03 showed there was no significant effect of differing yeast protein concentrate source (potable or bioethanol) on percentage foot ash (p=0.485). Although washing did not have a significant effect on bone mineralisation, all the washed YPC levels are numerically lower than the unwashed YPC. It may be that water soluble phosphorus is being removed during the washing process, but this has not been quantified in this study. However the levels of water soluble phosphorus would need to be considered in future studies to ensure there is not a detrimental environmental effect from feeding higher inclusion levels of YPC. Rate of inclusion of YPC had a significant effect on foot ash, with the highest inclusion levels having significantly higher percentage foot ash. This suggests that YPC may be potentially a good source of available phosphorus. The foot ash was subsequently analysed for phosphorus and calcium content and there were no significant differences found, whether expressed as a proportion of ash weight or dry foot weight. There were also no significant differences in Ca:P ratio of the bone, which were all around 2.25:1. Some authors have suggested a bone Ca:P ratio of 2:1 as optimum (Shafey, 1993; Skinner and Waldroup, 1992) and it needs to be considered as higher bone ratios can lead to leg weakness (Thorp and Waddington, 1997). Other authors have suggested that an expected ratio for bone would be 1.67:1, but that some bird strains reach 2.15:1 (Pellegrino and Blitz, 1968; Williams *et al*., 2000a). Widening the ratio can reduce growth but the Ca:P ratio was similar across all dietary treatments for BRC03 so although there may be a detrimental effect on bird performance, it does not affect the validity of the diet comparisons.

From the BRC03 finding that increasing dietary inclusion levels of YPC increased bone mineralisation, BRC07 diets were formulated with reducing MCP as the YPC inclusion increased and showed no significant differences in the bone mineralisation of the birds fed any of the trial diets. These results confirm that the YPC is a viable source of available phosphorus.

The 17.5% inclusion level of the YPC replaced 5g/kg of the di-calcium phosphate in the diet formulation with no adverse effects on bone mineralisation, which is equivalent to a 35% reduction in DCP use.

6.5 CONCLUSION

The validation study shows that both foot and tibia ash are appropriate measures of bone mineralisation for showing dietary P and Ca differences in birds up to 5 weeks of age. Toe ash lacked reproducibility on this occasion, and femur ash did not show dietary differences in younger chicks (less than 4 weeks of age). However femur ash appears to be the most appropriate bone for showing mineralisation differences in older chicks (over 5 weeks old), which may be due to this larger bone continuing to mineralise longer than the other bones in the leg. With the exception of toe ash, all the methods have low coefficients of variation so precision was not an issue. Foot ash is quicker and less labour intensive than other methods and this study suggests that it is a valid measure for assessing dietary phosphorus content in birds up to 5 weeks old.

The study BRC03 shows that increasing YPC inclusion in poultry diets increased bone mineralisation, suggesting a role for YPC as a source of available phosphorus. This was confirmed by study BRC07, where YPC replaced inorganic phosphorus in the diets with no adverse effects on bone mineralisation.

These findings increase the economic value of YCP as a feed ingredient, potentially reducing the need for inorganic phosphorus use, reducing economic cost to the industry and reducing the pressure on the limited mined phosphorus stocks.

CHAPTER 7: CONCLUSIONS

7.1 INTRODUCTION

This chapter is split into three sections to discuss the potential of YPC as a feed ingredient in the poultry feed market. Firstly, the success of the novel separation procedure will be discussed along with the variability of the final product. Subsequently, the effect of the distillery source on the YPC will be considered, and finally the effect of drying process on the YPC will be discussed.

The potential production volume of YPC is considerable. In 2011, bioethanol production in the EU was 4400 million litres (EU bioethanol fuel association, 2012), with every tonne of wheat producing 370 litres of ethanol (Bonnardeaux, 2007) and approximately 300kg DDGS (Shurson *et al*., 2005; Rausch and Belyea, 2006). Based on these figures, DDGS production in 2011 was around 3.5 million tonnes in the EU alone. Estimated production for the US is currently 43 million tonnes a year (AMRC, 2012). With conservative estimates of 4% yeast content in DDGS (Ingledew, 1999; Han and Lui, 2010), there is a potential production volume of 140 thousand tonnes of YPC per annum in the EU and 1.7 million tonnes in the US. Production of YPC is likely to be greater than this as the product does not contain yeast alone. This scale of production of sustainable protein could alleviate some of the pressures on other protein sources.

7.2 SUCCESS OF THE NOVEL YPC PRODUCTION PROCESS

There were considerable differences in the composition of the raw material in the pilot plant study, which is consistent with the large variation seen in DDGS (Spiehs *et al*., 2002). This also confirms that the variation not due to feedstock alone but also from variations in processing technique (Belyea *et al*., 2004). Belyea *et al*. (2010) found that fermentation batch was the greatest source of variation in DDGS, but the findings of the pilot plant study show that variation is also seen within the same batch of fermentation. Mixing of the material is crucial

to consistency for sampling. The yeast cream produced via the separation process proved to be consistent on a dry matter basis when considered across a whole separation run. However, contamination of the yeast cream with fibre at the decanter stage increased the dry matter content of the final product.

The separation is very dependent on the skill of the operator; the process requires trained operators and controls to be put in place in the plant to ensure the final product is as consistent as possible. This level of control is achievable: at the GEA Westfalia pilot plant in Oelde, the technicians use a spun volume centrifuge to assess the decanter separation and use a number of techniques to alter the cut depending on the characteristics of the stillage.

Fibre inclusion is a concern for processing as it has been shown to effect drying rate, due to reduction of small particle agglomeration, and more rapid drying of the larger fibrous particles. This inconsistency in particle size will make the material more difficult to dry evenly making Maillard reactions more likely. Particle size differences caused by fibre contamination may alter the flowability of the dry material (Barbosa-Conovas *et al*., 2005) leading to particle segregation as observed in DDGS (Ileleji *et al*., 2007). Larger particles can cause an overall reduction in bulk density with implications for transport costs and consistency. Another seen issue with DDGS is that it is hygroscopic with a tendency to cake, making it difficult to transport (Mathlouthi and Roge, 2003).

Large amounts of fibre contamination are undesirable as fibre is not highly digestible for poultry (Mangold, 1934), impairs other nutrient and energy digestibility (Halnan, 1930) and is therefore considered to be a dietary diluent (Jimenez-Moreno *et al*., 2010). This can be seen with the freeze dried YPCs which had much lower amino acid digestibility values than the spray dried YPCs. Most of the fibre in DDGS is insoluble dietary fibre (Parsons *et al*., 2006), and the high levels of this have been shown to reduce digestible energy in poultry (Thacker and Widyaratne, 2007). Most of the negative effects of fibre are due to its physicochemical properties which increase the rate of passage of digesta through the gastrointestinal tract

therefore limiting the time available for nutrient breakdown (Burkitt *et al*., 1972). Denstadli *et al*. (2010b) found that high inclusion levels of brewers spent grains in diets fed to broilers reduced bodyweight gain and increased FCR, but that the gizzard development was improved by the high fibre content. However, smaller inclusion levels of fibre may not be detrimental to poultry performance. Insoluble fibre has been shown to promote gizzard development and therefore starch digestibility (Sacranie *et al*., 2012) and can increase retention time in the gizzard (Hetland, *et al*., 2005). Improved gizzard activity and development can improve performance (Hetland *et al*., 2003) and reduce the entrance of pathogenic bacteria into the gastrointestinal tract (Bjerrum *et al*., 2005). Recent work has shown that inclusion levels of 3% insoluble fibre increased performance characteristics in 21 day old chicks (Jimenez-Moreno *et al*., 2009).

As seen in DDGS (Cromwell *et al*., 1993), the YPC is not consistent in amino acid content across potable and bioethanol sources. It may be that adding viscosity-reducing enzymes to the potable stillage may make it more consistent to separate the YPC. Kluth *et al*. (2009) found that xylanase addition reduced the variability of wheat in terms of amino acid digestibility, and it is possible that making all the stillage a consistent viscosity will improve reproducibility of separation runs. Variability in nutrient digestibility has been reduced with carbohydrase enzyme use in both wheat (Svihus and Gullord, 2002) and barley (Campbell *et al*., 1989).

The drying stage is not only very important for the nutritional value of the product as a feed ingredient, but also for the consistency of the product. Much of the cost of producing YPC is from the drying stage, but it is necessary to dry to improve shelf life of the product and make it easier to transport and mix into feed. Stock and Klopfenstein (1982) estimated that drying can account for 40% of energy costs in DDGS production. Freeze drying is the most costly method (Ratti, 2001), whereas spray drying can be costly is not carefully managed (Luna Solano *et al*., 2005) and ring drying is the least cost alternative (Vega Mercado *et al*., 2001). Drying has considerable effect on amino acid content of the YPC, which can be seen most clearly with the

ring dried product. This YPC was very dark in colour which suggests the formation of Maillard reactions (Martins *et al*., 2001). The correlation of colour to amino acid content and availability has been recorded with both maize (Shurson *et al*., 2005) and wheat DDGS (Cozannet *et al*., 2010).

In terms of nutritional composition of the YPCs compared in this thesis, the variation between distilleries does not appear large, as the freeze dried YPCs were both chemically similar, which reiterates the importance of the processing stage, as the freeze dried materials were separated and dried at the same time. The improvement in separation seen with the spray dried material (reduced fibre contamination) has led to an increase in both protein and fat in the bioethanol SD YPC and hence greater gross energy content. Washing was shown to increase protein considerably, confirming the work of Sharif *et al*. (2012) on distillery sludge. The inclusion of YPC into pelleted diets was successful but the high pellet quality may be due to small scale of the process used. Denstadli *et al*. (2010b) found that higher inclusion levels of brewers spent grains reduced pellet durability from 85% control to 68% at 40% inclusion, which is likely to be due to the reduced starch content. Similarly, Srinivasan *et al*. (2009) found that removing a proportion of the fibre from DDGS improved pellet durability. Loar *et al.* (2010) found that production rate for pellets decreased with increasing DDGS inclusion, which they suggested was due to the reduction in rock phosphate in the diets. Supplemental inorganic phosphates have a scrubbing effect through the pelleting die which improves pelleting rate, so the increased phosphorus in YPC may have cost implications for the pelleting process.

7.3 EFFECT OF DISTILLERY SOURCE ON THE NUTRITIONAL POTENTIAL OF YPC FOR POULTRY

Bioethanol sourced YPC was shown to have higher amino acid digestibility content than potable, which is likely due to the lack of exogenous enzyme addition, as potable production is constrained by legislation (Scotch Whisky Order, 1990). Wheat DDGS has been shown to

contain twice the amount of non-starch polysaccharides (NSPs) when compared to wheat (Zijlstra *et al*., 2007), and high levels of arabinoxylans increase viscosity and decrease enzyme diffusion. This is confirmed by the trend towards higher viscosity which found with increasing potable YPC inclusion for both spray and freeze dried material. This higher viscosity is likely to be due to the presence of non-starch polysaccharides from the parent grain forming a viscous gel in the gastrointestinal tract (Choct and Annison, 1992a; 1992b). The thickening of the unstirred layer in the intestinal tract impedes amino acid digestion by slowing absorption through the epithelial lining (Austin *et al*., 1999), and NSPs may physically encapsulate some proteins reducing amino acid digestibility (Selle *et al*., 2009). In bioethanol production exogenous carbohydrase enzymes are routinely used to improve the extract volume of the ethanol (Bamforth, 2009), and these will have broken down some of the NSPs present. The difference in amino acid digestibility was also seen in performance; for the freeze dried YPCs, the bioethanol sourced YPC was significantly better than potable YPC for both feed intake and bodyweight gain. This may be due to the faster gut transit of the less viscous material (Choct and Kocher, 2000) which would allow the birds to eat more and reduce intestinal fermentation. Exogenous enzymes have been shown to improve bird performance in wheat based diets (Yang *et al*., 2008). Previously work on brewer's grains has suggested that the NSP component in barley has been reduced during the malting process (Denstadli *et al*., 2010a; Wang *et al*., 2004), and Petterson *et al*. (1987) did not find any performance enhancing effect of enzyme addition with diets containing brewery waste. However the increases in viscosity seen with potable YPC suggest that the NSP content may be substantial. It is possible that NSPs have been partitioned with the YPC during separation, increasing their relative concentration. It certainly appears that the presence of NSPs in potable YPC has deleteriously affected both amino acid digestibility and bird performance.

Bioethanol derived yeast has been suggested to be of poorer quality than potable, as it is reused during the process and therefore could be considered to be exhausted of cellular

components (Yamada *et al*., 2005). Nonetheless, the current studies consistently demonstrate higher digestible amino acid content in the bioethanol YPC than the potable, suggesting the overwhelming detrimental impact of the NSP content. The high ethanol content of bioethanol processing may also toughen the cell wall, making it more resistant to proteolysis (Caballero and Sgarberi, 2000). However the disk stack process applies a mechanical shear to the yeast cream which has been shown microscopically to lyse the cell walls, which negates the digestive challenge of the toughened cell walls.

No significant differences were found in foot ash between potable and bioethanol sources of YPC, and both showed significant increases in bone mineralisation with rate of YPC inclusion. Yeast has been previously observed to contain substantial phosphorus (Sell *et al*., 1982), and it has been suggested that the alcohol production process may destroy phytic acid, thereby increasing available phosphorus (Cottril *et al*., 2007). It has also been postulated that yeast phytase during the process releases phytate (Gao *et al*., 2008), which would lead to increased phosphorus in the YPC from both sources, independent of parent grain.

7.4 EFFECT OF DRYING METHOD AND WASHING ON THE NUTRITIONAL POTENTIAL OF YPC FOR POULTRY

Although spray drying produced the YPC with the highest amino acid content and digestibility, the small particle size produced by this drying method (less than 200µM, Niessen, 2002) had a deleterious effect on bird performance. Very small particles are difficult for birds to manipulate, increasing feeding time and reducing feed intake (Waldroup, 1997) and can also reduce gizzard size to the point of hypotrophy (Nir *et al*., 1994; Nir and Ptichi, 2001). Fine mash feed has been shown to reduce FI and BWG in birds up to 3 weeks of age (Nir *et al*., 1994; Amerah *et al*., 2007a) and both spray dried YPCs showed reduced performance characteristics when compared with both freeze and ring drying. Both spray dried YPCs also caused increased

digesta viscosity, even though the bioethanol YPC would have benefited from the addition of exogenous enzymes earlier in the process. It is likely that the small particle size has also increased digesta viscosity in this case, as particles less than 0.5mm have been shown to increase NSP release and degradation (Yasar, 2003).

Although the ring dried YPC had a low amino acid digestibility values, it still provided acceptable performance parameters when included in bird diets, as issues of palatability and particle size effecting intake and digestion were addressed. Additionally, this YPC was pelleted and it is generally accepted that pelleted feed will result in improved performance when compared to mash (Amerah *et al*., 2011). It does appear easy to produce a very heat damaged material using the ring dryer - as can be seen in the very poor amino acid digestibility and very dark colour of the ring dried YPC. Overheating causes the formation of brown polymers (Martins *et al.,* 2001), and these Maillard reactions reduce the availability of amino acids (Moralez *et al*., 2007). The residence time in a ring dryer can vary from 1 to 300 minutes with extended residence times the cause of burning (Vega Mercado *et al*., 2001). Colour has been shown to reflect amino acid content and digestibility in DDGS (Ergul *et al*., 2003; Cozannet *et al*., 2011b), and it can be seen from the ring dried material that colour is also likely to be an indicator of amino acid digestibility in YPC.

The amino acid content of the washed YPC was high and comparable with soya, but this increase was not reflected in improved performance in bird studies. Performance characteristics reduced with higher inclusion levels of washed YPC and viscosity increased so it is possible be that washing did not remove all non-starch polysaccharides. A similar lack of efficacy for washing was shown in cereals by Antonoiu and Marquadt (1982). However Sharif *et al*. (2012) found that 8% inclusion of washed brewery sludge increased BWG and FI in broilers so it may be that washing would improve the nutritional content of YPC but that the decreased particle size resulting from the spray drying has negated any positive effects. It is

also possible that washing has removed some nutrients as suggested by Knott and Shurson (2004).

Freeze dried material should be the least damaged as the absence of air prevents oxidation and there is no heat present (Dalgeish, 1990) and this drying method has been shown to preserve anti-oxidants better than other methods (Lin Hsu *et al*., 2003). Santos *et al*. (2003) compared drying methods for brewer's grains and found that more protein was removed during freeze drying than during oven drying, which may be due to losses of volatile nitrogen compounds during freezing. In the case of the YPC material, is likely that the material was contaminated with fibre due to the inexperience of the operators during the separation process. This fibre contamination would have a deleterious effect on endogenous amino acid secretions and reduce the amino acid digestibility (Ravindren and Bryden, 1999; Adedokum *et al*., 2012). However this small fibre inclusion did not appear to negatively affect performance, which confirms the work of Jimenez-Moreno *et al*. (2010) who showed that fibre can improve nutrient digestibility by changing gizzard pH and improving gizzard development.

7.5 FUTURE DIRECTIONS

7.5.1 Large scale variability and drying studies

The consistency of any feed ingredient is vital for feed formulators to ensure reproducible results can be achieved with the final diets. Large scale studies need to assess the variability of YPC from different batches of feedstock and using different processing conditions. Belyea *et al*. (2010) found that fermentation batch was the greatest source of DDGS variation and this needs to be confirmed for YPC. Many of the processing factors will affect the final product including temperature and water quality (Rausch and Belyea, 2006), and the ethanol content and osmotic pressure will acts as stressors for the yeast (Zhao and Bai, 2009).

As well as consistency and chemical composition of the final product, cost implications of drying need to be considered. Ring drying is least cost option with freeze drying most costly

(Ratti, 2001) and spray drying needs to be carefully managed to reduce resource use (Luna-Solano *et al*., 2005). Studies to compare drying methods need to be carried out on the same batch of material wherever possible to allow a conclusive comparison to be made.

7.5.2 Uric acid as an antioxidant

The uric acid increase derived from the nucleic acid nitrogen content of yeast may have a previously unconsidered anti-oxidant effect. More assessment of this effect could include measurement of kidney and liver uric acid (Poo and Milan, 1990) in addition to measuring overall antioxidant status overall using the TEAC, which is a modified version of the total antioxidant capacity assay (Ou *et al*., 2001). These measures can be as used in conjunction with uric acid as shown by Cohen *et al.* (2007) to give an overall picture of the antioxidant status of the chicks when fed YPC including diets.

7.5.3 Enzyme addition

Carbohydrase enzyme addition pre-separation may have a positive effect on the consistency of the YPC separation, as enzymes have been used to reduce cultivar differences in both wheat and barley (Svihus and Gullord, 2002; Campbell *et al*., 1989). Although enzymes have not been shown to improve performance in diets containing DDGS in pigs (Jones *et al*., 2010), the viscosity increase seen with potable YPC suggests a substantial NSP inclusion. Enzymes are routinely used in broiler production diets (Huyghebaert *et al*., 2010), and have been shown to ameliorate negative effects on bird performance (Bedford and Classen, 1992). Future studies need to consider the effect of enzyme addition on YPC composition and bird performance, with particular emphasis on potable YPC from barley feedstock.

7.5.4 Phosphorus considerations

The effect of phytase addition needs to be quantified as it may further increase the available phosphorus in the YPC. Kim *et al.* (2008) showed that phytase could release 20% of non-available phosphorus in conjunction with citric acid. Phytase addition has been shown to improve total phosphorus digestibility of wheat DDGS in pigs (Yanez *et al*., 2011), although the baseline digestible phosphorus is higher in DDGS than in the parent grain, as the fermentation process appears to hydrolyse some of the phytate P. Water soluble phosphorus content of the YPC, and excreta phosphorus also need to be quantified to ensure that the use of YPC does not cause detrimental environmental effects. Leytem *et al*. (2008) recorded an increase in the excretion of water soluble phosphorus in chicks fed WDDGS and it may be that this will also be the case for YPC. Increases in water soluble phosphorus in manure has been shown to increase run off of phosphorus from soil (Sharply and Moyer, 2000) leading to increased eutrophication of water sources (Powers and Angel, 2008).

7.6 FUTURE RESEARCH AND DEVELOPMENT

7.6.1 Cost benefit analysis

A large bioethanol plant distilling 400 million litres of ethanol per annum will produce round 340 thousand tonnes of co-product in the form of DDGS. Using the novel separation process detailed in chapter 3, this co-product could be separated to produce 40K tonnes yeast which has the potential to be sold at £600 per tonne as a source of protein for animal feed. The production of YPC from a large bioethanol plant is therefore estimated at approximately £24 million per plant per year. There will be an initial outlay for the machinery and adaptions to the process. The extra processing step may slow down the whole production of the plant and this needs to be assessed on a commercial scale to get a true representation of the cost of the YPC production. A small reduction in DDGS output will result, and the DDGS will contain less protein, but tests show that it is still within specification for DDGS. Output of DDGS could reduce to around 300K t (from 340Kt) which reduces the DDGS revenues potentially by £10

million per year (based on £260 per tonne DDGS). However as production increases, the DDGS market will become more saturated so the value of DDGS may reduce. The financial potential for the product is very large, but a true profit value cannot be estimated until the process is upscaled to a commercial level.

7.6.2 Potential use as an additive

Additives made from mannan-rich fractions of yeast cell walls may have beneficial properties beyond the simple supply of nutrients; but claims for improved animal health and performance cause the product to be classified as a feed additive rather than a feed material and therefore bound to comply with EU regulations (EU, 2003b). Compiled evidence is presented in a dossier that may take 3 years to produce, at an approximate cost of 1 million euros, before authorisation to market the additive within the EU is granted for a specific animal species, under specific conditions of use, for ten year periods. These requirements are likely to heavily influence decisions on whether to present this novel YPC product as a feed material or feed additive.

7.6.3 Effect on consumer satisfaction

It is important to consider the bird from a market perspective and the effect of a new feed ingredient on the finished product. Taste can be considered by a consumer taste panel, and it may be that the incorporation of the YPC product into poultry diets may have enhance the flavour of the meat as yeast extract is widely used in the human food production to enhance flavour. Research on DDGS as shown no effect on consumer acceptance up to 12% inclusion, but above these levels there may be a negative effect on thigh meat as increased fatty acids may increase oxidation (Corzo et al., 2009; Schilling *et al*., 2010). The effect of YPC incorporation on meat yield, particularly for breast meat needs to be considered as Wang *et al*. (2007) noted a reduction in breast meat yield in broilers fed diets containing maize DDGS. The

texture of the meat may be affected and this can be quantified by textural analysis on the cooked and raw meat.

7.6.4 Potential for other poultry applications

The total feed production for the poultry sector in the UK was 210 thousand tonnes (kt) in July 2012, of which 75% is broiler feed (Defra, 2012). However there is significant production of both turkey (25 kt per month) and layer feed (13.5kt per month) and a small production of more specialist feeds (ducks and geese). Although the meat chicken industry is the main consumer of animal feed products in the poultry sector in the UK, there is still a significant market for YPC in other branches of poultry production. The turkey market in particular is appropriate for this product due to the issues with lameness also seen in turkeys due to their larger finishing weight. The layer market is the next largest but research has shown that egg weight has been reduced in layers fed higher levels (15%) of DDGS (Masa'deh *et al*., 2011), so this needs to be considered when looking at incorporating YPC into layer diets.

7.6.5 Product Contamination

The use of materials as animal feed is tightly governed by legislation ultimately aimed at protecting human and animal health. Legislation surrounding animal feed is most stringent in the EU (EU, 2009), and less so in the Asia-Pacific markets but western consumer awareness of food safety is placing pressure on countries exporting animal-based human food products to comply with western legislation. Specifically of relevance to bioethanol co-products, the EU restricts the use of medicated feed, i.e. inclusion of antibiotics (EU, 1990), and the legislation states maximum acceptable levels of microbial contamination and specific legislation on the marketing and use of feed additives (EU, 2003b).

Antibiotics

It is estimated that over half of US bioethanol plants are routinely and propylactically using

antibiotics (Olmstead, 2009), usually penicillin or virginiamycin (Hynes *et al*., 1997; Stroppa *et al*., 2000) to reduce microbial over growth. There are issues relating to the presence of antibiotic residues in co-products leading to possible resistance and public health consequences (Muthaiyan and Ricke, 2010). FEFAC states that antibiotic bactericides are not routinely used in the EU bioethanol industry, and not used at all in the spirit industry (FEFAC, 2008). Although there have been several instances in the EU where antibiotic residues have been found in DDGS from bioethanol, but these have been exclusively on Brazilian imports (Pol *et al.,* 2009). Other options to minimise contamination such as chlorine dioxide and natural hop-derived enzymes are becoming more widely used.

Mycotoxins

There are 400 recognised mycotoxins which may contaminate 25% of cereal feedstocks, but only one of these, AlflatoxinB1 has maximum permitted limit status (EU, 2002). This contamination is concentrated up to three times in DDGS, so contamination needs to be considered and treated in the feedstock and may have considerations for a YPC product. Wu and Munkvold (2008) estimated that contamination from a single mycotoxin could cost the pig industry alone up to 147 million dollars annually, depending on level of DDGS usage, so mycotoxin contamination needs to be assessed before YPC is marketed as a feed ingredient .

7.7 RECOMMENDATIONS

Spray drying produces YPC with the highest digestible amino acid content, but the small particle size is negative for production parameters. Ring dried material produces good performance with strong pellet quality, but care needs to be taken to ensure material is not over heated. Defining an acceptable level of residual fibre in the YPC is a fine balance between its deleterious effect on amino acid digestibility and its potential enhancement of bird performance. Consistency of the product is very important for transport and formulation and the separation stage and the decanter in particular is key to this. Operator training and in plant controls need to be put in place to ensure that the material is continually separated in a consistent manner.

Bioethanol YPC was preferable to potable YPC which appeared to be due to the use of exogenous enzymes during fermentation. It may be that the addition of enzymes to potable YPC pre or post separation would equalise the 2 products.

YPC appears to be a good source of available phosphorus. This finding adds financial and nutritional value to the product by reducing the pressure on limited inorganic phosphate stocks.

Yeast protein concentrate is an appropriate and useful protein source for feeding broiler chickens. If consistency of product can be achieved on a commercial plant scale, ring dried bioethanol YPC could be used to replace soyabean meal at levels of up to 17% with no deleterious effects on performance, while replacing 35% of supplemental phosphate.

REFERENCES

ABPR 1774/2002: Official Journal of the European Communities L273/1 Regulation (EC) No 1774/2002 of the European Parliament and Council of 3rd October 2002.

Adeola, O. and Bedford, M.R. (2004) Exogenous dietary xylanase ameliorates viscosity induced anti-nutritional effects in wheat based diets for white pekin ducks (*Anas platyrinchos domesticus*). *British Journal of Nutrition* **92**: 87-94

Adedokum, S.A., Ajuwon, K.M., Romero, L.F. and Adeola, O. (2012) Ileal endogenous amino acid losses: response of broiler chickens to fiber and mild coccidial vaccine challenge. *Poultry Science* **91**: 899-907

Agyekum, A.K., Slominski, B.A. and Nyachoti, C.M. (2012) Organ weight, intestinal morphology and fasting whole-body oxygen consumption of growing pigs fed diets containing distillers dried grains with solubles alone or in combination with a multi-enzyme supplement. *Journal of Animal Science* Published online May 14

Albright, J.A. (1987) Bone: physical properties. In *Scientific basis of orthopaedics*. J.A. Albright and R.A. Brand (*eds*) Appleton and Lang Publishers, Norwalk, CT. pp213-240

Almiral, M. and Esteve-Garcia, E. (1994) Rate of passage of barley diets with chromium oxide: influence of age and poultry strain and effect of B-glucanase supplementation. *Poultry Science* **73**: 1433-140

Amerah, A.M., Ravindran, V., Lentle, R.G., Thomas, D.G.(2007a) Feed particle size: Implications on the digestion and performance of poultry. *Worlds Poultry Science Journal* **63**: 439-456

Amerah, A.M., Ravindran, V., Lentle, R.G. and Thomas, D.G. (2007b) Performance and digestive tract characteristics of broilers as influenced by particle size and feed form. *Proceedings of the Australian Poultry Science Symposium* **19**: 85-88

Amerah, A.M., Gilbert, C., Simmins, P.H., Ravindran, V. (2011) Influence of feed processing on the efficacy of exogenous enzymes in broiler diets. *Worlds Poultry Science Journal* **67**: 29-46

AMRC: Agriculture Marketing Resource Centre statistics (2012) http://www.extension.iastate.edu/agdm/crops/outlook/dgsbalancesheet.pdf *accessed 23/7/12*

Angkanaporn, K., Choct, M., Bryden, W.L. and Annison, E.F. (1994) Effects of wheat pentosans on endogenous amino acid losses in chickens. *Journal of the Science of Food and Agriculture* **66**: 399-404

Annison, G. (1991) Relationship between the levels of non-starch polysaccharides and the apparent metabolisable energy of wheats assayed in broiler chickens. *Journal of Agricultural and Food Chemistry* **39**: 1252-1256

Antoniou, T.C. and Marquardt, R.R. (1982) Utilisation of rye diets by chicks as affected by lipid type and level and penicillin supplementation. *Poultry Science* **61**: 107-116

Applegate, T.J. and Lilburn, M.S. (2002) Growth of the femur and tibia of a commercial broiler line. *Poultry Science* **81**: 1289-1294

Association of Official Analytical Chemists (AOAC) (1990) Vitamin D_3 in poultry feed supplements. Method 932.16. Pages 1094-1095 in Official Methods of Analysis. 15th ed. AOAC, Arlington, VA.

Association of Official Analytical Chemists (AOAC) Official method 2001.11 (2007) Official methods of analysis, 18th Edition. Published by Association of official analytical chemists Inc., 2200 Wilson Boulevard, Arlington, Virginia, 222013301, USA.

Association of Official Analytical Chemists (AOAC) Official method 2003.05 (2007) Official methods of analysis, 18th Edition. Published by Association of official analytical chemists Inc., 2200 Wilson Boulevard, Arlington, Virginia, 222013301, USA.

Austin, S.C., Wiseman, J. and Chesson, A. (1999) Influence of non-starch polysaccharides structure on the metabolisable energy of UK wheat fed to poultry. Journal of Cereal Science **29**: 77-88

Aviagen (2007) Ross 308 Broiler performance objectives http://www.thepoultrysite.com/downloads/single/90/ accessed 31/5/2012

Azarfar , A., Jonker, A. and Hettiarachchi-Gamage, I.K. (2012) Nutrient profile and availability of co-products from bioethanol processing. *Journal of Animal Physiology and Nutrition* **96**: 450-458

Baird, F.D. and MacMillan, M.J. (1942) Use of toes rather than tibiae in A.O.A.C. chick methods of vitamin D determination. *Journal of the Association of Official Analytical Chemists* **25**: 518-524

Baker, D.H. and Molitoris, B.A. (1974) Utilization of Nitrogen from selected purines and pyrimidines and from urea by the young chick. *The Journal of Nutrition* **104**: 553-557

Bamforth, C.W. (2009) Current Perspectives on the role of enzymes in brewing. *Journal of Cereal Science* **50**: 353-357

Bandegan, A., Guenter, W., Hoehler, D., Crow, G.H and Nyachoti, C.M. (2009) Standardized ileal amino acid digestibility in wheat distillers dried grains with solubles for broilers. *Poultry Science* **88**: 2592-2599

Barbosa-Canovas, G.V., Ortega-Rivas, E., Juliano, P. and Yan, H. (2005). Food Powders: Physical Properties, Processing and Functionality. Kluwer Academic/Plenum Publishers, New York.

Batal, A. and Dale, N. (2003) Mineral Composition of Distillers dried grains with solubles. *Journal of Applied Poultry Research*. **12**: 400-403

Batal, A.B. and Dale, N.M. (2006) True metabolizable energy and amino acid digestibility of distillers dried grains with solubles. *Journal of Applied Poultry Research* **15**: 89-93.

Baurhoo, B., Ferket, P.R. and Zhao, X. (2009) Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations and carcass parameters in broilers. *Poultry Science* **88**: 2262-2272

Bedford, M.R. and Classen, H.L. (1992) Reduction of intestinal viscosity through manipulation of dietary rye and pentasanase concentration is effected through changes in carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and feed conversion efficiency of broiler chicks. *Journal of Nutrition* **122**: 560-569

Behnke, K.C. (2007) Feed manufacturing considerations for using DDGS in poultry and livestock diets. *Proceedings of the 5th Mid-Atlantic Nutrition Conference* Zimmerman, N.G. *ed*., University of Maryland pp 77-81

Behnke, K.C. and Beyer, R.S. (2002) Effect of feed processing on broiler performance. *VIII International Seminar on Poultry Production and Pathology*. Santiago, Chile.

Belyea, R.L., Rausch, K.D., Tumbleson, M.E. (2004) Composition of corn and distillers dried grains with solubles from dry grind processing. *Bioresource technology* **94**: 293-298

Belyea, R.L., Rausch, K.D., Clevenger, T.E., Singh, V., Johnston, D.B., Tumbleson, M.E. (2010) Sources of variation in composition of DDGS. *Animal Feed Science and Technology*. **159**: 122-130

Bennett, R.M., Christiansen, K. and Clifton-Hadley, R.S. (1999) Direct costs of endemic diseases of farm animals in Great Britain. *Veterinary Record* **145**: 376-377

Bethke, R.M., Steenbock, H. and Nelson, M.T. (1923) Fat soluble vitamins. XV. Calcium and phosphorus relations to growth and composition of blood and bone with varying vitamin intake. *Journal of Biological Chemistry* **58**: 71-103

Beyer, R.S., Moritz, J.S., Wilson, K.J. and Cramer, K.R. (2000) The effect of feed processing and feed from on animal performance. *Proceedings of the 61st Minnesota Nutrition Conference.* September 19-20, Bloomington, Minnesota pp225-235

Bhatty. R.S., MacGregor, A.W. and Rossnagal, B.G. (1991) Total and acid-soluble beta-glucan content of hulless barley and its relationship to acid-extract viscosity. *Cereal Chemistry* **68**: 221-227

Bilgili, S.F., Montenegro, G.I., Hess, J.B. and Eckman, M.K. (1999) Sand as a litter for rearing broiler chickens. *Journal of Applied Poultry Research* **8**: 345-351

Biofuels platform (2012) http://www.biofuels-platform.ch/en/infos/eu-bioethanol.php *accessed 28/5/2012*

Bjerrum , L., Pedersen, K. and Engberg, R.M. (2005) The influence of whole wheat feeding on salmonella infection and gut flora composition in broilers. *Avian Disease* **49**: 9-15

Boguhn, J. and Rodehutscord, M. (2010) Effects of nonstarch polysaccharide-hydrolyzing enzymes on performance and amino acid digestibility in turkeys. *Poultry Science* **89**: 505-513

Boling-Frankenbach, S. D., J. L. Snow, C. M. Parsons, and D. H. Baker (2001) The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets. *Poultry Science* **80**: 783-788.

Bonnardeaux, J. (2007) Potential uses for distillers grains. Department of Agriculture and Food, Western Australia. March 2007 *pp*3

Braun, E.J. and Dantzler, W.H. (1997) Vertebrate renal system. *In*: Handbook of Physiology. Bethesda, MD. *American Physiological Society* **1**: 481-576

Bringhurst, T.A., Fotheringham, A.L. and Brosnan J. (2003) Grain Whisky: Raw materials and processing. *In* Russel, I., Bamforth, C.W., Stewart, G. eds. Whisky: Technology, production and marketing. Academic press, London.

Brummer, M., Jansen van Rensburg, C. And Moran, C.A. (2010) *Saccharomyces cerevisiae* cell wall products: the effect on gut morphology and performance of broiler chickens. *South African Journal of Animal Science* **40**: 14-21

Burkitt, D.P., Walker, A.R. and Painter, N.S. (1972) Effect of dietary fibre on stools and transit times and its role in the causation of disease. *Lancet* **2**: 1408-1412

Butler, E.J. and Fenwick, G.R. (194) Trimethylamine and fishy taint in eggs. *World's Poultry Science Journal* **40**: 38-51

Caballero-Cordoba, G.M. and Sgarbieri, V.C. (2000) Nutritional and toxicological evaluation of yeast (Saccharomyces cerevisiae) biomass and a yeast protein concentrate. *Journal of the Science of food and Agriculture* **80**: 341-351

Calhoun, M. (1954). *Microscopic anatomy of the digestive system*. Ames: Iowa State College Press.

Campbell, G.L. and Bedford, M.R. (1992) Enzyme applications for monogastric feeds: A review. *Canadian Journal of Animal Science* **72**: 449-466

Campbell, G.L., Rossnagel, B.G. Classen, H.L. and Thacker, P.A.(1989) Genotypic and environmental differences in extract viscosity of barley and their relationship to its nutritive value for broiler chickens. *Animal Feed Science and Technology* **26**: 221-230

Campbell, G.L. and Bedford, M.R. (1992) Enzyme applications for monogastric feeds: A review. *Canadian Journal of Animal Science* **72**: 449-466

Carlson, M.S., Veum, T.L., Turk, J.R. (2005) Effects of yeast extract versus animal plasma in weanling pig diets on growth performance and intestinal morphology. *Journal of Swine Health and Production* **13** (4): 204-209

Chadd, S.A., Davies, W.P. and Koivisto, J.M. (2002) Practical production of protein for farm animals. From: *Protein sources for the animal feed industry, FAO proceedings*, Bangkok 29 April-3 May 2002

Chen, X.D., Mujunder, A.S. (2008) *Drying technologies in food processing*. Blackwell publishing, UK.

Cheng, T.K. and Coon, C.N. (1990) Research note: Calcium digestibility studies utilizing acid-insoluble ash measurements. *Poultry Science* **69**: 2228-2230

Chesson, A. (2001) Non-starch polysaccharide degrading enzymes in poultry diets: influence of ingredients on the selection of activities. *Worlds Poultry Science Journal* **57**: 251:263

Choct M. and Annison G. (1990) Antinutritive activity of wheat pentosans in broiler diets *British Poultry Science* **31**: 811-821

Choct, M. and Annison, G. (1992a) The inhibition of nutrient digestion by wheat pentosans. *British Journal of Nutrition* **67**: 123-132

Choct, M and Annison, G. (1992b) Anti-nutritive effect of wheat pentosans in broiler chickens: roles of viscosity and gut microflora. *British Poultry Science* **33**: 821-834

Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Morgan, A.J. and Annison, G. (1996) Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science* **37**: 609-621

Choct, M., Hughes, R.J. and Annison, G. (1999a) Apparent metabolisable energy and chemical composition of Australian wheat in relation to environmental factors. *Australian Journal of Agricultural Research* **50**: 447-451

Choct, M., Hughes, R.J. and Bedford, M.R. (1999b) Effects of a xylanase on individual bird variation, starch digestion throughout the intestine and ileal and caecal volatile fatty acid production in chickens fed wheat. *British Poultry Science* **40**: 419-422

Choct, M. and Kocher, A. (2000) Non-starch carbohydrates: Digestion and its secondary effects in monogastrics. *Proceedings of the 24th Annual meeting of the Nutrition Society of Australia*, Freemantle, Perth pp31-38

Choct, M. (2002) Non-starch polysaccharides: effect on nutritive value. In: *Poultry Feedstuffs*. McNab, J.M., Boorman,K.N. (*eds*). CABI Publishing, New York, USA.

Chodnik K.S. (1948) Cytology of the glands associated with the alimentary tract of the domestic fowl (*Gallus domesticus*). *Quarterly Journal of Microscopical Science* **89**: 75

Classen, H.L., Campbell, G.L. and Grootwassink, J.W.D. (1988) Improved feeding value of Saskatchewan-grown barley for broiler chickens with dietary enzyme supplementation. *Canadian Journal of Animal Science* **68**: 1253-1259

Classen, H.L. (1996) Cereal grain starch and exogenous enzymes in poultry diets. *Animal Feed Science and Technology* **62**: 21-27

Clementson, C.L. and Ileleji, K.E. (2010) Variability of bulk density of distillers dried grains with solubles (DDGS) during gravity-driven discharge. *Bioresource technology* **101:** 5459–5468

Cohen, A., Klasing, K. and Ricklefs, R. (2007) Measuring circulating antioxidants in wild birds. *Comparative Biochemistry and Physiology* **147**: 110-121

Correll, D. L. (1998) The role of phosphorus in the eutrophication of receiving waters: A Review. *Journal of Environmental Quality* **27**: 261-266.

Corzo, A., Schilling, M.W., Loar, R.E., Jackson, V.. Kin, S. and Radhakrishnan, V. (2009) Effects of feeding distillers dried grains with solubles on broiler meat quality. *Poultry Science* **88**: 432-439

Cottrill, B., Smith, C., Berry, P., Weightman, R., Wiseman, J., White, G., Temple, M. (2007) Opportunities and implications of using the co-products from biofuel production as feeds for livestock. *Research review No 66*. HGCA.

Coutteau, P., Lavens, P. and Sorgeloos, P. (1990) Baker's yeast as a potential substitute for live algae in aquaculture diets: Artemia as a case study. *Journal of the World Aquaculture Society* **21**: 1-9

Cowieson, A.J., Hruby,M. and Pierson, E.E.M. (2006) Evolving enzyme technology: impact on commercial poultry nutrition. *Nutrition Research Reviews* **19**: 90-103

Cozannet, P., Primot, Y., Gady, C., Metayer, J.P., Lessire, M., Skiba, F. and Noblet, J. (2010) Energy value of wheat distillers grains with solubles for growing pigs and adult sows. *Journal of Animal Science* **88**: 2382-2392

Cozannet, P., Lessire, M., Gady, C., Metayer, J.P., Primot, Y., Skiba, F. and Noblet, J. (2011a) Energy value of wheat distillers grains with solubles in roosters, broilers, layers and turkeys. *Poultry Science* **89**: 2230-2241

Cozannet, P., Primot, Y., Gady, C., Metayer, J.P., Lessire, M., Skiba, F. and Noblet, J. (2011b) Standardised amino acid digestibility of wheat distillers dried grains with solubles in force-fed cockerels. *British Poultry Science* **52**: 72-81

Crane, R., Davenport, R. and Vaughan, R. (2011) Farm business survey, poultry production in England 2009/10. Rural business research unit, Reading University, UK.

Cromwell, G.L., Herkelman, K.L. Stahly, T.S. (1993) Physical, Chemical and Nutritional characteristics of distillers dried grains with solubles for chicks and pigs. *Journal of Animal Science* **71**: 679-686

Cuirescu, G., Moldovan, I., Vasile, A. (2001) Bioproductive effect of a protein concentrate on the performance of broiler chicks. *Archiva Zootechnica* **6**: 83-88

Daghir, N.J. and Abdul-Baki, T.K. (1977) Yeast protein in broiler rations. *Poultry Science* **56**: 1836-1841

Daghir, N.J. and Sell, J.L. (1982) Amino acid limitations of yeast single-cell protein for growing chickens. *Poultry Science* **61** (2): 337-344

Dale, N. M. and Garcia, A. R. (2004) Evaluation of foot ash as an alternative to tibia bone ash for quantifying bone mineralization. *Abstracts International Poultry Scientific Forum*, Atlanta, GA. pp 29

Dalgleish, J. McN. (1990) Freeze drying for the food industries. New York, Elsevier.

Daniel, T. C., Sharpley, A. N. and Lemunyon, J. L. (1998) Agricultural phosphorus and eutrophication: A symposium overview. *Journal of Environmental Quality* **27**: 251-257.

Davis, M.E., Maxwell, C.V., Erf, G.F., Brown, D.C. and Wistuba, T.J. (2004) Dietary supplementation with phosphorylated mannans improves growth response and modulates immune function of weanling pigs. *Journal of Animal Science* **82**: 1882-1891

DEFRA (2011a) Crop areas, livestock populations and agricultural workforce 2011 England – final results. http://www.defra.gov.uk/statistics/files/defra-stats-foodfarm-landuselivestock-june-statsrelease-englandcropslivestocklabour-111024.pdf
accessed 21/12/11

DEFRA (2011b) GB animal feed statistical notice - October 2011
http://www.defra.gov.uk/statistics/files/defra-stats-foodfarm-food-animalfeed-statsnotice-111208.pdf
accessed 21/12/11

Defra (2012) Defra statistics September 2012. www.Defra-stats-foodfarm-food-poultry-meat-production-120927 accessed 09/10/12

Delacour, J.A., Van Win, H. and Grobet, P.J. (1999) Distribution and structural variation of arabinoxylans in common wheat mill streams. *Journal of Agricultural and Food Chemistry* **47**: 271-275

Denstadli, V., Westereng, B., Biniyam, H.G., Balance, S., Knutsen, S.H. and Svihus, B. (2010a) Effects of structure and xylanase treatment of brewer's spent grain on performance and nutrient availability in broiler chickens. *British Poultry Science* **51**: 419-426

Denstadli, V., Balance, S., Knutsen, S.H., Westereng, B. And Svihus, B. (2010b) Influence of graded levels of brewers dried grains on pellet quality and performance in broiler chickens.
Department for Transport (2012) Verified 2010/11 RTFO report
http://assets.dft.gov.uk/statistics/releases/verified-rtfo-biofuel-statistics-2010-11/year-3-verified-report.pdf
accessed 28/5/2012

Dilworth, B.C. and Day, E.J. (1965) Effect of varying dietary calcium: phosphorus ratios on tibia and femur composition of the chick. *Poultry Science* **44**: 1474-1479

Donkoh, A. and Attoh-Kotoku, V. (2009) Nutritive value of feedstuffs for poultry in Ghana: chemical composition, apparent metabolizable energy and ileal amino acid digestibility. *Livestock Research for Rural Development* **21**: 32

Donkoh, A. and Moughan, P.J. (1999)Endogenous ileal nitrogen and amino acid flows in the growing pig receiving a protein-free diet and diets containing enzymatically hydrolysed casein or graded levels of meat and bone meal. *Animal Science* **68**: 511-518

Dozier III, W.A., Purswell, J.L., Kidd, M., Corzo, A. and Branton, S.L. (2007) Apparent metabolizable energy needs of broilers from 2.0 to 4.0 kg as influenced by ambient temperature. *Journal of Applied Poultry Research* **16**: 206-218.

Duke, G.E. (1986a) Alimentary canal: anatomy, regulation of feeding and motility. In: *Avian physiology*, 4th edition, (ed. P.D. Sturkie) Springer Verlag, New York. 269-288.

Duke, G.E. (1986b) Alimentary canal: Secretion and digestion, special digestive functions and absorption. In: *Avian physiology*, 4th edition (ed. P.D. Stukie) Springer Verlag, New York. 289-302

Dunham, J. (2009) Economic impact study. www.meatfuelsamerica.com accessed 9/10/12

Elmusharaf, M., Peek, H.W., Nollet, L. and Beynen, A.C. (2007) The effect of an in feed mannanoligosaccharide preparation (MOS) of a coccidiosis infection in broilers. *Animal Feed Science and Technology* **134**: 347-354

Emiola, I.A., Opopeju, F.O., Slominski, B.A., Nyachoti, C.M. (2009) Growth performance and nutrient digestibility in pigs fed wheat DDGS-based diets supplemented with a multicarbohydrase enzyme. *Journal of Animal Science* **87**: 2315-2322

Ergul, T., Martinez Amezcua, C., Parsons, C.M., Walters, B., Brannon, J. and Noll. S.L. (2003) Amino acid digestibility in corn distillers dried grains with solubles. *Poultry Science* **82** (Supplement 1): 70

Essa, M.A., Mabrouk, H.A., Mohamed, R.A. and Michael, F.R. (2011) Evaluating different additive levels of yeast, *Saccharomyces cerevisiae*, on the growth and production performances of a hybrid of two populations of Egyptian African catfish, *Clarias gariepinus*. *Aquaculture* **320**: 137-141

EU (1990) Council Directive 90/167/EEC of 26 March 1990 laying down the conditions governing the preparation, placing on the market and use of medicated feeding stuffs in the Community. *Official Journal of the European Union.*

EU (2002) Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. *Official Journal of the European Union.*

EU (2003a). Directive 2003/30/EC of the European Parliament and of the Council of 8 May 2003 on the promotion of the use of biofuels or other renewable fuels for transport. Official Journal of the European Union.

EU (2003b) Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. *Official Journal of the European Union.*

European Bioethanol Fuel Association (2012) http://www.epure.org/statistics/info/Productiondata *accessed 12/7/12*

Evans, R.J. and Carver, J.S. (1944) The tie ash as a measure of calcification in chicks. *Poultry Science* **23**: 351-352

FAO (2010) Food and Agriculture Organisation statistics http://faostat.fao.org/site/339/default.aspx *accessed 28/5/2012*

FAO (2012) Oilseeds, oils and meals: Monthly price update No.35. May 2012. http://www.fao.org/fileadmin/templates/est/COMM_MARKETS_MONITORING/Oilcrops/Documents/MPPU_may_12.pdf *accessed 1/6/2012*

Farner, D.S. (1943) Biliary amylase in the domestic fowl. *Biology Bulletin*. (Woods Hol, Mass.) **84**: 240

Fastinger. N.D., Latshaw, J.D. and Mahan, D.C. (2006) Amino acid availability and true metabolisable energy content of corn distillers dried grains with solubles in adult cecectomised roosters. *Poultry Science* **85**: 1212-1216

FEFAC (2008) Antibiotic residues in Brazilian feed yeasts. *European feed manufacturers federation statement*, 28-8-2008.

Feltwell, R. and Fox, S. (1978). Poultry Nutrition: the process and the factory. In: *Practical Poultry Feeding*. Faber and Faber Ltd, London. Pp.15-35.

Fengler, A.I. and Marquardt, R.R (1988) Water-soluble pentosans from rye: II. Effects on rate of dialysis and on the retention of nutrients in the chick. *Cereal Chemistry* **65**(4): 298-302

Fernandez, S.R. and Parsons, C.M. (1996) Bioavailability of the digestible lysine and valine in cottonseed meal and soya bean meals for chicks. *Poultry Science* **75**: 216-223

Food Ethics Council (2007) Meat consumption: Trends and environmental implications. www.foodethicscouncil.org/system/files/businessforum201107.pdf accessed 28 June 2010

Fuller, M.F. (1988) Methods of protein evaluation for non-ruminants. In: *Feed Science*. Orskov, E.R. (Ed.) Elsevier Science Publishers: New York. pp. 81-101

Ganesan, V., Muthukumarappan, K. and Rosentrater, K.A. (2008) Effect of moisture content and soluble level of the physical, chemical and flow properties of distiller's dried grains with solubles (DDGS). *Cereal Chemistry* **85**: 466-472

Gao, J., Zhang, H.J., Yu, S.H., Wu, S.G., Yoon, I., Quigley, J., Gao, Y.P. and Qi, G.H. (2008) Effects of yeast culture in broiler diets on performance and immunomodulatory functions. *Poultry Science* **87**: 1377-1384

Garcia, A. R. and Dale, N. M. (2006) Foot ash as a means of quantifying bone mineralization in chicks. *Journal of Applied Poultry Research* **15**: 103-109.

Garcia, A.R., Batal, A.B. and Dale, N.M. (2007) A Comparison of Methods to determine amino acid digestibility of feed ingredients for chickens. *Poultry Science* **86**: 94-101

Garcia, M., Lazaro, R., Latorre, M.A., Gracia, M.I. and Mateos, G.G. (2008) Influence of enzyme supplementation and heat processing of barley on digestive traits and productive performance of broilers. *Poultry Science* **87**: 940-948

Gause, B. and Trushenski, J. (2011a) Replacement of fish meal with ethanol yeast in the diets of Sunshine Bass. *North American Journal of Aquaculture* **73**: 97-103

Gause, B. and Trushenski, J. (2011b) Production performance and stress tolerance of sunshine bass raised on reduced fish meal feeds containing ethanol yeast. *North American Journal of Aquaculture* **73**: 168-175

GEA Westfalia, 2011. http://www.westfalia-separator.com/products/decanters.html

Geier, M.S., Tork, V.A., Allison, G.E., Ophel-Keller, K. and Hughes, R.J. (2009) Indigestible carbohydrates alter the intestinal microbiota but do not influence the performance of broiler chickens. *Journal of Applied Microbiology* **106**: 1540-1548

Giec, A. and Skupin, J. (1988) Single cell protein as food and feed. *Die Nahrung* **32** (3): 219-229

Gilbert, R. (2002) The world animal feed industry. *From: Protein sources for the animal feed industry, FAO proceedings*, Bangkok 29 April-3 May 2002

GMO Compass (2012)
http://www.gmocompass.org/eng/grocery_shopping/processed_foods/153.animal_feed_genetic_engineering.html
Accessed 12/7/12

Graff, C., Clayton, D.A. and Larsson, N.G. (1999) Mitochondrial medicine – recent advances. *Journal of Internal Medicine* **246**: 11-23

Grosjean E., Rasmussen, R.A. and Grosjean D. (1998) Ambient levels of gas phase pollutants in Porto Alegre, Brazil. *Atmospheric environment* **32** (20):3371-3379

Gunaseelan, V.N. (1997) Anaerobic digestion of biomass for methane production: A review. *Biomass and Bioenergy* **13** (1-2): 83-114.

Gutierrez-Alamo, A., Perez de ayala, P., Verstegen, M.W.A., Den hartog, L.A. and Villamide, J. (2008) Variability in wheat : Factors affecting its nutritional value. *World's Poultry Science Journal* **64** : 20-40

Haldar, S., Ghosh, T. K., Toshiwati and Bedford M. R. (2011) Effects of yeast (*Saccharomyces cerevisiae*) and yeast protein concentrate on production performance of broiler chickens exposed to heat stress and challenged with *Salmonella enteritidis*. *Animal Feed Science and Technology* **168**: 61-71.

Hall, L.E., Shirley, R.B., Bakalli, R.I., Aggrey, S.E., Pesti, G.M. and Edwards Jr, H.M.(2003) Power of two methods for the estimation of bone ash. *Poultry Science* **82**: 414-418

Han, J. and Liu, K. (2010) Changes in composition and amino acid profile during dry grind ethanol processing from corn and estimation of yeast contribution towards DDGS proteins. *Journal of Agricultural and Food Chemistry* **58**: 3430-3437

Halnan, E.T. (1930) The role of fibre in poultry feeding. *Proceedings of the 4th World Poultry Congress*, pp221-228

Hardy, R.W. (2010) Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. *Aquaculture Research* **41**: 770-776
Heany, R.P. (1998) Excess dietary protein may not adversely affect bone. *Journal of Nutrition* **128**: 1054-1057

Hedemann, M.S., Theil, P.K., Bach-Knudsen, K.E. (2009) The thickness of the intestinal mucous layer in the colon of rats fed various sources of non-digestible carbohydrates is positively correlated with the pool of SCFA but negatively correlated with the proportion of butyric acid in digesta. *British Journal of Nutrition* **102**: 117-125

Hellstein, Y., Tullson, P.C., Richter, E.A. and Bangsbo, J. (1997) Oxidation of urate in human skeletal muscle during exercise. Free Radical Biology and Medicine **22**: 169-174

Hetland, H., Svihus, B. and Krogdahl, A. (2003) Effects of oat hulls and wood shavings on digestion in broilers and layers fed diets based on whole or ground wheat. *British Poultry Science* **44**: 275-282

Hetland, H., Svihus, B. and Choct, M. (2005) Role of insoluble fibre on gizzard activity in layers. *Journal of Applied Poultry Research* **14**: 38-46

Hevia, P. and Clifford, A.J. (1977) Protein intake, uric acid metabolism and protein efficiency ratio in growing chicks. *Journal of Nutrition* **107**: 959-964

Hew, L.I., Ravindran, V., Mollah, Y. And Bryden, W.L. (1998) Influence of exogenous xylanase supplementation on apparent metabolisable energy and amino acid digestibility in wheat for broiler chickens. *Animal Feed Science and Technology* **75**: 83-92

Hill, K.J. (1976). The anatomy and general physiology of the alimentary tract. In: *Digestion in the fowl*. (Eds K.N. Boorman and B.M. Freeman). Poultry Science Symposium, No.11. British Poultry Science Ltd. Edinburgh, 3-24.

Holtekjølen′ A.K., Uhlen, A.K., Brathen, E., Sahlstrom, S. and Knutsen, S.H. (2006) Contents of starch and non-starch polysaccharides in barley varieties of different origin. *Food Chemistry* **94** (3): 348-358

Hooge, D.M., Sims, M.D., Sefton, A.E., Connolly, A. and Spring, P.S. (2003) Effect of dietary mannanoligosaccharide, with and without bacitracin or virginiamycin, on live performance of broiler chickens at relatively high stocking density on new litter. *Journal of applied poultry research* **12**: 461-467

Huang, K.H., Ravindran, V., Li, X. And Bryden, W.L. (2005) Influence of age on the apparent ileal amino acid digestibility of feed ingredients for broiler chickens. *British Poultry Science* **46**: 236-245

Huang, K.H., Ravindran, V., Xiuhua, L., Ravindran, G. and Bryden, W.L. (2007) Apparent ileal digestibility of amino acids in feed ingredients determined with broilers and layers. *Journal of the Science of Food and Agriculture* **87**: 47-53

Huff, W.E. (1980) Discrepancies between bone as and toe ash during aflatoxicosis. *Poultry Science* **59**: 2213-2215

Hulan, H.W. and Bird, F.H. (1972). The effect of fat level in isonitrogenous diets on the composition of avian pancreatic juice. *Journal of Nutrition* **102**: 459-468

Huyghebaert, G., Ducatelle, R. and Van Immerseel, F.V. (2011) An update on alternatives to antimicrobial growth promoters for broilers. *The Veterinary Journal* **187**: 182-188

Hynes, S.H., Kjarsgaard, D.M., Thomas, K.C. and Ingledew, W.M. (1997) Use of virginiamycin to control the growth of lactic acid bacteria during alcoholic fermentation. *Journal of Industrial Microbiology and Biotechnology* **18**: 284-291

Ileleji, K.E., Prakash, K.S., Stroshine, R.L. and Clementson, C.L. (2007) An investigation of particle segregation in corn processed dried distillers grains with solubles (DDGS) induced by three handling scenarios. *Bulk Solids Powder Science Technology* **2**: 84–94.

Ileleji, K.E. and Rosentrater, K.A. (2008) On the Physical Properties of Dried Distillers Grains with Solubles (DDGS). *American Society for Agricultural Engineers* Paper No. 084576. ASAE, St. Joseph, MI.

Ingledew, W.M. (1999) Yeast – Could you base a business on this bug? In: Under the microscope – Focal points for the new millennium. Biotechnology in the feed industry. *Proceedings of the 15th Alltech Annual Symposium*. Nottingham University Press, Nottingham, UK. pp 27-50

Itoh, H. and Hatano, T. (1964) Comparison of calcium metabolism in various bones of growing chicks in varying states of vitamin D supplementation. *Poultry Science* **43**: 70-76

Jackson, M.E., Anderson, D.M., Hsiao, H.Y., Mathis, G.F. and Fodge, D.W. (2003) Beneficial effect of β-mannanase feed enzyme on performance of chicks challenged with *Eimeria sp*. and Clostridium perfringens. *Avian Diseases* **47**: 759–763.

Jacques, K.A. (2003) Ethanol Production and the modern livestock feed industry In: *The Alcohol Textbook*. (Eds. K.A., Lyons, T.P., Kelsall, D.R.) 4th Edition Nottingham University Press, Nottingham, UK.

Jagger, S., Wiseman, J., Cole, D.J.A and Craigon, J. (1992) Evaluation of inert markers for the determination of ileal and faecal apparent digestibility values in the pig. *British Journal of Nutrition* **68**: 729-739.

Jimenez-Moreno, E., Gonzalez-Alvarado, J.M., Gonzalez-Serrano, A., Lazaro, R and Mateos, G.G. (2009) Effect of dietary fibre and fat on performance and digestive traits of broilers from one to twenty-one days of age. *Poultry Science* **88**: 2562-2574

Jimenez-Moreno, E., Gonzalez-Alvarado, J.M., Gonzalez-Sanchez, D., Lazaro, R and Mateos, G.G. (2010) Effects of type and particle size of dietary fibre on growth performance and digestive traits of broilers from 1 to 21 days of age. *Poultry Science* **89**: 2197-2212

Jiranek, V., Langridge, P. and Henschke, P.A. (1995) Amino acid and ammonium utilization by *Saccharomyces cerevisiae* wine yeasts from chemically defined medium. *American Journal of Enology and Viticulture* **46**: 75-83

Jones, C. K., Bergstrom, J. R., Tokach, M. D., DeRouchey, J. M., Goodband, R. D., Nelssen, J. L. and Dritz, S. S. (2010) Efficacy of commercial enzymes in diets containing various concentrations and sources of dried distillers grains with soluble for nursery pigs. *Journal of Animal Science* **88**: 2084–2091.

Juanpere, J., Perez-Vendrell, A.M., Angulo, E. and Brufau, J. (2005) Assessment of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. *Poultry Science* **84**: 571-580

Karel, M. (1975) Principles of Food Science, Part II. *In*: Food process engineering (4th Edition) Englewood Cliffs, NJ: Prentice-Hall.

Kelshall D.R. and Lyons, T.P. (2003) Practical management of yeast : conversion of sugars to ethanol. *In* K.A. Jacques, T.P. Lyons, D.R. Kelshall eds. The Alcohol Textbook. Nottingham University Press.

Kim, W.K. and Patterson, P.H. (2003) Effect of minerals on activity of microbial uricase to reduce ammonia volatilization in poultry manure. *Poultry Science* **82**: 223-231

Kim, W. K., Donalson, L. M., Herrera, P., Woodward, C. L., Kubena, L. F., Nisbet, D. J. and Ricke, S. C. (2004) Effects of different bone preparation methods (fresh, dry, and fat-free dry) on bone parameters and the correlations between bone breaking strength and the other bone parameters. *Poultry Science* **83**: 1663-1666.

Kim, E.J., Martinez Amezcua, C., Utterback, P.L. and Parsons, C.M. (2008) Phosphorus bioavailability, true metabolizable energy, and amino acid digestibilities of high protein corn distillers dried grains and dehydrated corn germ. *Poultry Science* **87**: 700-705.

Kim, E.J., Parsons, C.M., Srinivasan, R. and Singh, V. (2010) Nutritional composition, nitrogen-corrected true metabolisable energy and amino acid digestibilities of new corn distillers grains with solubles produced by a new fractionation process. *Poultry Science* **89**: 44-51

Kim, E.J. and Corzo, A. (2012) Interactive effects of age, sex and strain on apparent ileal amino acid digestibility of soybean meal and an animal by-product blend in broilers. *Poultry Science* **91**: 908-917

Kim, E.J., Utterback, P.L. and Parsons, C.M. (2012) Comparison of amino acid digestibility coefficients for soybean meal, canola meal, fish meal and meat and bone meal among 3 different bioassays. *Poultry Science* **91**: 1350-1355

Kingsly, A.R.P., Ileleji, K.E., Clementson, C.L., Garcia, A., Maier, D.E., Stroshine, R.L., and Radcliff, S. (2010) The effect of process variables during drying on the physical and chemical characteristics of corn dried distillers grains with solubles (DDGS) – Plant scale experiments *Bioresource Technology* **101:** 193–199

Klose, A.A. and Fevold, H.L. (1945) Nutritional value of yeast protein to the rat and the chick. *The Journal of Nutrition* **29** (6): 421-430

Kluth, H., Mehlhorn, K. and Rodehutscord, M. (2005) Studies on the intestine section to be sampled in broiler studies on precaecal amino acid digestibility. *Archives of Animal Nutrition* **59**: 271-279

Kluth, H., Fricke, M. And Rodehutscord, M. (2009) Precaecal amino acid digestibility of different wheat cultivars in broilers. *Archiv für Geflügelkunde* **73** (2): 80-86

Kluth, H. and Rodehutscord, M. (2010) Effect of the duration of prefeeding on amino acid digestibility of wheat distillers dried grains with solubles in broiler chickens. *Poultry Science* **89**: 681-687

Knott, J.S. and Shurson, G.C. (2004) Nutritional value of yeast by-products from Ethanol Production for young pigs. *Proceedings of the 65th Minnesota Nutrition Conference*, St. Paul, MN. **65** : 142-159

Kogan, G. and Kocher, A. (2007) Role of yeast cell wall polysaccharides in pig nutrition and health protection. *Livestock Science* **109**: 161-165

Labuza, T.P. and Baisier, W.M. (1992) The kinetics of nonenzymatic browning. In: *Physical Chemistry of Foods.* Hartel, R.W. (*Ed*.) Dekker Marcel Publishers (p. 596).

Lan, Y., Opapeju, F.O., Nyachoti, C.M. (2008) True ileal protein and amino acid digestibilities in wheat dried distiller's grains with solubles fed to finishing pigs. *Animal Feed Science and technology* **140**: 155-163

Larbier, M. and Leclercq, B. (1994). Raw materials employed in poultry production. In: *Nutrition and Feeding of Poultry*. J. Wiseman (Ed.) (1994). Nottingham University Press, pp. 223-265

Lavinia, S., Dan, D., Eliza, S., Ducu, S., Calin, J. and Cosmin, P. (2010) Control of the anti-nutritive effect of non-starch polysaccharides from barley based combined forage in broilers. *Journal of Food Agriculture and Environment*. **8**: 369-375

Lei (2012) http://www.allaboutfeed.net/news/algae-as-sustainable-protein-alternative-for-animal-feed-12701.html *accessed 12/7/12*

Lemme, A., Cremers, S., Pallauf, J., Rostagno, H.S., Pack, M. and Petri, A. (2001) Apparent and true ileal amino acid digestibility of vegetable and animal protein of different origin in broilers. *Proceedings of the 13th European Symposium on Poultry Nutrition*, 30th Sept-4th Oct. Blankenberge, Belgium. pp 169-170

Lemme, A., Ravindran, V. and Bryden, W.L. (2004) Ileal digestibility of amino acids in feed ingredients for broilers *World's Poultry Science Journal* **60**: 423-437

Leytem, A.B., Kwanyuen, P., Thacker, P. (2008) Nutrient Excretion, Phosphorus characterisation, and phosphorus solubility in excreta from broiler chicks fed diets containing graded levels of wheat distillers grains with solubles. *Poultry Science* **87**: 2505-2511

Li, S., Sauer, W.C. and Fan, M.Z. (1993) The effect of dietary crude protein levels on amino acid digestibility in early weaned pigs. *Journal of Animal Physiology and Nutrition* **70**: 26-37

Li, Y.M., McAllister, T.A., Beauchemin, K.A., McKinnon, J.J. and Yang, W.Z. (2011) Substitution of wheat dried distillers grains with solubles for barley grain or barley silage in feedlot cattle diets: intake, digestibility and ruminal fermentation. *Journal of Animal Science* **89**: 2491-2501

Lin Hsu, C., Chen, W., Weng, Y.M. and Tseng, C.Y. (2003) Chemical composition, physical properties and antioxidant activities of yam flours as affected by different drying methods. *Food Chemistry* **83**: 85-92

Liu, K. (2011) Chemical composition of distiller's grains, a review. *Journal of Agricultural and Food Chemistry* **59**: 1508-1526

Liu, K. and Han, J. (2011) Changes in mineral concentrations and phosphorus profile during dry grind processing of corn into ethanol. *Bioresource Technology* **102**: 3110-3118

Loar, R.E., Srinivasan, R., Kidd, M.T., Dozier, W.A. and Corzo, A. (2009) Effects of elutriation and sieving processing (Elusieve) of distillers dried grains with solubles on the performance and carcass characteristics of male broilers. *Journal of Applied Poultry Research* **18**: 494-500

Loar, R.E., Moritz, J.S., Donaldson, J.R. and Corzo, A. (2010) Effects of feeding distillers dried grains with solubles to broilers from 0 to 28 days post hatch on broiler performance, fee manufacturing efficiency and selected intestinal characteristics. *Poultry Science* **89**: 2242-2250

Loar, R.E. and Corzo, A. (2011)Effects of feed formulation on feed manufacturing and pellet quality characteristics of poultry diets. *World's Poultry Science Journal* **67**: 19-28

Lopez, J.D. and Pascual, J.L.M. (1981) Influence of the drying process on the composition of brewers dried grains. *Animal Feed Science and Technology* **6**: 163-168

Low, A.G. (1990) Protein evaluation in pigs and poultry. In: *Feedstuff Evaluation*. Wiseman, J. and Cole, D. (Eds.) D. Butterworths, London. pp 91-114
Lumpkins, B., Batal, A. and Dale, N. (2004) Evaluation of distillers dried grains with solubles as a feed ingredient for broilers. *Poultry Science* 83: 1891-1896

Lumpkins, B., Batal, A., Dale, N. (2005) Use of Distillers dried grains plus solubles in laying hen diets. *Journal of Applied Poultry Research* **14**: 25-31

Lumpkins, B.S. and Batal, A.B. (2005) The Bioavailability of Lysine and Phosphorus in Distillers Dried grains with solubles. *Poultry Science* **84**: 581-586

Luna-Solano, G., Salgado-Cervantes, M.A., Ramirez-Lepe, M. *et al*., Garcia-Alvarado, M.A. and Rodriguez-Jimenes, G.C. (2003) Effect of drying type and drying conditions over fermentative ability of brewer's yeast. *Journal of Food Process Engineering* **26**: 135-147

Luna-Solano, G., Salgado-Cervantes, M.A., Rodriguez-Jimenes, G.C. and Garcia-Alvarado, M.A. (2005) Optimisation of brewer's yeast spray drying process. *Journal of Food Engineering* **68**: 9-18

Lynd, L.R.(1996) Overview and evaluation of fuel ethanol from cellulosic biomass: Technology, Economics, the Environment, and Policy. *Annual review of energy and the environment* **21**: 403-465

Magdelaine, P., Spiess, M. P. and Valceschini, E. (2008) Poultry meat consumption trends in Europe. *World's Poultry Science Journal* **64**: 53-63

Mangold, E. (1934) The digestion and utilization of crude fibre. *Nutrition abstract reviews* **3**: 647-656

Martinez-Amezcua, C., Parsons, C.M. and Noll, S.L. (2004) Content and relative bioavailability of phosphorus in distiller's dried grains with solubles in chicks. *Poultry Science* **83**: 971-975
Martins, S.I.F.S., Jongen, W.M.F. and van Boekel, M.A.J.S. (2001) A review of Malliard reactions in food and implications to kinetic modelling. *Trends in Food Science and Technology* **11**: 364-373

Martland, M.F. (1984) Wet litter as a cause of plantar pododermititis, leading to foot ulceration and lameness in fattening turkeys. *Avian Pathology* **13**: 241-252

Masa'deh, M.K., Purdum, S.E. and Hanford, K.J. (2011) Dried distillers grains with solubles in laying hen diets. *Poultry Science* **90**: 1960-1966

Mathlouthi, M., and Roge, B. (2003) Water vapour sorption isotherms and the caking of food powders. *Food Chemistry* **82**: 61–71.

McDonnell, P., O'Shea, C.J., Callan, J.J. and O'Doherty, J.V. (2011) The response of growth performance, nitrogen and phosphorus excretion of growing-finisher pigs to diets containing incremental levels of maize dried distiller's grains with solubles. *Animal Feed Science and Technology* **169**: 104-112

Mcfarland, D.C., Kenzy, S.G. and Coon, C.N. (1979) A micromethod for plasma uric acid determinations in companion birds. *Avian Diseases* **23** (3): 772-774

McGraw, K.J. and Ardia, D.R. (2004) Immunoregulatory activity of different dietary carotenoids in male zebra finches. *Chemoecology* **14**: 25-29

McNab, J.M. (1989) Measuring availability of amino acids from digestibility experiments. *Proceedings of the 7th European Symposium on Poultry Nutrition*, Girona, Spain pp. 45-53

McNab, J.M. (1995) Amino acid digestibilities: determination and application to poultry. In: *Recent Advances in Nutrition in Australia*. Rowe J.B. and Nolan J.V.. (Eds.) The University of New England, Armidale, NSW. pp7-13

Mendez, A. and Dale, N. (1998) Foot ash as a parameter to assay bone mineralization. *Poultry Science* **77** (Suppl. 1): 40. (Abstract).

Mendez, A., Dale, N. and Garcia, M. (1998) Comparison of parameters to assay bone mineralization. *Poultry Science* **77** (Suppl. 1): S176. (Abstract).

Meng, X., Slominski, B.A. and Guenter, W. (2004) The effect of fat type, carbohydrase and lipase addition on growth performance and nutrient utilisation of young broilers fed wheat based diets. *Poultry Science* **83**: 1718-1727

Meyers, W.D., Ludden, P.A., Nayigihugu, V. and Hess, B.W. (2003a) A comparison of titanium dioxide to chromic oxide as a site and extent of digestion marker in ewes. *University of Wyoming Annual Animal Science Research Report.* Pages 56-60.

Meyers, W.D., Ludden, P.A., Nayigihugu, V. and Hess, B.W. (2003b) A rapid analytical procedure for quantitative determination of titanium dioxide. *University of Wyoming Annual Animal Science Research Report*. Pages 61-65.

Miazzo, R.D., Peralta, M.F., Picco, M.L. and Nilson, A.J. (2005) Productive parameters and carcass quality of broiler chickens fed yeast (*S. cerevisiae*). *XVIIth European Symposium on the Quality of Poultry meat*, Doorwerth, The Netherlands. 23-26 May 2005

Miles, R.D. and Featherston, W.R. (1976) Uric acid excretion by the chick as an indicator of dietary protein quality. *Journal of Poultry Science* **55**: 96-102

Montgomery, R. and Smith, F. (1956) A review of carbohydrates of wheat and other cereal grains. *Journal of Agricultural and Food Chemistry* **4** (8): 716-720

Morales-Lopez, R., Auclair, E., Garcia, F., Esteve-Garcia, E. and Brufau, J. (2009) Use of Yeast cell walls : Beta-1, 3/1, 6-glucans; and mannoproteins in broiler chicken diets. *Poultry Science* **88**: 601-607

Moralez F.J., Acar, O.C., Serpen,A., Arribas-Lorenzo, G. and Gokmen, V. (2007) Degradation of free tryptophan in a cookie model system and its application in commercial samples. *Journal of Agricultural and Food Chemistry* **55**: 6793-6797

Moran, E.T.Jr. and Todd, M.C. (1994) Continual submarginal phosphorus with broilers and the effect of preslaughter transportation: carcass defects, further-processing yields, and tibia-femur integrity. *Poultry Science* **73**: 1448-1457

Morgan, N., Walk, C., Bedford, M.R. and Burton, E.J. (2012) Optimum methodology for determining broiler gastrointestinal pH. *British Poultry Abstracts* pp10

Morris, A., Barnett, A. and Burrows, O.J. (2004) Effect of processing on nutrient content of foods. *Cajanus* 37 (3): 160-164

Mustafa, A.F., McKinnon, J.J. and Christensen, D.A. (1999) Chemical characterisation and in vitro crude protein degradability of thin stillage derived from barley and wheat based ethanol production. *Animal Feed Science and Technology* **80**: 247-256

Muthaiyan, A. and Ricke, S.C. (2010) Current perspectives on detection of microbial contamination in bioethanol fermenters. *Bioresource Technology* 101: 5033–5042.

Nanney, R.L., Ferket, P.R., Santos, A.A., Oviedo, E.O., Grimes, J.L. and Parsons, C. (2007) Nutritional value of yeast extract Nupro in male broiler chicks and turkey poults. *International poultry Scientific forum*, Georgia Jan 22-23, 2007. Abstract M29

National Research Council (NRC) (1984) Nutrient Requirements of Poultry, 8th Edition. National Academy of Sciences, Washington.

National Research Council.(1994) 1. Components of Poultry Diets. *Nutrient Requirements of Poultry: Ninth Revised Edition* Washington, DC: The National Academies Press.

Niessen, W.R. (2002) Combustion and incineration processes. CRC Press. p. 588

Nir, I., Shefet, G. and Aaroni, Y. (1994) Effect of particle size on performance. 1. Corn. *Poultry Science* 73: 45-49

Nir,I. and Ptichi, I. (2001) Feed particle size and hardness: Influence on performance, nutritional behaviour and metabolic aspects. *Proceedings of the 1st World Feed Conference*, Utrecht, The Netherlands pp 157-186

Noll, S.L., Brannon, J. And Parsons, C. (2007) Nutritional value of corn distillers dried grains with solubles (DGS): Influence of solubles addition. *Proceedings of ADSA/ASAS Joint Annual Meeting*, San Antonio, Texas. Abstract M204

Novozymes (2009) Viscozyme. www.novozymes.com/en/MainStructure/ProductsAndSolutions/Fuel+ethanol/Viscosity+reduction/Viscozyme/Viscozyme.htm

Nyachoti, C.M., Haouse, J.D., Slominski, B.A., Seddon, I.R. (2005) Energy and nutrient digestibilities in wheat dried distillers grains with solubles fed to growing pigs. *Journal of the Science of Food and Agriculture*. **85**: 2581-2586

Oliva-Teles, A. and Goncalves, P. (2001) Partial replacement of fishmeal by brewer's yeast (*Saccharomyces cerevisiae*) in diets for sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture* **202**: 269-278

Olmstead, J. (2009) Fuelling Resistance? Antibiotics in ethanol production. *Institute of agricultural and trade policy*, Minneapolis, MN. July 2009.

Olukosi, O.A., Cowieson, A.J. and Adeola, O. (2007) Age-related influence of a cocktail of xylanase, amylase and protease or phytase individually or in combination in broilers. *Poultry Science* **86**: 77-86

Omar, S.S., Merrifield, D.L., Kuhlwein, H., Williams, P.E.V. and Davies, S.J. (2012) Biofuel derived yeast protein concentrate (YPC) as a novel feed ingredient in carp diets. *Aquaculture* **330-333** : 54-62

Onol, A.G. and Yalcin, S. (1995) The usage of baker's yeast in laying hen rations. *Veterinary Journal of Ankara University* **42** : 161-167

Orban, J. I., Roland, D. A., Bryant, M. M. and Williams, J. C. (1993) Factors influencing bone mineral content, density, breaking strength, and ash as response criteria for assessing bone quality in chickens. *Poultry Science* **72**: 437-446.

Ortin, W.G.N. and Yu, P. (2009) Nutrient variation and availability of wheat DDGS, corn DDGS and blend DDGS from bioethanol plants. *Journal of the Science of Food and Agriculture*. **89**: 1754-1761

Ou, B., Hampsch-Woodill, M. and Prior, R.L. (2001) Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry* **49**: 4619-4626

Papadopoulos,M.C. (1989) Effect of processing on high-protein feedstuffs: a review. *Biological Wastes* **29**: 123-138

Park, S.Y., Birkhold, S.G., Kubena, L.F., Nisbet, D.J. and Ricke, S.C. (2003) Effect of storage conditions on bone breaking strength and bone ash in laying hens at different stages of production cycles. *Poultry Science* **82**: 1688-1691

Parks, C.W., Grimes, J.L., Ferket, P.R. and Fairchild, A.S. (2001) The effect of mannanoligosaccharides , bambermycins and virginiamycin on performance of large white male market turkeys. *Poultry Science* **80**: 718-723

Parsons, C.M., Potter, L.M. and Brown, R.D. (1982) Effects of dietary protein and intestinal microflora on excretion of amino acids in poultry. *Poultry Science* **61**: 939-946

Parsons, C.M. (1986) Determination of digestible and available amino acids on meat meal using conventional and caecetomized cockerels or chick growth assays. *British Journal of Nutrition* **56**: 227-240

Parsons. C.M., Martinez, C., Singh, V., Radhakrishman, S. and Noll, S. (2006) Nutritional value of conventional and modified DDGS for poultry. *Multi-state poultry nutrition and feeding conference*. Indianapolis, IN, May 24-25, 2006

Patterson, T., Dinsdale, R. and Esteves, S. (2008) Review of energy balances and emissions associated with biomass-based transport fuels relevant to the UK context. *Energy and Fuels* **22**: 3506-3512

Payne, W.L., Combs, G.F., Kiefer, R.R. and Snider, D.G.(1968) Investigation of protein quality-ileal *recovery* of amino acids. *Federation Proceedings* **27**: 1199-1203.

Peddie, J., Dewar, W.A., Gilbert, A.B. and Waddington, D. (1982) The use of titanium dioxide for determining apparent digestibility in mature domestic fowls (*Gallus Domesticus*). *Journal of Agricultural Science* **99**: 233-236

Pederson, C. and Lindberg, J.E. (2010) Ileal and total tract nutrient digestibility in wheat wet distiller's solubles and wheat dried distillers grains with solubles when fed to growing pigs. *Livestock Science* **132**: 145-151

Pellegrino, E.D. and Biltz, R.M. (1968) Bone carbonate and the Ca to P molar ratio. *Nature* **219**: 1261-1262

Perez, V.G., Jacobs, C.M., Barnes, J., Jenkins, M.C., Kujhlenschmidt, M.S., Fahey, G.C. Jr., Parsons, C.M. and Pettigrew, J.E. (2011) Effect of corn distillers dried grains with solubles and *Eimeria acervulina* infection on growth performance and the intestinal microbiota of young chicks. *Poultry Science* **90** : 958-964

Perez-Vendrell, A.M., Angulo, E. and Brufau, J. (2001) Effects of microbial phytase on apparent retention of phosphorus, calcium and zinc in broilers, according to type of diet. *Cahiers Options Mediterraneennes* **54**: 191-195

Peterson, D.W., Hamilton, W.H. and Lilyblade, A.L. (1971) hereditary susceptibility to dietary induction of gout in selected lines of chickens. *Journal of Nutrition* **101**: 347-354

Petrou, E.C. and Pappis, C.P. (2009) Biofuels: A survey on pros and cons. *Energy and Fuels* **23**: 1055-1066

Pettersson D. and Aman P. (1988) Effects of enzyme supplementation of diets based on wheat, rye or triticale on their productive value for broiler chickens. *Animal Feed Science and Technology* **20**: 313-324

Pettersson, D., Hesselan, K and Aman, P. (1987) Nutritional value for chickens of dried distillers spent grain from barley and dehulled barley. *Animal Feed Science and Technology* **17**: 145-156

Pines, M., Hasdai, A. and Monsonego-Ornan, E. (2005) Tibial dyschondroplasia - tools, new insight and future prospects. *World's Poultry Science Journal* **61**: 285-297

Plakas, S.M., Lee, T.C., Wolke, R.E., Meade T.L. (1985) Effect of Maillard browning reaction on protein utilization and plasma amino acid response by rainbow trout. *Journal of Nutrition* **115**: 1589-1599

Pol, I., Driessen-van Lankveld, W., Van Egmond, H., Pikkemaat, M. and De Jong, J. (2009) Use of antibiotics in bioethanol production: Emerging risk in feed? *Proceedings of the European Feed microbiology organisation*, Rostock, Germany. Sept 21-23 2009.

Poo, M.E. and Millan, N. (1990) Effect of dietary concentration of *Saccharomyces carlsbergenesis* in yeast recovered from beer, in Warren male chicks. *Archives of Latin American Nutrition* **40**: 95-106

Powers, W. and Angel, R. (2008) A review of the capacity for nutritional strategies to address environmental challenges in poultry production. *Poultry Science* **87**: 1929-1938

Purlis, E. (2010) Browning development in bakery products – a review. *Journal of Food Engineering* **99**: 239-249

Rameshwari, K.S. and Karthikeyan, S. (2005) Distillery yeast sludge as an alternative feed resource in poultry. *International Journal of Poultry Science.* **4** (10): 787-789

Randall, K.M. and Drew, M.D. (2010) Fractionation of wheat distillers dried grains and solubles using sieving increases digestible nutrient content in rainbow trout. *Animal Feed Science and Technology* **159**: 138-142

Rath, N.C., Balog, J.M., Huff, W.E., Huff, G.R., Kulkarni, G.B. and Tierce, J.F. (1999) Comparative differences in the composition and biomechanical properties of tibiae of seven and seventy two week old male and female broiler breeder chickens. *Poultry Science* **78**: 1232-1239

Rath, N.C., Huff, G.E., Huff, W.E. and Balog, J.M. (2000) Factors regulating bone maturity and strength in poultry. *Poultry Science* **79**: 1024-1032

Ratti, C. (2001) Hot air and freeze-drying of high value foods: a review. *Journal of Food Engineering* **49**: 311-319

Rausch, K.D., Belyea, R.L., Ellersieck, M.R., Singh, V., Johnston, D.B., Tumbleson, M.E. (2005) Particle size distributions of ground corn and DDGS from dry grind processing. *Transactions of the ASBE*. **48** (1): 273-277

Rausch, K.D. and Belyea, R.L. (2006) The future of coproducts from corn processing. *Applied Biochemistry and Biotechnology*. **128** (1): 47-85

Ravindran, V., Kornegay, E.T., Potter, L.M., Ogunabameru, B. O., Welten, M. K., Wilson, J. H. and Potchanakorn, M. (1995) An evaluation of various response criteria in assessing biological availability of phosphorus for broilers. *Poultry Science* **74**: 1820-1830

Ravindran, V. and Bryden, W.L. (1999) Protein evaluation in poultry, *in vitro* and *in vivo* measurements. *Australian Journal of Agricultural Research* **50**: 889-908

Ravindran, V., Hew, L.I., Ravindran, G., Gill, R.J. , Pittolo, P.H. and Bryden, W.L. (1999) Influence of xylanase supplementation on the apparent metabolisable energy and ileal amino acid digestibility in a diet containing wheat and oats, and on the performance of three strains of broiler chickens. *Australian Journal of Agricultural Research* **50** (7): 1159-1163

Ravindran, V., Hew, L.I., Ravindran, G. and Bryden, W.L. (2004) Endogenous amino acid flow in the avian ileum: quantification using three techniques. *British Journal of Nutrition* **92**: 217-223

Ravindran, V., Hew, L.I., Ravindran, G. And Bryden, W.L. (2005) Apparent ileal digestibility of amino acids in dietary ingredients for broiler chickens. *Animal Science* **81**: 85-97

Reisinger, N., Ganner, A., Masching, S., Schatzmayr, G. And Applegate, T.J. (2012) Efficacy of a yeast derivative on broiler performance, intestinal morphology and blood profile. *Livestock Science* **143**: 195-200

RFA (2012) Renewable fuels agency statistics www.ethanolrfa.org/pages/statistics accessed 28/5/2012

Rodehutscord, M., Kapocius, M., Timmler, R. and Dieckmann, A. (2004) Linear regression approach to study amino acid digestibility in broiler chickens. *British Poultry Science* **45**: 85-92

Rodriguez, M.L., Rebole, A., Velasco, S., Ortiz, L.T., Trevino, J. and Alzueta, C. (2012) Wheat and barley based diets with or without additives influence broiler chicken performance, nutrient digestibility and intestinal microflora. *Journal of the Science of Food and Agriculture* **92**: 184-190

Rose, A.H. (1987) *In*: Biotechnology in the feed Industry. *Proceedings of the Alltech's Third Annual Symposium*. Alltech Technical Publications (T.P.Lyons ed). Alltech Inc. Nicholasville KY pp 113-118

Rosentrater, K.A. (2006) Some physical properties of distillers dried grains with solubles (DDGS). *Applied Engineering in Agriculture* **22** (4): 589–595.
RTFO Renewable Transport Fuels Obligation Order (2007)
www.opsi.gov.uk/si/si2007/draft/pdf/ukdsi_9780110788180_en.pdf

Rumsey, G.L., Hughes, S.G., Smith, R.R., Kinsella, J.E. and Shetty, K.J. (1991a) Digestibility and energy values of intact, disrupted and extracts from brewer's dried yeast fed to rainbow trout (*Oncorhynchus mykiss*). *Animal Feed Science and Technology* **33**: 185-193

Rumsey, G.L., Kinsella, J.E., Shetty, K.J. and Hughes, S.G. (1991b) Effect of high dietary concentrations of brewer's dried yeast on growth performance and liver uricase in rainbow trout (*Oncorhynchus mykiss*). *Animal Feed Science and Technology* **33**:177-183

Rutherford, S. M., Chung, T.K., Morel, P.C.H. and Mougham, P.J. (2004) Effect of microbial phytase on ileal digestibility of phytate phosphorus, total phosphorus and amino acids in a low-phosphorus diet for broilers. *Poultry Science* **83**: 61-68

Sacranie, A., Svihus, B., Denstadli, V., Moen, B., Iji, P.A. and Choct, M. (2012) The effect of insoluble fiber and intermittent feeding on gizzard development, gut motility, and performance of broiler chickens. *Poultry Science* **91**: 693-700

Sales, J. and Janssens, G.P.J. (2003) The use of markers to determine energy metabolizability and nutrient digestibility in avian species. *World Poultry Science Journal* **59**: 314-327.

Sanotra, G.S., Damkjer Lund, J. and Vestergaard, K.S. (2002) Influence of light-dark schedules and stocking density on behaviour, risk of leg problems and occurrence of chronic fear in broilers. *British Poultry Science* **43**: 344-354

Santin, E., Maiorka, A. and Macari, M. (2001) Performance and intestinal mucosa development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. *Journal of Applied Poultry Research* **10**: 236-244

Santos, M., Jimenez, J.J., Bartolome, B., Gomez-Cordoves, C. and del Nozal, M.J. (2003) Variability of brewer's spent grain within a brewery. *Food Chemistry* **80**: 17-21

Saragoni, P., Aguilera, J. M., and Bouchon, P. (2007) Changes in particles of coffee powder and extensions to cake. *Food Chemistry* **104**: 122–126.

Saulnier, L., Peneau, N. and Thibault, J-F. (1995) Variability in grain extract viscosity and water soluble arabinoxylan content in wheat. *Journal of Cereal Science* **22**: 259-264

Schaeffer, T.W., Brown, M.L. Rosentrater, K.A. and Muthukumarappan, K. (2010) Utilization of diets containing graded levels of ethanol production co-products by Nile tilapia. *Journal of animal Physiology and Animal Nutrition* **94**: 348-354

Schilling, M.W., Battula, V., Loar, R.E., Jackson, V., Kin, S. and Corzo, A. (2010) Dietary inclusion level effects of distillers dried grains with solubles on broiler meat quality. *Poultry Science* **89**: 752-760

Schiffman, H.R. (1968) Texture preference in the domestic chick. *Journal of Comparative and Physiological Psychology* 66: 540

Schingoethe, D.J. (2006) Feeding ethanol by-products to dairy and beef cattle. *Proceedings of the California Animal Nutrition Conference*, May 10-11, Fresno, CA. pp49-63

Scotch Whisky Order (1990) http://www.opsi.gov.uk/si/si1990/Uksi_19900998_en_1.htm

Scott, M.L., Nesheim, M.C. and Young, (eds) (1976). Methods for chemical and biological measurement of nutritive quality. In: *Nutrition of the Chicken*, 2nd edition, M.L. Scott and Associates, Publishers, New York. Pp. 518-554

Seeman, E. (1999) The structural basis of bone fragilty in men. *Bone* **25**: 143-147

Sell, J.L., Ashraf, M., Bales, G.L. (1981) Yeast single-cell protein as a substitute for soybean meal in broiler diets. *Nutrition reports international* **24** (2): 229-235

Sell, J.L., Angel, C.R., Piquer, F.J., Mallarino, E.G. and Al-Batshan, H.A. (1991) Development patterns of selected characteristics of the gastrointestinal tract of young turkeys. *Poultry Science* **70**: 1200-1205

Selle, P.H., Pittolo, P.H. Gill, R.J. and Bryden, W.L. (2002) Xylanase plus phytase supplementation of broiler diets based on different wheats. *Proceedings of the Australian Poultry Science Symposium* **14**: 141-144

Selle, P.H., Ravindran, V. and Partridge, G.G. (2009) Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility, energy utilisation, mineral retention and growth performance in wheat-based broiler diets. *Animal Feed Science and Technology* **153**: 303-313

Shafey, T.M. and McDonald, M.W. (1990) Effects of dietary calcium : available phosphorus on Ca tolerance of broiler chickens. *Australian Journal of Experimental Agriculture and Animal Husbandry* **30**: 483-490

Shafey, T.M., McDonald, M.W. and Dingle, G.J. (1991) Effects of dietary calcium and available phosphorus concentration on digesta pH and on the availability of calcium, iron, magnesium and zinc from intestinal contents of meat chickens. *British Poultry Science* **32**: 185-194

Shafey, T.M. (1993) Calcium tolerance of growing chickens: effect of ratio of dietary calcium to available phosphorus. *World's Poultry Science Journal* **49**: 5-18

Shareef, A.M. and Al-Dabbagh, A.S.A. (2009) Effect of probiotic (*Saccharomyces cerevisiae*) on performance of broiler chicks. *Iraqi Journal of Veterinary Sciences* **23**: 23-29

Sharif, M., Shahzad, M.A., Rehman, S., Khan, S., Ali, R., Khan, M.L. and Khan, K. (2012) Nutritional evaluation of distillery sludge and its effect as a substitute of canola meal on performance of broiler chickens. *Asian- Australasian Journal of Animal Science* **25**: 401-409

Shannon, D.W.F. and McNab, J.M. (1972) The effect of different dietary levels of an n-paraffin-grown yeast on the growth and food intake of broiler chicks. *British Poultry Science* **13**: 267-272

Sharpley A. and Moyer, B. (2000) Phosphorus forms in manure and compost and their release during simulated rainfall. *Journal of Environmental Quality*. 29: 1462-1469
Shim, M.Y., Pesti, G.M., Bakalli, R.I. (2011) Evaluation of corn distillers dried grains with solubles as an alternative ingredient for broilers. *Poultry Science* **90**: 369-376

Short, F.J, Gorton, P., Wiseman, J. and Boorman, K.N. (1996). Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Animal Feed Science and Technology* **59** : 215-221

Short, F. J., Wiseman, J. and Boorman, K. N. (1999) Application of a method to determine ileal digestibility in broilers of amino acids in wheat. *Animal Feed Science and Technology* **79**: 195–209.

Shurson, G.C. (2005) Issues and Opportunities Related to the production and marketing of ethanol by-products. *Agricultural Outlook Forum* 2005. <http://www.usda.gov/oce/forum/speeches/shurson.pdf>.

Shurson, J., Noll, S. and Goihl, J. (2005) Corn by-product diversity and feeding value to non-ruminants. *Proceedings of the 66th Minnesota Nutrition Conference*. St Paul, MN. Sept 20-21, 2005

Silva, V. K., Della Torre da Silva, J., Torres, K. A. A., de Faria Filho, D., Hirota Hada, F. and Barbosa de Moraes, V. M. (2009) Humoral immune response of broilers fed diets containing yeast extract and prebiotics in the prestarter phase and raised at different temperatures. *Journal of Applied Poultry Research* **18**: 530-540.

Simoyi, M.F., Van Dyke, K. and Klandorf, H. (2002) Manipulation of plasma uric acid in broiler chicks and its effect on leukocyte oxidative activity. *American Journal of Integrative Comparative* Physiology **282**: 791-796

Skinner, J.T. and Waldroup, P.W. (1992) Effects of Calcium and phosphorus levels in starter and grower diets on broilers during the finisher period. *Journal of Applied Poultry Research* **1**: 273-279

Skinner, J.T. and Waldroup, P.W. (1995) Allometric bone development in floor-reared broilers. *Journal of Applied Poultry Research* **4**: 265-270

Smith, T.C., Kindred, D.R., Brosnan, J.M., Weightman, R.M., Shepherd, M., Sylvester-Bradley, R. (2006) Wheat as a feedstock for alcohol production. Research review No.61. HGCA

Sorenson, H.R., Pederson, S., Meyer, A.S. (2006) Optimization of reaction conditions for enzymatic viscosity reduction and hydrolysis of wheat arabinoxylan in an industrial ethanol fermentation residue. *Biotechnology Progress* **22**: 505-513

Spark, M., Paschertz, H. and Kamphues, J. (2005) Yeast (different sources and levels) as protein source in diets of reared piglets: effects on protein digestibility and N-metabolism. *Journal of Animal Physiology and Animal* Nutrition **89** (3-6): 184-188
Speedy, A.W. (2002) Overview of world feed protein needs and supply. *Protein sources for the animal feed industry, FAO proceedings*, Bangkok 29 April-3 May 2002

Spencer-Martins, I. and Van Uden, N. (1977) Yields of Yeast growth on starch. *European Journal of Applied Microbiology*. **4** : 29-35

Spiehs, M.J., Whitney, M.H., Shurson, G.C. (2002) Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. *Journal of Animal Science*. **80**: 2639-2645

Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert, A. (2006) Commercial applications of microalgae. *Journal of Bioscience and Bioengineering* **101**: 87-96

Spring, P., Wenk, C., Dawson, K.A. and Newman, K.E. (2000) The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of Salmonella-challenged broiler chicks. *Poultry Science* **79**: 205-211

Srinivasan, R. and Singh, V. (2008) Physical properties that govern fiber separation from distiller's dried grains with solubles (DDGS) using sieving and air classification. *Separation and Purification Technology* **61**: 461-468

Srinivasan, R., Moreau, R.A., Parsons, C., Lane, J.D. and Singh, V. (2008) Separation of fiber from distillers dried grains (DDG) using sieving and elutriation. *Biomass and Bioenergy* **32**: 468-172

Srinivasan, R., To, F. and Columbus, E. (2009) Pilot scale fiber separation from distillers dried grains with solubles (DDGS) using sieving and air classification. *Bioresource Technology* **100**: 3548-3555

Sriperm, N., Pesti, G.M. and Tillman, P.B. (2011) Evaluation of the fixed nitrogen to protein (N:P) conversion factor (6.25) versus in gradient specific N:P conversion factors in feedstuffs. *Journal of the Science of Food and Agriculture* **91**: 1182-1186

Stanley, V.G., Gray, C., Daley, M., Krueger, W.F. and Sefton, A.E. (2004) An alternative to antibiotic based drugs in feed for enhancing performance of broilers grown on *Eimeria*-spp-infected litter. *Poultry Science* **83**: 39-44

Stein, H.H. and Shurson, G.C. (2009) The use an application of distillers dried grains with solubles in swine diets. *Journal of Animal Science* **87**: 1292-1303

Stock, R. and Klopfenstein, T. (1982) Feeding value of distillers grains for ruminants *In*: Feed and Fuel from ethanol production symposium. R.A. Parson (ed.) – *Northeast regional agricultural engineering service publication* **17** Ithaca NY

Stone, C.W. (1998) Yeast products in the feed industry: A practical guide for feed professionals. Diamond V Mills Inc., Cedar Rapids, IA, USA. pp1-16

Sturkie P.D. (ed.)(1965) *Avian Physiology*, 2nd edition, Bailliere, Tindel and Cassell Publishers, London.

Stroppa, C.T., Andrietta, M.G.S., Andrietta, S.R., Steckelberg, C. and Serra, G.E. (2000) Use of penicillin and monensin to control bacterial contamination of Brazilian alcohol fermentations. *International Sugar Journal* **102**: 78-82

Succi, G., Pialorsi, S., Di Fiore, L. and Cardini, G. (1980) The use of Methanol grown yeast LI-70 in Feeds for broilers. *Poultry Science* **59** (7): 1471-1479

Svihus, B. and Gullord, M. (2002) Effect of chemical content and physical characteristics on nutritional value of wheat, barley and oats for poultry. *Animal Feed Science and Technology* **102**: 71-92

Swanston, J.S., Smith, P.L., Gillespie, T.L., Brosnan, J.M., Bringhurst, T.A., Agu, R.C. (2007) Associations between grain characteristics and alcohol yield among soft wheat varieties. *Journal of the Science of Food and Agriculture*. **87**: 676-683

Swietkiewicz, S. and Koreleski, J. (2008) The use of distillers dried grains with solubles (DDGS) in poultry nutrition. *Worlds Poultry Science Journal*. **64**: 257-265

Tahir, M., Shim, M.Y., Ward, N.E., Smith, C., Foster, E., Guney, A.C. and Pesti, G.M. (2012) Phytate and other nutrient components of feed ingredients for poultry. *Poultry Science* 91(4): 928-935

Talaty, P.N., Katanbaf, M.N. and Hester, P.Y. (2009) Life cycle changes in bone mineralisation and bone size traits of commercial broilers. *Poultry Science* **88**: 1070-1077

Taylor, R.D. and Jones, G.P.D. (2004) The incorporation of whole grain into pelleted broiler chicken diet. 2. Gastrointestinal and digesta characteristics. *British Poultry Science* **45**: 237-246

Teirlynck, E., Bjerrum, L., Eeckhaut, V. Huyghebaert, G., Pasmans, F., Haesebrouck, F., Dewulf, J., Ducatelle, R. and Van Immerseel, F. (2009) The cereal type in feed influences gut wall morphology and intestinal immune cell infiltration in broiler chickens. *British Journal of Nutrition* **102**: 1453-1461

Ten Doeschate, R.A.H.M., Scheele, C.W., Schreurs, C.W., Shreurs, V.V.A.M. and Van der Klis, J.D. (1993) Digestibility studies in broiler chicks: influence of genotype, age, sex and method of determination. British Poultry Science 34: 131-146

Termine, J.D. and Gehron-Robey, P. (1996) Bone matrix proteins and the mineralisation process. In: *Primer on metabolic bone diseases and disorders of mineral metabolism* M.J. Favus (*ed*) Lippincott-Raven Publishers, Philadelphia, PA.

Terpstra, K, (1978) Total and digestible amino acids. *Proceedings of the 2nd European Symposium on Poultry Nutrition*. Beckbergen, The Netherlands. pp 97-101

Thacker, P.A and Widyaratne, G.P. (2007) Nutritional value of diets containing graded levels of wheat distillers dried grains with solubles fed to broiler chicks. *Journal of the Science of Food and Agriculture* **87** : 1386-1390

Thorp, B.H. (1992) Abnormalities in the growth of leg bones. *In* Bone Biology and Skeletal Disorders in Poultry. C.C. Whitehead (ed) Carfax, Abingdon, England.

Thorp B.H. and Waddington D. (1997) Relationship between the bone pathologies, ash and mineral content of long bones in 35 day old broiler chickens. *Research in Veterinary Science* **62**: 67-73

Titgemeyer, E.C., Armendariz, C.K., Bindel, D.J., Greenwood, R.H. and Loest, C.A. (2001) Evaluation of titanium dioxide as a digestibility marker for cattle. *Journal of Animal Science* **79**: 1059-1063.

TSE regulation (999/2001) http://ec.europa.eu/food/fs/bse/bse36_en.pdf *accessed 12/7/12*

Tumuluru, J.S., Tabil, L., Opoku, A., Mosqueda, M.R. and Fadeyi, O. (2010) Effect of process variables on the quality characteristics of pelleted wheat distiller's dried grains with solubles. *Biosystems Engineering*. **105**: 466-475

Vananuvat, P. (1977) Value of Yeast Protein for Poultry Feeds. *Critical reviews in food science and nutrition*. **9** (4): 325-343

Vananuvat, P. and Chiraratananon, R. (1977) The Use of Brewery yeast in commercial type ration for poultry. *World's Poultry Science*. **33** (2):88-99

V'ant Land, C.M. (1991) Industrial drying equipment. Marcel Dekker Inc., New York , USA. P21-25

Vega-Mercado, H., Gongora-Nieto, M.M., Barbosa-Canovas, G.V. (2001) Advances in dehydration of foods. *Journal of Food Engineering* **49**: 271-289

Ventura, M.R., Castanon, J.I.R. and McNab, J.M. (1994) Nutritional value of seaweed (*Ulva rigida*) for poultry. *Animal Feed Science and Technology* **49**: 87-92

Verdejo, H. Greig, D. and Castro, P. (2008) Uric acid, xanthine oxidase and heart failure: unresolved issues. *European Journal of Heart Failure* **10**: 1271-1272

Vohra, P. (1972) Evaluation of metabolisable energy for poultry. *World's Poultry science Journal* **28**: 204-214

Von Blottnitz, H. and Curran, M.A. (2007) A review of the assessments conducted on bioethanol as a transportation fuel from a net-energy, greenhouse gas and environmental life-cycle perspective. *Journal of Cleaner Production* **15** (7): 607-619

Waldroup, P.W., Hillard, C.M. and Mitchell, R.J. (1971) The nutritive value of yeast grown on hydrocarbon fractions for broiler chicks. *Poultry Science* **50**: 1022-1029

Waldroup, P. W. (1981) Energy Levels for Broilers. *Journal of the American Oil Chemists Society* **58**: 309-313.

Waldroup, P.W. (1997) Particle size reduction of cereal grains and its significance in poultry nutrition. *Technical Bulletin PO34-1997*, American Soybean Association, Singapore pp14

Waldroup, P.W. (2007) Biofuels and Broilers – Competitors or cooperators? *Proceedings of the 5th Mid-Atlantic Nutrition Conference.* Baltimore MD. March 29, 2007.

Wang, J., Zhang, G., Chen, J and Wu, F. (2004) The changes in β glucan content and β glucanase activity in barley before and after malting and their relationships to malt qualities. *Food Chemistry* **86**: 223-228

Wang, Z., Cerrate, S., Coto, C., Yan, F., Waldroup, P.W. (2007) Utilisation of Distillers dried grains with solubles (DDGS) in broiler diets using a standardised nutrient matrix. *International Journal of Poultry Science* **6** (7): 470-477

Wang, Z., Cerrate, C., Coto, P., Sacakli, P. Yan, F., Costa, F.G.P. and Waldroup, P.W. (2009) Evaluation of Nupro yeast product in diets for broilers. *International Journal of Poultry Science* **8**: 515-520

Weber, P. (1999) The role of vitamins in the prevention of osteoporosis. A brief status report. *International journal of vitamin nutritional research* **69**: 194-197

Weigel, J.C., Loy, D. and Kilmer, L. (1997) Feed co-products of the dry corn milling process. (pg 2-4) Renewable Fuels Association and National Corn Growers Association. Washington DC and St. Louis, MO.

Wheals, A.E., Basso, L.C., Alves, M.G. and Amorim, H.V. (1999) Fuel ethanol after 25 years. *Trends in Biotechnology* **17** (12) 482-487

White, W.B., Bird, H.R., Sunde, M.L., Prentice, N., Burger, W.C. and Martlett, J.A. (1981) The viscosity interaction of barley P-glucan with *Trichoderma viride* cellulase in the chick intestine. *Poultry Science* **62:** 853-862

Widyaratne, G.P. and Zijlstra, R.T. (2007) Nutritional value of wheat and corn distiller's dried grain with solubles: Digestibility and digestible contents of energy, amino acids and phosphorus, nutrient excretion and growth performance of grower finisher pigs. *Canadian Journal of Animal Science* **87**: 103-114

Wien, E.M. and Schartz, R. (1985) Dietary calcium exchangeability and bioavailability. *In*: Nutritional Bioavailability of calcium (*Eds*. Kies, C.) American Chemical Society, pp 1-16
Williams, B., Solomon, S., Waddington, D., Thorp, B. and Farquharson, C. (2000a) Skeletal development in the meat type chicken. *British Poultry Science* **41**: 141-149

Williams, P., Clarke, E. and Scholey, D. (2009). The production of a high concentration yeast protein concentrate co- product from a bioethanol refinery. *Proceedings of the 17th European Symposium on Poultry Nutrition*, Edinburgh, UK. P326

Williams, P.E.V. (2010) Protein Recovery. UK Patent application GB2010/000577.

Winkler, B., Tosi, H., Webster, A.J.F., Resende, F.D., Oliveira, A.A.M.A. and Villela, L.C.V. (2011) Dried yeast (*Saccharomyces cerevisiae*) as a protein source for horses. *Livestock Science* **137**: 168-177

Wohl, G.R., Loehrke, R.L., Watkins, B.A. and Zernicke, R.F. (1998) Effects if a high fat diet on mature bone mineral content, structure and mechanical properties. *Calcified tissue international* **63**: 74-79

Wu, F. and Munkvold, G.P. (2008) Mycotoxins in ethanol co-products: modelling economic impacts on the livestock industry and management practices. *Journal of Agriculture and Food Chemistry* **56**: 3900-3911.

Yalcin, S., Onol, A.G., Kocak, D. and Ozcan, I (1993) The usage of baker's yeast as a protein source in broiler rations. *Doga Turkish Journal of Veterinary Animal Science* **17**: 305-309

Yalcin, S., Erol, H., Ozsoy, B., Onbasilar, I and Yalcin S. (2008) Effects of the usage of dried brewer's yeast in the diets on the performance, egg traits and blood parameters in quail. *Animal* **2:12**: 1780-1785

Yamada, E.A. and Sgarbieri, V.C. (2005) Yeast (*Saccharomyces cerevisiae*) protein concentrate: preparation, chemical composition and nutritional and functional properties. *Journal of Agricultural and food chemistry* **53**: 3931-3936

Yan, F., Keen, C. A., Zhang, K. Y. and Waldroup, P. W. (2005) Comparison of methods to evaluate bone mineralization. *Journal of Applied Poultry Research* **14**: 492-498.

Yáñez, J. L., Beltranena, E., Cervantes, M. and Zijlstra, R. T. (2011) Effect of phytase and xylanase supplementation or particle size on nutrient digestibility of diets containing distillers dried grains with solubles co-fermented from wheat and corn in ileal-cannulated grower pigs. *Journal of Animal Science* **89**: 113–123.

Yang, Y., Iji, P.A., Kocher, A., Mikkleson, L.L. and Choct, M. (2008) Effects of xylanase on growth and gut development of broiler chickens given a wheat based diet. *Asian-Australasian Journal of Animal Sciences* **21**: 1659-1664

Yang, W.Z., McAllister, T.A., Mckinnon, J.J. and Beauchemin, K.A. (2012) Wheat distiller's grains with solubles in feedlot cattle diets: feeding behaviour, growth performance, carcass characteristics and blood metabolites. *Journal of Animal Science* **90**: 1301-10

Yasar, S. (2003) Performance, gut size and ileal digesta viscosity of broiler chickens fed with whole wheat added diet and the diets with different wheat particle sizes. *International Journal of Poultry Science* **2**: 75-82

Yoon, S. Y. , Yang, Y. X., Shinde, P. L., Choi, J. Y., Kim, J. S., Kim, Y. W., Yun, K., Jo, J. K., Lee, J. H., Ohh, S. J., Kwon, I. K. and Chae. B. J. (2010) Effects of mannanase and distillers dried grain with solubles on growth performance, nutrient digestibility, and carcass characteristics of grower-finisher pigs. *Journal of Animal Science* **88**: 181–191.

Yoshida, M. and Hoshii, H. (1983) Relationship between ash contents of the tibia bone and the toe of chicks. *Japanese Poultry Science* **20**: 51-54

Youssef, I.M.I., Westfahl, C., Sunder, A., Liebert, F., Kamphues, J. (2008) Evaluation of dried distillers grains with solubles (DDGS) as a protein source for broilers. *Archives of Animal Nutrition* **62** (5) : 404-414

Zerai, D.B., Fitzsimmons, K.M., Collier, R.J. and Duff, G.C. (2008) Evaluation of brewer's waste as partial replacement of fish meal protein in Nile tilapia, *Oreochromis niloticus*, diets. *Journal of the World Aquaculture Society***39**: 556-564

Zhao, X.Q. and Bai, F.W. (2009) mechanisms of yeast stress tolerance and its manipulation for efficient fuel ethanol production. *Journal of Biotechnology* **144**: 23-30
Zhang, B. and Coon, C.N. (1997) The relationship of various tibia bone measurements in hens. *Poultry Science* **76**: 1698-1701

Zhang, A.W., Lee, B.D., Lee, S.K., Lee, K.W., An, G.H., Song, K.B. and Lee C.H. (2005) Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality and ileal mucosa development of broiler chicks. *Poultry Science* **84**: 1015-1021

Zhou, B., Ileleji, K.E. and Ejeta, G. (2008) Physical property relationships of bulk corn stover particles. *Trans. ASABE* **51** (2): 581–590.

Zijlstra, R.T., Widyaratne, G., Beltranena, E. (2007) Characterisation of wheat DDGS and feeding to swine. *Proceedings of the 28th Western Nutrition Conference*. Sept 25-27 2007, Saskatoon, Sask.

APPENDIX 1

Vitamin and mineral premix breakdown:

Raw Material	g/kg
Limestone	736.72
Vitamin A	10.8
Vitamin D3	2.4
Vitamin E 50%	20
Vitamin B1	1.2
Vitamin B12 0.1%	12
Copper Sulphate 25%	16
Biotin 2%	2.5
Nicotinic acid	24
Vitamin B2 80%	5
Vitamin B6	1.2
Vitamin K 44%	4.56
Calcium Panthothenate 98%	6.12
Folic acid	0.6
iron Sulphate 20%	40
Manganese oxide 62%	64.52
Zinc oxide 74%	43.24
Calcium iodate 62%	0.65
Selenium 1%	8
Sodium molybdate 40%	0.48

APPENDIX 2

Proximate analysis of wheat, full fat soya and Hipro soya (g/kg)

Analysis	Wheat	FF Soya	Hipro Soya
Crude protein (based on N*6.25)	111	380	488
Crude Fat	9	190	13
Ash	4	45	58
Dry matter	880	894	882

Parts of this work have previously appeared as:

Williams, P., Scholey D.V. and Burton, E.J. (2009) The production of a high concentration yeast protein concentrate co-product from a bioethanol refinery. *Proceedings of the 17th European Symposium on Poultry Nutrition*, Edinburgh. p326.

Scholey, D.V., Williams, P. and Burton, E.J. (2011) Potential for alcohol co-products from potable and bioethanol sources as protein source in poultry diets. *British Poultry Abstracts* 7(1): 23-24

Scholey, D.V., Morgan, N., Williams, P. and Burton, E.J. (2011) Effects of incorporating a novel bio-ethanol co-product in poultry diets on bird performance. *British Poultry Abstracts* 7(1): 57

Scholey, D.V., Williams, P. and Burton, E.J. (2012) Welfare implications for broiler chicks fed diets containing graded levels of potable alcohol yeast protein. *Recent advances in animal welfare science III*, 21st June 2012, York.