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Abstract

The research was conducted to evaluate ileal and total tract digestibility of crude protein (CP), organic matter (OM) and energy in growing pigs supplied with dietary inclusion of commercially available wheat wet distillers grain with solubles (WWDGS) and wheat dried distillers' grain with solubles (WDDGS). Seven castrated male pigs with post valve t-caecum (PVTC) cannulas with an average initial body weight of 79 kg were fed two experimental diets (W and D) in a change-over design. Diet W was composed of 50% WWDGS and 50% basal diet, and diet D was composed of 50% WDDGS and 50% basal diet. The basal diet was composed of corn starch, sugar, premix and titanium dioxide. A casein-based diet was fed prior to and after the study to estimate the basal endogenous nitrogen and amino acid (AA) losses. Apparent ileal digestibility (AID) for CP in diet W was higher ($p<0.05$) than for diet D, whereas the AID for energy and OM remained similar ($p>0.05$) between the diets. Apparent total tract digestibility (ATTD) was recorded higher ($p<0.0001$) for CP in diet W than in diet D. In addition, higher ATTD was recorded for OM in diet W with no significant difference ($p>0.05$) in energy digestibility. Endogenous losses for each pig was evaluated separately and used for calculation of standardized ileal digestibility (SID) of CP and AA. The average endogenous losses of CP were from the pre- and post period was 8.53±0.95 g/kg DM of digesta. The SID value for CP, methionine and lysine in diet W was higher ($p<0.05$) than for diet D. There was significant increased variation in lysine digestibility in WWDGS than in WDDGS. Differences between the production plants and in chemical composition between grain sources were the main reasons for different nutritional values between the distillers grain products. Despite excellent nutritional properties, the low dry matter content, decreased P digestibility and high transportation cost, limits the use of WWDGS to a local area within reasonable distance from the factory.

Keywords: Apparent ileal digestibility, standardized ileal digestibility, endogenous losses

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1. Introduction

In the modern age, there is an increased emphasis in the production of alternative feed sources for livestock. Feed alternatives such as distillers' grain, has been proved as a major feed alternative for livestock including cattle, pig and poultry. Distillers' grain is a co-product produced during fuel ethanol production or by beverage industries (Pahm *et al.,* 2008). Different experiments have been conducted to evaluate nutrient efficiency of this co-product in different species (Cromwell, 1993; Pahm *et al.,* 2008; Knabe *et al.,* 1989; Ham et al., 1994; Batal and Dale, 2006). The co-product is a valuable asset with high nutritional value in animal nutrition. It has been extensively used in swine feeding for more than five decades, but with an increase in production of ethanol industries during the last two decades, the feeding ratio has dramatically increased (Stein, 2008). In the process, starch in the grain is completely fermented to ethanol and the co-product used in feed is rich in protein, fat, dietary fiber and minerals (Pedersen and Lindberg, 2010). Distillers' grain has been indicated as a valuable source of energy, protein, water soluble vitamins and minerals for livestock (Cromwell *et al.,* 1993).

The co-product, varies in composition with respect to the cereal seed sources used in the fermentation (Babcock et al., 2008). The co-product is used as wet distillers' grain (WDG) or in the dried form in animal feed (Pedersen *et al.,* 2003). When the co-product of the whole stillage is dried without adding solubles the product is called dried distillers' grain (DDG) (Pahm *et al.,* 2008). While stored, WDG harbors many fermentative microorganisms including lactic acid bacteria and yeasts. Positive effects including decreased feed pH, improved growth performance and decrease coliform count in the gastrointestinal tract are recorded (Scholten, *et al.,* 1999). Nutritional variability occurs among different production plants and factors like selection of grains, duration and temperature of drying and fermentation process also affects the composition (Spiehs *et. al.,* 2002, Stein *et. al.,* 2006).

Cereal grains are the major concentrate in pig nutrition, which contains 60-70% starch and is easily digestible to glucose for absorption. After fermentation of the grains, the co-product contains little easy available carbohydrates and relatively high concentration of non-starch polysaccharides. The content of neutral detergent fiber, acid detergent fiber and total dietary fiber, increases three fold compared with the original cereals. Digestibility of dietary fiber is limited in small intestine, thus contributing lower energy digestibility in the co-product (Stein, 2008). In the study by Pedersen *et al.* (2007), dried distillers' grain with solubles (DDGS) replaced corn in the diet with energy concentration in the diet slightly lower or not affected, either fed with DDGS or corn, but there was considerable increase in phosphorous (P) digestibility for DDGS. This also reduces need for supplemental inorganic phosphate in the diet. In addition, improved daily weight gain and efficient feed conversion ratio with good health, has also been shown when fed with DDGS (Brooks *et al.,* 2003). Diet can be replaced with 50% DDGS for gestating sows and 25% to lactating sows, without affecting sows and litter performance, it may even increase litter size and reproductive efficiency. Furthermore, there was satisfactory growth performance when adding 30% DDGS in the diets fed to nursery pigs or growing finishing pigs (Stein and Shurson, 2009).

Feeding pigs with fermented liquid feed improves their gastrointestinal health (e.g., decreased gastric pH, greater gastric lactic acid concentration, decreased number of coliform bacteria; Scholten, *et al.*, 1999) and decreased incidence of clinical diseases compared to feeding dry feed or non-fermented liquid feed (Mikkelsen and Jensen, 1998). WDG contains high amount of microorganism that may influence proliferation of beneficial microbial mass due to microbial activity in the feed (Scholten *et al.*, 1999, Olstorpe *et al.*, 2007).

Swine diets are formulated on the basis of amino acid (AA) requirement rather than crude protein (CP). Lysine, threonine and other sulphur containing AA are limiting in DDG while formulating swine diets (Liang *et al*., 2002). Nutrient and AA availability are influenced by digestive disturbances, digestive enzymes inhibitors and heat damage. Lysine is destroyed in heat treatment (Fastinger and Mahan, 2006, Pahm *et al.*, 2008, Cromwell *et. al*., 1993).

Despite drying causes reaction between carbohydrate and protein, which reduces energy digestibility in DDG (Stein, 2008), many of the researchers are focused in feeding WDG and DDG to livestock. A rationale behind this is higher economic benefits due to lower feed costs with high nutritional benefits (Pedersen et al., 2007). WDG is most often utilized locally because of low dry matter content and high transportation cost (Pedersen *et al.*, 2003).

The objective of the experiment was to determine the nutrient value of two different commercially sold distiller's grain in pigs.

2. Literature review

2.1 Non- starch polysaccharides

Carbohydrates are classified into sugars, oligosaccharides and polysaccharides. Sugars include monosaccharide such as glucose and fructose, and disaccharides such as sucrose and lactose. Oligosaccharides are three to nine monosaccharide linked with covalent bonds. Polysaccharides consist of 10 or more linked monosaccharide, which can further be classified into starch and non-starch polysaccharides (NSP). Starch is a homo-polymer of glucose, used in energy storage in plant, whereas NSP is composed of structural cell components which consist of cellulose, hemicelluloses and pectic substances (McDonald et al., 2009). Fructans are reserve carbohydrate present in roots, stems, leaves and seeds of plants mainly in the compositae and gramineae families. They are low molecular weight soluble fiber. Pectic substances are polysaccharides soluble in hot water. Galactans are also cell wall constituents made up of polymers of galactose and mannose. Cellulose is a single glucose polymer that forms the cell wall structures in plants (McDonald *et al*., 2009).

Carbohydrates are the major energy source for swine, and make up more than 80% of the dry matter in cereals. The major part of digestion and absorption of food particles takes

place in small intestine. Duodenum is the main site for secretions, whereas the jejunal area is the major site for absorption of nutrients (McDonald *et al.,* 2009). In case of insoluble NSP, digestion mainly occurs in the large intestine with formation of volatile fatty acid (VFA) and lactate, which is less efficiently utilized in the body (Kyriazakis and Whittemore, 2006).

NSP includes pectin, cellulose, hemicelluloses, β-glucans, fructans and inulin (Table 1). Starch is hydrolyzed by mammalian enzymes but NSP are not hydrolyzed, they are rather fermented in the hindgut by the microflora. The end products are VFA and lactate. NSP is mostly dependent on its residual sugars and the linkage between them. Cellulose and β -glucans are formed with linear 1-4 β-linked glucose polymers. Hemi-cellulose contains xylan, galactan or mannan with side chains of arabinose and galactose. Pectin contains chains of galacturonic acid with side chains of glucose, galactose and rhamnose (Lewis and Southern, 2001).

Table 1: Common non starch polysaccharides (NSP) in Pig (Lewis and Southern, 2001).

NSP	Constituents monomers	Common sources in pig diets
Cellulose	Glucose	Cereals, legumes & forages
Hemicellulose	Glucose, rhamnose, xylose, galactose, arabinose	Cereals, legume hulls
β-glucans	Glucose	Barley, oats, rye
Pectins	Uronic acids	Fruits, chicory & sugar beet pulp
Fructans & inulins	Fructose, glucose	yam, rye, chicory

Due to lack of endogenous enzymes in pigs, digestibility of these carbohydrates is restricted to microbial degradation in the large intestine. A major part of NSP digestibility occurs in large intestine in pigs. Digestibility of NSP is affected by different factors including animal species, solubility, chemical structure and their amount in the diet (Choct and Kocher, 2010). NSP solubility also affects digestibility, digestibility of insoluble NSP (Cellulose) is rather limited to 34-60%, whereas for soluble NSP (β-glucans) it can be almost complete. Increase in digesta viscosity is caused by β-glucans, which can be reduced by in-feed enzymes. More than 75% of β-glucans are digested as soon as it reaches small intestine, but insoluble NSP like cellulose, arabinoxylans and uronic acids are left undigested in the ileum. Digestibility also differs between processing; cellulose digestibility in whole wheat flour was 60%, whereas cellulose from wheat flour with pericarp and testa was 24%. Addition of fiber digesting enzymes like cellulases in feed to young pigs break down carbohydrates and improves digestibility. Commercially mixture of amylases, β-glucanases, pentosanases, general cellulases, lipases and proteases are available for dry feed incorporation in pig diet. This incorporation will improve digestibility of starch and NSP. The fermented products (VFA) after breakdown of insoluble NSP in the large intestine provides with 10-24% of the total energy for maintenance and some (1-4%) of the energy comes from the organic acid flow in ileum, depending upon the nature and quantity of carbohydrates in the pig diet (Bach Knudsen and Hansen, 2009).

Swine dysentery is mainly caused by *Treponaema spirochaete* and *Brachyspira hyodysentriae* in the large intestine. Disease transmission generally occurs through ingestion of infected fecal materials (Kyriazakis and Whittemore, 2006). Dietary changes influence the bacterial environment in gastro-intestinal tract. Structure and different forms of carbohydrates alters the substrate available in fermentation of gut microflora in the tract. Durmic *et al.* (1998) have shown that high concentration of NSP in pig diets are a likely cause of swine dysentery due to high fermentation in the large intestine, as confirmed by shedding of fecal *B. hyodysentria*. In contrast, in diets with low NSP content, low fermentation in caecum and colon occurs, which prevented microbial colonization of *B. hyodysentriae* due to reduction in substrate for growth of the bacteria or due to inhibition by the gut microflora. However, in the study by Lindecrona *et al*. (2003), feeding pigs with low NSP did not prevent swine dysentery; neither did an increase in NSP result in higher incidence of swine dysentery. Feeding fermented liquid feed with high NSP resulted in lower incidence of swine dysentery as compared with the other diets. The etiology behind these effects in the large intestine is yet to be found out (Pluske *et al.*, 1996).

2.2 Energy

Feed energy is used for maintenance of normal metabolic activity like muscle movement, respiration, digestion, blood circulation, recycling of existing body tissues, milk synthesis, thermoregulation, fatty tissue growth and reproductive function. Energy content of the feed is not completely utilized or available for metabolism, since some is not digested and left in the feces or some escapes in the form of gas. Gross energy (GE) in the feed can be calculated through measurement of heat of combustion. Digestible energy (DE) is remained after deducting undigested energy in the feces. Metabolizable energy (ME) is energy after removing energy from feces urine and gases. Net energy is energy retained in the body along with energy needed for basal metabolism. Net energy (NE) is value remained after deducting ME and energy lost as heat excluding heat associated with basal metabolism (Fig1) (Kyriazakis and Whittemore, 2006).

Energy is the product after oxidation of carbohydrates, protein and lipids in the feed. Estimated GE values are 17.5 MJ /kg in carbohydrates, 39.3 MJ /kg for lipid and oil and 23.6 MJ/kg for protein, respectively (Kyriazakis and Whittemore, 2006). Energy density of DDGS is estimated from around 12.6 MJ ME/kg DM (NRC, 1998) up to nearly 15.9 MJ ME/kg DM (Feedstuffs reference issue 1999; Distillers feed handbook, 2000) in USA. However based on chemical composition and different production plants ME differs between various DDGS (Spiehs *et al.*, 2002).

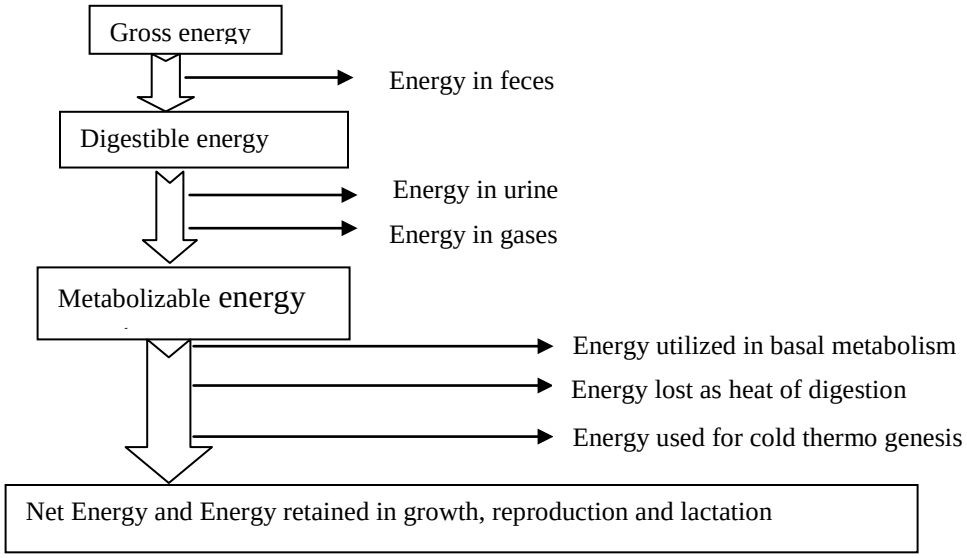

Fig 1: Flow chart of energy used in the pig body

2.3 Phosphorous in the diet

Soaking of a non-heat treated wheat based feed prior to feeding significantly reduced the level of P bound to inositol penta- and hexaphosphate (IP_5-IP_6) in the feed (Lyberg, 2006). Phytase enzyme in the feed will release more indigestible plant phosphorus and result in more available phosphorus (Kyriazakis and Whittemore, 2006). Retention of phosphorus is affected by P level in the diet and soaking tends to increase retention of phosphorus. Fermented or phytase treated feed has increased P digestibility compared to untreated or soaked feed. Fermentation also cause increased apparent ileal digestibility (AID) of P but there was no significant difference in apparent total tract digestibility (ATTD) in either soaked, fermented or phytase treated feed (Lyberg, 2006). In the experiment by Pedersen et al. (2007), P content in DDGS was 3 times higher than for corn. Only 14% of the total P in corn was digestible for swine. For DDGS, the apparent total tract P availability was increased to approximately 59% after fermentation.

2.4 Fermented feed and organic acids in pig diets

Liquid feeding is of great interest in pig nutrition. Non fermented liquid feed is a mixture of liquid and feed prior to feeding, whereas fermented liquid feeding is mixture of liquid and feed at certain temperature and time before feeding. A fermentation process starts with storing the mixture of feed and water with the initial phase having less of lactic acid and yeast, and high pH and higher enterobacterial counts, the process continues to a second stage with low pH and higher population of lactic acid bacteria, high yeast and low enterobacterial counts (Jensen and Mikkelsen, 1998). Fermented feed has high content of lactic acid and VFA, and low pH and contains large population of lactobacilli. Lactic acid bacteria are effective for reducing enterobacteriaceae and salmonella (Canibe and Jensen, 2003). It has also been reported that fermented feed with high protein content, has negative impact in gastrointestinal tract with production of harmful biogenic amines in the intestinal mucosa (Visek, 1978). In addition, significant loss of

supplemented AA (lysine in particular) has been recorded due to decarboxylation of AA during the fermentation process (Pedersen *et al*., 2002).

Organic acids are found in fermented feeds used for animal consumption. These include acetic, propionic, butyric and lactic acids. Organic acids in young piglets have beneficial effects in gut pH maintenance with the reduced incidence of post weaning diarrhea (Kyriazakis and Whittemore, 2006). At pH level above 6 in the gut, efficiency of enzyme action and degradation is lowered and increased susceptibility of pathogenic bacteria arises in gut. Therefore pig gut pH should be maintained at optimum level. There is marked reduction in gastric pH and coliforms counts in stomach when feeding fermented diets to weaned piglets (Jensen and Mikkelsen, 1998). Low gastric pH acts as bacteriostatic property that inhibits growth of undesirable bacteria such as salmonella, coliforms.

2.5 Dietary protein

AA supplementation of feed depends upon AA content in diet, AA digestibility and AA availability. Protein digestibility efficiency increases AA balance in the body with lower level of fecal nitrogen and low urea excretion.

Digestion of dietary protein starts in stomach by the action of gastric proteases and hydrochloric acid. Proteolytic enzyme pepsin in the gastric secretion starts the catabolism of larger protein to peptides. In the lumen of small intestine, pancreatic protease helps in the breakdown of large molecules protein to amino acids and oligopeptides. Pancreatic proteases are in the form of pro-enzyme, it comprises groups of endopeptidases which contain trypsinogen, chymotrypsinogen and proealstase and groups of exopeptidases which contains pro-carboxypeptidases A and B. In the duodenum activated form of trypsinogen (trypsin) is released from the membrane by enterokinase and enzyme liberated from duodenal mucosa. Thus trypsin further activates trypsinogen and other pancreatic proenzymes and digestion of protein by the pancreatic enzymes liberates AA and oligopeptides (McDonald *et al*., 2002). Finally brush border peptidase hydrolyzes oligopeptides having more than 3 AA in the extracellular region and both brush border and cytoplasmic peptidases hydrolyzes tri and di-peptides. These breakdown products are absorbed intact and transported through active transportation (Lewis and Southern, 2001).

Nearly 30-50% of the feed energy consumed is used for anabolism of lean tissue in growing pig. AA is required for protein building in body and replaces the lost protein during protein tissue turnover. Out of 20 essential AA, lysine, methionine, threonine, tryptophan, histidine, isoleucine, leucine, phenylalanine and valine are dietary essential AA. These AA are not synthesized in the pig's body. Cysteine and tyrosine are considered as semi-essential AA, because they are synthesized from methionine and phenylalanine, respectively. Lysine is the first limiting AA in a cereal based diets. In new born piglets, the digestive capacity is not well developed for endogenous synthesis of some AA, so dietary supplementation of glutamine and arginine must be given to meet up AA requirement. Dietary AA are also used as source of energy, after deamination, this supports to synthesize body lipids and fatty tissue accumulation. CP (CP = N x 6.25, assuming that protein contains 16% N) requirement in pig ration comes from cereals like

wheat, barley, corn sorghum or grain by-products, which are low in protein, more protein seed should be supplemented to meet requirement. Distillers' grains are concentrated protein supplement with high CP content (30-40% in DM).

2.6 Ileal vs. fecal digestibility

Determination of AA digestibility is often based on ileal digestibility rather than fecal digestibility (Fan *et al*., 1994) as this is considered to be more accurate and relevant for estimation of digestibility. By using this approach the modifying effect of the micro flora in the large intestine can be avoided (Knabe et al., 1989). Ileal digestibility coefficients can be determined by direct, difference and regression techniques. These methods are also used for the digestibility evaluation of other nutrients and dietary components.

a) Direct method: This is the most common method used to determine digestibility. This method is used in assay diets which can be formulated with a sole source of dietary AA. The direct method is not used with cereal grains, like wheat, corn, barley, rye, triticale and oats, because of the perplexing effects of endogenous losses. Low protein diets show low AID values (Fan and Sauer, 1995), due to large ileal endogenous losses (Fan *et al.,* 1995).

b) Difference method: This method should be used for low protein diets (< 18% CP) as discussed by Fan and Sauer (1995), and is applicable for cereal grains. This method requires use of at least two diets (semi-purified basal diet) having one protein source in each diet. Feedstuff of interest is mixed in one of the diets at the expense of starch and the other protein source. Both the diets should have similar level of protein. At last AID is calculated by direct method in both diet and AID in the interest feedstuff is calculated by difference.

c) Regression method: In this method digestibility is determined using a basal diet (barley or wheat) and assay diets with graded levels of the test ingredient. The basal diet is composed to have good palatability to avoid feed refusals. Digestibility in each the test ingredient is estimated through linear relationship between AID in the assay diets. This method is free from associative interaction between ingredients and is applicable for both low protein and high protein diets (Fan *et al.,* 1995).

Furthermore, the direct, difference and regression techniques are suitable for estimation of digestibility in high protein feedstuffs, whereas in case of low protein feedstuffs, regression and difference method are rather preferred, because endogenous losses is relatively higher at low dietary intake (Fan *et. al.,* 1995).

2.7 Ileal digestibility and endogenous losses

AID is measure of net disappearance of ingested dietary nutrients in the distal ileum (Stein et al., 2007). AID for AA is estimated by deducing total ileal outflow from the dietary intake of AA (Eq. 1) (Stein *et al.,* 2007). Same equation satisfies for the AID of other nutrients like OM, energy Ca, P and CP in the diet.

AID % = {(AA intake- ileal AA outflow)/AA intake} X 100 (Eq.1)

AID is dependent mainly on the nutrients contents in the assay diet. There is curvilinear increase in AID with increase in dietary contents, thus the values remains meaningful under standardized conditions with respect to nutrient in the diet (Fan *et. al.,* 1994). Different methods including AID produces some confounding ileal digestibility values because the sample collected in ileal digesta is not solely of dietary origin, it contains microbial protein, sloughed intestinal cells, mucosal protein and digestive enzymes (Moughan and Schuttert, 1992). True ileal digestibility is the proportion of dietary AA that disappears from the digestive tract within distal ileum and they do not include ileal endogenous AA (Eq. 2) (Stein *et al.,* 2007).

TID %= {(AA intake-(ileal AA outflow-total ileal endogenous AA))/AA intake}X 100 (Eq.2)

TID for feed ingredients is rarely used, due to difficulty in measuring total ileal endogenous losses. Ileal endogenous losses consist of basal and specific losses. Basal endogenous losses are the amount of nutrients being lost from the animal despite of the type of diet fed (Stein *et al.,* 2007). AID is corrected for the endogenous losses and the values obtained after deduction of the basal ileal endogenous losses is termed as SID (Stein et al., 2001). SID (Standardized ileal digestibility) is more precise and accurate measure of digestibility than AID. AID underestimates in feed ingredients with relatively low concentration of nutrient, because digesta collected at the distal ileum contains ileal endogenous losses along with other undigested dietary protein, whereas with a increase in dietary concentration there will be decrease in contribution of ileal endogenous losses, which in turn increases AID. Thus AID is fluctuating with dietary concentration of CP and AA and SID helps to measure ileal digestibility with more precise and greater accuracy (Moter and Stein, 2004). SID is calculated by deducing basal endogenous losses (Eq. 3) (Stein *et al.,* 2007).

SID%={(AA intake-(ileal AA outflow-basal ileal endogenous losses))/AA intake}X100 (Eq.3)

There are three different methods to estimate basal ileal recoveries endogenous, feeding nitrogen free diets, providing protein with 100% protein and AA digestibility or the regression method (Stein et al., 2007). CP and AA in casein is 100% digestible and the ileal recoveries of the pig fed with casein diet (as sole source of protein) determines the basal ileal endogenous losses. It increases with increased dietary CP contents thus represents a major methodological tool for SID estimation (Eklund et al., 2008). Specific ileal endogenous losses are losses induced by type and characteristic of the feed ingredients like concentration, type of fiber or anti nutritional factors. However no procedure is available for direct measurement of specific ileal endogenous losses, but the combined specific and basal ileal losses are being estimated by using homo arginine technique and isotope dilution technique. Thus specific endogenous losses are then calculated by deducing basal ileal endogenous losses from the total (Stein *et al.,* 2007).

There are different factors which affect digestibility, they are listed as follows:
Heat damage: Digestibility decreases when the protein in the feed is heated and denatured. In case of overheating, digestibility falls dramatically to 50% or less. Lysine

digestibility and availability is decreased after heating, which binds protein to sugar compound forming Maillard type reaction. Since lysine is limiting AA, its digestibility plays important role in other AA utilization.

Protein structure: Proteins in skin and feathers are resistant to enzymatic degradation by digestive enzymes.

Abrasion: Physical nature and condition of feedstuff may increase rate of tissue losses through abrasion or epithelial tissue sloughing. This prevents re-absorption of protein in the body. Highly fibrous and abrasive feeds like straw are associated with such activity.

Feeding level and passage rate: Digestibility is reduced with increase in passage rate of digesta through intestine.

Anti-nutritional factors: Factors that interferes with utilization of protein also decreases digestibility. These factors affects in growth, feed efficiency and animal health. Examples are: cyanogenic glycosides found in cassava meal; winter sown rapeseeds contain glucosinolates which are goitrogenic; lectins, protease inhibitors, tannins and phytates that affects protein digestibility; amylase inhibitors and polyphenols that affects carbohydrate utilization.

Feed processing: Digestibility is enhanced by effective particle size reduction, grinding, pelleting, and pre-heating before feeding. Mild heating in-activates anti-nutritional factors, whereas high heating reduces digestibility (Kyriazakis and Whittemore, 2006).

In the experiment by Pahm *et al*, (2008), CP digestibility in WDG varied with DDGS from ethanol production and high AA digestibility in whole stillage than in solubles was observed. Similarly Näsi (1985), also mentioned that, AID for CP was found lower in solubles than in DDG. Furthermore, Liang *et al*. (2002), also reported that AID of amino acid in corn DDG is lower than DDGS because of processing technique, where DDG is substrate free from concentration and evaporation of soluble fraction. Due to high amount of solubles added in distilled grains, there occurs a variability in AA digestibility particularly lysine because solubles contain some amount of residual sugars that escape fermentation and thus possibility through Maillard reaction occurs when it is dried, which results in higher digestibility of AA in DDG than in DDGS (Pahm et al., 2008). Crawshaw (2001) also explained that almost all starch with a lot of ash was found in the liquid phase, whereas protein and fat has been equally distributed in both liquid and solid fractions. AA digestibility in DDGS is slightly lower than corn, because not all AA in DDGS are utilized as well as in corn and high dietary fiber in DDGS reduces AA digestibility (Stein *et al.*, 2006).

2.8 Wet and dried distillers' grain

Corn is the major source of DDG used for ethanol production in United States of America, whereas wheat is predominantly used in Europe and Canada (Cozanet et. al., 2010). Ethanol, carbon dioxide and distillers grains are the obtained products from cereals during fermentation process. The co-products are marketed as WDG, distillers' solubles or DDG (after drying WDG and solubles). Dry grind process is generally used for the fermentation of large amount of grains. The process involves grinding, cooking, liquefaction, saccharification and fermentation. Starch in the cereals is broken down into simpler 6 carbon sugars before fermentation.

At Absolut AB, Sweden (Fig 2), the process starts when wheat enters mash preparation, where water and enzyme (Enzyme 1, amylase) is added and mixed for 60 minutes. The mash is then heat treated in two containers for 2 hours at 92 ºC. The mash is further diluted with water to the desired amount of fermentable carbohydrates. Mash is then pumped to fermentation chamber where yeast is added with the enzymes gluco-amylase and protease (Enzymes 2). Glucoamylase converts starch into glucose. It is called saccharification (Bothast and Schlicher, 2005). Fermentation lasts for 48 hours in 33ºC, and then the fermented mass is pumped in to distillation column. Distillation is separation of ethanol from the solid and water in the mass mixer. Remaining solid and liquid fractions after distillation is termed as whole stillage. It contains fiber, oil and protein components, and unfermented starch (Bothast and Schlicher, 2005).The thin stillage coming out from distillation column is heat exchanged and then recovered at the temperature of 38 ºC. After distillation, the stillage is taken either to the storage unit for wheat wet distillers' grain with solubles (WWDGS) that is sold to farmers or it is dried (Drying unit). At Lantmannen Agroetanol AB, Sweden, WWDGS is produced in a similar way (Fig 2) and further pelleting of Wheat dried distillers' grain with solubles (WDDGS) is done (Fig 3). Both methods of production need strict supervision to prevent bacterial contamination during mashing. This contamination may cause formation of acids that diverts glucose from ethanol production and interferes with the fermentation. Mouldy grains, improper storage, improper equipment and contaminated air or reintroduced stillage can cause bacterial contamination and decrease in ethanol production (Bothast and Schlicher, 2005).

Overall DM digestibility of wheat distiller's grain is recorded lower than in corn based diets (Pedersen *et al*., 2007). Commonly available distillers co-product is DDGS, which contains almost 70% of condensed soluble are produced after fermentation. If no soluble are added, the product is called as DDG. In processing, if grains are further de-hulled or de-germed before fermentation, the product is called high protein distillers dried grains with solubles, which contains high protein and less fiber and fat (Widmer et al., 2007).

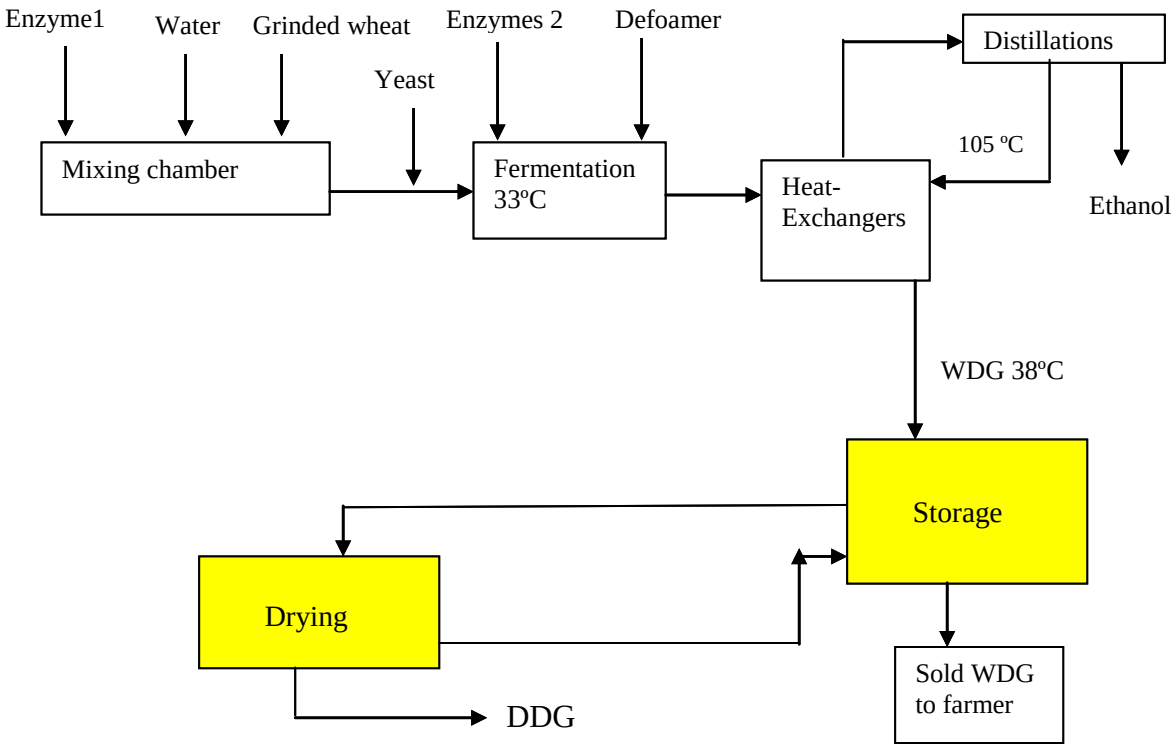

Fig 2: Schematic diagram for distillers grains byproducts formation in a plant from wheat (Absolute AB, Ahus, Sweden).

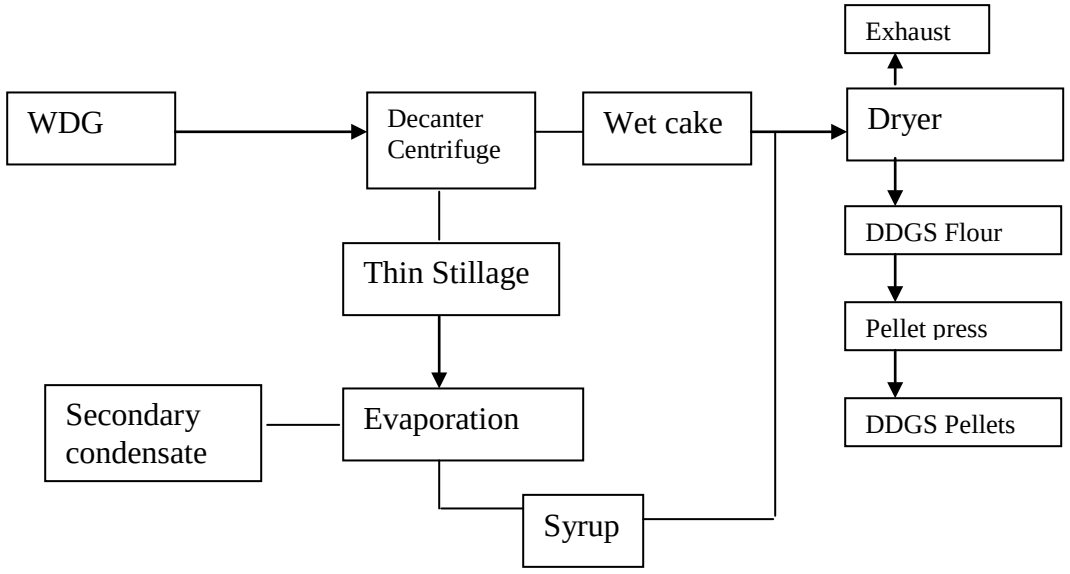

Fig 3: Process of formation of Dried distillers grain with soluble (DDGS) after similar principle as in Figure 2, Lantmannen Agroetanol AB, Norrkoping, Sweden.

3. Materials and methods

3.1 Animals and housing

Seven castrated male pigs (Swedish Landrace x Yorkshire) with initial average body weight of (79±9) kg were used for the experiment. The pigs were surgically fitted with post-valve t-caecum (PVTC) cannulas (van Leeuwen et. al., 1991) at an average weight of (22±0.73) kg, and they were used in another study prior to this experiment. Average final body weight was (116±10) kg.

Pigs were housed individually in a pen with rubber mats without bedding. The pigs were allowed to run in aisle once a day for observation and animal welfare. The temperature of the room was maintained in average at (22±2) ºC in the morning and (21±3) ºC in the evening. The light interval was maintained 14/10hr dark/artificial light cycle. Free access to water was provided all the times through low pressure water bowls and from nipple drinkers.

3.2 Experimental design

The experiment consisted of four experimental periods. The main study was arranged as a two period change-over design with seven pigs and two diets. Each experimental period comprised of 14 days in total and included 7 days of adaptation, 4 days of fecal collection and 2 days (day 12 and 14) of ileal digesta collection.

3.3 Diets and feeding

In one period before the main study and in one period after the main study a casein-based diet, with casein as the only protein source, was fed to allow estimation of the basal endogenous losses of AA and CP.

In the main experiment two experimental diets (W and D) was fed. Diet W was composed of 50% WWDGS and 50% of a basal diet, and diet D was composed of 50% WDDGS and 50% of a basal diet. The basal diet was composed of corn starch, sugar, premix (minerals and vitamins) and titanium dioxide (TiO_2) (Table 2). The WWDGS was the residue from ethanol production from Absolut AB (Ahus, Sweden) and WDDGS was the residue from biofuel ethanol production from Lantmannen Agroetanol AB (Norrkoping, Sweden). Diets were formulated to meet Swedish recommendations for growing pigs (Simonsson, 2006).

Daily feed intake was 2.4 kg DM for all pigs. Water was added respectively in proportion (6:1) to dry feed for a balanced DM content in the given treatments.

3.3 Sample collection

Feces were collected and pooled for each pig, twice a day from day 8 to 11 in each experimental period and then frozen in -20 ºC. Collection of ileal digesta through PVTC cannulas were carried out during 1 hr periods, on day 12 from 8:30-9:30, 10:30 to 11:30, 12:30 to 13:30 and 14:30- 15:30 and on day 14 from 9:30-10:30, 11:30-12:30, 13:30 to 14:30 and 15:30 to 16:30. Digesta were pooled for each pig and each experimental period and then frozen in -20 ºC. Feed samples from WWDGS, C-diet and D-diet were taken daily for chemical analysis. WWDGS samples were frozen in -20 ºC.

Table 2: Feed ingredients (g/kgDM) and chemical composition (g/kgDM) of experimental diets.

	C-diet	W-diet	D-Diet
Ingredients			
Wet distillers' grain		500	
Dried distillers' grain			500
Caesin	175		
Vegetable fat	30		
Cellulose	50		
Corn starch	667.5	425.5	425.5
Sugar	50	50	50
Premix 1*	25		
Premix 2[#]		22	22
TiO2	2.5	2.5	2.5
Analysed chemical composition			
Crude protein	172	200	179
Energy(MJGE/kg DM)	17	16	17

*Content per kg premix 1: retinol 162 400 IE, cholecalciferol 16261 IE, alpha-tocopherol 2436 mg, phytylmenaquinone 81 mg, thiamine mononitrate 81 mg, riboflavin 81 mg, pyridoxine hydrochloride 122 mg, cyanocobalamin 0.81 mg, pantothenic acid 406 mg, nicotinic acid 812 mg; minerals: Fe 3.08 g, Cu 0.61 g, Mn 1.05 g, Zn 3.07g, 10.01g, Se 0.016 g, Ca 256g, P 51 g, Mg 5.82 g, S 2.82g.

[#]Content per kg premix 2: vitamins: retinol 184500 IE, cholecalciferol 18500 IE, alpha-tocopherol 2800mg, phytylmenaquinone 92 mg, thiamine mononitrate 92 mg, riboflavin 92mg, pyridoxine hydrochloride 138 mg, cyanacobalamin 0.92 mg, pantotheni acid 461mg, nicotinic acid 923mg; minerals: Fe 2.71 g, Cu 0.70 g, Mn 1.14 g, Zn 3.48 g, 10.01 g, Se 0.018 g, Ca 288 g, P 0.44 g, Mg 4.31 g, S 3.20 g.

3.4 Sample analysis

Samples of diets, ileal digesta and feces were analyzed for dry matter (DM), ash (AOAC, 2000) and the GE was analyzed through Bomb calorimetry (Parr Instruments 1563, Moline IL). Nitrogen in the samples was determined through Kjeldahl method (Nordic

committee on feed analysis, 2003). Kjeldahl method is used for determination of the total amount of nitrogen in the sample. TiO_2 was used as marker and was analyzed according to Short et al. (1996). Ca and P were analyzed in diet, digesta and fecal samples according to Nordic committee on feed analysis (1998).

3.5 Calculations

AID was calculated using TiO_2 as indigestible marker in the feed and digesta by using the following equation (Fastinger and Mahan, 2006) (Eq.4).

AID % = 100 – ([ND/NF] x [TiF/TiD] x 100 (Eq.4)

ND = Nutrient concentration present in illeal digesta
NF = Nutrient concentration present in feed
TiF = TiO_2 concentration present in feed
TiD = TiO_2 concentration present in illeal digesta

Endogenous CP or AA losses (EAL) was calculated as described by Moughan et al. (1992) (Eq. 5) and SID according to Stein et al. (2007) (Eq. 3), where EAL of each pig was used.

EAL = (ND x [TiF/TiD]) (Eq. 5)

3.6 Statistical Analysis

Data in the experiment were analyzed in procedure mixed in the SAS program version 9 (SAS Institute, Inc, Cary, NC, USA). In the evaluation of AID and total tract digestibility (TTD), the model included treatment (D-diet and W-diet) and periods (1,2) as fixed effects and different pigs as random effect. Testing of carry over effect from the previous experiment was also included in the model. All the results were tabulated using least square mean and standard error of the differences (SED).

4. Results

During the experiment, average daily weight gain was recorded as (670± 8) g per day and one pig died due to blockage of intestine by cannula after the first period. Average concentration of GE in WWDGS was 20.7 MJ/kg DM and WDDGS was 19.6 MJ/kg DM of the feed respectively. W-diet in the experiment gave a higher AID ($p < 0.05$) for CP as compared to D-diet. AID for OM and energy in W diet was similar to D diet. TTD for CP was higher ($p < 0.0001$) in W diet than in D diet, whereas there was higher TTD for OM ($p < 0.05$) in W diet than D diet, but there was no significant difference in energy digestibility ($p > 0.05$) (Table 3).

Higher P digestibility ($p<0.05$) in W-diet than in D-diet was observed, whereas there is no significant difference in Ca digestibility ($p>0.05$) (Table 3). Higher methionine, CP

and lysine digestibility (P<0.05) was observed in WWDGS than WDDGS. SID values of CP, methionine and lysine for WWDGS and WDDGS correspond to the SID values for W-diet and D-diet. Significantly high lysine digestibility (P=0.0004) was observed in WWDGS than dried one (Table 4).

Table 3: Ileal and total tract digestibility of W diet and D diet and the calculations.

		D-diet	W-diet	SED	P-value
Ileal digestibility	Crude protein	69	76	2.06	0.02
	Organic matter	68	70	1.13	NS
	Energy	66	68	1.37	NS
	Ca	46	48	2.25	NS
	P	57	42	2.61	0.004
Total tract digestibility	Crude protein	69	83	0.82	<0.001
	Organic matter	81	82	0.34	0.04
	Energy	78	78	0.47	NS
	Ca	52	53	2.37	NS
	P	54	44	2.33	0.02

W-diet: WWDGS mixed with basal diet (starch mixture) D-diet: WDDGS mixed with basal diet (Satrch mixture) OM: Organic matter, ATTD: Apparent total tract digestibility, AID, Apparent ileal digestibility, SID: Standardized ileal digestibility.
NS indicate no statistical significance (p >0.05).

T able 4: AID and SID values of crude protein for WDDGS and WWDGS in the experiment

		WDDGS	WWDGS	SED	P value
AID	Methionine	66	73	1.18	0.006
	Lysine	37	66	2.72	0.004
	CP	69	76	2.06	0.023
SID	Methionine	67	73	1.18	0.0067
	Lysine	38	66	2.69	0.0004
	CP	69	76	2.07	0.028

AID Apparent ileal digestibility and SID standardized ileal digestibility, CP- crude protein WWDGS-Wheat Wet distillers grain with soluble, DDGS-Dried distillers' grain with soluble, CP: crude protein,

Endogenous losses for every pig were evaluated separately for SID calculations for the diets. The average endogenous loss of CP was 8.5 (±0.9) g/kg DM digesta. SID values for WWDGS and WDDGS respectively correspond to the SID values for W- and D-diet respectively as they are the only CP sources in the diets. SID values for CP in W-diet and D-diet was found higher than AID values. In addition, W-diet has higher SID values (p<0.05) than D-diet (Table 4).

Average lactic acid, acetic acid and propionic acid concentration in WWDGS were 1.4, 7.0 and 0.8 g/liter, respectively. Butyric acid and formic acid concentration in WWDGS were <0,01%. Average pH of WWDGS in the container was 3.86 (±0.02). Major constituents of NSP like xylose, mannose, galactose and glucose were 8.0%, 1.7%, 0.8%, 9.1% respectively in WDDGS and, 8.3%, 1.9%, 0.8% and 8.8% in WWDGS, respectively. Total neutral sugars in WWDGS were 25.6%, whereas it was 24.6 % in WDDGS.

5. Discussion

The study was conducted to estimate AID and TTD of energy, OM and CP values in WWDGS and WDDGS from different production plants. In the experiment the GE value in the WWDGS and WDDGS in the diets were similar with GE value of wheat WDG and wheat DDGS in the experiment by Pedersen and Lindberg (2010). The ME for corn DDGS by NRC (1998) was reported nearly 12.5 MJ ME/kg DM and up to 15.9 MJ ME/Kg DM by others (Feedstuffs reference issue, 1999; Distillers feed handbook, 2000) in USA. However based on chemical composition, ME differs between various DDGS. In the experiment by Stein *et al.* (2006), ME values were 1.25 MJ ME less then Spiehs *et al.* (2002). In the experiment by Pedersen *el al.* (2007), average ME in 10 different DDG samples from different plants in US, were 29% greater than current NRC (1998) values. However, these values were found similar to Spiehs *et al.* (2002). The underlying reason behind the greater value of ME in DDGS as compared with NRC(1998) may be that, modern ethanol plants use recent advances in production processes that differs from old one (Spiehs *et al.,* 2002). TTD of GE in W-diet and D-diet (78 and 77) in the experiment was pretty similar with energy digestibility of WWDGS and WDDGS (82 and 76) respectively in the experiment by Pedersen and Lindberg (2010) (Table 5).

Table 5: Comparision of digestibility measure of current experiment with Pedersen and Lindberg (2010)

		Pedersen and Lindberg, 2010		Present Study	
		W-Diet	D-diet	W-diet	D-diet
ATTD	OM	84	82	83	81
	Energy	82	76	78	77
AID	OM	68	62	69	67
	Energy	67	60	66	68
	CP	61	57	76	68
	Lysine	64	41	66	37
	Methionine	58	61	73	66
SID	CP	75	70	76	69
	Methionine	66	74	73	67
	Lysine	75	50	66	37

W-diet: WWDGS mixed with basal diet (starch mixture) D-diet: WDDGS mixed with basal diet (starch mixture) OM: Organic matter, ATTD: Apparent total tract digestibility, AID, Apparent ileal digestibility, SID: Standardized ileal digestibility.

The ATTD for OM and energy of the starch diets in the experimental diets is almost complete. The decrease in ATTD of OM and GE was exclusively due to addition of tested feedstuffs (WWDGS and WDDGS). ATTD of OM and energy in W-diet (83 and 78) were comparable with values from Pedersen and Lindberg (2010) (84 and 82 respectively in diet having 200 g CP/kg DM). In similar way, ATTD for OM and energy in D-diet were found 81 and 77 respectively, which was comparable with Pedersen and Lindberg (2010) (82 and 76 respectively in diet with 200 g CP/kg DM). ATTD for OM in W-diet was slightly higher than D-diet (Table 5). This difference in OM digestibility in this type of product is determined by insoluble fiber content in the diet (Pedersen and Lindberg, 2010).

The CP content of DDGS and WWDGS were 31% and 35% in the study, which was similar with CP content in the study by Pedersen and Lindberg (2010). SID value for CP in the present study agrees with the similar results shown by Pedersen and Lindberg (2010), whereas there was high variation in methionine and lysine digestibility. In addition, high SID variation in lysine for WWDGS than in WDDGS, may be due to reduction in lysine digestibility during drying process or nutritional composition differs between different production plants (Spiehs *et al.,* 2002). Higher AID for CP for fermented WWDGS in the experiment agrees with the observation showing that AID for nitrogen is enhanced if wheat barley/based diets is fermented before feeding (Lyberg et al., 2006). In the experiment by Pedersen and Stein (2010), there was reduced AID for CP, OM and energy, where pigs were fed with diet mixed with dry feed and water in ratio 1:3 immediately before feeding. Furthermore the experiment concluded that nutrient digestibility is not affected, if the dry feed and water is mixed in ratio (1:1) (Pedersen and Stein, 2010).

In this study, average acetic acid concentration was relatively high in WDG. The presence of high concentration of acetic acid in the WWDGS may make the feed less palatable (Shelef, 1994). In addition, natural fermentation may not be appropriate to produce safe and hygienic feed (Beal *et al.*. 2005). Decrease in feed pH improves gastrointestinal health (Scholten *et al.,* 1999). Growth of potential gut pathogens like coliforms and salmonella is reduced below pH 4.5 (Jensen and Mikkelsen, 1998). In the experiment by Lyberg et al. (2008), concentration of acetic acid, succinic acid and propionic acid were higher in WWDGS than in feed mixed with water or feed mixed with whey respectively, whereas lactic acid concentration was significantly lower in WWDGS than in the other two feed.

Fermented feed reduces gastric pH, reduces microbial activity and may change the microbial population in gastrointestinal tract, which may be more likely causes to stimulate pancreatic secretion and improve digestion and absorption of nutrients in the tract (Scholten *et al.,* 1999). Furthermore, the higher difference in AID and ATTD value of CP in W-diet than D-diet, may be due to difference in nutritional composition between different production plants (Spiehs *et. al.,* 2002). Digestibility is also affected by effective particle size reduction, grinding, pelleting and pre-heating before feeding (Kyriazakis and Whittemore, 2006) or different factors like grain selection, heating duration, temperature while drying and fermentation process can effect digestibility (Spiehs *et al.,* 2002; Stein

et al., 2006). SID of CP for WWDGS in the experiment was slightly higher than WDDGS. This may be due to higher dietary CP level and higher AID in WWDGS than WDDGS, which causes increase in endogenous losses (Eklund et al., 2008).

6. Conclusion

In the experiment there was difference in chemical composition and nutritive value of WWDGS and WDDGS, with higher AID and ATTD of CP and with higher SID of CP, methionine and lysine in WWDGS. However, due to low DM and high transportation cost of WWDGS, it is only feasible to use this feedstuff for pig producers within reasonable distance from the production plant. This study confirms that the nutritional properties of co-products from ethanol production plants may differ considerably due to difference in production technology.

7. References

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Nr	Titel och författare	År
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