THE EFFECT OF INCREASING DIETARY LEVELS OF CORN DISTILLERS DRIED GRAINS WITH SOLUBLES (CDDGS) ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN NURSERY PIGS.

by

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(Under the Direction of Michael J. Azain)

ABSTRACT

The objective of this paper was to evaluate the effect of diet formulation methods and addition of fiber degrading enzymes on growth performance, and nutrient utilization in nursery pigs fed high levels of CDDGS. In experiment 1, a total of 135 pigs were randomly selected for a 28 day study that was conducted in two trials. Pigs were randomly assigned to one of five treatments: positive control (Corn/SBM+0% CDDGS), negative control (30% CDDGS substituted for corn/SBM with no enzyme addition), negative control + 4000 unit/kg xylanase, negative control + 450 unit/kg β-glucanase, and negative control + xylanase + β-glucanase. Addition of xylanase and β-glucanase improved DM, CP, P, NDF, ADF, and hemicellulose digestibility, and ADG when compared to negative control. The results suggest that supplementing XYL, BGL, and XGL not only improved growth performance in pigs fed 30% CDDGS diet, but also altered the negative impact of CDDGS on nutrient digestibility. In second study, the first approach (Exp. 1) was based on formulating for total lysine in the diet and the second (Exp. 2), was based on balancing for standard ileal digestible (SID) lysine and maintaining a balanced amino acid pattern. In Exp 1, a total of 160 pigs were randomly assigned to one of four diets: 0% CDDGS, 10% CDDGS, 20% CDDGS, 30% CDDGS. In Exp 2, a total

of 145 pigs were assigned to one of five diets: 0% CDDGS, 10% CDDGS, 20% CDDGS, 30% CDDGS, 37.5% CDDGS, and 45% CDDGS. In Exp 1, pigs fed the 20% CDDGS did not reduced ADG (398 g/d), and those fed the 30% CDDGS diet had significantly lower ADG (361 g/d, $P<0.001$) as compared to the 0% CDDGS (416 g/d). In Exp. 2, however, increasing CDDGS up to 45% had no detrimental effect in ADG or ADFI ($P> 0.20$) when diet had balance amino acid. Apparent total tract digestibility of protein, fiber, energy and phosphorus were also evaluated in both phase 2 and 3 of Exp 2. In contrast, phosphorus digestibility increased with increasing dietary CDDGS. The results indicate that high levels of CDDGS can be used in nursery diets and are well tolerated if the diets are formulated on a constant SID lysine basis with a balanced amino acid pattern.

INDEX WORDS: CDDGS, Diet formulation, Fiber degrading enzymes, Growth performance, Nursery pigs, Nutrient digestibility.

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DEDICATION

I would like to dedicate this dissertation to my lovely wife, Chyong-Huoy Huang. Without her support and contribution to our family, I would not able to finish my PHD program. No matter what challenges in front of us, I deeply believe that we can pass that together. My future can not continue without you.

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CHAPTER 1

INTRODUCTION

Feed costs represent a major proportion of livestock farm expense, and those feed ingredients, which serve as energy sources in the diet, are responsible for approximately 70% of feed costs. Corn is used as a main feed ingredient for livestock in United States, and therefore the price of corn would be expected to affect the overall cost of pork production. In order to lower feed cost, DDGS, which is a byproduct of ethanol production and often an undervalued ingredient is being used to greater extent in livestock rations. Because of its high fiber content, DDGS is considered more applicable for feeding ruminant animals. Feeding DDGS to monogastric animals can be practiced but it is recommended that its inclusion in the ration be limited. Strategies that allow increased usage of DDGS in swine & poultry without compromising growth performance would provide these industries a more cost effective option to reduce feed cost particularly as corn and other feed ingredients are expensive.

The particle size of typical DDGS is greater than ground corn. Since particle size of dietary ingredients can affect pig growth performance, it may be useful to reduce DDGS to improve nutrient utilization of pigs fed high DDGS diets. Currently, there are no published reports on the effects of particle size on nursery pigs growth performance when fed diets containing DDGS.

Fiber degrading enzymes are more used as feed additives in European and Canadian livestock industries because high fiber grains such as barley are more prevalent feed ingredients. The fiber content of DDGS is relatively higher than corn. Therefore, addition of fiber degrading

enzymes to DDGS containing diets could also facilitate its digestion, and result in better growth performance, and nutrient retention in nursery pigs.

Corn contains relatively lower lysine (0.26%) than protein feed ingredients, and is not considered as a good balance in amino acids pattern for poultry and swine. Although DDGS (corn) has higher crude protein (CP) than corn, overheating during drying process in CDDGS production makes lysine even more unavailable to animals. Unbalanced amino acids could lead to lower feed intake and performance, and require extra metabolizable energy for urea cycle thus results in lower growth performance. Crystalline amino acids have been used to fortify swine diets and meet those essential amino acids needed for livestock. Crystalline amino acids could be especially useful to help balance amino acid profile in higher DDGS containing diets without compromising performance.

Hypothesis of this study is that reducing growth performance in high level of CDDGS fed to nursery pigs is due to high fiber content and imbalance amino acid in CDDGS.

CHAPTER 2

LITERATURE REVIEW

CURRENT ISSUES FOR FEEDING LIVESTOCK

Feed costs in swine industry represent 65-75% of the total cost of livestock production (Cromwell, 2002), so an increase in feed cost will either increase cost of pork meat to consumers or reduce the profitability to the producer. Corn & soy bean meal (SBM) are the major feed ingredients used in feeding livestock in United States. The reason is that the nutrient profiles and climate in U. S market make corn and SBM the gold standard feed for livestock animals. Corn is characterized as energy feed ingredient because of high starch content (71%) (Belyea et al., 2004). On the other hand, SBM is classified as protein feed ingredient as its range from 44% to 48% CP. In regards to amino acids profile, corn has relatively high methionine and low lysine. SBM, however, is high in lysine and low in methionine. Thus, corn and SBM are very complimentary nutritionally and cost-effective to produce supporting their extensive use in swine feeding. With the recent increase in corn price due to biofuel production, the U.S. swine producers is considering alternative feeds to substitute for corn and SBM as the main feed ingredient.

The U. S. government subsidy for blending ethanol with gasoline and using biodiesel as fuel has increased the demand for corn for ethanol and soybeans for biodiesel production. Ethanol production has increased dramatically for the past five years, and will most likely continue to rise in the future (30 billion gallons per year by 2020 and 60 billion gallons per year by 2030) (Harkin et al.; Vijay Singh, 2007). The demand for using soybeans for biodiesel

production has also increased in the past five years. An increase in the demand for biodiesel produced by using soybean oil, therefore, can result in an increase in the price of soybean (N.B.B., 2006). Thus, the need for corn for biofuel production has resulted in dramatic increase in the price of corn and other feed ingredients. Although petroleum cost has dropped, resulting in some ethanol plants closing, and the search for alternative sources of biomass for ethanol production is proceeding, corn will likely continue to be processed into ethanol as a biofuel additive into the future especially if petroleum costs rebound.

The other concern is that the human population is growing continuously, but the amount of land for crop is constant. Although crop production has increased per unit of land, the increase in human population makes it likely that it will be difficult to produce sufficient crops for both human and livestock needs. On the other hand, farmers are blamed for using up corn to feed livestock, and directly or indirectly leading to starvation in people (Henning Steinfeld. et al., 2006).

In order to lower feed costs, and maintain reasonable cost of production for pork and poultry, the feed industry must continue to evaluate economically alternative feed ingredients such as distiller dried grains with solubles (DDGS).

DISTILLERS DRIED GRAIN WITH SOLUBLES (DDGS)

Background

Using ethanol as fuel was first introduced more than a hundred year ago (1896). After that the demand for ethanol as biofuel has not increased much until 10 years ago in Unite States. During the past ten years, several change occurred in the climatic conditions, and petroleum

prices rose drawing people's attention to biofuel production. The United States currently is the world's largest ethanol producing country, while Brazil is second (Fox, 2008). Corn accounts for more than 80% of total ethanol production. The calculated amount of corn needed for biofuel production is around 4.2 billion bushel, which is about 21% of total corn yield of 2009/2010 marketing. Although using corn to produce ethanol is not very energy efficiency, compared to sugarcane, and is not environmental friendly because of higher fertilizer, such as N and P, needed per unit of energy gain from biofuel production from corn than SBM (Jason et al., 2006), until new generation of biofuel production can meet the demand, corn will continue to be used for ethanol production (Tokgoz Simla. et al., 2007).

Ethanol production process

There are two major procedures used in processing corn grain for producing ethanol: dry grind and wet milling. Processing corn by both dry grind and wet milling can generate ethanol, but dry grind can produce more ethanol per bushel of corn while wet milling generates more feed co-products.

Dry grind

More than 80% of ethanol plants use dry grind method to process corn. Part of the reason is because solvent (sulfur oxide) used in wet milling process is corrosive, and requires biohazard management. In dry grind process, the whole corn kernel is ground, and is slurried in water. Next, amylase is added to the fermentation tank to release sugars (glucose). Afterwards, yeast, such as Saccharomyces cerevisiae (Kuyper, 2005), converts sugars to ethanol and CO_2. Ethanol is collected for biofuel while CO_2 is used for beverage products subsequently. At the same time, the rest of the stillage is centrifuged to separate out wet grain and thin stillage. Thin stillage can be

condensed and added back to wet grain followed by dehydration and drying to produce DDGS (Figure 1) (Davis, 2001; Rausch, 2005a; Tao and Aden, 2009).

Wet milling

In the wet milling procedure, corn is soaked in sulfuric acid to separate germ, fiber, gluten, and starch. There separate fractions are then further processed: Corn oil is extracted from germ, gluten is processed to make corn gluten meal, which is a relatively high protein feed for livestock. The starch fraction is subjected to enzymatic hydrolysis using yeast fermentation to produce ethanol, CO_2, and DDGS. Wet milling can generate oil, corn gluten meal, high fructose corn syrup, and DDGS as by-products, whereas dry grind can produce DDGS, CO_2, and ethanol. Dry grind, however, can yield more ethanol than wet milling (2.8 gal/bushel vs. 2.5 gal/bushel). Additionally ethanol plants using dry grind process don't have to be concerned about the corrosiveness of sulfuric acid, used in wet milling process (figure 2.) (Davis, 2001; Rausch, 2005a; Tao and Aden, 2009).

Feed stocks of DDGS as a result of ethanol production will likely continue to increase into the future due to the government incentives for ethanol as biofuel. Introducing DDGS to livestock diets is more interesting, and acceptable to swine producers now than it was before (RFA, 2010). Instead of dry grind and wet milling, difference techniques have been created, and discussed for ethanol production that include dry milling, enzyme milling, quick de-germing, and more (Singh et al., 2007; Singh et al., 2005). These new methodologies provide co-product feeds with different nutrient patterns for animals. Proper characterizing of the variation associated with the nutrient content of co-products of different corn processing methods is needed to make DDGS a more standardized and acceptable feed ingredient on the market.

Corn distillers dried grain with soluble (CDDGS) usage in swine:

Corn distiller's dried grains with solubles (CDDGS), a byproduct from corn ethanol production, is an alternative ingredient that can be fed to livestock to lower feed cost (Stein., 2007). Thaler et al., (2002) reported that adding 10% of DDGS can lower feed price by $0.69/pig. In addition, Fabiosa et al., (2008) indicated that using new generation of DDGS can save up to $8/pig in feed cost. At present, DDGS is mainly fed to ruminant animals in United States, and only small proportion of DDGS is supplemented in poultry and swine diet (Fox, 2008). NASS (2007) reported that only 12% of swine farmers add DDGS in their diet, while around 38% of dairy farmers adopted DDGS to their diet. In 2008 (2008), Fox reported that United States' food animal DDGS utilization after excluding export could be attributed to: dairy cattle (42%), beef cattle (42%), swine(11%), and poultry(5%). Renewable Fuel Association at (2010) showed that ratio of DDGS usage in 2009 was 39% by dairy cattle, 38% by beef cattle, 15% by swine, and 7% by poultry. This indicated that more swine farmers accepted DDGS as an alternative feed ingredient since it is cost effective source of nutrient.

DDGS in swine research

DDGS was first tested as a feed ingredient in research conducted in 1943. Krider et al., (1944) fed nursery pigs weighting 11 kg with 0%, 6% and 12% of DDGS diet to market weight. The result showed that DDGS addition improved growth performance (ADG: 422, 458, 517 g/day). Later on, Fairbanks et al., (1945) fed 50kg pigs with 6% DDGS to market weight, that resulted in 18% increase in ADG than control diet. The effect of adding CDDGS to feed during different phase of production in pigs is discussed in the following sections.

Effect of CDDGS in nursery phase

The inclusion rate of CDDGS depends on animals' status. The maximal CDDGS supplementation for nursery pigs is suggested as 10% to 20% of diet (Stein., 2007), but there are relatively few studies where higher levels of CDDGS have been fed to nursery pigs (Spencer et al., 2007; Whitney et al., 2006d). Burkey et al., (2008) reported that nursery pigs (initial BW 7kg) fed 30% DDGS diet had decreased ADG by 22% in phase 2 and 8% in phase3 when compared to a corn-SBM diet. Weber et al., (2008), on the other hand, demonstrated that 7.5% of CDDGS addition to nursery pigs (5kg) did not reduce growth rate. Also, Spencer et al., (2007) reported that 7.5% and 15% CDDGS can be added to the diet of early nursery pigs (6.4kg) without negative effect. Whitney and Shurson (2004) fed 7 kg in Exp. 1 and 5 kg pigs in Exp. 2 with 0, 5, 10, 15, 20, and 25% CDDGS and reported no significant effect on growth performance. However, a linear trend for decreasing ADG was observed in pigs with 7kg initial BW fed with diet containing 15% and higher CDDGS (P=0.09). However, Barbosa et al., (2008) showed that pigs (11kg) fed with up to 22.5% DDGS did not have negative effect on growth performance. Furthermore, better feed efficiency was observed in DDGS diet. In another experiment, Jones et al., (2008) reported that in pigs weighting 9kg, 30% CDDGS diet did not significantly lower ADG, compared to positive control. The variable results in feeding CDDGS to nursery pigs seems due to multiple factors including: the differences in initial BWs of pigs, variable amounts of CDDGS in diet, how diet was formulated, and the quality of DDGS used in the studies.

Effect of CDDGS withdrawal

Addition of CDDGS in diet for finishing pigs may lower carcass quality, but it does not seem to be a problem if CDDGS is used only in the nursery phase. Burkey et al., (2009) reported

that the negative effects of including 30% CDDGS in the nursery pigs can be reversed by exposing nursery pigs to 30% CDDGS and gradually withdrawing CDDGS from diet with an increase in age. Thus, carcass quality because of CDDGS included in diet should not be an issue when CDDGS is only fed to pigs early in their production cycle.

Conclusion:

Overall, Dritz et al., (2007) suggested that 5% CDDGS can be added to the diet of nursery pigs while Stein (2007) suggested that if a good source of CDDGS can be obtained, feeding late nursery pigs with up to 20% of DDGS should be possible without detrimentally effecting growth rate.

Effect of CDDGS on pigs in growth-finishing phase

It is suggested that higher level of DDGS can be used more effectively in mature pigs. Diet containing 0% to 20% CDDGS did not show negative effect on growth performance in 17.6 kg pigs (Wahlstrom et al., 1970). However, Cromwell et al., (1993) fed 20% CDDGS to 16 kg pigs, that resulted in decrease growth performance. Whitney et al., (2006d) conducted a long term DDGS feeding experiment. This experiment was designed to have four treatments 0, 10, 20, and 30 % DDGS included in diet fed to pigs from 28 kg to market weight. The results showed that diets containing CDDGS linearly decreased ADG (861, 855, 821, 806g/d), and feed conversion ratio (0.38, 0.38, 0.37, 0.36) ($P<0.01$). Likewise, Whitney et al., (2006), and Hinson et al., (2007) also demonstrated that feeding pigs (32 kg to market weight) with increasing levels of CDDGS (0%, 10%, 20%) reduced growth rate significantly ($P<0.01$). Moreover, Linneen et al., (2008) conducted two trials, which included 0, 10, 20, and 30 % of CDDGS for trial 1, and 0, 5, 10, 15, and 20 % of CDDGS for trial 2. Pigs weighing 48 kg through finishing under commercial

environment were fed with these diets, and results showed that a linear decrease in ADG (trial 1: P=0.09, trial 2: P=0.02) and ADFI (trial 1: P=0.05, trial 2: P=0.04) with increasing levels of CDDGS in diet. Cook et al., (2005) found that pigs (47kg to market weight) fed up to 30% CDDGS did have detrimental effects on growth performance. In contrast, Widmer et al., (2008) fed pigs (22 kg) with 20% of CDDGS, and showed no decrease in growth performance, which agreed with Yoon et al., (2009) who included 10% and 15% of DDGS in diet fed to grower (60.5 kg) and finisher (86.5 kg) pigs and failed to observe any detrimental effects in growth performance (862 vs. 844g/d).

The performance of pigs fed DDGS in growth-finishing phase is as variable as those reported in the nursery phase. It is still unclear if finishing pigs are able to utilize nutrients from DDGS better than pigs in growing phase. Xu et al., (2010b) observed a linear decrease in ADG in growing phase pigs (initial 20kg) fed gradual incremental levels of CDDGS, up to 30% CDDGS ($P<0.05$), but no negative effects were noticed in either the finishing or overall time period. However, in a different study, pigs (from 30 kg to market weight) fed with 0, 15, and 30 % DDGS showed a decrease in ADG by 0.92, 0.91, 0.87 kg/d ($P<0.05$), respectively (Xu et al., 2010a).

Effect of DDGS on carcass quality:

In ruminant animals, DDGS dietary unsaturated fatty acids become saturated during the rumen fermentation process. In monogastric animals unsaturated fatty acids from DDGS are absorbed and can have a detrimental effect on carcass quality. Averette Gatlin et al., (2002) reported that increased unsaturated fatty acids in the diet fed to pigs and observed that carcass fatty acids composition is following the same pattern as dietary fatty acids only two weeks after

feeding. CDDGS contains high unsaturated fatty acids (~50% linoleic acid, ~25% oleic acid) (Martinez-Amezcua et al., 2007). Therefore, feeding higher levels of DDGS consumption by finishing pigs leads to a concern regarding the carcass quality. Whitney et al., (2006d) observed that linear decreased in dressing percentage, loin depth, belly firmness, and thickness ($P<0.02$) and a linear increase in iodine volume (IV) ($P<0.02$), when 0, 10, 20, and 30 % CDDGS was fed to finishing pigs. In a later study, Widmer et al., (2008) demonstrated that 20% DDGS decreased belly firmness and increased IV, but didn't affect dressing percentage, and palatability of pork chop. Even more recent, Leick et al., (2010) reported that pigs fed 0 to 60% CDDGS showed a linear increases in linolenic acids (C18:2), iodine volume (IV), unsaturated (USFA)/saturated (SFA) fatty acids ratio in jowl and belly fat; as well as linear decreases in dressing percentage, loin depth, and oxidative stability of pork chop fatty acids during storage (especially with feeding level over 15% CDDGS) were also observed. Recently, Xu et al., (2010b) also reported that 30% CDDGS in diet fed to finishing pigs lowered dressing percentage, decreased belly firmness, lowered SFA:USFA ratio, but did not affect taste characteristics. However, Lineen et al., (2008) observed that 15% CDDGS didn't have negative impact in carcass quality. Thus it appears that feeding levels of approximately 20% CDDGS or less can be fed to finishing pigs without detrimental effect on carcass quality.

Several strategies were suggested to alleviate the negative impact of feeding higher levels of DDGS to finishing pigs on carcass fat. Xu et al., (2010a) suggested that the desired effect of reducing the C18:2 content and IV of pork fat could be elicited in as little as 3 wk after withdrawing DDGS from the diet before slaughter. White et al., (2009) recommended that a supplement of 6% CLA could also potentially alleviate negative effect on carcass quality caused

by feeding 20% or more CDDGS. Carcass quality of feeding CDDGS, therefore, may be revered by adding CLA or withdrawing CDDGS 3 weeks prior to slaughter.

Effect of CDDGS in sow diets

Hill et al., (2008) reported that adding 15% CDDGS in lactation diet did not affect the reproductive performance of sows. Wilson et al., (2003) concluded that feeding sows in gestation with 50% DDGS and lactating sows with 20% DDGS did not affect reproductive performance.

Conclusion:

The reports of feeding DDGS to growth-finishing, gilt and sows are variable because of the source (quality) of DDGS, level of DDGS inclusion, method of feed formulation, and status of pigs. According to literature, 20% CDDGS can be added in growth-finishing phase diet without sacrificing growth and carcass quality, whereas Stein et al., (2008) suggested that up to 30% DDGS can be included in diet without a negative impact on growing pigs, and 30%, and 50% DDGS can be used in gestation and lactation diet respectively, without any detrimental effects on reproductive performance. Although high levels of CDDGS may lower ADG and prolong the time required for finishing pigs to reach market weight, if its price is favorable enough to overcome the loss, producers may still be willing to put more CDDGS in the diet.

NUTRIENT CONTENT OF CDDGS

The benefit of feeding DDGS was first reported in 1940s. Fairbanks et al., (1944) reported that post-weaning to market weight pigs fed with 6, and 12 % DDGS had improved ADG by 26 and 5 %. Moreover, Krider et al., (1944) concluded that with supplementing 0, 6, 12 % DDGS

to the diets, pigs (10.5 kg to market weight) had better growth rate (422, 459, 518 g/day ADG). The authors suggested that the increased in growth performance of pigs fed diets containing DDGS could be attributable to higher water soluble vitamins (vitamin B) in DDGS, and other unknown factors. Although Fairbanks et al., (1945) showed that supplementing crystalline B-vitamins in the diet had better growth performance than 6% DDGS addition in diet (531 g/d vs. 422 g/d), 6% DDGS still had better ADG than control fed pigs (422 g/d vs. 359 g/d). Due to the higher nutrient content in DDGS than corn, the addition of DDGS in diet could have advantages on growth performance.

CDDGS chemical composition:

The corn kernel contains around 61% to 71% of starch, 3420 kcal/g ME, 4% fat, 8% crude protein, 0.28% P (14% available), 9.6% NDF, and 2.8% ADF (NRC, 1998). During its manufacture of DDGS, most of starch from corn is extracted and fermented to produce ethanol, and its remaining nutrients from corn DDGS (CDDGS) are concentrated (Singh et al., 2007). CDDGS contains 6% of starch, 3350 kcal/g ME, 9% fat, 29% CP, 0.65% P, 32% NDF, and 11% ADF (Pedersen et al., 2007; Urriola et al., 2009). Pedersen et al., (2007) suggested that the apparent total tract (ATTD) of gross energy (GE) (90.4% vs. 82%) was higher in pigs fed only corn as compared to those fed CDDGS, which could be due to higher fiber content in CDDGS. Unlike ATTD of GE, the ATTD of N (82% vs. 83%), and P (20% vs. 50%) were similar to or higher in pigs fed CDDGS supplemented diets than those fed corn only.

Protein and fat are normally considered higher cost nutrients in dietary feed ingredients. Moreover, higher levels of available P in DDGS may have the benefit of reducing P excretion and potentially lessen the environmental impact of land-applied swine waste on pollution and

eutrophication a fresh water supplies. Addition of DDGS to diet, therefore, provides higher levels of bio-available amino acids, fat, and minerals to animals compared to a corn basal diet. Thus, adding CDDGS to diet to replace corn and SBM should be beneficial to animals and producers.

Limitations of DDGS in swine diets

In spite of the potential nutrient benefits provided by CDDGS, producers have to be aware of certain limitations, such as its variation in quality (between batches and ethanol plants), pattern of amino acids, concentration of fiber, and potential levels of mycotoxins. This is particularly the case when CDDGS from a dry grind ethanol plant is used.

QUALITY VARIABILITY

The variation in quality and nutrient availability in DDGS from different sources has been reported previously (Cromwell et al., 1993; Pedersen et al., 2007). Inconsistent quality of DDGS from batch to batch and from plant to plant may limit its use in the diet. The quality of DDGS can be affected by the processing procedure used for ethanol production, by the particular batch of corn (agronomic and harvesting influences, and method of processing), and other factors (Olentine, 1986; Shurseon et al., 2004). Beylea et al., (2004) examined corn and DDGS from 1997 to 2001 for fat, starch and protein content. The results demonstrated that the nutritional content of corn differed between each year, and that fat and protein in corn were more constant than in DDGS (actual variation, %/unit: 0.32% vs. 1.6%, 0.29% vs. 5%), whereas the coefficient of correlation was similar for fat and protein in corn and DDGS (4.71% vs. 6.54%, 3.62% vs. 4.69%). Hence, the author suggested that the reason for inconsistency in DDGS is mainly due to

the process during ethanol production rather than the source of corn itself. Thus, in order to avoid feed formulation problems that could occur with DDGS variability, Stein, (2007) suggested that it is better to purchase DDGS from the same reliable ethanol plant. The color of DDGS can be used as a quality index (Cromwell et al., 1993; Fastinger and Mahan, 2006) when nutrient component profile is not available.

AMINO ACID IMBALANCE

Corn is known as a relatively low quality protein feed ingredient (low in lysine, high methionine). During the process of drying wet distillers grains in ethanol production, the Maillard reaction may result in lowered bio-availability of lysine (Bemiller, 1997). Therefore, a dietary amino acids (AA) imbalance is a consequence of feeding higher level of CDDGS. Overheating during the drying process could damage lysine bio-availability, and results in low feed value co-product to animals (Hancock et al., 1990).

The Maillard reaction, also called the browning reaction, occurs when epsilon of amide group, especially lysine and arginine, binds to the hydroxyl constituents of aldehydes, and reducing sugar, and then forms a brown color. The heating time, heating temperature, heating duration, and amount of reacting compounds can affect degree of Maillard reaction. Once AAs are bound to reducing sugar, they are no longer available for animals to uptake, and utilize (Hegedus, 1990). The mallard reaction has an irreversible action on amino acid availability. Standard ileal digestible (SID) lysine of corn and SBM are 78% and 90%. In CDDGS, however, SID lysine was reported at around 62% (NRC, 1998; Urriola et al., 2009).

Importance of the amino acid pattern

For muscle development, the body requires all 20 amino acids present at the site of protein synthesis. The negative impact of feeding diets with an imbalanced amino acid content is well recognized (Harper et al., 1970). Lenis et al., (1999) reported that increasing the total N in diet (18.8, 22.9, and 30 %) and increasing essential (EAA) versus non essential NEAA ratio (35, 45, and 56 %) significantly improved ADG ($P<0.001$, $P=0.08$). However, N retention was greater in animals fed lower N diets (18.8%, 22.9%) with lower EAA:NEAA ratios, 35% (49.5% vs. 56.6%), and 45% (46.4% vs. 56.8%). Thus, feeding of higher levels of CP of less AA balance diets to animals may improve ADG but results in increased N excretion into the environment.

Cereal grains and oilseeds (corn and soybean meal) are major feed ingredients in typical swine diets. As a result their higher cost, animal protein sources are usually limited in creep feeds and early nursery phase diets to improve nutrient quality, palatability, and survival rate. In plant based diets, providing sufficient CP is not a problem, but providing a diet balanced in amino acids is. The most limiting amino acids are lysine, threonine, methionine, and tryptophan in a plant based diet.

After the development of amino acid analysis techniques, the ideal protein ratio discovery in swine is definitely one of the biggest contribution to the improvement of livestock nutrition in the past century (Cromwell, 2008). The concept of an ideal pattern of amino acid for pigs was first introduced by Wang and Fuller (1989). Later Chung and Baker (1992) conducted an animal trial, and came out with a improved amino acid pattern. The NRC (1998) summarized ideal protein pattern for different stages of pigs based on several research studies. Balanced amino acids in swine diet can reduce N excretion (Chung and Baker, 1992), and increase lean gain (N

in the diet is used for muscle growth instead of fat deposition)(Kropf et al., 1959). It can also improve growth performance (an imbalance in amino acids increases urea cycle, which requires energy), avoid amino acid antagonism (lysine vs. arginine or branch chain amino acids)(Harper et al., 1970; Southern and Baker, 1982), and most importantly increases profitability (CP feed ingredient is usually the most expensive).

Color of CDDGS

The color of CDDGS can provide an index for the quality of CDDGS if chemical analysis is not possible. Basically, the darker the brown color of DDGS is, the lower the bio-availability of lysine in the DDGS. Cromwell et al., (1993) reported that the dark colored DDGS products (beer distillers and fuel alcohol) have a characteristic odor, and lesser availability of lysine (0.8% vs. 0.44%), arginine (1.12% vs. 0.9%), and cystine (0.61% vs. 0.44%). Further, Fastinger and Mahan et al., (2006) examined five CDDGS (from different dry grind ethanol plants), and observed that dark colored CDDGS tends to have lower total lysine content (0.75% vs. 0.48%), reduced AAs, and poor lysine digestibility (AID, SID of lysine 24.6 vs. 52.3% and 38.2 vs. 61.5%, $P<0.05$) when compared to lighter colored CDDGS. Therefore, the author concluded that the color of CDDGS may be a good indicator of overall amino acid, and particularly lysine's bio availability. The color of DDGS from both studies did not affect CP concentration, indicating that diets formulated with DDGS using digestible amino acids is needed if the data is available.

In conclusion, typical corn and SBM basal diets have highly digestible amino acids, so formulating diets based on total or ileal digestible amino acids does not have a major effect on N utilization. However, if alternative feed ingredients, such as DDGS and other high fiber diets by-

product feed ingredients are used it is well advised to formulate diets based on an ileal digestible amino acid requirement (Shannon and Allee., 2010).

FIBER

Fiber classification

Fiber, also known as roughage, includes dietary fiber, and added fiber. There is no standard definition for fiber in any country. Dietary fiber is a carbohydrate (plant original) which can not be broken down and utilized by the endogenous enzymes of the animal. Added fiber, on the other hand, is a isolated indigestible carbohydrate, which may have physiological benefit to human health (IOM, 2001). Another definition for fiber depends on its physiological characteristics in GI tract, where it exists as soluble or insoluble fiber. Soluble fiber is defined as the fiber that absorbs water in the GI tract, thus increasing viscosity of digestible food, which is fermented in large intestine to produce short chain fatty acid (SCFA). Insoluble fiber, on the other hand, increases bowel movement, increases passage rate, thus can optimize laxation, and is incompletely fermented in hindgut. Earlier in chemical analysis, the AOAC procedure was used to represent crude fiber content in feed samples. Van Soest and McQueen (1973) demonstrated that using the AOAC method could lead to under estimation of cellulose (50%-80% recovery), hemicelluloses (20% recovery), and lignin (10%-50% recovery), and thus modified this method of analysis in 1991 (Van Soest et al.). Since then, instead of representing fiber content in diet with crude fiber, newer methods to analyze fiber are suggested, and reported as NDF, ADF, and hemicellulose if HPLC is not available.

Function of fiber in plants

The fiber in plants is mainly found in the cell wall (figure 3) (McDougall et al., 1996) for supporting the structure (such as cellulose, hemicelluloses, pectin), and also serves as a form of stored energy (fructan, glucomannans, galactomannans) (Selvendran, 1990). Lignin is a polyphenol compound that was known to have antioxidant properties. The concentration of lignin is typically low in swine diets (Dreher, 1999).

Effect of fiber to body health

The beneficial effects of fiber on human health were widely discussed (Roberfroid, 1993), including the effects on decreasing blood sugar (mainly by viscous fiber)(Anderson and Ward, 1979), and increasing insulin sensitivity (Fukagawa et al., 1990), preventing cardiovascular disease (by lower blood cholesterol)(Anderson et al., 2000), managing obesity and BW loss (results are variable)(Appleby et al., 1998; Baron et al., 1986; Miller et al., 1994), prevention of breast cancer(results vary) (Rock et al., 2004; Rose et al., 1991), antioxidant effect, and promoting colon health (insoluble fiber improve constipation laxation, and help prevent colon cancer) (Birkett and Jones, 1997; Brodribb and Humphreys, 1976; Trock et al., 1990).

Effect of fiber on pigs

Dietary fiber is considered a beneficial feed ingredient in human diets because it enhances water holding capacity of digesta, stimulates intestinal peristalsis and digesta passage rate, and also non-covalently binds to dietary cholesterol, thus effectively lowering blood cholesterol. However, dietary fiber is seen as negative factor in swine and poultry diets. Fiber is normally

considered as indigestible by monogastric animal's endogenous enzymes. High fiber diets, therefore, could interfere with other nutrient digestibility by either increasing passage rate (Kass et al., 1980; Owusu-Asiedu, 2006; Pond et al., 1988) or increasing viscosity.

The effect of dietary fiber on nutrient digestibility, and growth performance has been discussed (Dierick et al., 1989). Diets containing a high percentage of fiber lower dietary energy density, which leads to higher feed intake to meet the energy requirement. However, very high dietary fiber (more than 10% or 15%) can cause feed intake reduction (excess bulk, low palatability) (NRC, 1998).

Effect of fiber on nursery pigs

Feeding pigs with diets containing 3.8% and 9% total fiber (from oat and wheat bran together) from post-weaning to market age reduced ADG significantly (7%, 22%, 7% and 17%)($P<0.05$)(Drewry, 1981). Increasing pectin (soluble fiber), or barley hull (insoluble fiber) level (7%, 10% and 14.5%) in the diets of pigs weighing 8kg showed different results. For the pectin diet, decreased ADFI, and ADG ($P<0.001$) was observed. In the pigs fed the barley hull diet, however, ADG was similar (186, 166, 204g/d), and gradually, ADFI increased (302, 283, 322g/d)(Hedemann et al., 2006). Similarly, increasing peanut hull (0%, 8%, and 16%) linearly in the diet fed to nursery pigs decreased ADG (431, 411, 407g/d), G:F ratio(0.6, 0.57, 0.55), and digestibility of DM, N, and NDF ($P<0.02$). Soybean hull inclusion in diet (0% vs. 8%) did not decrease ADG, while higher ADFI was observed (728 vs. 799 g/d) (Kornegay et al., 1995). Addition of oat hull (15%), soybean hull (15%), alfalfa meal (20%) to the diet fed to pigs weighing 9.7 kg did not decrease growth performance, but digestibility of DM, energy, and N was decreased significantly ($P<0.05$). In addition, pigs consuming oat hull and alfalfa meal in

diet showed significantly reduced NDF, ADF, hemicellulose, and cellulose digestibility ($P<0.05$)(Moore et al., 1988). DDGS, soybean hull, and citrus pulp (7%) fed to pigs weighing 5.2 kg did not affect growth performance (Weber et al., 2008).

Effect of fiber on growth-finishing pigs

Adding alfalfa (NDF:26.2%) to 8 week old pig's diets decreased growth performance regardless of the pig's genotype (Pond et al., 1988). Including 10% dehydrated alfalfa (NDF:14%) in the diet of 24 kg weighing pigs increased ADFI (1.93 vs 2.05 kg/d)($P<0.01$), and decreased ADG (796 vs. 776g/d) ($P<0.1$) at 22.5 ^{0}C (Stahly and Cromwell, 1986). Increasing dietary fiber from 16% to 27% by wheat bran significantly reduced apparent total tract digestibility(ATTD) of crude protein (CP), ash, crude fat (EE), dry matter (DM), and energy, but did not affect apparent ileal digestibility(AID) in 30kg pigs (Wilfart et al., 2007). Similarly, feeding pigs (32kg) three diets containing 0% (NDF:ADF)(8%, 3.48%), 7.5% (13.5%, 6.25%), and 15% (18.9%, 8.5%) corn cob reduced ADG by 3.6%, and 6%, and nitrogen digestibility by 3.2%, 3.7% compared to the 0% corn cob diet (Frank et al., 1983). However, when increasing soybean hulls in diet during the growth phase (24kg), Kornegay (1981) did not observe any detrimental effects on growth performance, but noticed dried fecal output($P<0.01$), energy digestibility($P<0.001$), and CP digestibility ($P<0.001$) was decreased linearly as the level of SB hull increased in the diet. Adding increasing level of corn germ meal (6%, 10%, 14%, 18%), which contains higher level of hemicellulose, to diet fed to 30kg gilts reduced ADG (0.84, 0.79, 0.66, 0.59 g/d) and suppressed ADFI (1.69, 1.55, 1.48, 1.41 kg/d) quadratic ($P<0.03$), but growth performance was not affected during weeks 2 to 4 (Weber et al., 2010). For synthetic cellulose, 0, 10, 20, 30, and 40% cellulose inclusion in diet fed to pigs (52kg) decreased ADG (0.78, 0.68, 0.48, 0.25g/d), ADFI(2.63, 2.42, 2, 1.5kg/d), and G:F (0.29, 0.28, 0.24, 0.17)(Baker et al., 1968).

Effect of fiber on reproductive gilt and sow

It is recommended to add fiber (alfalfa, wheat bran) to the diet of gestating and lactating pigs to increase satiety (Ramonet et al., 1999), and prevent constipation post partum (Reese E. Duane. et al., 2000). After delivery, sows fed with high levels of fiber (12.42%) resumed full feed earlier (Guillemet et al., 2006), had higher feed intake (Farmer et al., 1996), diminished stereotypic behavior (Robert et al., 1997), and improved reproductive performance (Matte et al., 1994; Quesnel et al., 2009).

Effect of fiber on mineral retention

The information on the effect of fiber on mineral digestibility is limited and unclear. Adding wheat bran to diet (increased NDF from 13.5% to 20.2%) did not affect P, Ca, Zn, Mg, Mn, Na, K digestibility (Ravindran et al., 1984). Pigs fed diets containing oat hull, soybean hull or alfalfa meal decreased Mg (17.8% vs. 7.3%) retention, but increased iron (3.23% vs. 5.36%) and Mn (0.18% vs. 0.32%) retention ($P<0.1$). In addition, various types of fiber affect mineral retention differently (Moore et al., 1988). Phytic acid, however, is consistently shown to reduce P, and Ca digestibility (Kerr et al., 2010; Lei et al., 1993). In spite the effect of fiber on the utilization of minerals, the amount of minerals and their content in feed ingredients should be considered when formulating the diet. If mineral content is low, exogenous minerals can be added to diet to meet the requirement. If minerals in feed ingredient are exceeded the requirement, it can be a limitation for using that particular feed ingredient.

The effect of fiber on ADG and nutrient digestibility was variable because of the various types of fiber (Chabeauti et al., 1991; Dierick et al., 1989), source of fiber (plant intact or isolated), age of pigs (Noblet and Shi, 1993), concentration of fiber in the diets, and

concentration of other nutrients concentration in the diet. Organic matter that is not digested in foregut will be fermented in hindgut by microbial bacteria.

Hindgut fermentation

Indigestible fiber (especially soluble fiber), and protein (diet origin, endo- exo-geneous enzymes, and sloughed epithelial wall) can be fermented by microbes in the intestines, and produce volatile fatty acids(VFA), methane, carbon dioxide, ethanol, hydrogen, and ammonia. High fiber diet increases microbial bacteria count, increases weight of distal intestine, decrease pH at caecum and colon, and have more ATP produced in hindgut, compared to low fiber diet (Jensen and Jorgensen, 1994). Cummings and Macfarlane(1991) suggested that fiber is the main source for hindgut fermentation although protein also contributes to short chain fatty acid (SCFA) production (Macfarlane and Gibson., 1995). The fermentation mostly takes place at proximal large intestine, and VFA are absorbed rapidly before the contents enter lower large intestine (Ehle et al., 1982). Carbohydrate and protein fermentation mainly generates acetate, propionate, and butyrate (around 57:22:21 ratio). Valerate, isobutyrate, isovalerate, on the other hand, are formed during catabolism of branched chain amino acids (valine, leucine, isoleucine)(Mackie et al., 1998). The formation of SCFA depends on chemical and physical properties and the availability of the substrate. NO_3^- (Allison and Macfarlane, 1988), and sulfate(Gibson et al., 1993), which serves as electron recipient, also influences the amount of SCFA production. In weanling pigs, SCFA concentration was increased after pigs were introduced to diet, and increased fiber level resulted in lower ammonium, and branched chain fatty acid production, which indicated that increasing fiber level in diet can reduce proteolytic activity (Awati et al., 2006). Differences in polysaccharides content can also result in different ratios of SCFA. A simplified SCFA pathway was reported by Macfarlane (2003)(figure 5).

Energy provided from microbial fermentation

Energy from anaerobic fermentation is less efficient at providing energy for animal as compared to aerobic. Moreover, bacteria also use energy for their growth. Thus, even though indigestible nutrients can contribute to the production of energy after hindgut fermentation (Jensen and Jorgensen, 1994), energy derived from VFA by fermentation is less compared to the energy from digestible nutrients in the foregut. It was reported that VFA produced from 6% of crude fiber fed to 60kg pig was provided 30% of the energy requirement for maintenance (Rerat et al., 1987). Increasing microbial fermentation by adding gradually alfalfa meal (0, 20, 40, and 60 %) generates 6.9, 11.3, 12.5 and 12.0% of the energy required for maintenance in the 48 kg weighing pigs and 4.8, 11.4, 14.0 and 12.0% in the 89 kg weighing pig (Kass et al., 1980). Auguita et al., (2006) found that increased fiber (7.7, 16, and 24%) reduced foregut energy digestion (3.5, 2.9, 2.6 Kcal/kg DM). Hindgut fermentation, however, provided only 7, 13 and 17 % (0.38, 0.71, 0.84Kcal/kg of DM) of maintenance energy, which was not enough to compensate the loss in foregut. Therefore, increasing fiber results in higher VFA production, but high SCFA can only can provide 5% to 30% maintenance energy. The energy provided by SCFA, on the other hand, can't compensate for the energy loss due to the interference of fiber in the digestion of nutrient.

According to the literature, the beneficial effects of fiber in human health are clear. In swine, however, dietary fiber is associated with a decrease in digestibility and ADG. In order to use CDDGS as an alternative feed ingredient, understanding CDDGS's composition of fiber is necessary.

CDDGS fiber composition

Chemical composition of corn fiber was analyzed and reported by Knudsen (1997). Corn fiber contains 9% soluble non cellulose polysaccharides, and 66% insoluble non cellulose polysaccharides, 1% β-glucan, and 22% cellulose, which is mainly located in bran. Carbohydrate composition of fiber in CDDGS has higher arabinose, xylose, mannose, galactose, and cellulose than corn (table 1)(Shurson, 2008a). Since nutrients in corn other than starch are condensed in DDGS the amount of fiber is also concentrated (table 2). Fiber content in CDDGS is around 4 times and 3 times higher than in corn and SBM respectively. Stein and Shurson (2008) observed that DDGS contains 25.3% NDF, 9.9% ADF, and 42.1% total dietary fiber, which was three time higher than corn.

Although fiber from CDDGS has some beneficial effects on GI tract infection (Whitney et al., 2006a, b, c), the results are variable. High fiber content in CDDGS may decrease digestibility of other nutrients, and challenge the amount of CDDGS that can be added in diet. So, using different strategies to expand the usage of CDDGS without decreasing the growth performance, and nutrient digestibility can eliminate environmental impact caused by excretion of excess nutrient are necessary to increase producers cost returns.

MYCOTOXINS

Introduction

Most of the mycotoxins belong to four genera: *Penicillium* (P), *Aspergillus* (A), *Claviceps* (C), and *Fusarium* (F). Aflatoxins are isolated from *A. flavus*, and *A. parasiticus*: Orchratoxin is isolated from *A. niger, A. ochraceus,* and *P. verrucosum*: ergot alkaloids are isolated from *C.*

purpurea, C. paspali, and *C. fusiformis*: deoxynivaenol(DON) (*F. graminearum, F. culmorum*), T-2 toxins (*F. sporotrichioides*), fumonisin (*F. verticillioides, F. proliferatum*) are isolated from *fusarium* family and are nonestrogenic trichothecenes while zearalenol (*F. graminearum, F. culmorum*) is also isolated from *F.* family, but is a mycoestrogens (Richard, 2007). Corn is mainly contaminated by aflatoxins, DON (also called vomitoxins), zearlenone, T-2 toxin, and fumonisin. Contamination of feedstuffs is one of major threats to human and animals' health, and also causes large economic loss. The economic impact on swine industry can be affected by mycotoxin level in grain, DDGS processing, DDGS inclusion rate in the diet, number of animals fed DDGS, and the market value of pigs. The value of expected annual loss because of reduced weight gain can be $4 million with 5% DDGS inclusion rate and 12% of producers used DDGS causing an annual loss of $147 million dollars (20% inclusion rate and 100% farmers used DDGS)(Wu and Munkvold, 2008). Therefore, the problem of mycotoxins should not be ignored.

CDDGS from 11 ethanol stations during three crop years was collected, and analyzed for mycotoxins (Zhang et al., 2009). The results showed that the concentration of total fumonisins (none detectable vs. 8.6ppm), and zearalenone (none detectable vs. 0.14ppm) varied between stations. Moreover, DON level did not show correlation with time, and storage with overseas shipment. The DDGS from the same stations had similar result regardless of when the sample was collected. If corn contains high toxicants, DDGS is most likely to have levels of toxins. FDA issued a guidance for maximum level of aflatoxin (FDA, 2000), DON (FDA, 2010), and fumonisine (FDA, 2001) in feed (table 3): aflatoxin in corn: 2ppm for finishing or pigs weighing more than 200lb, 1 ppm for breeding pigs, 0.02 ppm for immature pigs, DON (grain or grain byproduct): 5 ppm for pigs (not to exceed 20% inclusion rate in diet), fumonisine (corn or by-product): 20 ppm for swine.

Effect of mycotoxins in nursery pigs

For DON, 5ppm allowance with 20% inclusion rate gives us 1ppm acceptable concentration for immature swine. Diaz-Llano et al., (2010) found diet contaminated with 3.5 ppm natural mycotoxins (DON) lowered ADFI and BW, but didn't affect serum ammonia, serum urea nitrogen, and muscle DNA, RNA, and protein levels in late gestation and lactation sows. In addition, supplemented glucomannan mycotoxin adsorbent (GMA) did not reverse toxicity effect. In contrast, when higher level of DON was used (5.5ppm), detrimental effects were noticed (Diaz-Llano and Smith, 2007). In nursery pigs weighing 7 kg, including DON at levels more than 0.34 ppm decreased ADG, ADFI, and G:F ratio linearly. Furthermore, pigs started to lose weight with diet contaminated with 11.9 ppm DON (Young et al., 1983). DON (0.5ppm) in the diet of pigs weighing 10 kg significantly decreased ADG (35%, $P<0.001$), reduced ADFI (33%, $P<0.001$), increased serum IgA, IgM ($P<0.05$), and reduced liver and kidney weight (Swamy et al., 2002). Similarly, pigs weighing 9 kg, fed 4, or 8 ppm DON showed a significant decreased in ADG, and ADFI (Swamy et al., 2003). GMA supplementation reversed serum IgA, and IgM levels. Diet contaminated with 0, 0.42, 0.8 ppm of natural aflatoxin B1 fed to weaning pigs (10kg) decreased growth performance significantly (Lindemann et al., 1993). Diet contaminated with 0.9 ppm aflatoxin B1 decreased ADG (22%) and ADFI (20%) significantly in nursery phase, but only ADFI was reduced in growth phase (Schell et al., 1993). Similarly, Rustemeyer et al., (2010) observed that diet contaminated with 0, 0.25ppm, 0.5ppm aflatoxin B1 fed to pigs (14 kg) reduced ADG, and ADFI ($P<0.001$), but didn't affect serum urea nitrogen, asparate transferase, alanine transferase, bilirubin. Fusarium B1 contaminated diet decreased ADG (471 vs. 327g/d) and G:F ratio(0.49 vs. 0.33), but did not affect ADFI (0.96 vs. 1kg/d) in pigs weighing 7 kg (Piva and Casadei, 2005). Combination of aflatoxin and orchratoxins in diet reduced the growth by

about 52%, while the feed contaminated with individual toxins caused only 25% decreased in the growth of pigs (Huff et al., 1988).

Methods to alleviate toxic effect

The ameliorators that can be used to reverse the negative impact of mycotoxins were evaluated by several researchers. The compound that have the ability to eliminate the aflatoxin B1 effect on growth, feed intake and serum chemistry are: 0.5% sodium bentonite (SB), 0.5% hydrated sodium calcium aluminosilicate (HSCA), 2 ppm of folic acid(FA), and 0.6 ppm of Se (Schell et al., 1993). Adding 1% sodium betonite significantly reversed the negative impact of aflatoxin contaminated diet. Washing the damaged corn for 48hrs normalized the growth performance and nutrient digestibility in pigs (Forsyth et al., 1976). Including higher CP level (18% vs. 20%) in the diet of nursery pigs weighing 7 kg reduced the effect of aflatoxins (0.182 ppm) on growth performance. However, increasing CP concentration in the diet could increase more feed cost and environmental impact by excess N excretion (Coffey et al., 1989).

Conclusion

According to literature, DON (as low as 0.26 ppm), aflatoxin (0.25ppm) affected ADG, and ADFI while diet contaminated with fusarium B1 only affected ADG, and G:F ratio. The impact of synthetic and natural toxicants on livestock depends on age, species, and exposure level (Diekman and Green, 1992). Premature animals are more susceptible to toxin effects than mature animal. Moreover, the biological effect of toxins varies depending on the different types of toxins (Shull and Cheeke, 1983). The feed additive such as HCFA, and SB can significantly decreased the negative effect of toxicants. Both ethanol stations and producers need to be aware

of the effect of mycotoxins on livestock, and human health. Hence, choosing a reliable ethanol station is absolutely important.

COSTS EFFECTIVENESS

Ingredients costs vary by climate and by region in the US. Typically, DDGS is priced lower than corn and thus, may provide cost effective alternative. The benefit of including CDDGS in the diet depends on energy and protein feed ingredients used, such as corn and SBM. Stein (2007) suggested that the maximum price that can be paid for DDGS is the difference in the cost of corn and SBM without increasing cost of complete feed (table 4). A software program developed at the Ohio State University (SESAME, http://www.sesamesoft.com) can calculate the value of nutrients based on current feed prices. Breakeven price of DDGS ($/ton) = {Corn ($/bu) x 17.85} + {SBM ($/ton) x 0.5}48% SBM (Bill Weiss. et al., 2007)

STRATEGIES THAT CAN BE USED TO INCREASE CDDGS DIET

Particle size

Feed particle size has been reported to affect animal's growth performance, feed efficiency, nutrient digestibility, digesta passage rate, and GI tract weight and length in chickens (Amerah et al., 2008) and pigs (Healy et al., 1994; Lahaye et al., 2007; Maxwell, 1970). There are different factors that contribute to particle size of feed.

Factors affecting particle size:

The particle size is of diet is dependent on: the type of grains, hardness of endosperm, processing methods, pellet or mash form when the same size of screen is used. Sola-Otriol et al., (2009) examined a total 127 of cereal, protein, lipid, and fiber source by replacing 1 main ingredient from basal diet. The diet was ground through 3 mm screen, and a difference in particle size was observed by replacing the source of cereal, protein and fiber in the diet, except lipid. Soft wheat endosperm has higher mean particle size and wider distribution than hard wheat endosperm (Dobraszczyk et al., 2002). Different cultivars of wheat have different particle size even when ground through the same size screens (Lentle et al., 2006).

Milling methods

The most common methods to reduce the particle size of grain are: hammer milling, and rolled milling (Koch, 2002). Using hammer milling is relatively less energy efficient and not as uniform as rolling milling does. However, it costs less to purchase and maintain hammer mill. Reducing particle size can enhance diet mixing. Hammer milling has higher geometric standard deviation than roller milling with same size of sieve (particularly smaller than 0.5mm and lager than 1.6mm), and has no difference in terms of energy requirement (Svihus et al., 2004). Hammer milling had higher geometric mean diameter (GMD) (0.46 vs 0.45 mm), and stability ratio (0.95 vs. 0.86), which indicates less uniform diet than rolling milling (Costa et al., 2007), which agreed with (Wondra et al., 1995b), who also observed less uniform in hammer mill (GSD: 2.3 vs. 1.9) than rolling mill in 0.8 mm. Particle size of 0.4 mm, however, reduced the difference in GSD between two milling types (GSD: 1.7 vs. 1.9). Feed composition also affects particle size.

Costa et al., (2007) found that sow diet had higher GMD (0.43 vs 0.48 mm) than finishing pig diet (ground through 2 mm screen).

Pelleting

Grinding corn (0.3 vs. 0.53) and wheat (0.28 mm, 0.89 mm) through the size of screen (1 mm, 7 mm) results in difference GMD. Pelleting, however, reduces the difference between corn and wheat (Amerah et al., 2008). Similar result was also observed by Amerah et al., (2007b) and Svihus et al., (2004). Svihus et al., (2004) found that pelleting procedure evened out diet particle size and eliminated significant effect on growth performance, AME, and gizzard weight in broilers. Person et al., (2005), however, found that particle size distribution was not affected by pelleting when wheat/SBM basal diet was ground through 3mm and 6mm screen (GMD: 0.4mm and 1mm). Moreover, pelleting has benefits with regards to nutrient's digestibility, which agrees with the finding of Lentle et al., (2006). Therefore, the effect of pelleting on particle size is controversial between studies because of types of grains, size of screen. In order to study particle size effect in animals it is better to use mash form from the same type of grain instead of pelleting.

Effect of reducing particle size in diet

Decreasing particle size can increase the surface area of diet, and thus has more exposure area accessible to endogenous enzymes (Lawrence, 1983). Therefore, improved digestibility, and better feed efficiency are expected (Fastinger and Mahan, 2003; Healy et al., 1994). In addition, improving feed conversion ratio by reducing diet particle size lowers the cost of production (Goodband et al., 2002).

Effect of particle size in chicken

Reducing the particle size (GMD: 0.4mm vs. 1mm) improves the digestibility of DM, AME, lipid, and starch in broiler (d7-21), but does not affect their growth performance (Peron et al., 2005). Broiler (d1-21) fed with coarse mash diet had better growth, and feed efficiency than the ones fed with fine and very coarse diet. However, in d21-42 broiler, very coarse diet gave best growth (Hamilton and Proudfoot, 1995). Similarly, broilers (d1-21) fed coarse corn (GMD: 0.3 vs. 0.53 mm) and wheat (0.28 mm vs. 0.89 mm) had higher feed intake, better feed efficiency, and heavier gizzard weight (Amerah et al., 2007b). Amerah et al., (2007a) suggested that broiler chickens fed with GMD 0.9 mm to 1.4 mm from d1 to market age should have better feed efficiency. For broilers, diet with coarse particle size (>0.9 mm) results in better growth performance. As chickens get mature, switching diet to larger particle size is needed to obtain maximum growth. Nutrient digestibility, however, is better with fine particle size.

Effect of particle size in swine

Although benefits with coarse feed particle size were observed (Canibe et al., 2005; Hedemann et al., 2005), reducing particle size is known to have an effect on pig performance, and nutrient utilization in pigs (Healy et al., 1994; Hedde et al., 1985; Ivan et al., 1974). Nursery pig growth was increased as particle size of corn decreased from 0.9 to 0.3 mm, and corn with 0.5 to 0.7 mm particle size had lower cost of gain (Healy et al., 1994). Similar results were observed in barley basal diet (Goodband and Hines, 1988). Reducing particle size from 1 mm to 0.5 mm in corn and wheat fed to pigs improved SID of protein (63.3% vs. 69.5%) numerically. SID of protein, however, was similar (74.5% vs. 74.8%) in wheat (Lahaye et al., 2007). Reducing particle size in wheat/SBM basal diet (GMD: 1.3 vs. 0.6mm) improves nutrient

digestibility, increases growth performance, and reduces ADFI in nursery pigs (Mavromichalis et al., 2000). In finishing pigs, however, lower particle size from 1.2 mm to 0.4 mm does not affect growth, but digestibility of DM, N, and GE increases linearly with both pellet and meal form diet ($P<0.001$)(Wondra et al., 1995a; Wondra et al., 1995b). Nevertheless, Hedde et al., (1985) found that fine particle diet improved ADG, and feed efficiency in pigs from 27 kg to market weight ($P<0.01$). In growing pigs, Frank et al., (1997) reported no effect of particle size on performance of pigs fed corn with particle size ranging from 1 mm to 0.4 mm. In contrast, Lahaye et al., (2007) observed that decreasing corn particle size increased N digestibility that was attributed to lower endogenous losses. Fine diet improves ADG (728 vs. 699 g/d), G:F ratio (0.48 vs. 0.44) numerically, but decreases ADFI (1.54 vs. 1.59kg/d)(Canibe et al., 2005). Semi-purified SBM diet (33%) with decreasing particles size (0.95, 0.6, 0.33, and 0.19 mm) increased AID and TID of amino acids linearly, especially isoleucine, leucine, methionine, phenylalanine, and valine (Fastinger and Mahan, 2003), These reports are in agreement with Owsley et al., (1981) who found that coarse, medium, and fine sorghum (91% of diet) fed to growth-finishing pigs resulted in better DM, N, starch, and GE digestibility in fine sorghum diet($P<0.05$), and both AID and ATTD of amino acids except lysine ($P>0.1$) was better with fine particle diet ($P<0.05$). In order to obtain optimum growth increasing the particle size as pigs get mature is needed (Healy et al., 1994; Seerley et al., 1988). In primiparous lactating sow, feeding diet with decreasing corn particle size from 1.2 to 0.4 mm linearly increased ADFI (4.19, 4.24, 4.4, 4.43kg/d) ($P=0.04$), litter weight gain (34.9, 36.7, 38.2, 38.6kg)($P=0.05$), and digestibility of DM, N, GE ($P<0.001$) (Wondra et al., 1995d). Similar results for nutrients digestibility was observed in second parity sows(Wondra et al., 1995c). Increasing the uniformity of diet (GSD: 2.5 vs. 2) improved

digestibility of DM (80.2% vs. 83.1%), N (72.4% vs. 78.5%), and GE (79.6% vs. 82.6%) in growth-finishing pigs (Wondra et al., 1995b).

In general, the effect of particle size on the growth performance of pigs has not been consistent. Unlike chickens, which prefer coarse diet, pigs, however, can have better growth with 900 to 500 mm GMD. But the effect of particle size on nutrient utilization is more consistent in studies and species.

Effect of particle size on ulcer

Stomach ulcer is a concern when feeding fine particle diet, which causes ulcer by increasing the acidity and pepsin activity (Reimann et al., 1968) in the unprotected esophageal region of the stomach (Mahan, 1966; Maxwell, 1970). Genetic effect on stomach ulcer is also reported (Reimann et al., 1968). Ulcers were not observed in nursery pigs fed 1.3 mm and 0.6 mm particle size in diet, but ulcers increased significantly when diet particle size was 0.4 mm (Healy et al., 1994; Mavromichalis, 2000). These reports are in agreement with Wondra et al., (Wondra et al., 1995c; 1995d) , who also observed an increasing incidence in stomach ulcers and keratinization in sows fed a diet with fine particles. Wondra et al. (Wondra et al., 1995a; 1995b), however, reported that no significantly increase in stomach ulcer incidence with decreasing particle size in growth-finishing pigs. Hammer mill and pellet diet could increase the chances of stomach ulcer regardless of particle size (Wondra et al., 1995b), which agreed with Flatandsmo and Slagsvold (1971). Thus, stomach ulcer incidence depends on diet particle size, age and the status of pigs, genetic background, and how diet is processed and prepared.

CDDGS particle size

There have been few or no studies related to the impact of DDGS particle size on pig's growth performance. The physical appearance of CDDGS suggests that it may be greater than the ingredients it is replacing in the standard diets. Rausch et al., (2005b) observed that particle size between CDDGS obtained from difference ethanol plants was not difference, and had more than 86% located at >600 mm sieve. However, there were only three sieves used (2mm, 0.8mm and 0.6mm). Nevertheless, Knott and Shurson (2004) reported that CDDGS particles vary from 0.6 to 2.1 mm GMD with an average of 1.3 mm GMD. Thus, decreasing particle size in CDDGS may help increase growth performance and nutrient digestibility when high CDDGS is fed to pigs.

Fiber degrading enzymes

The market for exogenous enzymes market is expected to grow to 300 million dollars by 2013 (Clark, 2009). Recently interest in adding co-products to the diets of monogastric animals may bloom the market for enzymes even quicker (Shurson, 2008a). Fiber degrading enzymes have been used as feed additive (Bedford and Classen, 1992; Carneiro et al., 2008; Inborr et al., 1993; Omogbenigun et al., 2004) not only to improve growth performance, feed efficiency, and nutrients digestibility in monogastric animal species fed diets with higher concentration of non starch polysaccharides, but also to alleviate the negative impact due to excess nutrients being excreted into environment (Mackie et al., 1998).

Effect of age on fiber degrading enzymes

Reports of pigs fed diets with fiber degrading enzymes are mostly focused on wheat, barley, and rye basal diets (Partridge, 2001). The reason is that, corn and SBM contains relatively less

fiber (NRC, 1998). Age of animals is also a consideration when including enzymes in the diet because young pigs have less ability to utilize the nutrients from diet. In addition, endogenous enzymes, such as pancreatic trpsin increases 826% while amylase increases 180%, from weaning to the end of nursery phase (Jensen et al., 1997). Development of pig's GI tract in younger animal is not as well developed as in growth phase pigs. Xylanase addition into wheat based diet fed to nursery pigs (4.1kg) improved AID of DM, CP, GE, CF, NDF, ADF and amino acids except lysine, threonine, cysteine, and proline ($P<0.05$). However, the benefit of xylanase was diminished in ATTD of nutrient. In addition, older pigs had better ability to digest nutrients (Diebold et al., 2004), which is in agreement with Li et al., (1996b) and Woyengo et al.,(2008). Similarly, Baidoo et al., (1998) found that cellulose, beta glucanase, and xylanase added to hullless barley diet showed better ADG (511 vs. 461g/d) in pigs weighing 9 kg, while the benefit of adding enzymes seems to be diminished in pigs weighing 40-60 kg. Thus, supplementing enzymes in young pigs should have more beneficial effects than in growth-finishing pigs.

Effects of fiber degrading enzymes supplementation in nursery pigs

Addition of xylanase to wheat/barley diet improved ADG (462 vs. 354 g/d), F:G(1.66 vs. 1.92), and reduced viscosity (18.7 vs. 32.4 mPas)(Dusel et al., 1997). In contrast, Kiarie et al., (2007) observed no beneficial effects on growth performance by supplementing young pigs weighing 6 kg with multiple enzymes when the same basal diet was used. However, the digestibility of DM was higher in enzyme treatment group. B-glucanase addition to hull less barley diet fed to pigs (10 kg) increased growth rate, and protein digestibility (Bedford et al., 1992). Supplementing xylanase in barley/wheat/rye diet to pigs weighing 9 kg improved feed efficiency ($P=0.04$), and DM digestibility ($P=0.036$)(Dersjant-Li et al., 2001). Similarly, better growth in pigs (10 kg) fed a diet containing xylanase was reported by He et al., (2010).

Combining xylanase/β-glucanase with barley/SBM basal diet for pigs weighing 13 kg improved ADG (418 vs. 486 g/d), F:G (2.53 vs. 2.37) (Fan et al., 2009). Cadogan et al., (2003) indicated that xylanase increased ADG and ADFI by 50.6% and 42.8% in pigs (7 kg) with low quality wheat diet($P<0.01$). However, Officer et al., (1995) and Thacker et al., (1992) failed to observe the effect of fiber degrading enzymes supplemented with wheat or hullless barley diet in young pigs. For corn/wheat middling/SBM diet, supplement xylanase, amylase, and protease did not improve growth, but did have better ATTD of DM, energy, and P in pigs weighing 10 kg (Olukosi et al., 2007). The reports on supplementing fiber degrading enzymes in young swine fed corn/SBM diet are few. Kim et al., (2003) observed no beneficial effect in pigs weighing 6 kg when supplemented with galactosidase, mannanase, and mannosidase combination in corn/SBM diet.

Benefits of enzymes supplementation in different species and substrates

Species

The response to carbohydratase addition is more consistent in chickens than in pigs. This is likely due differences in gastrointestinal anatomy between the species. The pig has a more capacious GI tract, particularly in the hindgut, which enables a longer digesta retention time, and more hindgut fermentation than in birds. Hindgut microbes in pigs are able to degrade fiber into VFA, which can be utilized as energy source by pig (Wrong, 1995). Effect of exogenous fiber enzymes is more consistent in wheat, barley, sorghum, and oat than in corn/SBM diet. Moreover, the different sources of the same types of enzymes have dissimilar effects (Inborr et al., 1993; Vahjen and Simon, 1999).

Substrate sensitivity

It is suggested that the benefits of enzymes can only can be seen when enzymes match the nutrient limitation in substrates (Zijlstra et al., 2010). Unlike barley, which contains high β-glucan, wheat mainly contains arabinoxylan with small amount of β-glucan. Li et al., (1996b) demonstrated that β-glucanase added to SBM and different cereal grains: barley, wheat, corn and rye (0.32%, 0.79%, 0.26%, 0.71% β-glucan) diets fed to nursery pigs only benefit nutrients digestibility in barley/SBM diet. In contrast, β-glucanase addition to either hullless barley/SBM or wheat/SBM diet not only improve nutrient digestibility in barley/SBM diet but also increased CP, GE, and β-glucan digestibility in wheat/SBM diet (by 0.8%) (Li et al., 1996a). For corn/SBM diet, including β-glucanase, and protease combination had better ATTD of DM, energy, CP, and minerals while AID of NDF and hemicelluloses were improved by enzymes (Ji et al., 2008). Thus, although β-glucan content in substrate is low and viscous, it may not be as severe problem in pigs when compared to chicks. Further, β-glucanase addition may help release the negative impact from substrate or other feed ingredients, such as SBM (Bedford et al., 1992). Hence, endogenous and exogenous enzymes can work on substrates better.

Fiber degrading enzymes in DDGS

Removal of starch to produce ethanol results in the fiber concentration being increased in DDGS. Arabinose (5.69%) and xylose (8.1%)(mostly insoluble) are higher in CDDGS than corn/SBM(Jones et al., 2010). Therefore, supplementing DDGS with fiber degrading enzymes may reduce or eliminate the negative effects of fiber on nutrient digestion in growing swine (Shurson, 2008b), and consequently increase the level of DDGS usage in pig's diets. The benefit of fiber degrading enzymes in growth performance and nutrients digestibility in wheat DDGS

was reported (Emiola et al., 2009). Adding combination of xylanase/ β-glucanase/protease/amylase in 30% CDDGS diet did not benefit ADG (1.16 vs. 1.19 kg/d) or F:G (1.41 vs. 1.39). ATTD of DM, N, and GE, however, was improved with enzymes supplementation pigs (8 kg) (Feoli et al., 2008), which is in agreement with the finding of Spencer et al., (2007). Similarly, Jones et al., (2010) observed no benefit in adding enzymes to CDDGS diet on growth performance in pigs weighing 9 kg. In contrast, Yoon et al., (2009) observed better growth and digestibility of CP when CDDGS supplemented with mannanase was fed to pigs weighing 56 kg. The benefit of supplementing fiber degrading enzymes with CDDGS to younger pigs is less clear. More research is needed to evaluate the effect of these enzymes on CDDGS diet.

Types of enzymes that can be used in CDDGS

Combination of xylanase and β-glucanase (XG) may be needed for nutrient digestibility when higher levels of co-products are used in diet (Zijlstra et al., 2010). The benefit of adding XG on growth and nutrients utilization was reported (Cowieson and Adeola, 2005; Nortey et al., 2008). The NSP in wheat bran is mainly insoluble fiber (29.6% NSP and 17.6% are insoluble arabinose and xylose), which is similar to CDDGS (Jones et al., 2010). XG supplement can increase the solubility of wheat bran, protein, and minerals, which make these nutrients available to animal. Furthermore, XG addition increases monosaccharides, such as arabinose, xylose, glucose, and mannose, from soluble and insoluble parts of wheat bran disappeared in stomach and small intestine (Aulrich and Flachowsky, 1998, 2001). Although arabinose and xylose are considered less digestible in pigs (Yule and Fuller, 1992), breaking down its linkage by enzymes should decrease the negative impact on nutrient utilization by altering the activity of endogenous enzymes (Fan et al., 2009; Jensen et al., 1997). In contrast, Fang et al., (2007) found no additive

effect in combination enzymes versus xylanase alone, which both improved growth rate and nutrient digestibility. These results are in consensus with the findings of Vahjen et al., (2007) in wheat base diet.

Thereafter, using fiber degrading enzymes, such as xylanase and beta glucanase alone or in combination, in CDDGS diet fed to young pigs should have beneficial impact on nutrient utilization.

Balance in amino acid profile

The importance of ideal protein ratio is discussed previously. Poor amino acid (AAs) quality of CDDGS is discussed previously, and increasing CDDGS in diet will lead to the AAs imbalance situation getting even worse. Crystalline amino acids (CAA) could be used to alleviate the negative impact of high CDDGS fed to animals without excess N excretion into environment.

The diet formulation for DDGS study is variable, and is based on three methods: meeting lysine requirements by increasing protein feed ingredients, meeting CP requirements regardless of lysine level, meeting lysine requirement by increasing crystalline amino acid (CAA) and holding CP the same or similar. CAA is ready to be absorbed in the body (Chung and Baker, 1992), and absorption rate is quicker than intact protein (Yen et al., 2004). Due to the differences in absorption times, an imbalance in AAs can be seen in protein synthesis site, which can be a detrimental effect of including CAA in the diet.

Adding only one CAA can: have toxicity effect, especially methionine (Brudevold and Southern, 1994; Edmonds et al., 1987), increase fat accretion (De la Llata et al., 2002), and have antagonism effect (Edmonds and Baker, 1987b; Southern and Baker, 1982). Pigs tend to choose a diet balance in AAs over an imbalanced one, even a high concentrate CAA (lysine-hcl:0.7%) is

used in diet (Henry, 1987). The main purpose of feeding DDGS is to reduce feed cost. Therefore, increasing protein ingredients to meet AA requirement, however, is less economical and not environmental friendly.

Crystalline amino acid usage

CAA is used to: determine the limit of AA in feed ingredients (Brudevold and Southern, 1994), to meet AA requirement in lower CP diet in order to eliminate protein excretion without affecting growth (Frantz et al., 2005; Kendall, 2003; Kerr and Easter, 1995; Knowles et al., 1998; Kropf et al., 1959), to alleviate N pollution, such as lesion and odor (Le, 2007; Obrack et al., 1997), and to lower feed cost (Funderburke, 2008). In the recent time, CAA is available at reasonable prices and sources for producers than before (Lewis, 2001). Thus, supplementing crystalline lysine (HCl salt) and formulating diets to meet the bioavailable level of lysine in the diet can restore the essential AA profile in the diet, containing DDGS.

Amine acid composition in CDDGS

CDDGS is higher in sulfur AAs and previous studies have shown the potential for amino acid toxicity with pigs fed the diet contain 4% methionine (Edmonds et al., 1987), whereas supplementation of diets with only 0.5% to 1% methionine (Edmonds and Baker, 1987a) or feeding diets containing 0.948% of total sulfur amino acids (Matthews et al., 2001) did not negatively impact the performance of nursery pigs. Although young pigs are more susceptible to methionine toxicity, Brudevold and Southern (1994) suggested that if other AAs are supplemented, methionine toxicity will be alleviated. Typically, CDDGS contains around .7% methionine (only 0.18% in corn), and the total sulfur amino acids in 45% DDGS diet is around 1.034%, which is only about 0.25% higher than corn and SBM basal diet. Thus, the relatively

higher sulfur amino acids content in 45% CDDGS diets should still be acceptable and should not have detrimental effects on pig's growth performance as long as the other essential amino acids are supported. The amount of CAA that can be used to reduce CP level is unclear. Diet with a decrease in CP by 4% did not have negative effects when essential amino acids (EAAs) were supplemented (Kerr and Easter, 1995; Lunchick et al., 1978). Figueroa et al., (2002), however, found that although EAAs were added(0.55% lysine), reducing CP by 5% decreased growth rate in pigs weighing 19 kg. Supplementing wheat based diet with lysine (0.68%), threonine (0.12%), and methionine(0.05%) totally replaced SBM, and had similar growth performance (Barrera et al., 2004). Similarly, Frantz et al., (2005) observed no negative effect with pigs (6 kg) fed with diet containing up to 0.52% lysine-HCL with other EAAs together to meet the lysine requirement. Moreover, adding 0.48% synthetic lysine and other EAAs together in diet fed to pigs (11 kg) did not reduce growth (Kendall, 2003). In addition, using CAA (0.625% of lysine-hcl) to replace fish meal fed to 8-15 kg weighing pigs did not affect growth rate (Ratliff, 2005). Therefore, as long as AA pattern is balanced by other CAA, including high levels of CAA should not have detrimental effect on pig's performance.

Supplementing CAA in high level in diet containing CDDGS can balance out the AA ratio, and should alleviate the detrimental effects due to poor CP quality in CDDGS.

CONCLUSION

Based on the literature, the number of studies investigating the effects of CDDGS in young pigs is limited, and in addition, the results are inconsistent. Further research is required in order to better understand the effect of CDDGS in young pigs. The price of CDDGS increases the producers' interest in adding more CDDGS in diet. However, the limitations of CDDGS restrict the level of CDDGS that can be included in swine diet.

CDDGS has not only higher levels of nutrients, such as CP, P, and fat, but is also higher fiber. Although CP in CDDGS is higher than corn, the quality of AAs ratio is poor. CAA are available and easily used in swine diets. Adding CAA to CDDGS diet can improve AA pattern, thus neutralizing the detrimental effects. Fiber degrading enzymes have been added to high fiber diets to reduce the effects of fiber content on performance. The effect of higher fiber in CDDGS containing diets, therefore, could be alleviated by adding fiber degrading enzymes to the diet, so that there will be better retention of nutrients. Reducing particle size may also increase the substrate surface area. Furthermore, increasing the surface area of particle size can help the pigs' endogenous enzymes to digest nutrients. Thus, decreasing the particle size in CDDGS may be able to eliminate the impact of negative effect of CDDGS. The following studies will focus on using different strategies to increase CDDGS utilization in the nursery diet.

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Figure 2.1 DDGS dry grind process diagram (Rausch, 2005a).

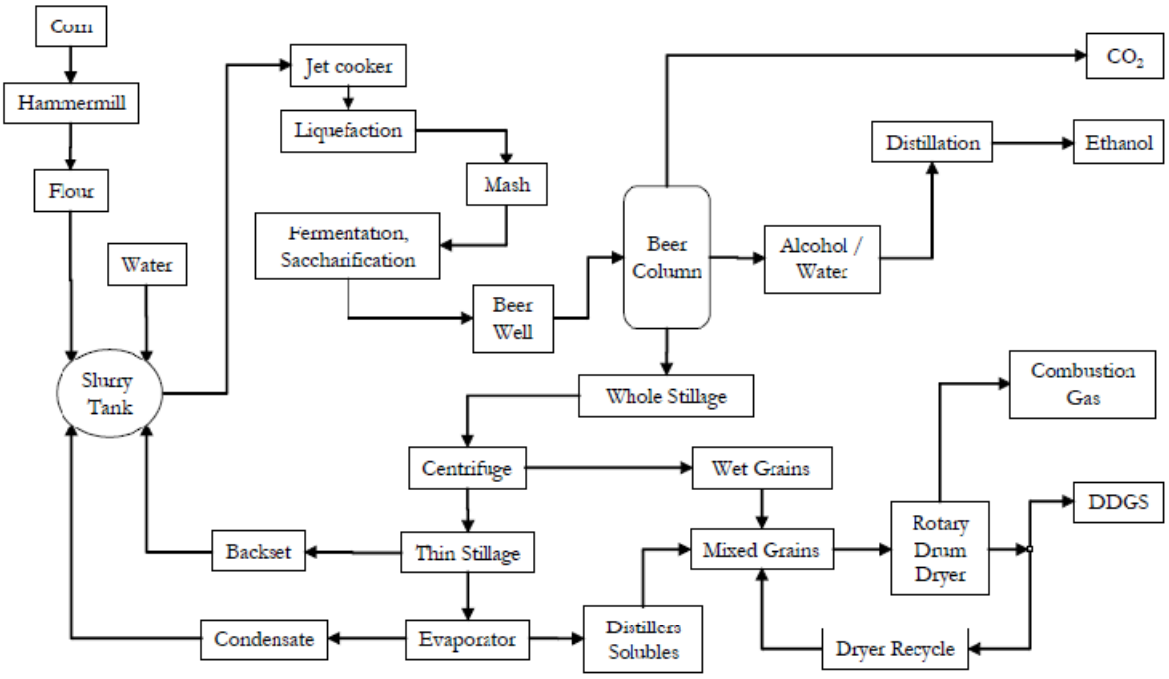


Figure 2.2 DDGS wet milling process diagram (Rausch, 2005a).

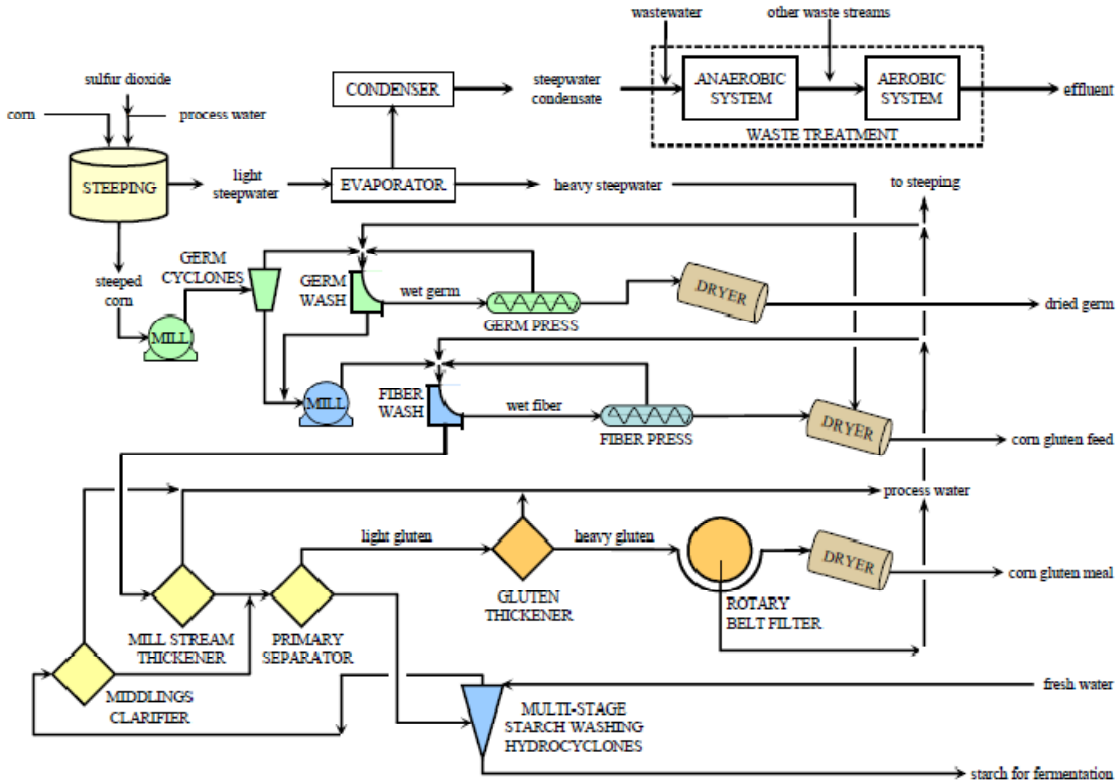
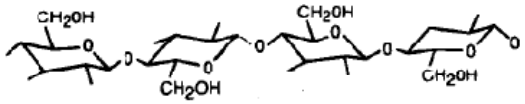
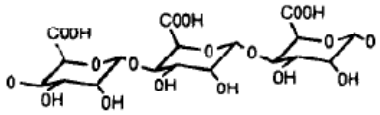
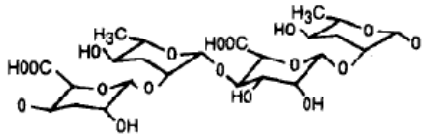
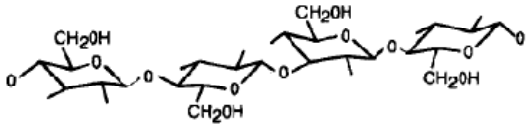
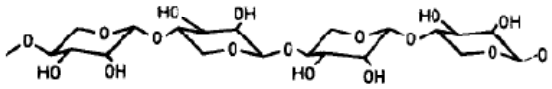
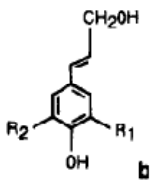


Figure 2.3 Structure of major component of plant cell walls(McDougall et al., 1996).

Polymer	Backbone	Sidechains	Comments
Cellulose	[Glc β(1-4)Glc β(1-4)]$_n$..... 	None	Forms crystalline microfibrils
Galacturonan	[GalAα(1-4)GalAα(1-4)]$_n$..... 	None	Interacts with Ca methoxylated methylated acetylated
Rhamnogalacturonan	GalA α(1-2)Rha α(1-4)GalA α(1-2)Rha 	Arabinans Galactans	Rhamnogalacturonan II as minor component
Mixed linkage Glucans	Glc β(1-4)Glc β(1-3)Glc β(1-4)Glc 	None	c 30% (1-3) linkages
Xylans	[Xyl β(1-4)Xyl β(1-4)]$_n$..... 	Arabinose Glucuronic acid	Methylated acid groups acetylated feruloyl groups
Xyloglucan	as cellulose	Xylose Galctose Fucose as Xyl(-Gal)-(Fuc)[a]	Acetylated
Extensin	polypeptide rich in Ser(Hyp)4 repeats	Arabinans linked to Hyp, galactose to Ser	Tyrosines coupled to form isodityrosine cross-links
Lignin	polymer of cinnamyl alcohols 	None	Cross-linked to proteins and polysaccharides

Glc = glucose; GalA = galacturonic acid; Rha = rhamnose; Xyl = xylose; Gal = galatose; Fuc = fucose.
a - xyloglucan may be substituted with α(1-6) Xyl or α(1-6) Xyl β(1-2) Gal or α(1-6) Xyl β(1-2) Gal α(1-2) Fuc units.
b - where R1 = R2 = H; p-coumaryl alcohol; where R1 = H and R2 = OCH_3; coniferyl alcohol; where R1 = R2 = OCH_3; sinapyl alcohol.

Figure 2.4 Diagram of fermentation that occur in human's large intestine(Varel, 1987).

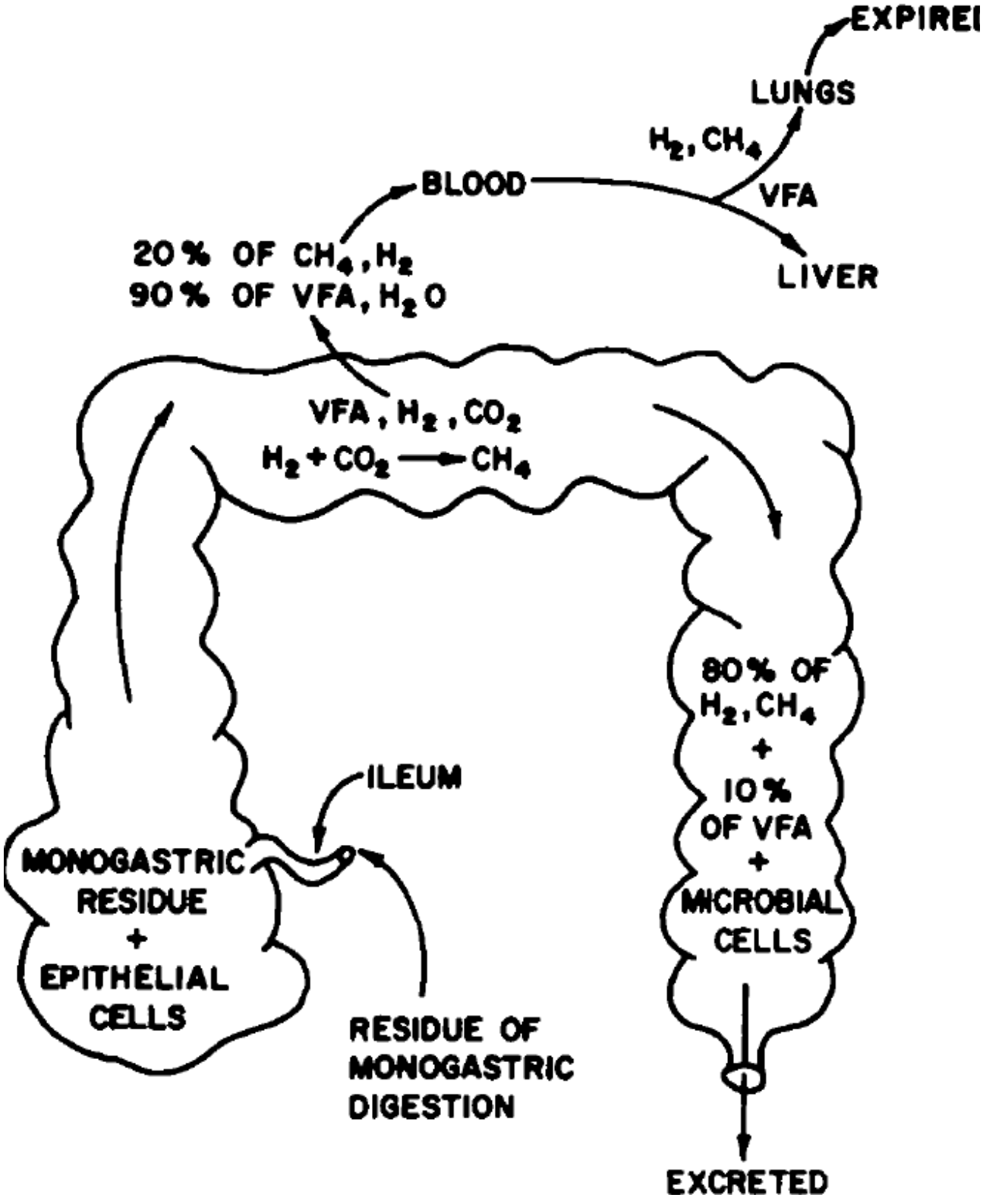


Figure 2.5 Simplified diagram of polysaccharide breakdown and main route of carbohydrate fermentation (Macfarlane and Macfarlane, 2003).

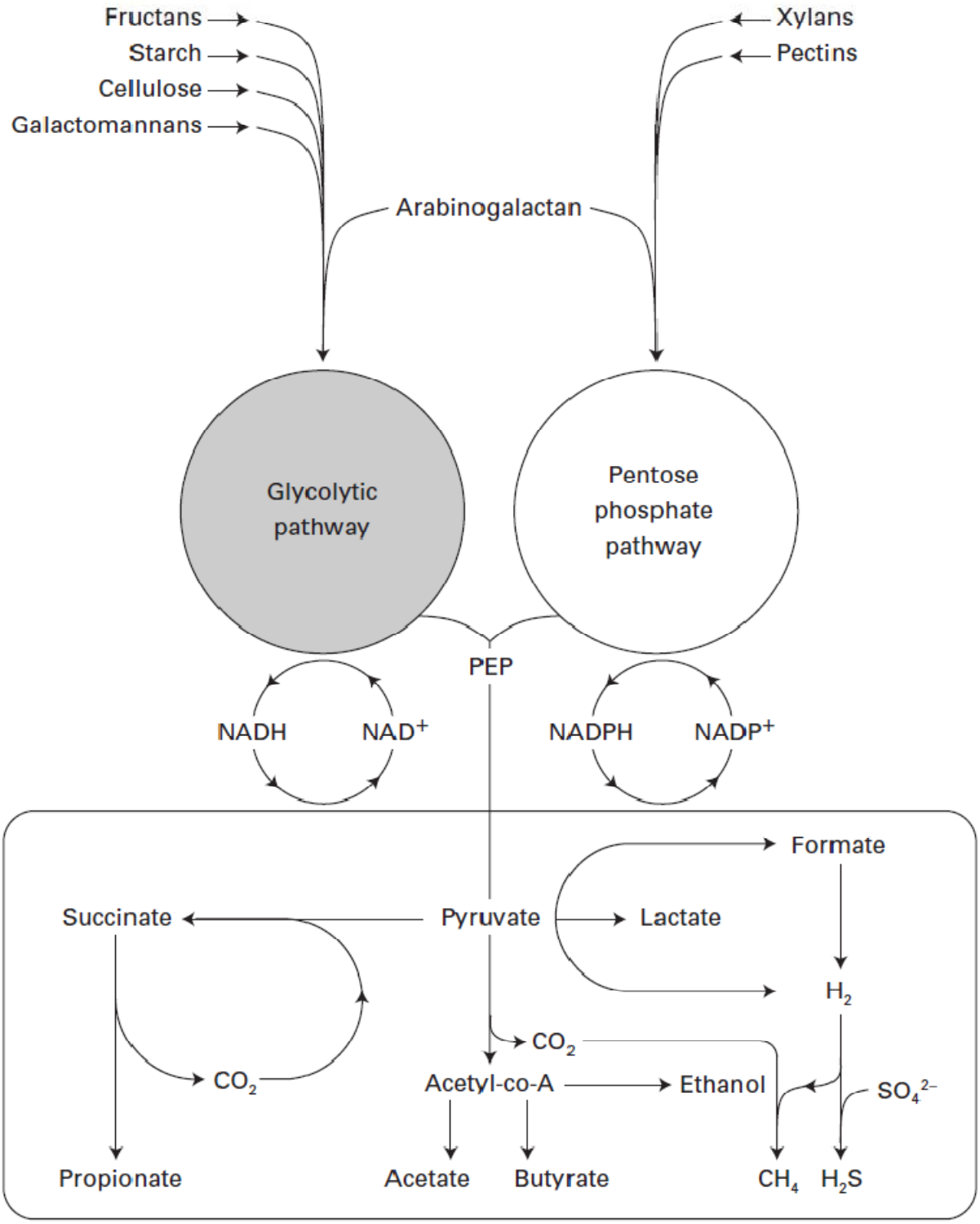


Table 2.1 Carbohydrate composition of fiber in corn, SBM and CDDGS(%) (Shurson, 2008a).

%	Arabinose	Xylan	Mannose	Galactose	Cellulose	Dietary fiber
Corn	1.54	2.42	0.44	0.66	3.74	11.89
SBM	1.98	1.32	1.10	5	3.96	21.15
CDDGS	5.07	7.49	1.98	5	8.59	32.60

Table 2.2 Nutrient composition of DDGS, which will be used in study.

	Corn Grain[1]	DDGS UGA[2]	Soybean Meal 49%[1]
Crude Protein	7.31	27.64	49.42
Fat	3.92	10.54	0.55
Fiber	1.68	6.8	2.87
Ash	1.3	5.81	6.13
Moisture	12.42	12.88	9.98
Lysine	0.26	0.96	3.02
Methionine + Cystine	0.36	1.47	1.39
Threonine	0.29	1.01	1.87
Tyrptophan	0.06	0.24	0.74
Energy, kcal ME/kg	3420	3390	3380
Phosphorous, total	0.28	0.75	0.62
Phosphorous, available	0.1	0.6	0.24

1. NRC(1998)
2. University of Missouri Agriculture Experiment Station Chemical Lab

Table 2.3 FDA guidance for mycotoxins level in animal feed (Wu and Munkvold, 2008).

mycotoxin	FDA standard for animal feed
total fumonisin (FB_1 + FB_2 + FB_3)[b]	5 mg/kg for equids and rabbits (<20% of diet[c]) 20 mg/kg for swine and catfish (<50% of diet) 30 mg/kg for breeding ruminants, breeding poultry and breeding mink (<50% of diet) 60 mg/kg for ruminants >3 months old raised for slaughter and mink raised for pelt production (<50% of diet) 100 pm for poultry raised for slaughter (<50% of diet) 10 mg/kg for all other species or classes of livestock and pets (<50% of diet)
total aflatoxin (AFB_1 + AFB_2 + AFG_1 + AFG_2)[d]	300 μg/kg for maize and peanut products intended for finishing (i.e., feedlot) beef cattle 300 μg/kg for cottonseed meal intended for beef cattle, swine, or poultry (regardless of age or breeding status) 200 μg/kg for maize or peanut products intended for finishing swine of 100 lb or greater 100 μg/kg for maize and peanut products intended for breeding beef cattle, breeding swine, or mature poultry 20 μg/kg for maize, peanut products, and other animal feeds and feed ingredients, but excluding cottonseed meal, intended for immature animals 20 μg/kg for maize, peanut products, cottonseed meal, and other animal feeds and feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known
deoxynivalenol[e]	10 mg/kg for ruminating beef and feedlot cattle> 4 months, chickens (<50% of diet) 5 mg/kg for swine (<20% diet) 5 mg/kg for all other animals (<40% diet)

[a] The standards for total fumonisins and DON are guidelines for industry, whetrsd those for total aflatoxins are action levels. [b] Source: http://www.cfsan.fda.gov/~dms/fumongu2.html. [c] Assuming the feed item (e.g., maize) makes up no more than this proportion of the animal's diet. [d] Source: http://www.cfsan.fda.gov/~lrd/fdaact.html. [e] Source: http://www.cfsan.fda.gov/~dms/graingui.html.

Table 2.4 maximum price can be paid for DDGS with difference cost of corn and SBM without increasing cost of complete feed (Stein., 2007).

	Corn, $/bushel			
SBM, $/ton	2.5	3	3.5	4
175	109	119	128	138
200	120	130	140	150
250	131	141	151	161

CHAPTER 3

THE EFFECT OF ADDING XYLANASE OR B-GLUCANASE TO STARTER DIETS WITH CORN DISTILLERS DRIED GRAIN WITH SOLUBLE (CDDGS) ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN NURSERY PIGS

Tsai, T. 2010. To be submitted to Journal of Animal Science.

ABSTRACT

The objective of current study was to evaluate the effect of Xylanase (XYL) and β-glucanase (BGL) alone or in combination (XGL) on growth performance and nutrient digestibility in nursery pigs fed 30% corn distillers dry grain with soluble (CDDGS) diet. A total of 135 pigs (PIC42xPIC280), weaning at 21 day age, (with initial body weight 5.5±1kg) were randomly selected for a 28 day study that was conducted in two trials. Pigs were randomly assigned to one of five treatments: positive control (Corn/SBM+0% CDDGS)(PC), negative control (30% CDDGS substituted corn/SBM with no enzyme addition)(NE), negative control + 4000 unit/kg xylanase (XYL), negative control + 450 unit/kg β-glucanase (BGL), and negative control + xylanase + β-glucanase (XGL). All nutrients met or exceeded the NRC requirement for this age pigs. Titanium dioxide (0.1%) was added as indigestible maker for determination of nutrient digestibility. All pigs were fed a common phase 1 diet (DE: 3.58 kcal/g, CP: 21.68%, Lys: 1.5%) (d0-7) for one week before the experiment started, and were switched to phase 2 (d7-21) (CP: 22.8%, Lys: 1.38%) and 3 (d21-35) (CP: 22.5%, Lys: 1.25%) (two weeks each phase) experimental diets. The concentration of NDF, ADF, and hemicellulose were increased 120% in phase 2 whereas were 88% higher in phase 3 in 30% CDDGS diets, compared to PC. Pigs fed the NC diet had reduced growth rate and lowered nutrient digestibility. Pigs fed BGL diet increased ADG 7.7%. Those fed the XGL diet had increased ADG (9.3%) over NC diet during d21-35

(P<0.001). Overall, XGL diet improved ADG by 6.6%, compared to NC diet (P<0.001). Digestibility of DM, was improved by 1.6 and 1.4% in pigs fed XYL and XGL, respectively. Energy digestibility was improved 5.5% in pigs fed enzymes supplemented diets (P=0.007). Similarly, CP digestibility was 5.9% greater in pigs fed diet supplement with enzymes as compared to the NC (P=0.005). Addition of XYL, BGL, and XGL increased NDF, ADF, and hemicellulose digestibility by 33%, 30%, and 34% when compared to NC diet (P<0.01). Supplementation of XYL, BGL, and XGL not only improved P digestibility (46%) when compared to NC diet (P<0.001), but also resulted in no difference in P digestibility when compared to PC diet (46% vs. 42%). ADFI, G:F ratio, and VFA concentration were not affected by diets. The results suggests that supplementing XYL, BGL, and XGL not only improved growth performance in pigs fed 30% CDDGS diet, but also alter negative impact of CDDGS on nutrient digestibility.

Key words: CDDGS, growth performance, nursery pigs, nutrient digestibility, xylanase, β-glucanase.

INTRODUCTION

Fiber degrading enzymes have been used as feed additive to improve growth performance, feed efficiency, and nutrient digestibility in monogastric animal species fed diets with higher concentrations of non starch polysaccharides (Bedford and Classen, 1992; Carneiro et al., 2008;

Inborr et al., 1993; Omogbenigun et al., 2004). Fiber degrading enzymes have also been shown to alleviate the negative impact due to excess nutrients being excreted in environment (Mackie et al., 1998). Enzyme supplementation has more benefit in young pigs than growth-finishing pigs (Diebold et al., 2004; Li et al., 1996b; Woyengo et al., 2008) because GI tract has less capacity to produce sufficiency quantity of endogenous enzymes to digest nutrients from diet (Jensen et al., 1997).

The benefit of adding xylanase (XYL) and β-glucanase (BGL) for growth and nutrient utilization was reported previously (Cowieson and Adeola, 2005; Nortey et al., 2008). Xyl is mainly added to wheat based diets or substrate that contains high arabinoxylan, BGL, on the other hand, is introduced to hull-less barley or substrate which has high β-glucan (Bedford et al., 1992; He et al., 2010; Partridge, 2001). However, the benefit of BGL addition in wheat/SBM or Corn/SBM basal diet in nutrient digestibility was also reported (Ji et al., 2008; Li et al., 1996a). Supplementing XYL and BGL combination (XGL) increased nutrients solubility, and disappearance in stomach and small intestine (Aulrich and Flachowsky, 1998, 2001), and consequently improved animal nutrient digestibility.

After starch is removed from corn to produce ethanol, other nutrients are concentrated, including fiber. Fiber in CDDGS is around 4 times and 3 times higher than in corn and SBM

(Stein and Shurson, 2008). Fiber in CDDGS is mainly arabinose (5.69%) and xylose (8.1%)(mostly insoluble)(Jones et al., 2010). Adding XGL in diet may be needed for nutrient digestibility when higher co-product is used in diet (Zijlstra et al., 2010).

The objective of current study was to evaluate the effect of XYL and BGL alone or in combination on growth performance and nutrient digestibility in nursery pigs fed 30% CDDGS diet.

MATERIALS AND METHODS

The experimental protocols used in this study were approved by the Animal Care and Use Committee of the University of Georgia.

Animals

A total of 135 pigs (with initial body weight 5.5±1kg) were randomly selected from the University of Georgia Animal & Dairy Science Swine Unit for a 28 day study that was conducted in two trials. Pigs were weaned at approximately 21 days of age (PIC42xPIC280), and were housed in environmentally controlled nursery. Each pen ($1.68\times1.83mm^2$) was equipped with one stainless steel water nipple, a four hole feeder, and expanded crossed plastic floor. Temperature was initially set at 27°C, and decreased subsequently to 23°C at the end of each trial. Light/dark cycle was set at 12 hours interval (0600/1800). Body weight and feed intake

were monitored weekly. In the first trial, a total of 60 pigs with 4 pigs per pen (two barrows, and two gilts), and 3 weight blocks were randomly assigned to one of five treatments. A total of 75 pigs with 5 pigs per pen (two gilts, three barrows), and 3 weight blocks were selected for second trial.

Diet

There were three feeding phases during the trials. All pigs were fed a common phase 1 diet (d0-7)(DE: 3.58 kcal/g, CP: 21.68%, Lys: 1.5%) for one week before the experiment started, and were switched to phase 2 (d7-21) and 3 (d21-35) (two weeks each phase) experiment diets (table 1). Diets were formulated on a total lysine basis. Pigs were randomly assigned to one of five treatments: positive control (Corn/SBM+0% CDDGS)(PC), negative control (30% CDDGS substituted corn/SBM with no enzyme addition)(NE), negative control + 4000 unit/kg xylanase (XYL), negative control + β-glucanase 450 unit/kg (BGL), and negative control + xylanase + β-glucanase (XGL) post-weanling, and were remained in the same pen during phase 2 and 3. XYL is produced from a strain of *Trichoderma ressei* whereas BGL is produced from a strain of *Trichoderma longibrachiatum*. Enzymes were replaced with equally amount of corn, and were premixed with corn, and indigestible marker. Titanium dioxide (0.1%) was used as indigestible marker for nutrient digestibility determination. All nutrients met or exceeded the NRC

recommend for this age of pigs except metabolism energy (ME). In order to show benefit of fiber degrading enzymes in energy utilization, the ME was formulated 3% and 4.7% lower in phase 2 and phase 3 in NC, XYL, BGL, and XGL diet than PC. Feed and water were provided ad libitum during the experiment.

Sample collection

Body weight and feed intake were monitored every week. Diet samples from three phases were reserved and kept in a -20 ^{0}C freezer for further analysis. Fresh fecal samples from each pen were collected on 3 consecutive days at the end of phase 2 and 3, and stored in a -20 ^{0}C freezer for further analysis.

Sample analysis

Diets were sent to Danisco Lab for enzyme activity determination.

A sample of CDDGS was sent to the Agricultural and Environmental Services Laboratories (AESL) of University of Georgia for approximate analysis, and the mineral content was measured by inductively coupled atomic emission spectroscopy (ICP-AES). The amino acid profile of CDDGS was analyzed by Agricultural Experiment Station Chemical Laboratories of

University of Missouri-Columbia. The results were used in diet formulation. The ME in CDDGS was obtained from Pederson et al., (2007).

Fecal volatile fatty acids concentration

A total of 25g fecal sample was obtained from each pen, and mixed with 100g distilled water by blender. The entire solution was then filtered through cheese cloth. A 50 ml subsample was stored frozen. A 1 ml of phosphoric acids (2.5%) was added, and was brought up to 6 ml from subsample in 10ml plastic tube. After freezing overnight, samples with phosphoric acid were centrifuged (IEC Centra GP8R, Thermo Fisher Scientific Inc., Suwanee, GA) at 1320 × g for 20 minutes at 4^{o}C. The supernatant from each tube was collected, and volatile fatty acids were analyzed by gas chromatography (3400 Gas Chromatography, Varian Instrument Group, Walnut, CA).

DM, CP, GE, NDF, ADF, hemicelluloses determination

Diet and fecal samples were oven dried at 55 ^{0}C for 72 hours to a constant weight in a Grieve Shelf oven SA-350 (The Grieve Cooperation, Round Lake, IL) for DM determination. After drying, diets and feces were ground through a 1mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) for other analyses. Diets and feces were analyzed for CP by a Leco FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Gross energy concentration in

diets and fecal was examined by bomb calorimeter (Parr 1261, Parr Instrument Co., Moline, IL). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured by Van Soest procedure (Van Soest et al., 1991) using a Ankom 200 fiber analyzer (Ankom technology, Macedon, NY). Hemicellulose was determined by difference between NDF and ADF. Crude fat from diets and feces samples were analyzed by Labconco Goldenfisch Fat Extractor (Labconco CO. Kansas City, MI) using the AOAC procedure (AOAC, 2000).

Ti and P determination

Diet (2 g) and fecal (1 g) samples were ashed 12 hours at 600 ^{o}C in a Isotemp Muffler Furnace (Fisher Scientific, Suwanee, GA) for determination of titanium and phosphorous. Ash samples were cooked with sulfuric acid for 1 hour, and then were brought up to 50ml with distiller water. Titanium dioxide concentration analysis was modified from Short et al., (1996). Samples were centrifuged at 1320 x g for 20 minutes. Solutions were analyzed by visible light spectrophotometer and were measured absorbance at 410 nm. Phosphorous concentration procedure was modified from Augspurger et al., (2003), and Kim et al., (2005). Solutions were analyzed by visible light spectrophotometer and measured absorbance at 660 nm. Apparent total digestibility (ATTD) of nutrient by marker was calculated by following equation:

$$\text{ATTDM}=1-\frac{([mar\,\text{ker}]_{feed}\times[Nuti.]_{feces})}{([mar\,\text{ker}]_{feces}\times[Nuti.]_{feed})}$$

Statistical Analysis

The SAS GLM procedure was used to analyze the data (SAS Inst. Inc, Cary, NC). A randomized block design was used to conduct the experiment with five diets in each of the three BW groups, with the three BW groups being the blocks. The diets were the main effects and covariates between the experimental trial and initial BW were used in the statistical model. The individual pen was the experimental unit for ANOVA. The results represented the least square mean for the diet effect, with significance for diet effect at $P<0.05$. The difference between NC and enzyme treatment group (XYL, BGL, and XGL) was determined using a simple contrast.

RESULT

There were four pigs removed from two trial periods (2 from PC diet, 1 from XYL diet and 1 from XGL diet) because of illness, and illness situation was not associated with diet treatments.

Diet

Feed composition is shown in table 1. Proximate analysis resulted in similar values as calculation in diet formulation (table 2). Concentration of NDF, ADF, and hemicellulose were increased 122%, 112%, 126% in phase 2, and were 88%, 83%, 93% higher in phase 3 30% CDDGS diets as compared to PC. The result of enzyme activity is shown in table 3. Xylanase and β-glucanase activities in PC and NC diets, which were not supplemented enzymes, were low as expected. Any activity detected was likely of plant origin. In diet with added xylanase (such as XYL and XGL), xylanase activity was approximately 2700 U/kg in XYL diet, and 2900 U/kg in XGL diet. β-glucanase activity, on the other hand, was about 290 U/kg in BGL diet, and 440 U/kg in XGL diet.

Growth performance

The result for growth performance is shown in table 4 and figure 1. Dietary treatments did not affect BW from d7-21. Pigs fed PC diet had highest BW than other treatment in d28 and d35 ($P<0.05$). In contrast, the pigs fed the NC diet had the lowest BW at d35, compared to other treatments ($P<0.001$). In overall, addition of XYL, BGL, and XGL in diet improved BW by 0.5%, 2.7%, and 4% when compared to NC. There was no treatment difference in ADG at first two weeks of study ($P>0.25$). Higher ADG was observed in pigs fed PC diet in d21 to 35

significantly, compared to NC diet (P<0.001). Pigs fed XYL, and BGL diets had improved ADG (2.7% and 5.3%) numerically for d21-28. Pigs fed the XGL diet, however, had increased ADG by 10.8%, and resulted in no difference when compared to those pigs fed PC diet (0.501 vs. 0.509kg/d) for d21-28. Pigs fed BGL and XGL diet had significantly better ADG than NC diet in d21-35 (P<0.001). In overall, pigs fed NC diet, which contained 30% CDDGS, had lower ADG by 12.3% than PC diet. XYL and BGL supplement diet did not increase ADG. XGL diet, on the other hands, improved ADG by 6.6%, compared to NC diet (P<0.001). Therefore, pigs fed BGL and XGL diet did not have effect on ADG in d7-21. BGL and XGL diet, however, increased ADG significantly in d21-35.

There was no difference in ADFI between diets through the whole study period. Pigs tended to have numerically higher feed intake in NC, XYL, BGL and XGL diet, which could be due to lower ME value was used to formulate those diets. Pigs fed the PC diet had better feed efficiency than other diets through the whole study except d28-35 (P<0.02). Although pigs fed the BGL diet had numerically better feed efficiency, enzyme supplementation to NC diet did not have effect on feed efficiency overall.

Nutrient digestibility

The result of nutrient digestibility is showed in table 5. In phase 2(d7-21), digestibility of DM, energy, and CP was higher in pigs fed the PC diet, whereas those fed the NC diet had lowest digestibility. Addition of XYL, and XGL improved DM digestibility 1.6% and 1.4% when compared to those fed the NC diet (P=0.003). Digestibility of ash was not different between diets (P=0.73). However, in enzyme supplemented diets, especially BGL and XGL, there was 26% higher ash digestibility than in the NC diet (P=0.005). Digestibility of energy was improved 5.5% when enzymes were added to diets (P=0.007). Similarly, CP digestibility was 5.9% better in diets supplemented with enzymes (P=0.005). Digestibility of NDF, ADF, and hemicellulose was lower in PC and NC diet (P<0.02). Addition of fiber degrading enzymes to the diet increased NDF, ADF, and hemicellulose digestibility by 33%, 30%, and 34% when compared to the NC diet (P<0.01). Digestibility of P was lower in NC diet than other diets (P=0.006). Addition of XYL, BGL, and XGL in the diet not only improved P digestibility (46%) when compared to NC diet (P<0.001), but also resulted in no difference in P digestibility when compared to PC diet. In phase 3 (d21-35), there was no effect in diets supplemented with enzyme on nutrient digestibility.

Fecal VFA concentration

The result of fecal VFA concentration is shown in table 6. There was no difference in fecal VFA concentration between diets in phase 2 or 3.

DISCUSSION

The results of current study demonstrated that use of 30% CDDGS in phase 2 and 3 nursery diet decreased growth performance and nutrient utilization. Supplementing the diets with BGL and XGL, however, improved ADG, especially in phase 3. In addition, the combination of XYL and BGL had additive effect in overall ADG. Including XYL, BGL and XGL in diet improved nutrient digestibility in diets containing 30% CDDGS, especially in phase 2.

Addition of BGL and XGL in this study improved BW and ADG in nursery pigs fed diets with 30% CDDGS, which agreed with: Emiola et al., (2009) (multienzymes in wheat DDGS diet in 36kg pigs) , Spencer et al., (2007) (multienzymes in CDDGS diet in 9kg pigs), and Yoon et al., (2009)(mannanase in CDDGS in 53kg pigs). However, opposite results were also reported (Feoli et al., 2008; Jones et al., 2008). Adding XYL, BGL and XGL in the current study did not improve feed efficiency. In contrast, Emiola et al., (2009) observed better feed conversion ratio in pigs fed wheat DDGS diet supplement multienzymes. There are several notable differences in the present study. First, the ME in this study was formulated at 3 to 4.7% lower in 30% CDDGS

diets than PC. Although there was no significant different in intake, ADFI was about 9.3% higher in pigs fed diets with 30% CDDGS diet as compared to the standard corn/SBM based PC. Thus, this explain why the G:F ratio was not improved after exogenous enzyme addition. Secondly, BGL supplementation was relatively lower in this study than Emiola et al., (2009) (450 U/kg vs. 780 U/kg). Third, substrates are different (CDDGS vs. wheat DDGS). There was a few paper discuss fiber degrading enzymes effect on CDDDS in early nursery pigs(Feoli et al., 2008; Jones et al., 2008; Spencer et al., 2007). Therefore, more studies are needed to evaluate the effect of different level and various types of fiber degrading enzymes on high level CDDGS diet for early nursery pigs in growth performance.

During phase 2, Supplementing XYL, BGL, and XGL improved digestibility of: DM by 1.3%, CP by 4.4%, energy by 5.1%, ash by 2.5%, and P by 46% when compared to the NC, which is in agreement with other reports (Emiola et al., 2009; Feoli et al., 2008; Yoon et al., 2009). Moreover, digestibility of NDF, ADF, and Hemicellulose was also improved in XYL (33%), BGL (30%), and XGL (34%) diet significantly. In phase 3, nutrient digestibility, was not improved by enzyme supplementation, which is in agreement with other research (Diebold et al., 2004; Li et al., 1996b; Woyengo et al., 2008). It is suggested that as the pig matures, the digestive system is more developed, and can produce sufficient endogenous enzymes to release nutrients in lumen and mucosa, and the pigs has larger and longer GI tract, which provides more

capacity and longer time for endogenous enzymes to digest nutrients (Cera et al., 1988a; Lindemann et al., 1986; Owsley et al., 1986; Yen, 2000). Jensen et al., (1997) reported that SI length increased from weaning (10.5m) to end of nursery (14.4m), and pancreatic trpsin increases 826% while amylase increases 180%, from weaning to end of nursery. Lindemann et al., (1986) observed that pancreas weight increased from weaning (7.36g) to two weeks post weaning (12.28g). In the same time, trpsin and amylase activity increased 116% and 28.4%. Similarly, Cera et al., (1990) found that pancreas increased two fold (1.44 vs. 2.21 g/empty BW, kg), and pancreatic lipase activity increased from 193 to 218 U/g from d7 to d28 postweaning. Therefore, supplementing exogenous enzymes should have more benefit in young pigs than in mature pigs (Partridge, 2001).

After starch is extracted for ethanol production, fiber concentration in CDDGS is around 4 times and 3 times higher than in corn and SBM (Stein and Shurson, 2008). The NDF, ADF, and hemicellulose in 30% CDDGS diet in this study were 18%, 5%, and 13% respectively, which is similar to other reports (Song et al., 2010; Urriola and Stein, 2010; Xu et al., 2010). The fiber in CDDGS is mainly insoluble fiber, which contains high level of arabinose (5.5%), xylan (8%), mannose (1.5%) and cellulose (8.5%) (Jones et al., 2008; Shurson, 2008). It is suggested that the benefit of enzymes can only be seen when enzymes match the nutrient limitation in substrates (Zijlstra et al., 2010). The benefit of XYL supplementation has been reported in diets,

which contain high arabinoxylan such as wheat (Cadogan et al., 2003; Dusel et al., 1997; He et al., 2010), whereas adding BGL has been showed to improve performance and nutrient utilization in high β-glucan diet, such as barley(Bedford et al., 1992; Li et al., 1996b). However, BGL supplementation in diets, which had lower level of β-glucan also showed better nutrient digestibility, such as wheat or corn SBM basal diet (Ji et al., 2008; Li et al., 1996a). Bedford et al., (1992) indicated that although β-glucan content is low, and viscosity may not be as a severe problem in pigs as in chicks, β-glucanase addition may help releasing negative impact from substrate or other feed ingredients, such as SBM. Hence, endogenous and exogenous enzymes can work on substrates better. This may explain why BGL addition had a benefit in this study. Adding XYL should help break down arabinoxylan in CDDGS. Although arabinose and xylose are considered less digestible in pigs (Yule and Fuller, 1992), breaking down the linkage by exogenous enzymes should decreasing negative impact on nutrient utilization by altering endogenous enzymes activity (Fan et al., 2009; Jensen et al., 1997; Seri et al., 1996). Moreover, Aulrich and Flachowsky, (1998) found that XGL addition to wheat bran, which had higher insoluble arabinoxylans, increased protein, mineral, and monosaccharides release from insoluble part of wheat bran. In addition, adding XGL increased nutrient's hydration in vitro. Nutrient utilization, therefore, should increase after XGL supplementation.

Growth performance was improved significantly during phase 3 of this study. Nutrient digestibility, however, were better with XYL, BGL, and XGL addition in phase 2. Other than rapid development in GI tract during weaning, which discussed previously, the lack of effect of enzymes on digestibility in phase 3 could be fat concentration in CDDGS diet. Crude fat concentration was lower in the PC diet than other treatments (3.66% vs. ave. 4.7%) in phase 2. However, similar fat concentration was observed in phase 3 (5.4% vs. ave. 5.04%). CDDGS has higher fat concentration than corn or SBM (Widmer et al., 2008). Unlike fiber, which lowers diet energy density, fat increases energy density, which in term lowers digesta passage rate, and result in an "extra caloric effect". Therefore, endogenous enzymes have more time to prepare nutrients for body to uptake and utilize (Azain, 2001). Li and Saucer, (1994) found that increased dietary fat from 4% to 13.9% reduced DM disappearance from 178.9 to 152.3 g/kg of DMI whereas ATTD of DM and CP increased linearly as percentage of fat increased. The benefit of fat on nutrient digestibility depends on fat characteristics: unsaturated fat better than saturated fat, median chain fatty acids better than long chain fatty acids, vegetable fat better than animal fat (Cera et al., 1989, 1988b; Li et al., 1990; Li and Sauer, 1994). Thus, once the negative impact of fiber in CDDGS is diminished by enzymes, highly unsaturated fat in CDDGS could benefit nutrient utilization. In contrast to the effect of dietary fat on nutrient digestibility, reports on the effect of fat in ADG were variable (Li et al., 1990; Tokach et al., 1995). Thus, this may explain

why digestibility was better in phase 2 with XYL, BGL, and XGL diets, but ADG was not improved in this study.

Concentration of VFA was not affected between treatments during phase 2 and 3. Recently, Urriola et al., (2009) also observed no difference in VFA production between 0 and 30% CDDGS diets in digesta of ileal, cecum, and feces. Hindgut has more microbial activity, and has more fermentation processes than foregut (Castillo et al., 2006; Jensen and Jorgensen, 1994). After being produced by microbes, VFA are absorbed rapidly (Bergman, 1990; Ehle et al., 1982), and only 10% of VFA is excreted in feces (Wolin and Miller., 1983). Thus, measurement of VFA production in both ileal digesta and feces may be needed to understand the effect of diet on VFA utilization. Adding exogenous fiber degrading enzymes did not affect VFA concentration in both ileal and post-ileal GI tracts (Carneiro et al., 2008; Diebold et al., 2004; Yin et al., 2000). Increasing fiber in the diet increases VFA concentration, and profile in hindgut (Anguita et al., 2006; Kass et al., 1980), which, however, can be altered by adding fiber degrading enzymes (Carneiro et al., 2008; Diebold et al., 2004; Yin et al., 2000). Exogenous fiber degrading enzymes release monosccharide from NSP (Aulrich and Flachowsky, 1998), but most free monosaccharides disappear in the SI (Anguita et al., 2006). Moreover, monosacchrades released in 30% CDDGS diet may be not high enough to result the differences in VFA concentration or pattern.

It was suggested that soluble fiber has more effect on growth performance whereas both soluble and insoluble fiber contribute to the decreased nutrient digestibility (Chabeauti et al., 1991; Dierick et al., 1989; Hedemann et al., 2006; Kornegay et al., 1995; Partridge, 2001). Supplementing fiber degrading enzymes in high fiber diets in nursery pigs as a means to improve growth performance has been discussed previously (Partridge, 2001). The results are controversial between studies. This may be due to differences in types of substrates, concentration of substrate, types of enzymes, single or mixed enzymes, enzymes concentration, and age of animals. Supplementing XYL or BGL in the diet has been shown to increase ADG in nursery pigs fed: wheat basal diet (Cadogan et al., 2003; Fang et al., 2007; Vahjen et al., 2007), wheat and barley basal diet (Dusel et al., 1997), corn, wheat, and SBM basal diet (He et al., 2010), and hull-less barley diet (Bedford et al., 1992). However, other studies report no benefit of XYL or BGL addition (Officer, 1995; Olukosi et al., 2007; Thacker et al., 1992). The same type of enzymes with different strains and preparation methods can result in differently (Inborr et al., 1993; Vahjen and Simon, 1999). The XYL, BGL, and XGL used in this study showed ability to work on fiber from CDDGS.

Due to rapid changes in the GI tracts of young animals, measuring nutrient digestibility based on twice fecal collections from whole study may not be sufficicnet to evaluate digestibility. More frequently sample collection may be needed, such as at weekly intervals. In this study, we

only collected feces to measure nutrient digestibility. However, in order to understand more of treatments effect on nutrient utilization, ileal digestibility measurement may also be needed (Dierick and Decuypere., 1996).

In conclusion, most of DDGS studies were mainly focused on growing and finishing pigs with very few studies in nursery pigs (Stein and Shurson, 2008), which is because of the assumption that young pig would not perform well in the high fiber content of DDGS. The results of this study indicated that diet where 30% CDDGS replaced corn and SBM, resulted in decreasing growth performance and nutrient digestibility. Adding XYL, BGL, and XGL in 30% CDDGS not only improved growth performance in pigs fed 30% CDDGS diet, but also altered the negative impact of CDDGS on nutrient digestibility. Further study is needed to investigate the effect of different concentration of enzymes in diet with high levels of CDDGS. Moreover, using ileal cannulas to evaluate the effect of enzymes on nutrient disappearance in the GI tracts of in nursery pigs fed high level CDDGS diet is needed.

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Table 3.1 Feed composition(as-fed basis).

Diet	Phase 2		Phase 3	
Ingredient , %	PC[9]	NC[9]	PC[9]	NC[9]
Corn, Grain	51.58	41.39	55.76	47.43
Soybean Meal, 48%	27.32	10	36.52	18.3
CDDGS[1]	-	30	-	30
Poultry Fat	2.5	-	3.5	-
Menhaden Meal	2.5	2.5	-	-
Blood, spray-dried	2.5	2.5	-	-
Whey, Dehydrated	10	10	-	-
Limestone	0.75	1.21	0.99	1.46
Dical. Phos.[2]	0.97	0.3	1.25	0.55
Zinc oxide	0.25	0.25	-	-
Common salt	-	-	0.35	0.35
Vitamin Premix[3]	0.25	0.25	0.25	0.25
Mineral Premix[4]	0.15	0.15	0.15	0.15
DL-Methionine	0.13	-	0.1	-
L-Lysine HCl	-	0.33	0.03	0.4
L-Tryptophan	-	0.025	-	0.015
Titanium oxide[5]	0.1	0.1	0.1	0.1
Antibiotic[6]	1	1	1	1
Calculated Analysis				
ME[7], Kcal/g	3.34	3.24	3.40	3.24
CP, %	22.89	22.78	22.51	21.47
Calcium, %	0.8	0.8	0.75	0.75
Total P, %	0.65	0.60	0.59	0.55
Available P, %	0.40	0.41	0.35	0.35
Total Lys, %	1.39	1.38	1.25	1.25
SID[8] Lys, %	1.2	1.15	1.12	1.06

1. Corn original DDGS.
2. Dicalcium phosphate.
3. Supplied per kg of premix: vitamin A 4400 IU; vitamin D 660000 IU; vitamine E 17600 IU; vitamin K 1760 IU; riboflavin 3960 mg; niacin 22000 mg; vitamin B12 17600 μg .
4. Supplied per kg of premix: iron 110000 mg; copper 11000 mg; manganese 26400 mg; zinc 110000 mg; iodine 198 mg; selenium 198 mg.
5. 0.1% of Titanium dioxide was premixed with corn, and fiber degrading enzymes.
6. Mecadox.
7. Metabolism energy.
8. Standard ileal digestible. Data was obtained from NRC (1998).
9. Positive control (Corn/SBM+0% CDDGS)(PC), negative control (30% CDDGS substituted corn/SBM with no enzyme addition)(NE), negative control + 4000 unit/kg xylanase (XYL), negative control + β-glucanase 450 unit/kg (BGL), and negative control + xylanase + β-glucanase (XGL).

Table 3.2 Diet approximate analysis (DM basis).

	Diet[1]				
	PC	NC	XYL	BGL	XGL
Phase 2					
Gross energy, Kcal/g	4.1	4.2	4.2	4.3	4.3
Dry matter, %	90.63	91.31	90.82	90.50	90.76
Crude protein, %	22.44	21.21	22.13	22.10	21.47
Ether extract, %	3.66	4.54	4.20	5.07	4.94
Ash, %	6.29	6.12	6.09	6.53	6.20
Total P, %	0.63	0.51	0.52	0.53	0.51
NDF[2], %	7.66	16.06	16.48	18.09	17.64
ADF[2], %	2.36	4.57	4.68	5.47	5.00
Hemicellulose, %	5.30	11.49	11.80	12.62	12.64
Phase 3					
Gross energy, Kcal/g	4.1	4.1	4.1	4.2	4.2
Dry matter, %	89.23	89.57	89.55	89.07	89.36
Crude protein, %	22.23	21.41	20.75	19.81	20.80
Ether extract, %	5.40	4.96	4.89	5.62	4.62
Ash, %	6.14	6.01	6.08	6.09	6.12
Total P, %	0.55	0.48	0.51	0.52	0.48
NDF[2], %	9.90	18.07	18.72	18.84	19.17
ADF[2], %	2.89	5.71	5.34	5.21	5.20
Hemicellulose, %	7.01	12.36	13.38	13.62	13.97

1. Positive control (Corn/SBM+0% CDDGS)(PC), negative control (30% CDDGS substituted corn/SBM with no enzyme addition)(NE), negative control + 4000 unit/kg xylanase (XYL), negative control + β-glucanase 450 unit/kg (BGL), and negative control + xylanase + β-glucanase (XGL).
2. Neutral detergent fiber and acid detergent fiber.

Table 3.3 Xylanase and β-glucanase activity between diets (DM basis).

	Diet				
	PC	NC	XYL	BGL	XGL
Phase 2					
Xylanase, U/kg	180	ND	2832	474	3090
B-glucanase, U/kg	164	196	ND	306	467
Phase 3					
Xylanase, U/kg	363	ND	2644	760	2880
B-glucanase, U/kg	<100	<100	ND	282	403

1. Positive control (Corn/SBM+0% CDDGS)(PC), negative control (30% CDDGS substituted corn/SBM with no enzyme addition)(NE), negative control + 4000 unit/kg xylanase (XYL), negative control + β-glucanase 450 unit/kg (BGL), and negative control + xylanase + β-glucanase (XGL).
2. ND=not detectable.

Table 3.4 Least square means of with or without fiber degrading enzymes on growth performance in nursery pigs fed 30% CDDGS diet (DM basis).

	Diet[1]						
	PC n=25[2]	NC n=27	XYL n=26[2]	BGL n=27	XGL n=26[2]	SEM	P-value
BW, kg							
Day 7	7.09	7.10	7.21	7.33	7.25	0.13	0.623
Day 14	8.52	8.41	8.30	8.39	8.44	0.07	0.274
Day 21	11.39	11.08	10.97	11.03	11.14	0.14	0.231
Day 28	14.96^{b}	14.24^{a}	14.21^{a}	14.36^{a}	14.65^{b}	0.19	0.035
Day35	19.33^{c}	17.83^{a}	17.93^{ab}	18.31^{ab}	18.54^{b}	0.25	<0.001
Daily Gain, kg/d							
Day 7-14	0.185	0.169	0.153	0.167	0.173	0.010	0.274
Day 14-21	0.411	0.382	0.382	0.377	0.387	0.013	0.403
Day 21-28	0.509^{b}	0.452^{a}	0.464^{ab}	0.476^{ab}	0.501^{b}	0.016	0.072
Day 28-35	0.624^{b}	0.513^{a}	0.531^{a}	0.565^{a}	0.555^{a}	0.021	0.004
Day 7-35	0.432^{c}	0.379^{a}	0.382^{ab}	0.396^{ab}	0.404^{b}	0.009	<0.001
Feed Intake, kg/d							
Day 7-14	0.283	0.334	0.322	0.329	0.323	0.023	0.559
Day 14-21	0.630	0.719	0.731	0.700	0.743	0.035	0.201
Day 21-28	0.882	0.911	0.912	0.919	0.970	0.021	0.081
Day 28-35	1.019	1.024	1.001	1.063	1.101	0.038	0.394
Day 7-35	0.702	0.745	0.739	0.758	0.828	0.034	0.154
G:F ratio							
Day 7-14	0.652^{b}	0.496^{a}	0.495^{a}	0.505^{a}	0.542^{a}	0.033	0.011
Day 14-21	0.656^{b}	0.532^{a}	0.531^{a}	0.552^{a}	0.530^{a}	0.028	0.017
Day 21-28	0.578^{b}	0.494^{a}	0.505^{a}	0.527^{a}	0.521^{a}	0.015	0.007
Day 28-35	0.604b	0.504^{a}	0.526^{a}	0.543^{ab}	0.516^{a}	0.026	0.095
Day 7-35	0.611^{b}	0.505^{a}	0.516^{a}	0.535^{a}	0.503^{a}	0.019	0.003

The results are LS means for 3 pens of 5 pigs each diet in trial 1 whereas there are LS means for 3 pens of 4 pigs each diet in trial 2.

Within a row (or column), means without a common superscript differ ($P < 0.05$).

1. Positive control (Corn/SBM+0% CDDGS)(PC), negative control (30% CDDGS substituted corn/SBM with no enzyme addition)(NE), negative control + 4000 unit/kg xylanase (XYL), negative control + β-glucanase 450 unit/kg (BGL), and negative control + xylanase + β-glucanase (XGL).
2. Two pigs were removed from both trials. It was not due to treatment.

Table 3.5 Least square means of fiber degrading enzymes on nutrients ATTD in nursery pigs fed 30% CDDGS diet (DM basis).

	Diet[1]						P-Value	
Digestibility, %	PC	NC	XYL	BGL	XGL	SEM	Diet	NC vs. E[2]
Phase 2								
Dry matter	93.1^{c}	90.0^{a}	91.4^{b}	90.9^{ab}	91.3^{b}	0.003	<0.001	0.003
Ash	49.7^{ab}	41.6^{a}	50.8^{b}	52.7^{b}	52.4^{b}	0.029	0.073	0.005
Energy	80.4^{c}	72.1^{a}	76.1^{b}	75.0^{ab}	76.2^{b}	0.011	<0.001	0.007
CP	75.2^{c}	66.1^{a}	71.6^{b}	69.6^{ab}	70.2^{b}	0.012	<0.001	0.005
NDF	36.2^{a}	37.0^{a}	48.1^{b}	51.6^{b}	47.9^{b}	0.030	0.003	0.002
ADF	34.0^{a}	40.7^{a}	50.2^{b}	56.4^{b}	52.1^{b}	0.027	<0.001	<0.001
Hemicellulose	37.1^{ac}	35.6^{a}	47.3^{b}	49.5^{b}	46.2^{bc}	0.032	0.015	0.003
Phosphorous	41.6^{b}	31.6^{a}	44.6^{b}	46.0^{b}	46.1^{b}	0.028	0.006	<0.001
Phase 3								
Dry matter	93.3	92.4	92.7	92.2	92.3	0.004	0.204	0.875
Ash	52.8	61.7	60.5	56.2	56.5	0.032	0.291	0.288
Energy	82.0	78.5	79.6	78.2	78.5	0.010	0.063	0.808
CP	78.7	77.2	77.6	74.3	75.3	0.013	0.144	0.316
NDF	50.2	55.4	58.2	53.8	57.1	0.028	0.287	0.752
ADF	48.9^{a}	62.0^{b}	60.1^{b}	55.2^{ab}	57.1^{b}	0.026	0.016	0.147
Hemicellulose	50.7	52.1	57.4	53.3	57.1	0.029	0.398	0.269
Phosphorous	33.7	36.6	46.8	43.4	39.5	0.039	0.156	0.155

The results are LS means for 3 pens of 5 pigs each diet in trial 1 whereas there are LS means for 3 pens of 4 pigs each diet in trial 2.
Within a row (or column), means without a common superscript differ (P< 0.05).
A total 0.1% titanium dioxide was used as indigestible marker.

1. Positive control (Corn/SBM+0% CDDGS)(PC), negative control (30% CDDGS substituted corn/SBM with no enzyme addition)(NE), negative control+ 4000 unit/kg xylanase (XYL), negative control + β-glucanase 450 unit/kg (BGL), and negative control + xylanase + β-glucanase (XGL).
2. A simple contrast was used to determine difference between NC vs. enzymes addition diets (XYL, BGL, and XGL).

Table 3.6 Effect of fiber degrading enzymes on VFA concentration in nursery pigs fed 30% DDGS diet.

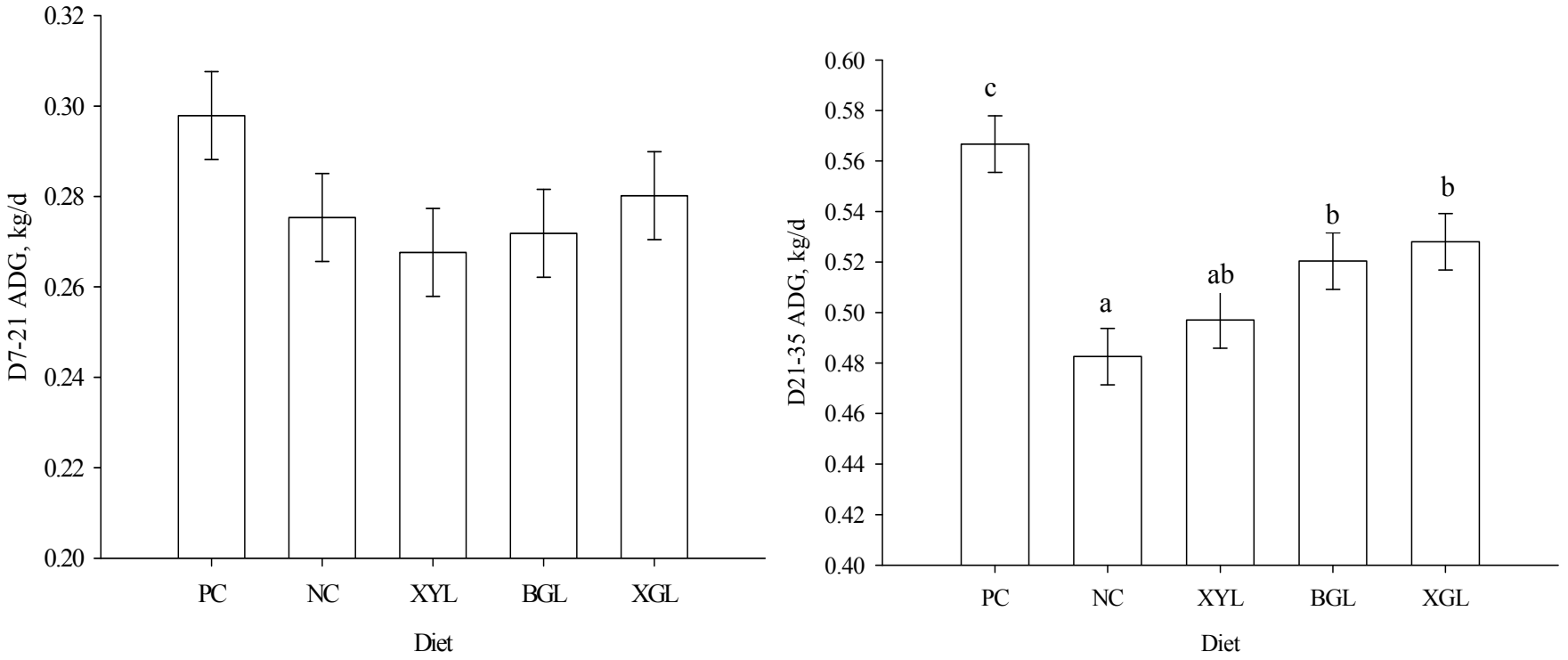
	Diet						
% of DM	PC	NC	XYL	BGL	XGL	SEM	P-Value
Phase 2							
Acetate	0.830	0.747	0.755	0.790	0.702	0.047	0.397
Propionate	0.528	0.478	0.595	0.582	0.549	0.047	0.433
Butyrate	0.698	0.816	0.845	0.907	0.970	0.111	0.512
Isobutyrate	0.239	0.282	0.293	0.252	0.303	0.034	0.615
Isovalerate	0.196	0.124	0.153	0.167	0.147	0.016	0.111
Valerate	0.200^{b}	0.127^{a}	0.158^{ab}	0.156^{ab}	0.146^{a}	0.018	0.043
TotalVFA	2.666	2.558	2.780	2.744	2.841	0.197	0.863
Phase 3							
Acetate	0.917	0.817	0.776	0.740	0.777	0.043	0.079
Propionate	0.627	0.581	0.537	0.521	0.548	0.031	0.167
Butyrate	0.199	0.169	0.161	0.185	0.182	0.016	0.504
Isobutyrate	0.599	0.525	0.505	0.598	0.576	0.067	0.801
Isovalerate	0.132	0.108	0.093	0.131	0.110	0.030	0.864
Valerate	0.169	0.152	0.135	0.133	0.117	0.013	0.084
TotalVFA	2.643	2.352	2.206	2.308	2.309	0.164	0.416

The results are LS means for 3 pens of 5 pigs each diet in trial 1 whereas there are LS means for 3 pens of 4 pigs each diet in trial 2.

Within a row (or column), means without a common superscript differ ($P < 0.05$).

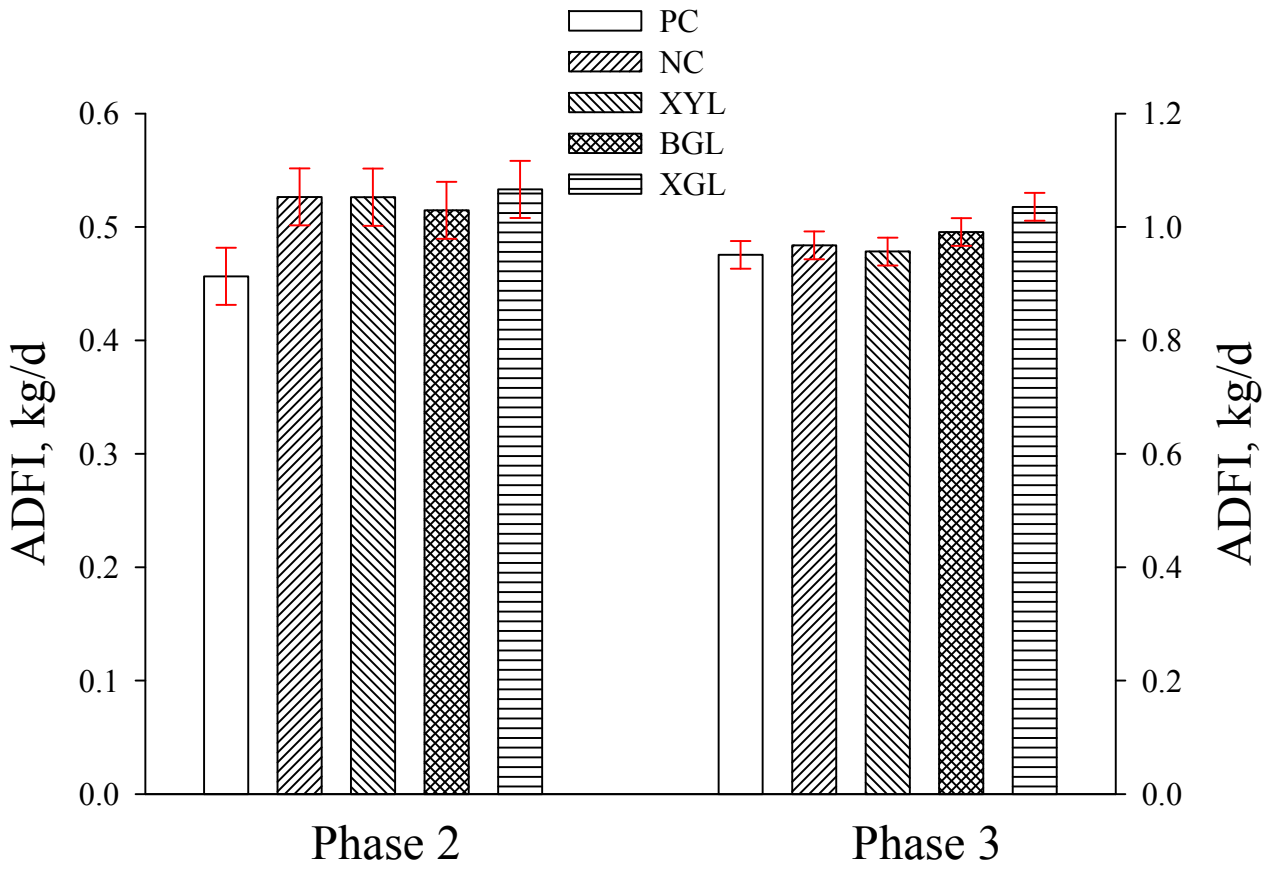
1. Positive control (Corn/SBM+0% CDDGS)(PC), negative control (30% CDDGS substituted corn/SBM with no enzyme addition)(NE), negative control + 4000 unit/kg xylanase (XYL), negative control + β-glucanase 450 unit/kg (BGL), and negative control + xylanase + β-glucanase (XGL).
2. Two pigs were removed from both trials. It was not due to treatment.

Figure 3.1 Mean ADG of day 7-21 and day 21-35 in nursery pigs fed PC, NC, XYL, BGL, and XGL.



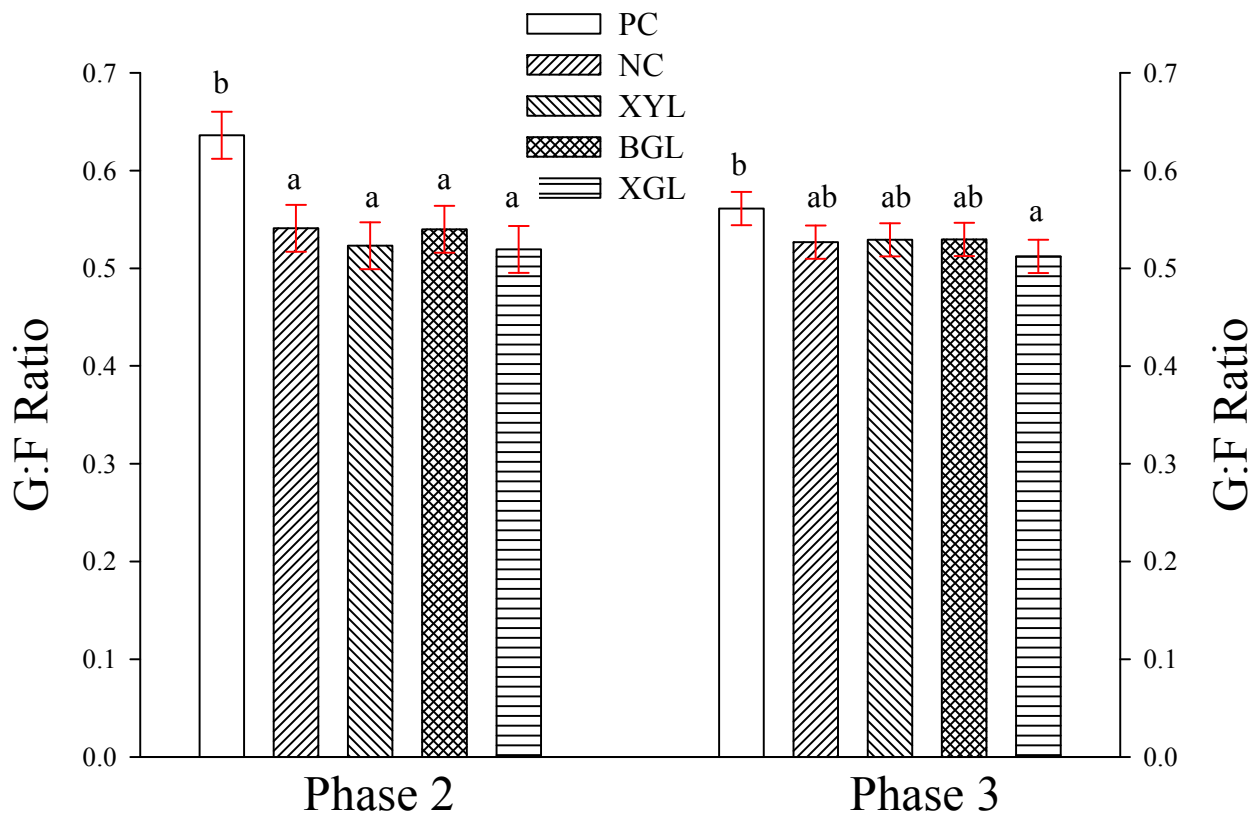
Mean are least square means ± SEM. Means without a common letter differ ($P < 0.05$). There was no significant diet effect in d7-21 ADG (P=0.231). Pigs fed PC had better ADG in d21-35, and those fed enzyme supplementation diets improved ADG in d21-35 significantly (P<0.001).

Figure 3.2 Mean ADFI of day7-21 and day 21-35 in nursery pigs fed PC, NC, XYL, BGL, and XGL.



Mean are least square means ± SEM. Means without a common letter differ ($P < 0.05$). There was no significant diets effect on ADFI in both d7-21 (P=0.216) and d 21-35 (P=0.135).

Figure 3.3 Mean feed efficiency of day 7-21 and day 21-35 in nursery pigs fed PC, NC, XYL, BGL, and XGL.



Mean are least square means ± SEM. Means without a common letter differ ($P < 0.05$). Pigs fed PC diet had better feed efficiency in both phase 2 (P=0.001) and 3(P=0.002).

CHAPTER 4

THE EFFECT OF DIET FORMULATION METHOD IN NURSERY PIGS FED GRADUAL LEVEL OF CORN DISTILLERS DRIED GRAIN WITH SOLUBLES (CDDGS) DIET IN GROWTH PERFORMANCE, AND NUTRIENTS DIGESTIBILITY

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ABSTRACT

The objective of these experiments was to evaluate the effect of increasing CDDGS in starter diets on growth performance using different diet formulation approaches. The first approach (Exp 1) was based on formulating for total lysine in the diet and the second (Exp 2), was based on balancing for SID lysine and maintaining a balanced amino acid pattern. In both experiments, pigs were weaned at 21 d and fed a common phase I starter diet (DE: 3.58 kcal/g, CP: 21.68%, Lys: 1.5%) for d 0-7 post-weaning. In Exp 1, a total of 160 pigs (80 pigs per trial) with initial body weight 6.61±0.5kg were randomly assigned to one of four diets: 0% CDDGS, 10% CDDGS, 20% CDDGS, 30% CDDGS. Test diets were fed in two phases (phase I: 7-21 d, 1.38% lysine; Phase 2: 21-35 d, 1.25% Lysine). In Exp 2, a total of 145 pigs (65pigs in trial 1 and 80 pigs in trial 2) with initial body weight 6.77±0.5kg in trial 1 and 7.91±0.5kg in trial 2 were assigned to one of five diets: 0% CDDGS, 10% CDDGS, 20% CDDGS, 30% CDDGS, 37.5% CDDGS, and 45% CDDGS, that were fed in 2 phases (phase 1: 7-21 d; 1.20% SID lysine; Phase 2: 21-35 d, 1.01% SID lysine). [Pigs had free access to feed and water. All diets met or exceeded the NRC nutrient requirements for this age of pigs.] In exp 1, pigs fed the 20% CDDGS had numerically reduced ADG (398 g/d, -4.5%), and those fed the 30% CDDGS diet had significantly lower ADG (361 g/d, $P<0.001$) as compared to the 0% CDDGS (416 g/d). There were diet x trial interactions noted in Exp 2, which were determined to be related to dietary mycotoxin contamination in the second trial. In trial 1 of exp 2, increasing CDDGS up to 45% had no detrimental effect in ADG or ADFI ($P> 0.20$). In trial 2 of exp 2, ADG and ADFI were linearly decreased as CDDGS in the diet increased ($P < 0.001$). Apparent total tract digestibility of protein, fiber, energy and phosphorus were also evaluated in both phase 2 and 3 of Exp 2. In general, energy and protein digestibility decreased as CDDGS level increased in

both phase 2 and 3. In contrast, phosphorus digestibility increased with increasing dietary CDDGS. The effect of diet on fiber digestibility was not consistent. The results indicate that high levels of CDDGS can be used in nursery diets and are well tolerated if the diets are formulated on a constant SID lysine basis with a balanced amino acid pattern and are screened for mycotoxin contamination.

Key words: CDDGS, diet formulation, growth performance, nursery pigs, nutrient digestibility.

INTRODUCTION

Ethanol production has increased dramatically for the past ten years, and will most likely continue to rise in the future (30 billion gallons per year by 2020 and 60 billion gallons per year by 2030) (Harkin et al.; Vijay Singh, 2007). Although the search for alternative sources of biomass for ethanol production is proceeding, corn will likely continue to be processed into ethanol as a biofuel additive into the future especially if petroleum costs rebound. Therefore, the feed industry must continue evaluate economic alternative feed ingredients such as corn original dry distiller grain of soluble (CDDGS). After the starch is fermented for ethanol production, other nutrients that remain in the corn are concentrated (Singh et al., 2007), which should result in a higher value for CDDGS as a feed ingredient. However, the results from studies where CDDGS was fed to nursery pigs were variable. The recommend level of CDDGS in a nursery diet ranges from 5% (Dritz et al., 2007) to 20% (Hans H. Stein, 2007). In order to increase the level of CDDGS usage in diet an understanding of the basis for limitations in its use are necessary. The heating process during ethanol production can result in severe damage to amino acid bio-availability, particularly for lysine (Hancock et al., 1990). Imbalanced amino acid patterns can cause lower lean gain (Kropf et al., 1959). The amino acid profile in CDDGS may

contribute to amino acid antagonisms (Harper et al., 1970; Southern and Baker, 1982), and also result in excess N excretion (Chung and Baker, 1992). It has been suggested that if alternative feed ingredients, particularly those with high fiber content are adopted, formulation of diets on an ileal digestible amino acid basis is necessary (Shannon and Allee., 2010). Diets using CDDGS have been formulated in various ways. Some have been based on meeting the total lysine requirement (Cromwell et al., 1993; Whitney and Shurson, 2004), while others have been based on meeting the SID lysine requirement (Jones et al., 2008; Linneen et al., 2008). The main value of feeding CDDGS is to reduce feed cost. If the use of CDDGS, results in greater use of other high protein ingredients to balance the diet, then the economic advantage is lost and there will likely be excess N excretion. Several crystalline AA are available at cost-effective prices and can be used to offset feeding high protein ingredients (Lewis, 2001). Supplementing diets with crystalline lysine (HCl salt) and formulating diets to meet the bioavailable level of lysine in the diet can restore the essential AA profile in the diet containing CDDGS. The objective of these studies was to evaluate the effect of increasing CDDGS level in nursery diets. In the first experiment, diets were based on formulation for total lysine, while in the second, diets were formulated based on SID lysine and a balanced amino acids pattern.

MATERIAL AND METHODS

The experimental protocols used in this study were approved by the Animal Care and Use Committee of the University of Georgia.

Animals

All pigs were randomly selected from the University of Georgia, Animal & Dairy Science Department Swine Unit for a 28 day study that was conducted in two trials for each experiment. Temperature was set at 27°C, and decreased subsequently to 23°C at the end of trial. Lighting was set on a 12 hours schedule (Lights on: 0600/1800). Body weight and feed intake were monitored weekly.

In Exp. 1:

A total of 160 pigs (weaning at day 21, with initial body weight 6.61±0.5kg, PIC42xPIC280) were randomly selected, and were housed in an environmentally controlled nursery. Each pen ($1.68\times1.83m^2$) was equipped with one stainless steel water nipple, a four hole feeder, and expanded plastic flooring. In the first trial, a total of 80 pigs with 5 pigs per pen (two barrows, and three gilts), and 4 weight blocks were randomly assigned to one of four dietary treatments within the block, while a total 80 pigs with 5 pigs per pen (two gilts, and three barrows), and 4 weight blocks were selected for the second trial.

In Exp. 2:

A total of 145 pigs were randomly selected, and were housed in environmentally controlled nursery in the large animal research unit of University of Georgia. Each pen ($1.8\times0.9m^2$) equipped with one stainless steel water nipple, and a four hole feeder. In the first trial, a total of 65 pigs (weaning at day 21, with initial body weight 6.77±0.5kg) with 3 pigs per pen for pen 1 to 15 (3 weight blocks) and 2 pigs per pen for pen 16 to 25 (2 weight blocks) were used. A total of 80 pigs (weaning at day 28, with initial body weight 7.91±0.5kg) with 4 pigs per pen for pen 1 to 15(3 weight blocks) and 2 pigs per pen for pen 16 to 25 (2 weight blocks) were

selected for second trial. Pens within each weight block were randomly assigned to one of five dietary treatments.

Diet

There were three feeding phases during both expermients. All pigs were fed a common phase 1 diet (d0-7) for one week before the experiment started (DE: 3.58 kcal/g, CP: 21.68%, Lys: 1.5%), and were switched to the experimental diets (Table 1 for Exp. 1 and Table 3 for Exp. 2) for phase 2 (d7-21) and 3 (d21-35) (two weeks each phase). Pigs had free access to water and feed in both experiments. All diets met or exceeded the NRC requirements for pigs in this weight range (NRC, 1998).

In Exp. 1:

The diets were formulated, and balanced based on total lysine (Table 2). Crystalline lysine level was increased as CDDGS increased in diet to obtain similar total lysine level among the diets. The ME level for CDDGS was based on that reported by Pederson et al., (2007). At the end of phase 1, pigs were randomly assigned to one of four diets: 0% DDGS, 10% DDGS, 20% DDGS, 30% DDGS.

In Exp. 2:

Diets were formulated based on standard ileal digestible (SID) lysine (table 4). SID amino acids for CDDGS were based on those reported by Urriola et al., (2009), whereas SID of corn and SBM were based on those reported in the NRC (1998). At the end of phase 1, pigs were randomly assigned to one of five diets: 0% DDGS, 15% DDGS, 30% DDGS, 37.5% DDGS,

45% CDDGS. SID lysine and metabolism energy (ME) ratio and amino acids pattern were controlled at similar level between diets. Diets, which contained 15%, 30%, and 37.5% CDDGS, were mixed from 0% and 45% CDDGS diets proportionally. A total 0.1% titanium dioxide was added to all diets as indigestible marker for nutrient digestibility determination.

Sample collection

Body weight and feed disappearance were monitored every week in both experiments. Experiment 1 was a growth performance trial. Thus, there was no titanium dioxide added in diets, and no determination of digestibility was performed. Diet samples from three phases were reserved and kept in -20 ^{0}C freezer for further analysis. Fresh grab fecal sample from each pen in experiment 2 were collected on 3 days consecutive prior to the end of phase 2 and phase 3, and stored in -20 ^{0}C freezer for further analysis.

Sample analysis

CDDGS was sent to the Agricultural and Environmental Services Laboratories (AESL) of University of Georgia for proximate analysis, and the minerals profile (inductively coupled atomic emission spectroscopy (ICP-AES). The amino acid profile of CDDGS was analyzed by Agricultural Experiment Station Chemical Laboratories of University of Missouri-Columbia. The results were used for diet formulation.

Fecal volatile fatty acids concentration

A total of 25g fecal sample was obtained from each pen, and mixed with 100g distilled water by blender. The entire solution was then filtered through cheese cloth. A 50 ml subsample was stored frozen. A 1 ml of phosphoric acids (2.5%) was added, and was brought up to 6 ml

from subsample in 10ml plastic tube. After freezing overnight, samples with phosphoric acid were centrifuged (IEC Centra GP8R, Thermo Fisher Scientific Inc., Suwanee, GA) at 1320 × g for 20 minutes at 4^{o}C. The supernatant from each tube was collected, and volatile fatty acids were analyzed by gas chromatography (3400 Gas Chromatography, Varian Instrument Group, Walnut, CA).

DM, CP, GE, NDF, ADF, hemicelluloses determination

Diet and fecal samples were oven dried at 55 ^{0}C for 72 hours or until reaching a constant weight (Grieve Shelf oven SA-350, The Grieve Cooperation, Round Lake, IL) for DM determination. After drying, diets and feces were ground through 1mm screen (Wiley mill, Thomas Scientific, Swedesboro, NJ) for other analysis. Diets and feces were analyzed for CP by A Leco FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). Gross energy concentration in diets and feces was examined by bomb calorimeter (Parr 1261, Parr Instrument Co., Moline, IL). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured using the Van Soest procedure (Van Soest et al., 1991) in an Ankom 200 fiber analyzer (Ankom technology, Macedon, NY). Hemicellulose content was determined by difference between NDF and ADF. Crude fat from diets and feces samples were analyzed by Labconco Goldenfisch Fat Extractor (Labconco CO. Kansas City, MI), using the AOAC procedure (AOAC, 2000).

Ti and P determination

Diet (2 g) and feces (1 g) were ashed 12 hours at 600 ^{o}C for titanium and phosphorous determination. Ash samples were heated with sulfuric acid for 1 hour, and then were brought up to 50ml with distilled water. Titanium dioxide concentration analysis was modified from Short et al., (1996). Samples were centrifuged at 2500 rpm for 20 minutes. Solutions were analyzed by

visible light spectrophotometer and were measured absorbance at 410 nm. Phosphorous concentration was determined by modification of the procedure described by Augspurger et al., (2003), and Kim et al., (2005). Solutions were analyzed by visible light spectrophotometer and measured absorbance at 660 nm. Apparent total digestibility (ATTD) of nutrient by marker was calculated by following equation:

$$\text{ATTDM}=1-\frac{([mar\,\text{ker}]_{feed}\times[Nuti.]_{feces})}{([mar\,\text{ker}]_{feces}\times[Nuti.]_{feed})}$$

Mycotoxin analysis:

Diets with 0% CDDGS and 45% CDDGS in Exp. 2 from both trials were sent to Veterinary Medical Diagnostic Lab (VMDL) of University of Missouri for mycotoxin analysis, and HPLC method was used to measure aflatoxin, orchratoxin, zearalenone, and vomitoxin concentration in diets.

Statistical Analysis

The SAS GLM procedure was used to analyze the data (SAS Inst. Inc, Cary, NC). A randomized block design was used to conduct the experiment with: four diets in each of the four BW groups, with the four BW groups being the blocks in Exp. 1, five diets in each of the five BW groups, with the five BW groups being the blocks in Exp. 2. The diets were the main effects and covariances between the experimental trial, initial BW, and BW blocks were used in the statistical model. The individual pen was the experimental unit for ANOVA. The results represented as least square mean for the diet effect in Exp. 1, whereas for trial and diet interaction effect in Exp. 2, with significance for diet effect at $P<0.05$. Turkey test was used for multiple comparison between diets. The proc IML procedure was used to obtain coefficient for

linear, quadratic and cubic polynomial. For linear, quadratic and cubic effects, orthogonal contrast was used.

RESULTS

There was no significant difference between trials in experiment 1. Thus, results were pooled. In exp. 2, however, there was significant trial by diet interactions. Thus, results were shown separately for the trials.

Diets:

In Exp. 1:

The results of diet chemical analysis are shown in table 2. The CP level and calculated total lysine was similar between diets, but SID lysine decreased (4%) from 1.21% in 0% CDDGS diet to 1.16% in 30% CDDGS diet in phase 2, and from 1.12% in 0% CDDGS diet to 1.05% in the 30% CDDGS diet in phase 3. Crude fat (CF), however, increased 33% in phase 2 and 47% in phase 3 as the level of CDDGS in the diet increased. NDF, ADF, and hemicellulose concentration were increased gradually in both phase 2, and phase 3. In addition, phase 3 diets had higher NDF, ADF, and hemicellulose level than phase 2 diets, respectively.

In Exp. 2:

The results of chemical analysis of the diets are shown in table 4. GE, CP, and DM were similar between diets in both phases. Calculated total lysine increased as CDDGS level increased. SID lysine, on the other hand, was constant between diets. Calculated CF increased linearly as CDDGS level increased. Similarly, NDF, ADF and hemicelluloses concentrations were increased 69%, 63%, 72% in phase 2 and 112%, 92%, 110% in phase 3 from 0% CDDGS diet to 45% CDDGS diet. Total P level was similar between the analyzed and calculated result.

Growth performance

In Exp. 1:

The effect of CDDGS level on growth performance is shown in table 5. There was no significant difference in body weight on d 0, 7, and 14 between diets. Pigs fed 10 % CDDGS showed no difference in BW during overall study period, compared to the 0% CDDGS diet. There was a numerical decrease in BW observed in pigs fed 20% CDDGS at d 21, 28, and 35 by 0.7%, 3%, 2.87%, whereas with 30% CDDGS in the diet, pigs tended to decrease BW by 3.5% at d 21 ($P=0.098$), and significantly decreased by 6.23%, 7.72% at d 28 ($P=0.014$), 35 ($P<0.001$). There was a linear decrease BW at d 28 ($P=0.022$) and 35 ($P=0.004$) as level of CDDGS increased.

There was no difference in overall ADG between control and 10% CDDGS treatments. Pigs, fed diets with 20% and 30% CDDGS, decreased ADG by 2.8% and 15.3% for d 7-21 ($P=0.0155$), and 3.5%, 13.5% for d 21-35 ($P<0.001$) as level of CDDGS increased from 10% to 30% (figure 1). Overall, pigs fed the 20% CDDGS diet reduced BW gain numerically (4.5%), and those fed the 30% CDDGS diet had significantly lower weight gain ($P<0.001$). Therefore, ADG was decreased linearly ($P<0.001$), particularly from 20% to 30% CDDGS.

Feed consumption was increased linearly for d 7-14 ($P=0.025$), and was linearly increased in phase 2 ($P=0.0387$) as CDDGS level increased in the diet (figure 2). However, ADFI was not different in d 21-35 or overall.

During phase 2 (d 7-21), feed conversion ratio was linearly decreased by 17.36%, 20.58%, and 35.9% ($P<0.001$). Similarly, feed efficiency was 1.6%, 6.8%, and 10.4% lower in phase 3 (d 21-35) ($P=0.002$) in pigs fed diets with 10%, 20%, and 30% of CDDGS content in

diet (figure 3). Feed efficiency was not significantly different between diets for d28-35 (P=0.237). Overall, pigs fed the control diet (0% CDDGS) had numerically improved feed conversion (7.66%) compared to pigs fed 10% DDGS diet. Those fed the 20% and 30% DDGS diet had lower efficiency (P<0.001).

In Exp. 2:

The results of growth performance were shown in table 6, 7, and 8. Initial BW was heavier in trial 1 than trial 2 (P<0.001). After initial BW was used as a covariate, there was no trial effect in BW through study. However, a significant diet x trial interaction for BW was observed during the whole study (P<0.001). In trial 1, BW decreased linearly at d 14 (P=0.008) and 21 (P=0.014), but no linear effect was observed at d28 (P=0.616) or 35(P=0.965). Nevertheless, quadratic effect in BW was observed through the whole study in trial 2 (P<0.02). Overall, pigs fed diets with increasing level of CDDGS had no detrimental effect on BW in trial 1. BW in trail 2, on the other hand, was decreased 2%, 9.3%, 13.8%, and 21.5% quadratic, following the level of CDDGS in diet.

There was no trial effect on ADG during the study although a significant diet x trial interaction for ADG was observed (P<0.01). In trial 1, ADG decreased linearly in d 7-14 (P=0.008), which resulted in linearly decreased in phase 2 as level of CDDGS increased in diets (P=0.014) (figure 4). Although ADG in d 21-28 was decreased linearly (P=0.025), no diet effect was observed in d 28-35 (P=0.492) and overall (P=0.965). Unlike trial 1, a linear effect in ADG was observed through the whole period (P<0.001) in trial 2. ADG was decreased in phase 2 by 0.6%, 14.9%, 24.4%, and 47.4% (P<0.001), and in phase 3 by 5%, 13.8%, 19%, and 22.4% (P<0.001) when CDDGS increased from 0% to 45%. Overall, pigs fed diets with increasing

levels of CDDGS had no detrimental effect in ADG in trial 1 (P=0.965). In trial 2, on the other hand, ADG was decreased 3%, 14.3%, 21.3%, and 33.1%, following the level of CDDGS in the diet (P=0.012).

There was no trial effect in ADFI during the study (table 7), but a significant diet x trial interaction for ADFI was observed during the whole study. In trial 1, ADFI was decreased linearly in phase 2 as level of CDDGS increased in diets (P=0.032) (figure 6). However, no diet effect was observed for the rest of study. Unlike trial 1, a linear effect for ADFI was observed through the whole period (P<0.002) in trial 2. ADFI was decreased linearly in phase 2 by 6.2%, 17.2%, 23.2%, and 41% (P<0.001), and in phase 3 by 9.7%, 14.16%, 16.1%, and 24.9% (P<0.001) when CDDGS increased from 0% to 45%. Overall, pigs fed diets with increasing levels of CDDGS had no effect on ADFI in trial 1 (P=0.441). ADFI in trial 2, on the other hand, was decreased 8.4%, 15.3%, 18.9%, and 31.2% linearly, following the level of CDDGS in diet (P<0.001).

A significant diet x trial interaction in G: F ratio was observed for d 7-14 (P<0.001), and also in phase 2 (P=0.01). In trial 1, a cubic effect was observed for d 7-14 (P=0.001), and d 7-21(P=0.008). G: F ratio was increased linearly at d 21-28, but was not different between levels of CDDGS in phase 3. Similarly, a cubic effect was observed in d 7-14 (P=0.04). A quadratic effect, on the other hand, was observed for: d 14-21(P=0.026), d 7-21(P<0.005). CDDGS did not affect the G: F ratio for the rest of study. Overall, increasing the level of CDDGS in the diet had no effect on G: F ratio in both trials.

Nutrient digestibility:

Nutrient digestibility is shown in tables 9, 10 and 11.

In phase 2(d 7-21):

Pigs responded to CDDGS level differently between trials in nutrient digestibility (Diet x Trial, P<0.05). However, the effects of diet on digestibility were in the same direction in both trials. Only the magnitude varied. Increased CDDGS in the diet resulted in a linear decrease in DM digestibility in trial 1 (P=0.028) whereas quadratic effect was observed in trial 2 (P<0.001). Digestibility of energy was decreased by: -1.4%, 2.1%, 4%, and 11.3% in trial 1 (P=0.022), and 10.9%, 17.4%, 11.8%, and 8.4% in trial 2 (P<0.001). Digestibility of CP was not affected by level of CDDGS in trial 1. In contrast, CP digestibility was decreased quadratic by 9.76%, 18.37%, 14.99%, and 9.52% when CDDGS level increased in trial 2 (P=0.008). Digestibility of NDF and hemicellulose was decreased linearly in trial 1 (P=0.05, P=0.007), but affected quadratic in trial 2 (P<0.001, P<0.001). P digestibility was increased linearly by 25.7%, 32.7%, 46.8% and 44.8% following the level of CDDGS in diet in trial 1(P=0.001). In trial 2, However, P digestibility had a cubic effect (P=0.031) from 0% to 45% CDDGS diet.

In phase 3(d 21-35):

There was no diet and trial interactions for nutrient digestibility except digestibility of P. DM, energy and CP digestibility were decreased linearly when the level of CDDGS increased in diets in both trials (P<0.003). Increasing CDDGS in the diet had no significant affect on NDF, and hemicellulose digestibility. Pigs responded to CDDGS level differently for P digestibility between the two trials (P=0.009). CDDGS level had a cubic effect on P digestibility in trial 1 (P<0.001), whereas a quadratic effect was observed in trial 2 (P=0.009).

VFA:

In phase 2, there was no significant difference between diets or trials in fecal VFA concentration. Iso-valerate concentration was lowered 9.2%, 14.7%, 33.7%, and 48.3% when CDDGS inclusion rate increased from 0% to 45% in trial 1 (P=0.015). In phase 3, however, fecal VFA concentrations were higher than trial 1 (P<0.004). In addition, increasing CDDGS inclusion rate decreased fecal iso-butyrate by: 17.1%, 39.1%, 45.2%, and 34.7% in trial 1 (P=0.054), and 9.7%, 17.9%, 30.4%, 30.7% in trial 2 (P=0.04). Similarly, fecal iso-valerate was 6.9%, 37.8%, 41.8%, 44.3% lower in trial 1 (P=0.002), and was 20.4%, 23.2%, 32.6%, and 34.6% lower in trial 2 (P=0.011). Total fecal VFA concentration was linearly decreased in trial 1 as CDDGS increased from 0% to 45% in diet (P=0.035), but no effect of diet was observed in trial 2.

Mycotoxin:

Diet with 0% and 45% CDDGS in both phase 2 and 3 from both trials were analyzed for mycotoxin concentration. The level of mycotoxin in phase 2 or 3 diets in trial 1 was not detectable or below 0.75 ppm. In trial 2, however, vomitoxin was 0.47 ppm in 0% CDDGS diet and 4.7 ppm in 45% CDDGS diet (data not shown).

DISCUSSION

The objective of this study was to evaluate the effect of increasing CDDGS inclusion in diet on nursery pig growth performance when diets were formulated based on either a total lysine basis or by formulating diets for SID lysine with a balanced amino acid ratio. In Exp 1, diets were formulated based on total lysine, and CDDGS inclusion rate increased from 0% to 30%. There was a linear decrease in BW, ADG, and G:F ratio as CDDGS concentration increased. However, this was mainly due to decrease performance at the higher inclusion rates. Diets

containing 10% CDDGS had no effect on growth performance when compared to the 0% CDDGS diet. Feeding diets with 30% CDDGS, however, lowered growth performance significantly as compared to the lower levels of CDDGS. This is in agreement with other studies (Burkey et al., 2008; Feoli, 2008). However, based on the results Whitney and Shurson (2004), feeding up to 25% CDDGS did not lower growth rate if diets were formulated by simply replacing CDDGS for corn. Using this method, the CP of the diet increased from 21 to 27% with the high CDDGS, but there was no negative effect on performance. Although this work suggests that the detrimental effects of high CDDGS can be avoided by providing higher CP diet, the benefit of using CDDGS could be diminished because of excess N excretion and a reduced saving in feed cost (High CP ingredients are normally more costly). Crystalline amino acids can also be used to meet the amino acid requirements without increasing CP. In trial 1 of experiment 2, diets were formulated with SID lysine and other crystalline amino acids were added to the diet to maintain an ideal amino acid pattern. Although growth performance reduced in phase 2 with high (45%) CDDGS, there was no detrimental effect on growth performance in phase 3 or overall. Jones et al., (2008) found that 30% CDDGS diet fed to 9kg pigs did not reduce growth rate when diets contained similar CP, and ideal protein ratio was maintained. Similarly, Spencer et al., (2007) also reported no different in growth performance with 9kg pigs were fed up to 30% CDDGS in diets with crystalline lysine added to diet to balance based on a digestible amino acid basis. Thus, the imbalanced amino acid in CDDGS is a limitation of CDDGS. Corn has lower quality amino acid profile relative to the needs of the pig. The DDGS that originates with corn has a similar pattern of amino acids. In addition, the heating process used in DDGS production can favor the Malliard reaction, which can further affect lysine availability (Bemiller and Whistler, 1997; Hancock et al., 1990). Thus, an unbalanced amino acid pattern is further

exacerbated, particularly when high levels of the ethanol by-product is introduced in diets. Pigs fed diets with unbalanced amino acid profiles may have reduced growth rate, and lowered lean gain (Kropf et al., 1959; Lenis et al., 1999). Amino acid digestibility in corn and SBM is typically greater than 80% and often close to or at 90% (NRC, 1998). However, if alternative feed ingredients, such as CDDGS or other high fiber ingredients are used, formulation on an ileal digestible amino acid basis is warranted (Shannon and Allee., 2010). Amino acid digestibility in ingredients such as CDDGS is often lower and in the range of 60-70% (Urriola et al., 2009). Crystalline amino acids are available at prices that favor their use in practical diets (Lewis, 2001). Thus, supplementing crystalline lysine (HCl salt) and formulating diets to meet the bioavailable level of lysine in the diet can restore the essential AA profile of diets which contain DDGS. Hence, growth performance on diets with higher CDDGS should not be affected. Furthermore, based on current prices, formulation of nursery diets with high CDDGS levels is likely to result in lower feed costs.

Although growth performance was not affected in pigs fed diets with high CDDGS levels, digestibility of DM, energy, and CP was reduced significantly in both trials of experiment 2. This is in agreement with other studies (Emiola et al., 2009; Feoli, 2008; Urriola and Stein, 2010). The decrease in energy and DM from CDDGS was suggested because of high insoluble fiber concentration (Urriola and Stein, 2010). Unlike soluble fiber, fiber in CDDGS has a lower impact on digesta viscosity as compared to wheat based DDGS (Yang et al., 2010). The fiber contained in CDDGS is approximately 4 times and 3 times higher than in corn and SBM, which is mainly arabinose, xylose, mannose, galactose, and cellulose, and mostly is insoluble form (Shurson, 2008; Stein and Shurson, 2008). The amount of arabinoxylan degraded before hindgut should be limited because of low microbial activity (Elsden et al., 1946). Even though

arabinoxylan is degraded in foregut, it was reported that arabinose and xylan lowered mucosal sucrase activity (Seri et al., 1996), which can affect energy digestibility in higher CDDGS diet. Moreover, high insoluble fiber in diet can increase digesta passage rate (Kass et al., 1980b; Owusu-Asiedu et al., 2006; Pond et al., 1988), and reduce the time that endogenous enzymes have to release nutrients. Therefore, lowered nutrient digestibility can be expected (Kornegay et al., 1995; Moore et al., 1988). CDDGS also has relatively high fat concentration (Spiehs et al., 2002). Dietary fat has an extra caloric effect, which increased diet density and increase nutrient digestibility because of longer chyme exposure time to endogenous enzymes (Azain, 2001). Since viscosity is not a concern in pigs (Bedford et al., 1992) and CDDGS contains small amount of soluble fiber, fat in CDDGS should slow passage rate, and reverse the negative impact in nutrient digestibility from fiber. Urriola and Stein (2010) reported that digesta disappearance time was not different when 30% CDDGS was added to diet, but ATTD of nutrient was reduced significantly in high CDDGS diet. The reason could be: first, pigs lack of fiber degrading enzymes to break down fiber polymers. Therefore, even though CDDGS contains mostly insoluble fiber, there may still be interference with utilization of other nutrients. Secondly, the fiber in CDDGS has a high concentration of arabinoxylan, which is less fermentable (Knudsen, 1997). Third, although fermentation process can provide ME to pigs, energy derived from fermentation is less efficiently utilized than that of dietary origin (Anguita et al., 2006; Kass et al., 1980a). Therefore, fiber from high CDDGS diet can reduce nutrient utilization. The quadratic effect of DM, energy and CP digestibility in trial 2 of experiment 2 could be at least partially explained by lower feed intake. Reduced feed intake can result in higher nutrient digestibility. Haydon et al., (1984) found that lowering feed intake from ad libitum to 3% of BW (1520g to 810g/d) increased energy (82.5 vs. 86.4%) and DM (83.5 vs. 87.2%0) digestibility ($P<0.1$).

Similarly, Moter and Stein (Moter and Stein, 2004) reported that pigs fed diets with energy content equal to maintenance increased SID AA significantly as compared to pigs fed diets with energy equal to 3 times maintenance. This likely accounts for our observation that digestibility of DM, energy and CP had quadratic effects in trial 2 of experiment 2.

Digestibility of P was increased when level of CDDGS increased in both phase 2 and 3 diets. Available P in CDDGS is higher than corn or SBM (approximately 60% digestible, Pedersen et al., 2007). Therefore, it is expected that as CDDGS level increases in the diet, P digestibility will improve. Excess P in manure can have a negative impact on the environment (Knowlton et al., 2004). Adding inorganic P to the diet to meet the requirement is logical, but it contributes significantly to diet cost and can result in increased P excretion into the environment. Thus, exogenous phytase is use as a means to improve the efficiency of P utilization (Augspurger et al., 2003; Lei et al., 1993; Veum et al., 2006). In this study, diets that contained 45% CDDGS did not require further supplementation with inorganic P, and had no difference in growth performance, compared to 0% CDDGS diet. Thus, CDDGS can be used in swine diet to lower P excretion.

Content of NDF and hemicellulose in the diet increased 73% and 76% in phase 2, and 100% and 110% in phase 3 from 0% to 45% CDDGS diet. However, digestibility of NDF and hemicellulose were reduced significantly when CDDGS inclusion rate increased in phase 2 diets, but there was no difference in NDF, ADF, and hemicellulose digestibility in phase 3. Similarly, ADG in trial 1 of experiment 2 was linearly decreased as level of CDDGS diet increased for d7-14, but there was no detrimental effect in ADG for rest of the study. Although feed efficiency was lower in phase2, the negative effect of CDDGS was less in phase 3. It is believed that the digestive system is more developed (larger, longer, more enzyme secretion) in the older pigs and

is able to produce sufficient endogenous enzymes to overcome any negative effects of fiber (Cera et al., 1988; Jensen et al., 1997; Lindemann et al., 1986; Owsley et al., 1986; Yen, 2000). In addition, increasing dietary fiber concentration can increase the concentration of hindgut bacteria (Jensen and Jorgensen, 1994), and stimulate growth of the GI tract(Pond et al., 1988). The larger hindgut in mature pigs provides more space for microbial population. Therefore, higher VFA concentration can be produced (Imoto and Namioka, 1978; Kass et al., 1980a) and utilized by animals (Macfarlane and Macfarlane, 2003). This may be the reason why the growth performance and the fiber digestibility response to CDDGS were different between phase 2 and phase 3 in experiment 2.

Fecal VFA concentration was not affected by CDDGS inclusion rate in phase 2, except for the branch chain fatty acids, and the two trials had similar total VFA concentration. However, significantly higher VFA concentration was observed in trial 2 than trial 1 in phase3. Pigs used in trial 2 were one week older than trial 1, which should be the reason of this observation. Iso-butyrate, and iso-valerate were decreased linearly following CDDGS level. Although amino acid fermentation produces lower VFA than fiber (Cummings and Macfarlane, 1991), amino acids from endogenous loss or dietary origin also contributed to VFA production (Macfarlane and Macfarlane, 2003). Branch chain fatty acids are derived from amino acid fermentation, especially branch chain amino acids (Macfarlane et al., 1992; Mackie et al., 1998). There have been a few reports of investigations of VFA concentration in CDDGS containing diets fed to pigs. Urriola and Stein (2010) reported that 30% CDDGS fed to pigs did not affect ileal, cecal and fecal VFA, but pH was higher in CDDGS diet. Moreover, branch amino acids were mainly produced in hindgut. Increasing fiber level resulted in lower ammonium and branch chain fatty acids production, which indicated that increasing fiber level in the diet can reduce microbial

proteolytic activity (Awati et al., 2006). This may explain why increasing CDDGS in diet in this study lowered iso-valerate and iso-butyrate significantly. Fiber is considered indigestible in monogastric animals. Therefore, fiber in CDDGS diets needs to be digested by microbial in the GI tract to generate VFA, which can be absorbed by animals, and used as energy sources. Although pigs have relatively higher fermentation ability than other single stomach animals, VFA are absorbed rapidly after produced by microbial (Bergman, 1990; Ehle et al., 1982), and only 10% of VFA is excreted in feces (Wolin and Miller., 1983). Thus, determination of VFA production by collection of samples from ileal, ceacum, colon and anus may provide better understanding of the effects of high fiber diets.

The results of Exp 2, trial 1 suggested that when diet was formulated based on SID lysine and use of an ideal amino acids ratio, increasing level of CDDGS did not have a detrimental effect on growth performance. In trial 2, however, ADG was reduced 3%, 14%, 21%, and 33%, and ADFI was decreased 8%, 15%, 19%, 31% when CDDGS increased from 0% to 45% in overall. In order to determine the basis for the response in trial 2, mycotoxin levels in the diets were determined. The results indicate that there was undetectable level of mycotoxin in trial 1. In trial 2, however, deoxynivaenol was 0.47ppm in 0% CDDGS diet, and was 4.7ppm in 45% CDDGS diet. Deoxynivaenol(DON), also called vomitoxin, are isolated from Fusarium family and is nonestrogenic trichothecenes (Richard, 2007). It was reported that naturally contaminated vomitoxins in diets fed to nursery pigs resulted in decreased ADG, ADFI, and G:F ratio, with level as low as 1.3 ppm (Swamy et al., 2002; Swamy et al., 2003; Young et al., 1983). Mature animals are less susceptible to these toxic effects (Diaz-Llano and Smith, 2007; Diaz-Llano et al., 2010). It is likely that this accounts for the different results between two trials. The FDA issued a guidance for maximum level of DON (FDA, 2010) 5ppm for swine from grain or grain

byproduct, and should not exceed 20% inclusion rate of contaminated substrate, which suggests that DON level should not exceed 1 ppm in diet. Contamination of feedstuffs threatens human and animal health, and also causes large economic loss. Economic impact on livestock industry can be affected by mycotoxins level from original grain, processing, DDGS inclusion rate, number of animals fed DDGS, and pig's market value. The value of expected annual loss from reduced weight gain can be greater than $4 million with 5% DDGS inclusion rate and 12% producers used DDGS to $147million (20% inclusion rate and 100% producers used DDGS)(Wu and Munkvold, 2008). Therefore, the problem of mycotoxins should not be ignored. Zhang et al., (2009) found that mycotoxin was varied between stations. Moreover, the level of toxicity was not affected by time if stored properly. The DDGS from the same station resulted in similar results regardless of time when sample was collected. However, if corn contains high levels of toxin, the resulting CDDGS is most likely to have a higher toxin level. Therefore, obtain a good source of CDDGS from the same ethanol plant is important.

There are several limitations of CDDGS, such as poor amino acid profile and high fiber level. As a result Dritz et al., (2007) suggested that a limit of 5% CDDGS can be added to nursery pigs diet, while Stein (2007) suggested that if a good source of CDDGS can be obtained, feeding late nursery pigs with up to 20% of DDGS should not affect growth rate. There are no other reports of feeding nursery pigs more than 30% CDDGS. Formulating CDDGS diet to meet SID lysine level and following an ideal amino acid pattern in this study demonstrated that it is possible to feed up to 45% CDDGS without decreasing growth performance in nursery pigs. Use of high levels of CDDGS in finishing feeds can increase content of unsaturated fatty acids in the carcass. Feeding nursery pigs with high levels of CDDGS does not raise concerns for carcass quality as expected with the use of CDDGS in finishing diets. Xu et al., (2010) suggested that

the desired effect of reducing the C18:2 content and iodine value of pork fat could be elicited in as little as 3 wk after withdrawing DDGS from the diet before slaughter. Nutrient digestibility, however, decreased with level of CDDGS in diets, especially at 45% CDDGS. Thus, investigation of strategies to increase nutrient utilization, and reduce environmental impact is needed when high CDDGS is adopted in diet. Diets formulated diet to meet the amino acid requirement by adjusting crystalline amino acids level in CDDGS diet can help lower excess N excretion. Lenis et al., (1999) reported that increasing total N in diet (18.8%, 22.9%, 30%) and EAA and NEAA ratio (35%, 45%, 56%) significantly improved ADG ($P<0.001$, $P=0.08$). However, N retention was significantly improved only in low N (18.8%, 22.9%) diet with 35% (49.5% vs. 56.6%), and 45% (46.4% vs. 56.8%) EAA: NEAA ratio group. Thus, higher CP fed to animals results in more N excretion in environment. Additional studies evaluating the use of CDDGS in nursery diets are needed to confirm these studies and to further investigate strategies to increase CDDGS inclusion rate as a means to lower feed cost without negatively affecting the environmental impact of the diet on nutrient excretion.

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Table 4.1 Feed composition for Exp. 1(as-fed).

	Phase 2				Phase 3			
Ingredient, %	0%	10%	20%	30%	0%	10%	20%	30%
Corn	51.71	48.60	44.66	40.41	58.90	53.71	48.42	43.40
Soybean Meal, 48%	27.30	21.34	15.55	10.00	33.38	28.70	24.00	19.00
CDDGS[1]	0.00	10.00	20.00	30.00	0.00	10.00	20.00	30.00
Poultry Fat	2.50	1.60	1.35	1.15	3.50	3.50	3.50	3.50
Menhaden Meal	2.50	2.50	2.50	2.50	0.00	0.00	0.00	0.00
Whey, Dehydrated	10.00	10.00	10.00	10.00	0.00	0.00	0.00	0.00
Blood, spray-dried	2.50	2.50	2.50	2.50	0.00	0.00	0.00	0.00
Zinc oxide	0.25	0.25	0.25	0.25	0.00	0.00	0.00	0.00
Limestone	0.76	0.98	1.18	1.36	1.08	1.22	1.38	1.53
Dical. Phos.[2]	0.96	0.62	0.30	0.00	1.12	0.90	0.65	0.42
Common Salt	0.00	0.00	0.00	0.00	0.35	0.35	0.35	0.35
Vitamin Premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix[4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.12	0.09	0.06	0.04	0.13	0.00	0.00	0.00
Lysine-hcl	0.00	0.12	0.25	0.38	0.14	0.22	0.30	0.39
Tryptophan	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.01
Antibiotic[5]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Amino acids pattern[6]								
SID lysine:ME[7], g/Kcal	3.62	3.57	3.54	3.51	3.29	3.22	3.14	3.07
SID TSAA:lysine	0.64	0.62	0.60	0.59	0.65	0.56	0.57	0.59
SID Thr:lysine	0.67	0.65	0.63	0.61	0.64	0.64	0.63	0.63
SID Trp:Lysine	0.21	0.19	0.18	0.18	0.22	0.21	0.20	0.19

1. Corn original DDGS.
2. Dicalcium phosphate.
3. Supplied per kg of premix: vitamin A 4400 IU; vitamin D 660000 IU; vitamine E 17600 IU; vitamin K 1760 IU; riboflavin 3960 mg; niacin 22000 mg; vitamin B12 17600 μg .
4. Supplied per kg of premix: iron 110000 mg; copper 11000 mg; manganese 26400 mg; zinc 110000 mg; iodine 198 mg; selenium 198 mg.
5. Mecadox.
6. Standard ileal digestible amino acids level was obtained from NRC (1998).
7. Standard ileal digestible lysine : metabolism energy ratio.

Table 4.2 Diet calculate and analysis results for Exp. 1.

	Phase 2				Phase 3			
	0%	10%	20%	30%	0%	10%	20%	30%
Calculated (as-fed)								
DM, %	87.8	87.6	87.5	87.4	86.5	86.3	86.2	86.0
ME, Kcal/kg	3.34	3.31	3.30	3.30	3.40	3.40	3.41	3.41
CP, %	22.87	22.85	22.86	22.96	21.58	21.55	21.58	21.51
Crude fat, %	5.09	5.08	5.69	6.35	6.07	6.87	7.66	8.46
Lysine, %	1.39	1.38	1.38	1.38	1.25	1.25	1.25	1.25
Calcium, %	0.80	0.80	0.80	0.80	0.75	0.75	0.75	0.75
Total P, %	0.65	0.63	0.61	0.60	0.58	0.57	0.56	0.54
Available P, %	0.40	0.40	0.40	0.41	0.35	0.35	0.35	0.35
Crude fiber, %	2.24	2.83	3.41	3.99	2.60	2.98	3.36	3.74
SID lysine, %	1.21	1.18	1.17	1.16	1.12	1.10	1.07	1.05
Analyzed (DM basis)								
Gross Energy, Kcal/kg	4.25	4.28	4.38	4.42	4.37	4.46	4.39	4.47
DM, %	92.2	92.0	92.2	92.1	92.4	92.4	92.5	92.5
CP, %	22.87	22.85	22.86	22.96	21.58	21.55	21.58	21.51
Crude fat, %	5.30	5.28	6.27	7.03	6.11	6.96	8.10	8.98
NDF, %	11.96	14.30	16.19	17.94	16.00	19.42	21.51	23.31
ADF, %	3.08	3.38	4.04	4.21	2.93	4.29	4.34	4.83
Hemi-cellulose, %	8.88	10.91	12.15	13.73	13.07	15.12	17.17	18.48

1. Metabolism energy.
2. Neutral detergent fiber
3. Acid detergent fiber

Table 4.3 Feed composition for Exp. 2 (As-fed).

	Phase 2 diet		phase 3 diet	
Ingredient, %	0%	45%	0%	45%
Corn, Grain	53.29	24.60	59.78	31.42
Soybean Meal, 48%	26.39	10.9	33	16.65
CDDGS[1]	0	45	0	45
Poultry Fat	1.5	1	3.3	2.85
Menhaden Meal	2.5	2.5	0	0
Blood, spray-dried	2.5	2.5	0	0
Whey, Dehydrated	10	10	0	0
Zinc oxide	0.25	0.25	0	0
Limestone	0.84	1.35	1.03	1.8
Dical. Phos.[2]	0.95	0	0.97	0
Vitamin Premix[3]	0.25	0.25	0.25	0.25
Mineral Premix[4]	0.15	0.15	0.15	0.15
Salt	0.18	0	0.35	0.35
Lysine-hcl	0.02	0.34	0	0.35
DL-Methionine	0.075	0.004	0.075	0.011
Threonine	0	0.009	0	0.022
Trptophan	0	0.046	0	0.05
TiO_2[5]	0.1	0.1	0.1	0.1
Antibiotic[6]	1	1	1	1
Amino acid pattern[7]				
SID lys:ME[8], g/Kcal	3.66	3.66	2.97	2.97
SID TSAA:lysine	0.60	0.60	0.67	0.67
SID Thr:Lysine	0.67	0.67	0.71	0.71
SID Trp:Lysine	0.21	0.21	0.24	0.24

Diet with 15%, 30%, and 37.5% CDDGS was archived by mixing 0% and 45% CDDG diet proportionally.

1. Corn original DDGS.
2. Dicalcium phosphate.
3. Supplied per kg of premix: vitamin A 4400 IU; vitamin D 660000 IU; vitamine E 17600 IU; vitamin K 1760 IU; riboflavin 3960 mg; niacin 22000 mg; vitamin B12 17600 μg .
4. Supplied per kg of premix: iron 110000 mg; copper 11000 mg; manganese 26400 mg; zinc 110000 mg; iodine 198 mg; selenium 198 mg.
5. A total 0.1% Titanium dioxide was added in diet as indigestible maker.
6. Mecadox.
7. Standard ileal digestible amino acids level was obtained from NRC (1998).
8. Standard ileal digestible lysine : metabolism energy ratio.

Table 4.4 Diets calculate and analysis result for Exp. 2.

	Phase 2					Phase 3				
CDDGS level	0%	15%	30%	37.50%	45%	0%	15%	30%	37.50%	45%
Calculate(as-fed)										
ME[1], Kcal/kg	3.28	3.28	3.29	3.29	3.29	3.39	3.39	3.40	3.39	3.39
Crude Protein, %	22.55	23.32	23.77	24.01	24.24	21.31	21.85	22.37	22.36	22.67
Crude fat, %	4.12	5.12	6.19	6.75	7.26	5.90	7.01	8.03	8.55	9.10
Crude fiber, %	2.24	2.83	3.41	3.69	3.98	2.60	3.18	3.76	4.03	4.32
Lysine, %	1.38	1.42	1.47	1.49	1.51	1.13	1.18	1.22	1.24	1.27
Calcium, %	0.80	0.80	0.80	0.80	0.80	0.70	0.70	0.69	0.70	0.75
Total P, %	0.64	0.63	0.62	0.62	0.64	0.55	0.55	0.54	0.53	0.55
Available P, %	0.40	0.40	0.40	0.40	0.42	0.32	0.33	0.32	0.32	0.34
SID lysine, %	1.20	1.20	1.20	1.20	1.20	1.01	1.01	1.01	1.01	1.01
Analysis(DM basis)										
Gross Energy, Kcal/kg	4.1	4.2	4.3	4.3	4.3	4.2	4.2	4.4	4.5	4.5
Dry Matter, %	90.37	91.74	92.24	90.81	91.03	90.49	90.50	90.62	90.78	89.87
Crude Protein, %	23.83	23.16	23.80	23.68	23.78	21.74	22.70	23.66	22.94	22.94
Crude fat, %	3.46	4.46	4.89	6.18	6.92	5.85	6.60	7.85	8.70	8.33
NDF[2], %	11.29	13.96	16.55	18.31	19.49	12.16	16.76	20.18	22.63	25.72
ADF[3], %	3.41	4.37	4.96	5.08	5.64	4.21	5.39	6.97	7.90	8.99
Hemi-cellulose, %	7.88	9.59	11.59	13.22	13.85	7.96	11.37	13.21	14.73	16.73
Ash, %	6.68	7.17	7.18	6.89	6.53	5.93	5.74	6.09	6.44	6.08
Total P, %	0.60	0.64	0.62	0.64	0.62	0.57	0.50	0.52	0.54	0.54

1. Metabolism energy.
2. Neutral detergent fiber.
3. Acid detergent fiber.

Table 4.5 Least square mean of increasing level of CDDGS diet in growth performance in nursery pigs.

	Diet					P-value			
CDDGS Level	0%	10%	20%	30%	SEM	Diet	Linear	Quadratic	Cubic
BW, kg									
Day 0	6.53	6.55	6.52	6.54	0.04	0.966			
Day 7	7.74	7.88	7.72	7.80	0.09	0.596	0.983	0.873	0.519
Day 14	8.86	9.17	8.91	8.88	0.13	0.272	0.731	0.350	0.328
Day 21	11.58	11.88	11.49	11.19	0.20	0.098	0.187	0.264	0.516
Day 28	15.24^{b}	15.34^{b}	14.77^{ab}	14.29^{a}	0.25	0.014	0.022	0.379	0.613
Day 35	19.40^{b}	19.50^{b}	18.85^{b}	17.90^{a}	0.30	<0.001	0.004	0.175	0.782
Daily Gain, kg									
Day 7-14	0.160	0.186	0.171	0.154	0.009	0.092	0.503	0.081	0.470
Day 14-21	0.388^{b}	0.387^{b}	0.369^{ab}	0.331^{a}	0.014	0.011	0.030	0.312	0.987
Day 21-28	0.523^{b}	0.493^{b}	0.468^{a}	0.443^{a}	0.013	<0.001	0.001	0.892	0.927
Day 28-35	0.595^{b}	0.596^{b}	0.582^{b}	0.516^{a}	0.014	<0.001	0.011	0.118	0.686
Day 7-35	0.416^{b}	0.415^{b}	0.398^{b}	0.361^{a}	0.009	<0.001	<0.001	0.080	0.962
Intake, g/d									
Day 7-14	0.324	0.409	0.444	0.442	0.080	0.091	0.025	0.244	0.951
Day 14-21	0.611	0.657	0.666	0.671	0.028	0.406	0.138	0.489	0.750
Day 21-28	0.792	0.793	0.808	0.785	0.044	0.862	0.951	0.539	0.549
Day 28-35	0.996	0.971	0.998	0.926	0.030	0.315	0.199	0.457	0.281
Day 7-35	0.681	0.707	0.724	0.706	0.022	0.609	0.379	0.362	0.874
G:F ratio									
Day 7-14	0.499^{b}	0.470^{b}	0.412^{ab}	0.354^{a}	0.033	0.020	0.007	0.696	0.863
Day 14-21	0.632^{c}	0.593^{bc}	0.574^{b}	0.492^{a}	0.017	<0.001	<.0001	0.228	0.305
Day 21-28	0.661^{c}	0.622^{bc}	0.581^{ab}	0.565^{a}	0.015	<0.001	<0.001	0.444	0.695
Day 28-35	0.600	0.612	0.587	0.559	0.018	0.237	0.080	0.291	0.662
Day 7-35	0.614^{c}	0.590^{c}	0.556^{b}	0.512^{a}	0.011	<0.001	<0.001	0.362	0.969

Results were represented as LS mean with diet as main effect, and weight block and initial BW as covariance.
A total 80 pigs, 5 pigs per pen, 4 pens per treatment, with initial body weight 6.4kg ± 0.5kg were used per trial and two trials were conducted.
Means within a row lacking a common superscript letter differ (P<0.05).
One pig was removed from trial 1 study due to health problem (not related to treatment effect).

Table 4.6 Least square mean of CDDGS level in nursery pigs in BW gain in Exp. 2.

	DDGS						P-value		
	0%	15%	30%	37.5%	45%	SEM	Trial	Diet	Int.
BW, kg									
bw7									
trial 1	7.68	8.05	7.55	7.85	8.19	0.50	<0.001	0.865	0.992
trial 2	9.23	9.64	9.47	9.54	9.63				
bw14									
trial 1	11.37	11.04	11.04	11.01	10.78	0.16	0.847	<0.001	<0.001
trial 2	11.77	11.65	10.88	10.91	9.93				
bw21									
trial 1	15.30	14.69	14.61	14.70	14.38	0.28	0.603	<0.001	<0.001
trial 2	15.86	15.81	14.81	14.14	12.53				
bw28									
trial 1	18.90	18.04	18.41	18.62	18.38	0.39	0.879	<0.001	<0.001
trial 2	20.30	20.01	18.66	17.72	15.86				
bw35									
trial 1	23.00	22.52	22.45	22.98	22.84	0.50	0.831	<0.001	<0.001
trial 2	25.18	24.67	22.85	21.70	19.77				
ADG, kg/d									
d 7-14									
trial 1	0.36	0.32	0.32	0.31	0.28	0.02	0.847	<0.001	<0.001
trial 2	0.42	0.40	0.29	0.30	0.16				
d 14-21									
trial 1	0.56	0.52	0.51	0.53	0.51	0.02	0.476	<0.001	<0.001
trial 2	0.58	0.59	0.56	0.46	0.37				
d 21-28									
trial 1	0.51	0.48	0.54	0.56	0.57	0.03	0.245	0.347	<0.001
trial 2	0.63	0.60	0.55	0.51	0.48				
d 28-35									
trial 1	0.59	0.64	0.58	0.62	0.64	0.03	0.807	0.064	0.008
trial 2	0.70	0.67	0.60	0.57	0.56				
d 7-35									
trial 1	0.51	0.49	0.49	0.51	0.50	0.02	0.831	<0.001	<0.001
trial 2	0.58	0.57	0.50	0.46	0.39				

Results were represented as LS mean with diet, trial and interaction as main effect, and weight block and initial BW as covariance.
A total 65 pigs (ave. BW: 7.9kg ± 0.5kg) for trial 1 and 80 pigs (ave. BW: 9.5kg ± 0.5kg) were used for trial 2. Three pigs from trial 1 and one pig from trial 2 were removed from study due to health problem (not related to treatment effect).

Table 4.7 Least square mean of CDDGS level in nursery pigs in ADFI and feed efficiency in Exp. 2.

	DDGS						P-value		
	0%	15%	30%	37.5%	45%	SEM	Trial	Diet	Int.
ADFI, kg/d									
d 7-14									
trial 1	0.53	0.52	0.40	0.45	0.42	0.03	0.445	<0.001	0.059
trial 2	0.60	0.57	0.47	0.44	0.33				
d 14-21									
trial 1	0.86	0.82	0.73	0.77	0.78	0.05	0.135	0.001	0.029
trial 2	0.88	0.82	0.76	0.70	0.55				
d 21-28									
trial 1	0.98	0.95	0.89	0.94	1.00	0.05	0.270	0.083	0.050
trial 2	1.03	0.96	0.89	0.89	0.78				
d 28-35									
trial 1	1.11	1.16	1.05	1.05	1.21	0.05	0.905	0.036	0.003
trial 2	1.30	1.14	1.10	1.06	0.96				
d 7-35									
trial 1	0.85	0.86	0.77	0.80	0.85	0.04	0.611	0.001	0.003
trial 2	0.95	0.87	0.81	0.77	0.66				
G:F ratio									
d 7-14									
trial 1	0.71	0.62	0.80	0.70	0.66	0.03	0.033	<0.001	<0.001
trial 2	0.70	0.70	0.64	0.73	0.49				
d 14-21									
trial 1	0.65	0.64	0.69	0.69	0.68	0.02	0.037	0.056	0.524
trial 2	0.66	0.71	0.76	0.71	0.70				
d 21-28									
trial 1	0.54	0.50	0.60	0.61	0.59	0.03	0.012	0.368	0.066
trial 2	0.63	0.63	0.61	0.60	0.63				
d 28-35									
trial 1	0.55	0.55	0.56	0.60	0.53	0.03	0.617	0.933	0.316
trial 2	0.56	0.59	0.56	0.55	0.59				
d 7-35									
trial 1	0.59	0.57	0.63	0.64	0.60	0.02	0.124	0.292	0.076
trial 2	0.62	0.65	0.63	0.62	0.61				

Results were represented as LS mean with diet, trial and interaction as main effect, and weight block and initial BW as covariance.

A total 65 pigs (ave. BW: 7.9kg ± 0.5kg) for trial 1 and 80 pigs (ave. BW: 9.5kg ± 0.5kg) were used for trial 2. Three pigs from trial 1 and one pig from trial 2 were removed from study due to health problem (not related to treatment effect).

Table 4.8 Result of linear, quadratic and cubic effect in Exp. 2 growth performance.

	Trial 1			Trial 2		
	P-Value			P-Value		
	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic
BW, kg						
bw7	0.735	0.384	0.352	0.144	0.481	0.436
day14	0.008	0.918	0.209	<0.001	0.008	0.387
day21	0.014	0.550	0.286	<0.001	<0.001	0.296
day28	0.616	0.339	0.198	<0.001	0.002	0.367
day35	0.965	0.466	0.698	<0.001	0.012	0.584
Daily Gain, kg/d						
bw7	0.008	0.918	0.209	<0.001	0.008	0.387
day14	0.145	0.388	0.602	<0.001	0.166	0.241
day21	0.025	0.261	0.238	<0.001	0.502	0.846
day28	0.492	0.988	0.227	<0.001	0.889	0.619
day35	0.965	0.466	0.698	<0.001	0.012	0.584
Feed Intake, kg/d						
bw7	0.004	0.556	0.202	<0.001	0.343	0.836
day14	0.118	0.649	0.816	<0.001	0.166	0.241
day21	0.742	0.346	0.767	0.001	0.706	0.401
day28	0.833	0.315	0.045	<0.001	0.619	0.339
day35	0.441	0.711	0.245	<0.001	0.570	0.310
G:F ratio						
bw7	0.607	0.351	0.001	0.004	0.027	0.040
day14	0.286	0.778	0.226	0.149	0.026	0.726
day21	0.022	0.511	0.145	0.370	0.832	0.345
day28	0.837	0.513	0.337	0.722	0.876	0.153
day35	0.248	0.829	0.065	0.779	0.076	0.522

Orthogonal contrast was used to determine the linear, quadratic and cubic trend. Proc IML procedure was used to obtain coefficient for contrast.

A total 65 pigs (ave. BW: 7.9kg ± 0.5kg) for trial 1 and 80 pigs (ave. BW: 9.5kg ± 0.5kg) were used for trial 2. Three pigs from trial 1 and one pig from trial 2 were removed from study due to health problem (not related to treatment effect).

Table 4.9 Least square mean of CDDGS level in nursery pigs in phase 2 nutrient digestibility in Exp. 2.

	CDDGS						P-value		
Digestibility[1], %	0%	15%	30%	37.5%	45%	SEM	Trial	Diet	Int.[2]
Phase 2									
Dry matter									
trial 1	92.84	93.62	92.58	92.06	90.65	0.45	0.642	<0.001	<0.001
trial 2	95.03	92.34	90.56	92.13	92.56				
Ash									
trial 1	53.16	65.36	63.91	66.10	58.46	2.83	0.036	0.535	0.007
trial 2	60.11	53.16	54.08	56.47	58.12				
Energy									
trial 1	77.97	79.08	76.32	74.84	69.15	1.73	0.821	<0.001	<0.001
trial 2	83.94	74.76	69.33	74.07	76.86				
Crude protein									
trial 1	75.71	77.18	76.83	73.03	68.42	2.42	0.022	0.014	0.009
trial 2	77.68	70.10	63.41	66.03	70.28				
NDF[3]									
trial 1	44.38	44.26	36.05	34.86	26.78	3.80	<0.001	0.035	0.004
trial 2	60.15	47.41	44.27	54.51	59.91				
ADF[4]									
trial 1	30.67	38.94	37.56	32.32	30.41	4.23	<0.001	0.672	0.048
trial 2	51.15	45.53	38.69	43.39	54.28				
Hemicellulose									
trial 1	50.86	46.77	35.18	36.01	24.83	3.75	<0.001	0.001	<0.001
trial 2	63.77	48.29	46.39	58.28	61.88				
Phosphorous									
trial 1	46.07	57.91	61.13	67.65	66.73	2.65	<0.001	<0.001	0.007
trial 2	45.45	46.47	40.58	54.16	61.23				

A total 65 pigs (ave. BW: 7.9kg ± 0.5kg) for trial 1 and 80 pigs (ave. BW: 9.5kg ± 0.5kg) were used for trial 2. Three pigs from trial 1 and one pig from trial 2 were removed from study due to health problem (not related to treatment effect).

1. A total 0.1% titanium dioxide was added in diet as indigestible marker.
2. Trial and diet interaction.
3. Neutral detergent fiber.
4. Acid detergent fiber.

Table 4.10 Least square mean of CDDGS level in nursery pigs in phase 3 nutrient digestibility in Exp. 2.

	DDGS						P-value		
Digestibility, %	0%	15%	30%	37.5%	45%	SEM	Trial	Diet	Int.
Phase 3									
Dry matter									
trial 1	93.84	92.40	92.39	87.06	90.67	1.52	0.374	0.038	0.544
trial 2	94.11	92.53	92.08	91.35	91.32				
Ash									
trial 1	42.02	46.61	56.02	53.25	50.70	3.02	0.163	0.020	0.078
trial 2	55.78	45.55	53.68	56.67	52.73				
Energy									
trial 1	83.04	77.57	78.31	76.17	71.52	1.52	0.076	<0.001	0.281
trial 2	81.84	75.77	74.50	71.38	72.93				
Crude protein									
trial 1	78.86	74.12	78.13	74.44	69.54	1.69	0.147	<0.001	0.244
trial 2	81.24	77.71	77.25	73.27	74.81				
NDF									
trial 1	48.98	44.06	54.54	53.27	49.41	3.50	0.583	0.727	0.139
trial 2	50.35	49.73	47.64	43.38	52.03				
ADF									
trial 1	46.87	42.39	58.67	59.31	57.43	3.13	0.693	<0.001	0.122
trial 2	51.91	50.13	55.22	52.28	59.72				
Hemicellulose									
trial 1	50.23	44.98	52.18	49.73	44.56	3.80	0.421	0.664	0.162
trial 2	49.61	49.55	43.88	38.97	48.32				
Phosphorous									
trial 1	29.54	26.78	50.29	45.75	44.29	3.55	0.079	<0.001	0.009
trial 2	37.30	25.15	31.63	36.54	42.47				

A total 65 pigs (ave. BW: 7.9kg ± 0.5kg) for trial 1 and 80 pigs (ave. BW: 9.5kg ± 0.5kg) were used for trial 2. Three pigs from trial 1 and one pig from trial 2 were removed from study due to health problem (not related to treatment effect).

1. A total 0.1% titanium dioxide was added in diet as indigestible marker.
2. Trial and diet interaction.
3. Neutral detergent fiber.
4. Acid detergent fiber.

Table 4.11 Result of linear, quadratic and cubic effect in nutrient digestibility in Exp. 2.

	Trial 1			Trial 2		
	P-Value			P-Value		
Digestibilty[1], %	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic
Phase 2						
Dry matter	0.028	0.096	0.777	<0.001	<0.001	0.546
Ash	0.053	0.018	0.638	0.930	0.075	0.541
Energy	0.022	0.131	0.773	0.001	<0.001	0.500
Crude protein	0.139	0.165	0.837	0.012	0.008	0.270
NDF[2]	0.050	0.542	0.891	0.875	<0.001	0.992
ADF[3]	0.987	0.431	0.661	0.961	0.001	0.411
Hemicellulose	0.007	0.612	0.973	0.409	<0.001	0.522
Phosphorous	<0.001	0.502	0.769	<0.001	<0.001	0.031
Phase 3						
Dry matter	<0.001	0.423	0.248	<0.001	0.241	0.769
Ash	0.279	0.382	0.086	0.956	0.090	0.011
Energy	<0.001	0.808	0.251	<0.001	0.108	0.940
Crude protein	0.002	0.334	0.172	0.001	0.395	0.898
NDF[2]	0.845	0.631	0.713	0.544	0.376	0.270
ADF[3]	0.040	0.906	0.180	0.199	0.247	0.849
Hemicellulose	0.209	0.529	0.957	0.213	0.435	0.163
Phosphorous	<0.001	0.290	<0.001	0.440	0.009	0.417

Orthogonal contrast was used to determine the linear, quadratic and cubic trend. Proc IML procedure was used to obtain coefficient for contrast.
A total 65 pigs (ave. BW: 7.9kg ± 0.5kg) for trial 1 and 80 pigs (ave. BW: 9.5kg ± 0.5kg) were used for trial 2. Three pigs from trial 1 and one pig from trial 2 were removed from study due to health problem (not related to treatment effect).

1. A total 0.1% titanium dioxide was added in diet as indigestible marker.
2. Neutral detergent fiber.
3. Acid detergent fiber.

Table 4.12 Least square mean of CDDGS level in nursery pigs in phase 2 fecal VFA concentration.

	CDDGS						P-value		
	0%	15%	30%	37.5%	45%	SEM	Trial	Diet	Int.[1]
Phase 2, % DM									
Acetate									
trial 1	1.00	1.09	0.86	0.95	0.98	0.10	0.4284	0.9399	0.2968
trial 2	0.99	0.77	0.98	0.95	0.87				
Propionate									
trial 1	0.61	0.60	0.53	0.57	0.58	0.05	0.6978	0.8292	0.7925
trial 2	0.61	0.61	0.61	0.60	0.54				
Butyrate									
trial 1	0.26	0.33	0.26	0.23	0.37	0.08	0.2309	0.457	0.9371
trial 2	0.37	0.46	0.35	0.30	0.38				
Iso-Butyrate									
trial 1	0.02	0.03	0.02	0.02	0.01	0.01	0.4384	0.1306	0.7995
trial 2	0.03	0.04	0.02	0.01	0.02				
Iso-valerate									
trial 1	0.08	0.07	0.07	0.05	0.04	0.01	0.3635	0.087	0.232
trial 2	0.06	0.06	0.07	0.04	0.06				
Valerate									
trial 1	0.11	0.10	0.08	0.10	0.09	0.01	0.6852	0.1111	0.5363
trial 2	0.11	0.10	0.09	0.08	0.07				
Total VFA									
trial 1	2.08	2.22	1.82	1.93	2.08	0.19	0.8695	0.8158	0.7316
trial 2	2.17	2.04	2.12	1.98	1.95				

A total 65 pigs (ave. BW: 7.9kg ± 0.5kg) for trial 1 and 80 pigs (ave. BW: 9.5kg ± 0.5kg) were used for trial 2. Three pigs from trial 1 and one pig from trial 2 were removed from study due to health problem (not related to treatment effect).

1. Trial and diet interaction.

Table 4.13 Least square mean of CDDGS level in nursery pigs in phase 3 fecal VFA concentration.

	DDGS						P-value		
Digestibility, %	0%	15%	30%	37.5%	45%	SEM	Trial	Diet	Int.
Phase 3, % DM									
Acetate									
Trial 1	1.01	0.96	0.84	0.90	0.82	0.10	<0.001	0.5645	0.8483
Trial 2	1.52	1.49	1.53	1.55	1.41				
Propionate									
Trial 1	0.69	0.67	0.55	0.52	0.65	0.09	<0.001	0.818	0.4133
Trial 2	0.97	1.07	0.98	1.08	0.92				
Butyrate									
Trial 1	0.50	0.53	0.49	0.47	0.58	0.06	<0.001	0.8202	0.605
Trial 2	0.75	0.81	0.74	0.71	0.68				
Iso-Butyrate									
Trial 1	0.08	0.06	0.05	0.04	0.05	0.01	0.0031	0.0176	0.9453
Trial 2	0.10	0.09	0.08	0.07	0.07				
Iso-valerate									
Trial 1	0.12	0.11	0.08	0.07	0.07	0.01	<0.001	<0.001	0.8056
Trial 2	0.17	0.14	0.13	0.12	0.11				
Valerate									
Trial 1	0.13	0.12	0.08	0.08	0.10	0.03	<0.001	0.5112	0.7101
Trial 2	0.29	0.27	0.26	0.29	0.24				
Total VFA									
Trial 1	2.53	2.45	2.08	2.09	2.27	0.22	<0.001	0.4609	0.6185
Trial 2	3.82	3.87	3.72	3.82	3.44				

A total 65 pigs (ave. BW: 7.9kg ± 0.5kg) for trial 1 and 80 pigs (ave. BW: 9.5kg ± 0.5kg) were used for trial 2. Three pigs from trial 1 and one pig from trial 2 were removed from study due to health problem (not related to treatment effect).

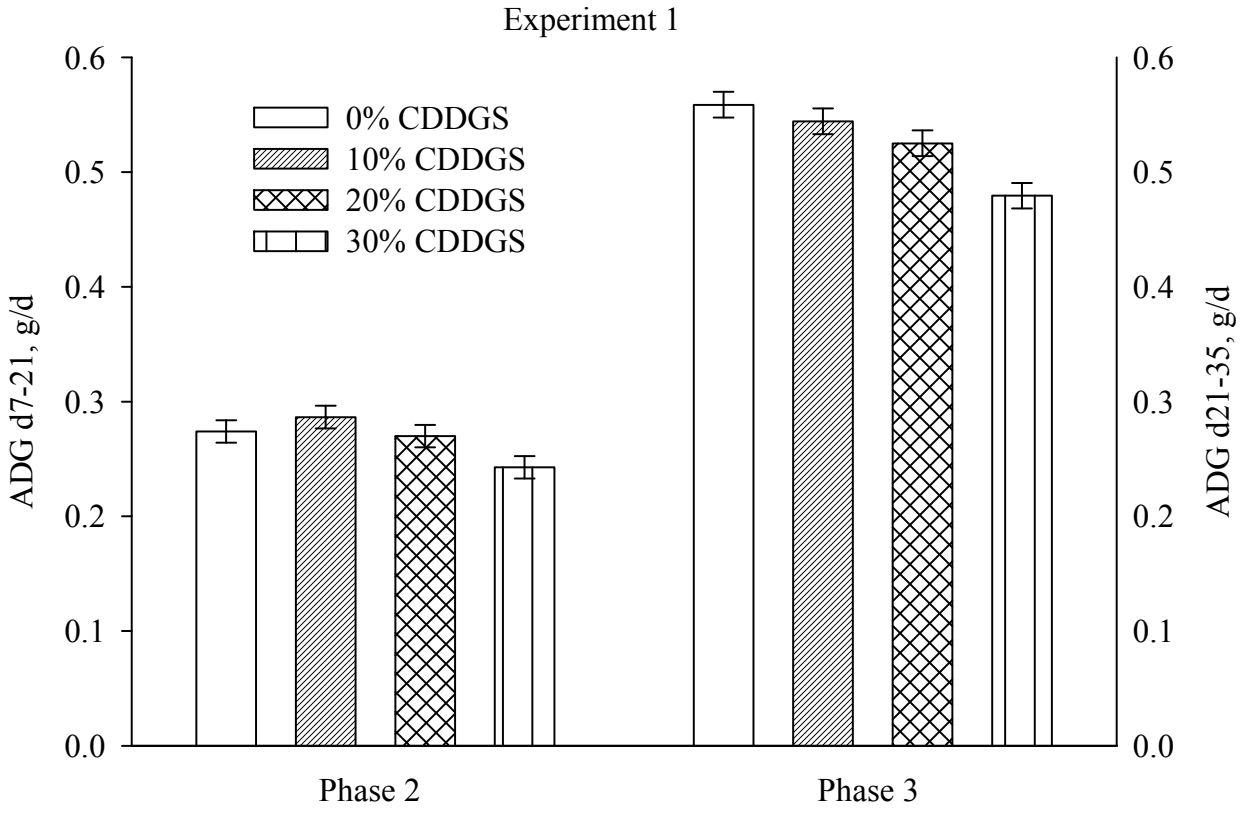
1. Trial and diet interaction.

Table 4.14. Result of linear, quadratic and cubic effect in fecal VFA concentration in Exp. 2.

	Trial 1			Trial 2		
	P-Value			P-Value		
	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic
Phase2, % DM						
Acetate	0.411	0.937	0.223	0.610	0.884	0.082
Propionate	0.441	0.471	0.621	0.962	0.272	0.622
Butyrate	0.414	0.153	0.092	0.682	0.625	0.152
Iso-butyrate	0.248	0.864	0.280	0.021	0.882	0.155
Iso-valerate	0.015	0.651	0.657	0.928	0.680	0.625
Valerate	0.082	0.815	0.323	0.074	0.508	0.824
Total VFA	0.465	0.368	0.103	0.914	0.623	0.673
Phase 3, % DM						
Acetate	0.017	0.704	0.870	0.826	0.421	0.432
Propionate	0.091	0.485	0.090	0.966	0.401	0.916
Butyrate	0.790	0.843	0.175	0.497	0.330	0.631
Iso-butyrate	0.054	0.360	0.490	0.040	0.901	0.891
Iso-valerate	0.002	0.748	0.418	0.011	0.619	0.624
Valerate	0.059	0.593	0.318	0.586	0.915	0.548
Total VFA	0.035	0.806	0.216	0.631	0.318	0.701

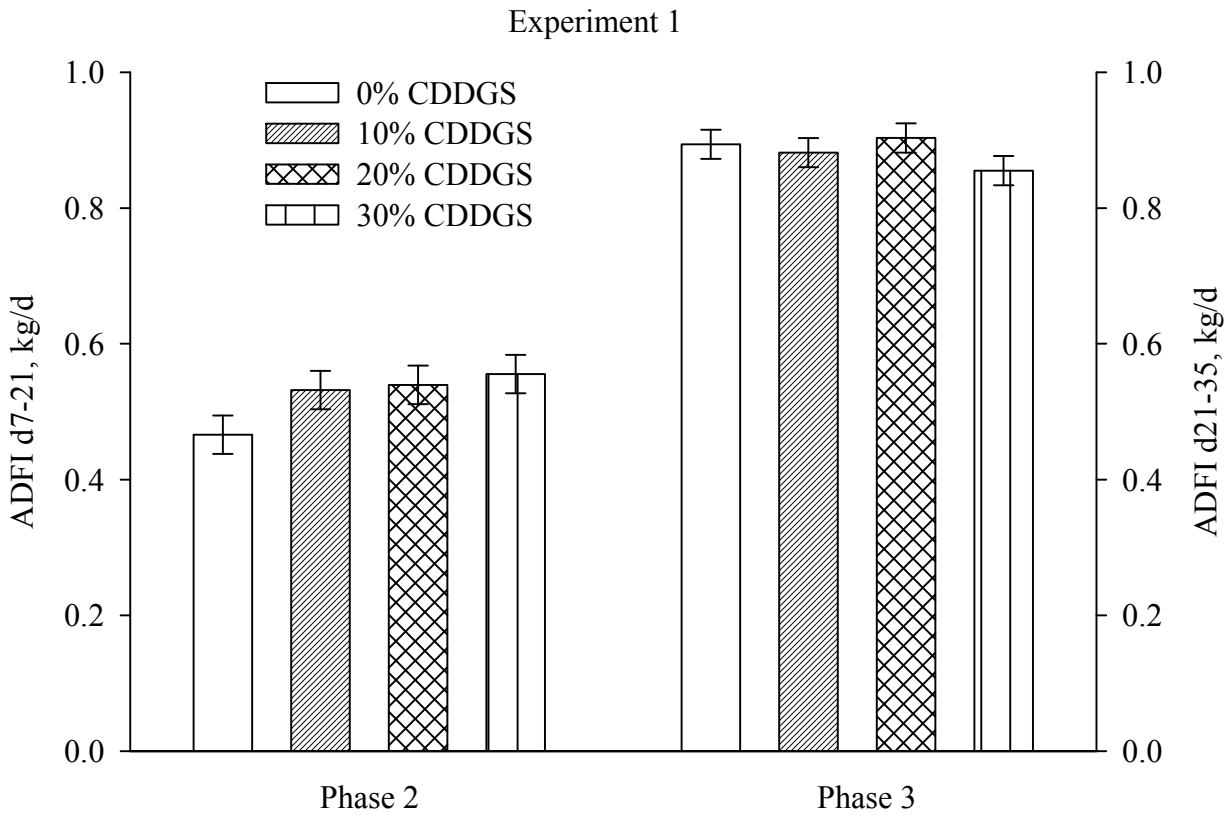
Orthogonal contrast was used to determine the linear, quadratic and cubic trend. Proc IML procedure was used to obtain coefficient for contrast.
A total 65 pigs (ave. BW: 7.9kg ± 0.5kg) for trial 1 and 80 pigs (ave. BW: 9.5kg ± 0.5kg) were used for trial 2. Three pigs from trial 1 and one pig from trial 2 were removed from study due to health problem (not related to treatment effect).

Figure 4.1 Least square mean of CDDGS diet in d7-21, and d21-35 ADG in nursery pigs fed increased level CDDGS in experiment 1.



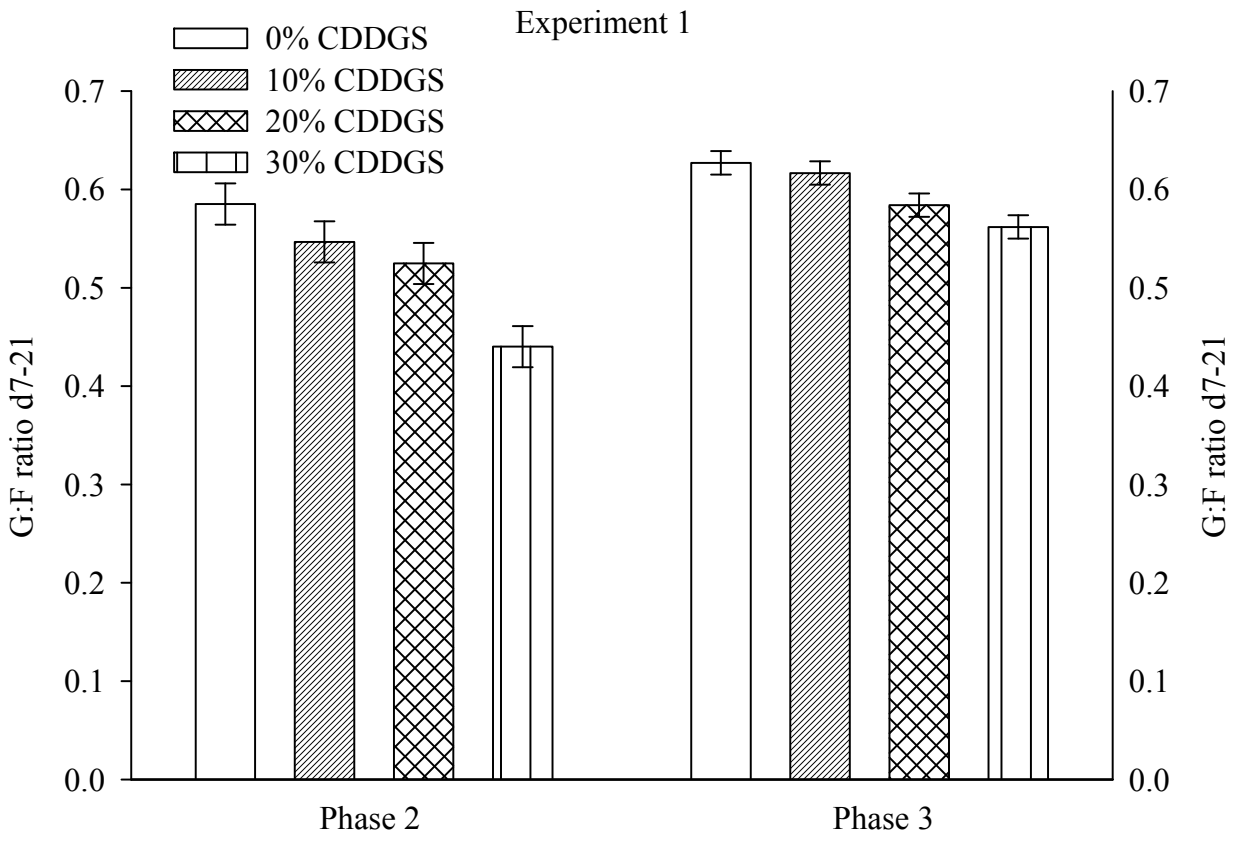
Results were represented as LS mean with diet as main effect, and weight block and initial BW as covariance. A total 80 pigs, 5 pigs per pen, 4 pens per treatment, with initial body weight 6.4kg ± 0.5kg were used per trial and two trials were conducted. One pig was removed from trial 1 study due to health problem (not related to treatment effect). The linear effect was observed at d21-35 (P=0.0004).

Figure 4.2 Least square mean of CDDGS diet in d7-21, and d21-35 ADFI in nursery pigs fed increased level CDDGS in experiment 1.



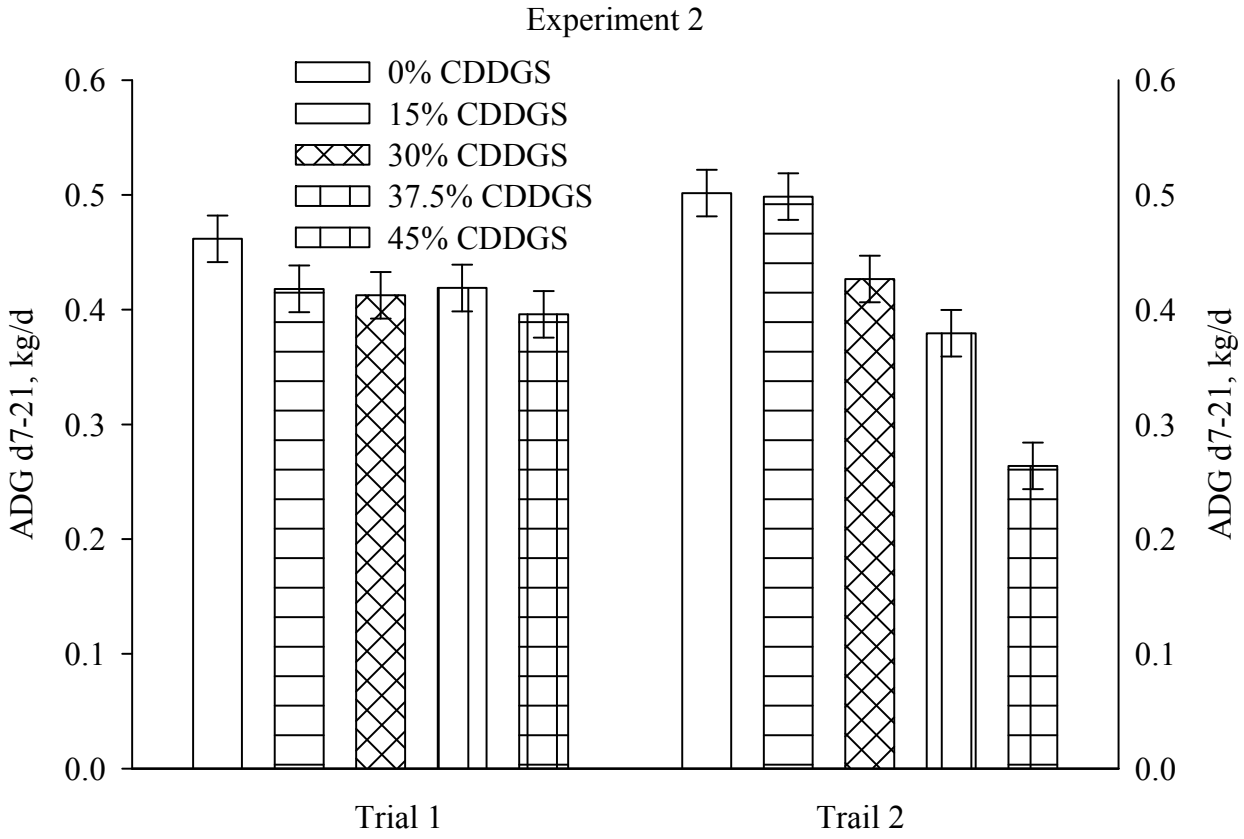
Results were represented as LS mean with diet as main effect, and weight block and initial BW as covariance. A total 80 pigs, 5 pigs per pen, 4 pens per treatment, with initial body weight 6.4kg ± 0.5kg were used per trial and two trials were conducted. One pig was removed from trial 1 study due to health problem (not related to treatment effect).

Figure 3. Least square mean of CDDGS diet in d7-21, and d21-35 feed efficiency in nursery pigs fed increased level CDDGS in experiment 1.



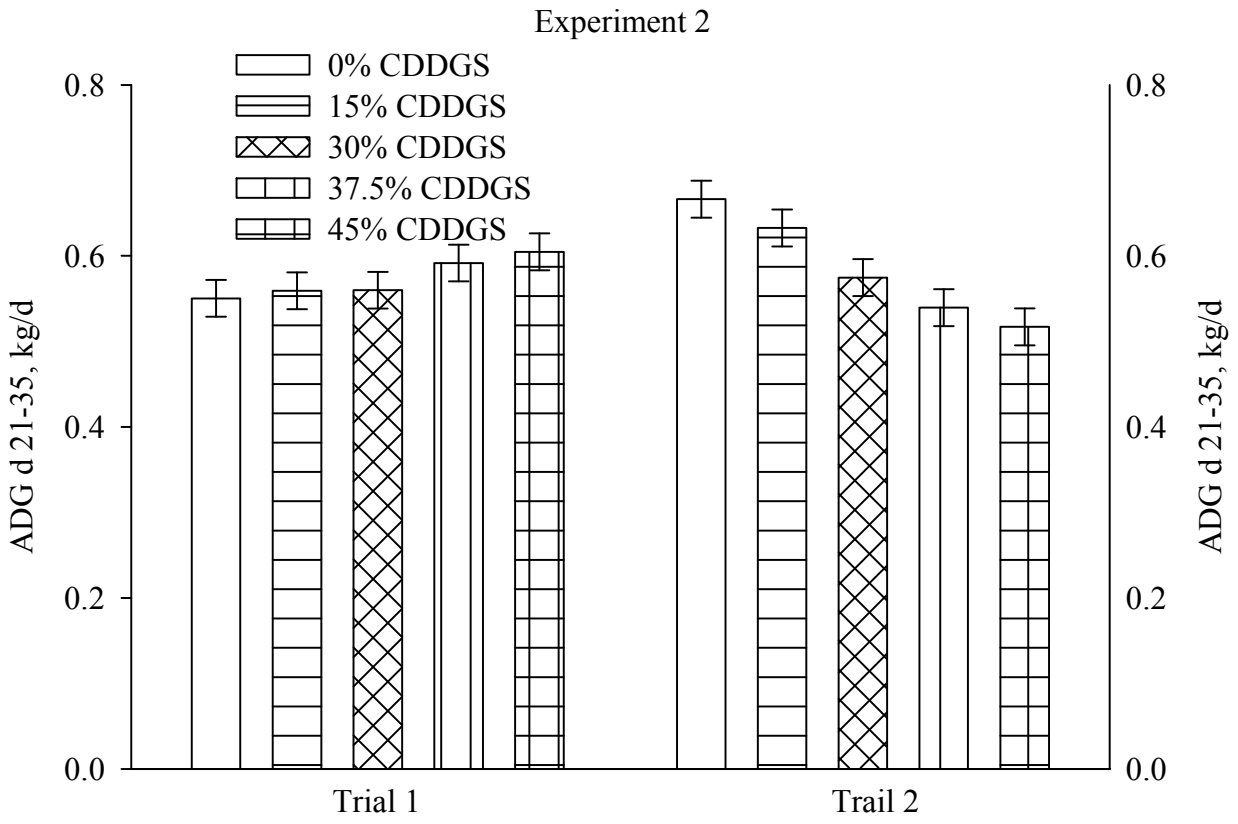
Results were represented as LS mean with diet as main effect, and weight block and initial BW as covariance. A total 80 pigs, 5 pigs per pen, 4 pens per treatment, with initial body weight 6.4kg ± 0.5kg were used per trial and two trials were conducted. One pig was removed from trial 1 study due to health problem (not related to treatment effect).

Figure 4.4 Least square mean of diet and trial interaction in d 7-21 and d 21-35 ADG.



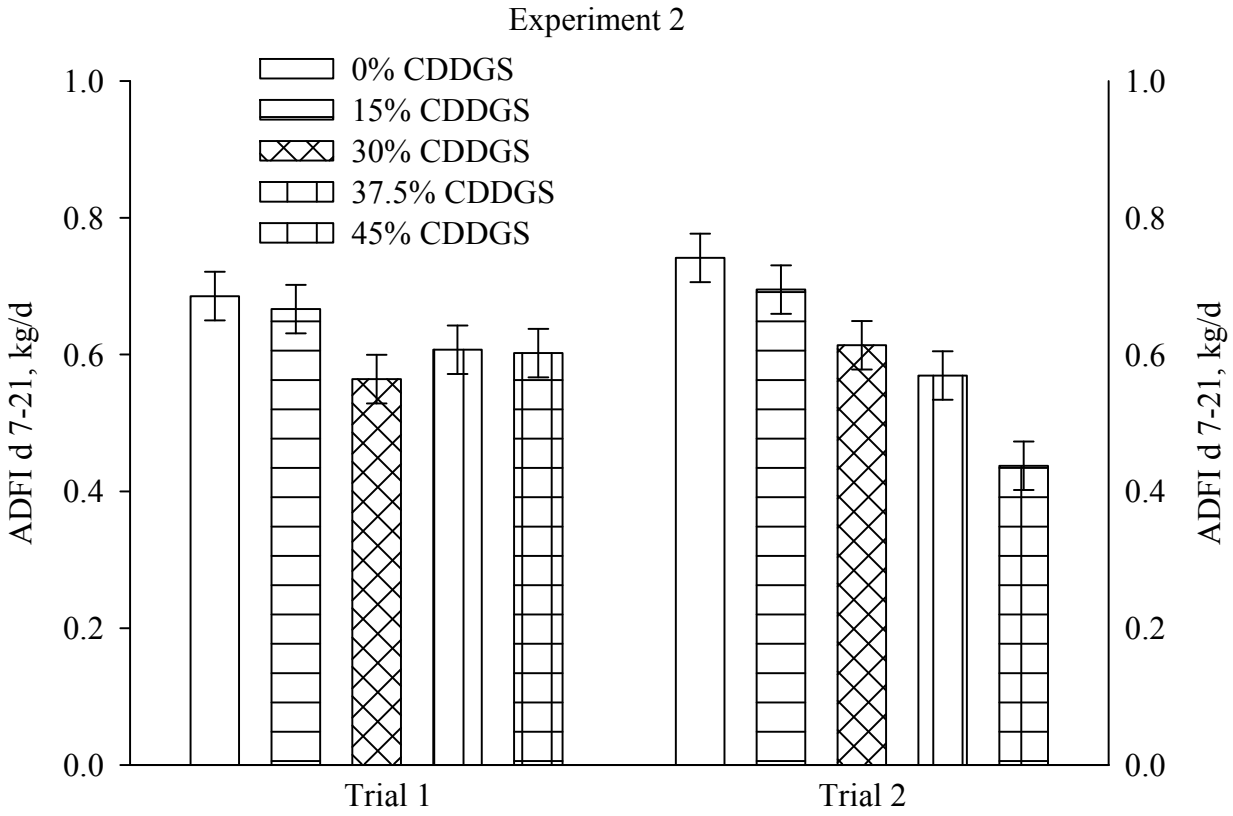
Significant interaction was observed in d7-21 (P=0.001). ADG in d 7-21 was linear decreased in trial 1 (P=0.0142), whereas was quadratic decreased in trial 2 (P=0.003). Results were represented as LS mean with diet, trial and interaction as main effect, and weight block and initial BW as covariance. A total 65 pigs (ave. BW: 7.9kg ± 0.5kg) for trial 1 and 80 pigs (ave. BW: 9.5kg ± 0.5kg) were used for trial 2. Three pigs from trial 1 and one pig from trial 2 were removed from study due to health problem (not related to treatment effect).

Figure 4.5 Least square mean of diet and trial interaction in d 21-35 ADG.



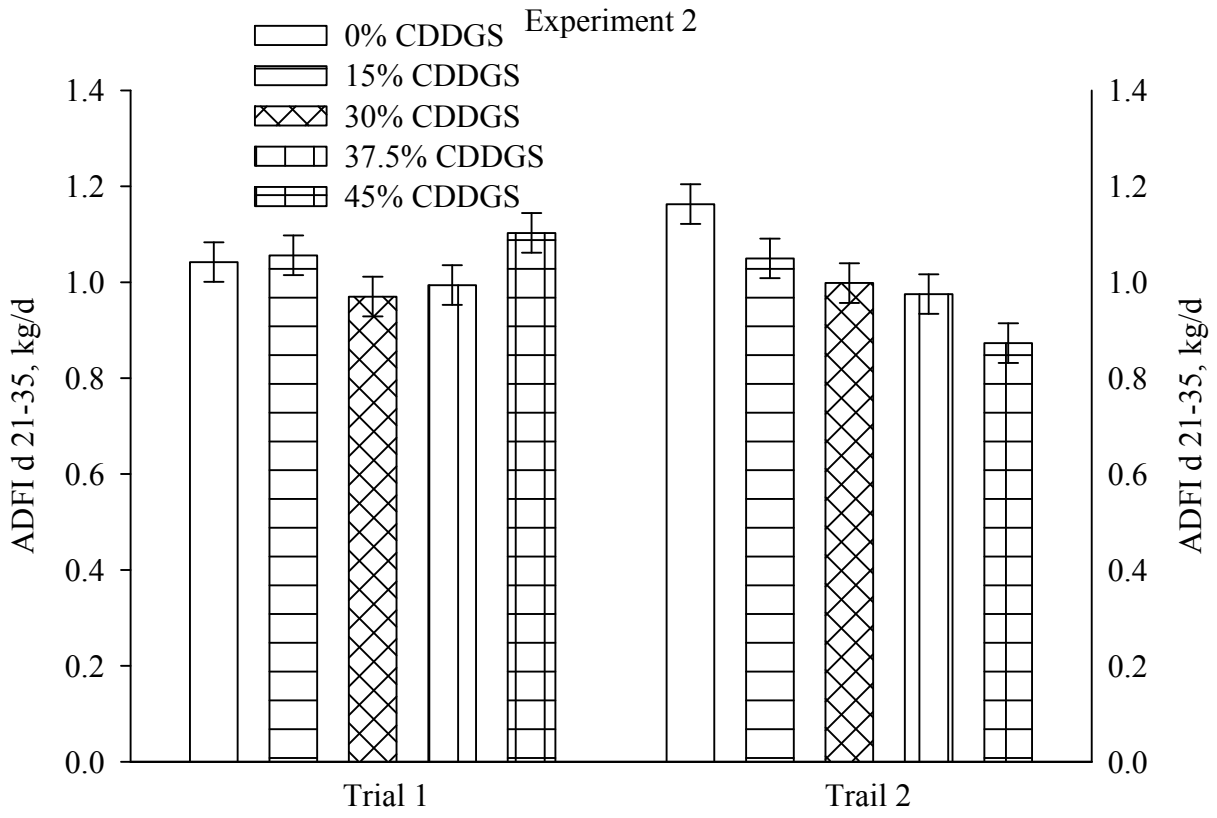
There was significant different between trials (P=0.036), but interaction was no different in d21-35 (P=0.127). ADG in d 21-35 was no different between diets in trial 1 (P=0.0917), whereas was linear decreased in trial 2 (P<0.001). Results were represented as LS mean with diet, trial and interaction as main effect, and weight block and initial BW as covariance. A total 65 pigs (ave. BW: 7.9kg ± 0.5kg) for trial 1 and 80 pigs (ave. BW: 9.5kg ± 0.5kg) were used for trial 2. Three pigs from trial 1 and one pig from trial 2 were removed from study due to health problem (not related to treatment effect).

Figure 4.6 Least square mean of diet and trial interaction in d7-21 ADFI in pigs fed increasing level of CDDGS diets.



There was significant diet and trial interaction in d7-21 ADFI (P=0.016). ADFI was linear decreased more dramatically in trial 2 (P<0.001) than trial 1 (P=0.0317). Results were represented as LS mean with diet, trial and interaction as main effect, and weight block and initial BW as covariance. A total 65 pigs (ave. BW: 7.9kg ± 0.5kg) for trial 1 and 80 pigs (ave. BW: 9.5kg ± 0.5kg) were used for trial 2. Three pigs from trial 1 and one pig from trial 2 were removed from study due to health problem (not related to treatment effect).

Figure 4.7 Least square mean of diet and trial interaction in d21-35 ADFI in pigs fed increasing level of CDDGS diets.



There was significant diet and trial interaction in d21-35 ADFI (P=0.003). ADFI was not affected by diet CDDGS level in trial 1 (P=0.945). In trial 2, however, increased CDDGS level in diets linear decreased ADFI (P<0.001). Results were represented as LS mean with diet, trial and interaction as main effect, and weight block and initial BW as covariance. A total 65 pigs (ave. BW: 7.9kg ± 0.5kg) for trial 1 and 80 pigs (ave. BW: 9.5kg ± 0.5kg) were used for trial 2. Three pigs from trial 1 and one pig from trial 2 were removed from study due to health problem (not related to treatment effect).

CHAPTER 5

CONCLUSIONS

The U. S. government subsidy for blending ethanol with gasoline and using biodiesel as fuel has increased the demand for ethanol and biodiesel production, which increases corn price dramatically. Feed cost represents more than 60% of total farm expanse. Thus, in order to lower feed cost, corn dry distillers grain with soluble (CDDGS), by product of ethanol production can be fed to monogastric animals, but it is recommended that its inclusion in the ration be limited. Limitation of CDDGS includes imbalance amino acids and high fiber content. Strategies that allow increased usage of DDGS in swine & poultry without compromising growth performance would provide these industries a more cost effective option to reduce feed cost particularly when corn or other feed ingredients are expensive. After starch is extracted for ethanol production, concentration of fiber, such as arabinoxylan, in CDDGS increases. Moreover, CDDGS has poor amino acids pattern because of ethanol processing, and corn origin. Therefore, high level of CDDGS adding in diet can have negative impact on growth and nutrient utilization in nursery pigs. Adding fiber degrading enzymes, such as xylanase, and β-glucanase, in high CDDGS diet fed to nursery pigs partially reserved the negative effect on growth performance and nutrient digestibility. In addition, supplementing crystalline amino acids to balance standard ileal digestible lysine level and having diet formulating with ideal protein ratio can increase CDDGS inclusion rate up to 45% without decreasing growth performance, but nutrient digestibility still decrease by levels of CDDGS. CDDGS can be added to nursery pigs diet to lower the feed cost.

In conclusion, 30% of CDDGS inclusion rate can be archived, and growth performance was improved (still less than PC), and nutrient digestibility was better when fiber degrading enzymes are added. The results indicate that high levels (45%) of CDDGS can be used in nursery diets and are well tolerated if the diets are formulated on a constant SID lysine basis with a balanced amino acid pattern and are screened for mycotoxin contamination. However, excess nutrient excretion with high CDDGS in the diet needs to be aware. Further study is necessary to evaluate the effect of different type and concentration of fiber degrading enzymes on growth performance and nutrient utilization when 45% or higher CDDGS, which balances in ideal protein ratio, is added to diet fed to nursery pigs. Moreover, investigating other strategies to increase DDGS usage in diet fed to nursery pigs is needed to lower feed cost.