

**ROLE OF DIETARY FACTORS ON NUTRIENT EXCRETION AND MANURE
CHARACTERISTICS OF GROWING PIGS**

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Dedication

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Chapter 1

Literature review

INTRODUCTION

The U.S. pork industry is among the largest in the world with an output of 10.4 million metric tonnes of carcass weight in 2014, which accounts for approximately 10% of the global pork production (USDA - FAS, 2015). The number of swine produced and marketed has increased on average 5% every year from 1990 to 2014, and as a result, the U.S. pork industry generated \$26.5 billion in gross income in 2014 (USDA - NASS, 1991; USDA - NASS, 2015a). This production increase is the result of the combination of high production efficiency and large scale pork production operations. The market share of U.S. pig farms with 2,000 pigs or more increased from less than 30 % to more than 88 % from 1992 to 2014 (USDA - NASS, 2015b). In 2014, approximately 57% of the pigs marketed in the U.S. were raised on large farms under controlled environmental conditions (temperature, humidity, light and air flow; National Pork Board, 2014). These types of confinement production systems allow for better monitoring and management of animals, resulting in improved feed efficiency (25% improvement in growing-finishing pigs) than production facilities without controlled environments (Berton et al., 2015). However, those large confinement production systems concentrate manure in their storage systems, which require a large amount of land for proper manure application (Hatfield et al., 1998).

In 2007, the U.S. pork industry generated approximately 1.1 billion tons of manure (USDA - NASS, 2009), which contains high levels of nitrogen (N) and phosphorus (P;

Daniel et al., 1994). Although the use of proper manure management procedures and application methods can capture the fertilizer value (e.g. N and P; Jackson and Keeney, 2000) of swine manure, the relatively concentrated N and P content in manure can also have a negative environmental impact. Application of excess N and P to crop land may lead to accumulation in soil, and excess P can lead to river or lake eutrophication, which results in excessive, and potentially harmful growth of plants and algae (Duda and Finan, 1983).

Pig diets are often supplemented with supranutritional concentrations of copper (Cu) and zinc (Zn) as growth promoters, in amounts that exceed the pig's nutritional requirements (Smith et al., 1997; Carlson et al., 1999; Hill et al., 2000). As a consequence, swine manure may contain high levels of Cu and Zn. Copper and Zn can accumulate in the water and soil, causing intermediate to long-term toxicity risk to plants, and the suppression of microorganism activity in the water and soil (McGrath et al., 1995).

Excess minerals are not the only challenge when dealing with manure. Manure also contains pathogens including parasites, viruses, and bacteria that may infect pigs and humans (Cotruvo et al., 2004). Parasites like *Cryptosporidium* (20 to 90 oocysts per gram swine wastewater) and *Giardia* (500 to 1,075 cysts per gram swine wastewater) have been observed in swine lagoons compared to undetectable levels in soil or lakes (Thurston-Enriquez et al., 2005). *Cryptosporidium* and *Giardia* are common causes of protozoan infection in humans, causing gastrointestinal (GI) illness known as cryptosporidiosis (Rose, 1997) and giardiasis (Perdek et al., 2003). A number of GI illnesses related to viruses, including norovirus and sapoviruses, are also associated with

swine manure (Wang et al., 2006). Moreover, *Salmonella* spp., *E. coli* O157:H7, and *Campylobacter* spp. are three common zoonotic pathogenic bacteria that can cause serious waterborne or foodborne diseases in humans that are associated with swine manure (Hutchison et al., 2004; Hutchison et al., 2005).

Another major concern related to manure produced in animal production systems is the presence of antimicrobial residues and the development of antimicrobial resistant bacteria. It is estimated that 89% of U.S. pork producers administer antimicrobials to growing-finishing pigs and 85% of pork producers use antimicrobials in the feed for nursery pigs (USDA - APHIS, 2008). Most of the antimicrobial compounds used in the swine industry are aimed at improving animal growth rather than for therapeutic purposes (disease treatment and prevention; Zimmerman, 1986; Cromwell, 2001). Since many of the antimicrobials used in swine production are the same as those used in human therapeutic treatments (Mellon et al., 2001), antimicrobial resistant bacteria may develop in the animals or in the environment due to antimicrobial residues in the manure (Sapkota et al., 2007; Zounková et al., 2011). Chlortetracycline is the antimicrobial of highest annual use in the pork industry (533,973 kg/year), followed by tylosin and oxytetracycline with 165,803 and 154,956 kg/year, respectively (Apley et al., 2012). Antimicrobial products like chlortetracycline, oxytetracycline, lincomycin and sulfadimethoxine, have been detected in the water and soil near swine farms (Campagnolo et al., 2002). Therefore, an effective manure management system is required to minimize the potentially negative environmental and potential animal and human health impact of swine manure.

MANURE MANAGEMENT SYSTEMS

In general, there are 3 systems to manage manure: solid, semisolid, and liquid systems (Hatfield et al., 1998). The major difference among these 3 manure management systems is the moisture content. Solid systems handle mainly dry feces, while the semisolid systems are design to handle a slurry mixture of feces and urine, and liquid systems are designed to handle feces and urine that are flushed with water to storage facilities (Hatfield et al., 1998). Swine manure has to be processed into acceptable end products by decomposing organic matter (Hatfield et al., 1998). Each of these manure management systems decompose manure in different ways and differ in the capability of providing biological treatment to minimize the potentially negative environmental impact (Burton and Turner, 2003). Biological treatment makes use of manure microorganisms to decompose manure organic matter under aerobic or anaerobic conditions, and proper temperature affects biodegradation rate (Burton and Turner, 2003).

The solid system is most commonly used on small operations, which represent less than 15% of swine farms in U.S. (Hatfield et al., 1998). Some of these farms use housing systems that collect and handle solid manure, while others make use of pastures or open feedlots , which result in inadequate capability to control environmental contamination (Hatfield et al., 1998).

Liquid manure systems are used in 20 to 30% of swine production facilities in the U.S. (Hatfield et al., 1998). Lagoons are used for storage and treatment of swine manure.

Odors, leakage, overflow, and over application of lagoon sewage are the major environmental concerns associated with this system (Hatfield et al., 1998).

Semisolid manure management systems are the most popular in the U.S. pork industry (Key et al., 2011), where it is estimated that 50 to 60% of pork producers use these types of semisolid manure systems (Hatfield et al., 1998). There are two types of semisolid manure systems used in different climates and geographic regions in the U.S. The earthen basin system used in warm climates (southern U.S.) are similar to lagoons, but have less capacity and they do not provide significant dilution or biological treatment of manure (Hatfield et al., 1998). The storage period for earthen basins usually range from 6 to 12 months, and a permanent minimal volume of sludge must remain at the bottom of basins to preserve the clay liner from drying and cracking (Fulhage and Pfof, 2000).

Anaerobic deep storage pits are the most common semisolid indoor storage system used in swine farms in the Midwestern U.S. because of the low temperature during winter time (Hatfield et al., 1998). In this system, manure typically falls through a slotted floor into an underground storage pit, which allows pork producers to store manure for relatively long periods of time (3 to 12 months) before pumping out of pits for land application (Hatfield et al., 1998).

HEALTH AND SAFETY CONCERNS ABOUT ANAEROBIC DEEP PIT MANURE STORAGE

There are important health and safety issues associated with air quality in buildings with anaerobic deep storage pits dedicated to long-term indoor manure storage when compared to other outdoor manure management systems. Ammonia (NH₃), carbon dioxide (CO₂), hydrogen sulfide (H₂S), and methane (CH₄) gases from the manure and manure fermentation processes can cause health and safety problems in swine barns (Muechling, 1970; Mikesell et al., 2004; Zhao et al., 2005). In a confined area, NH₃ at concentrations of 50 ppm, CO₂ concentration at concentrations of 5,000 ppm, and H₂S at concentrations as low as 10 ppm are dangerous and become toxic to workers and animals (OSHA, 1996; OSHA, 2010). Methane is not soluble in water, but it is highly flammable (Muechling, 1970) once it is mixed with air. When methane concentrations rise to 50,000 to 150,000 ppm, any small spark or flame can cause a dangerous explosion (Muechling, 1970).

FOAMING MANURE PITS AND BARN EXPLOSIONS

Since 2008, several barn explosions have been reported in the Midwestern U.S. and Quebec, Canada that coincided with observations of foam accumulation in manure pits (Theisen, 2009; Vansickle, 2010). The manure foam is described as a dark brown to grey and viscous fluid with bubbles (Andersen, 2014). A pork producer survey conducted by the University of Minnesota showed that of the 225 pork producers who responded, about 25% of swine farms in Minnesota, Illinois, and Iowa had experienced manure foaming in

their facilities between September 2012 and May 2013 (Akdeniz et al., 2013).

Two obvious problems caused by excessive accumulation of foam in manure pits are dirty pigs and reduced manure pit storage volume. The reduced manure storage volume results in the need to pump and remove manure more frequently during untimely seasonal weather conditions to prevent the overflow of storage, which significantly increases manure handling cost. The foam can raise through the slotted floors and can also threaten the respiratory and overall health of the personnel and pigs since gases are released and manure contains a number of pathogens (Cotruvo et al., 2004). An even greater concern is that H₂S gas is trapped in the foam (180 – 220 ppm; Moody et al., 2009), and when released by handling manure, the gas concentration in the barn can easily exceed Occupational Safety and Health Administration permissible exposure limits (50 ppm for more than 10 min; OSHA, 2010), which are lethal to both humans and pigs. Handling swine manure with foaming bubbles was considered to be the cause of five human deaths in confined swine barns that occurred in both 2010 and 2015 (Associated Press, 2010; KTTC, 2010; Brumm, 2015). Methane gas is also trapped in this foam (60 – 66%; Moody et al., 2009), and when disrupted, CH₄ is released and result in very high concentrations (50,000 to 150,000 ppm; Muechling, 1970). Methane is flammable and has been implicated as the cause of several barn explosions, resulting in farm worker injuries, death in pigs, and severe structural damage to buildings (Theisen, 2009; Vansickle, 2010).

PRODUCTION OF MANURE FOAM AND POTENTIAL CAUSES

The knowledge regarding the causes of foaming in swine manure is limited. The

theory of “froth flotation” first introduced by Leja (1982) suggests that foam formation in wastewater generally requires 3 components, including biogas, surfactants, and stabilizers (Figure 1). First, a mixture of biogas is produced as a result of the decomposition of organic matter by microbial activity in the liquid. Then, gas bubbles reach the air-liquid interface, where surfactants can decrease the surface tension of manure. Finally, gases are trapped in foam at the interface, which is stabilized by small particles. If all 3 phases occur simultaneously, foam bubbles will occur and accumulate. Petrovski et al. (2011) further investigated whether these 3 factors were necessary for foam formation. In their experiments, only unstable foam was generated in the absence of small particles. Without surfactants, only a greasy surface scum was generated. After adding surfactants into the mixture, stable foam was generated.

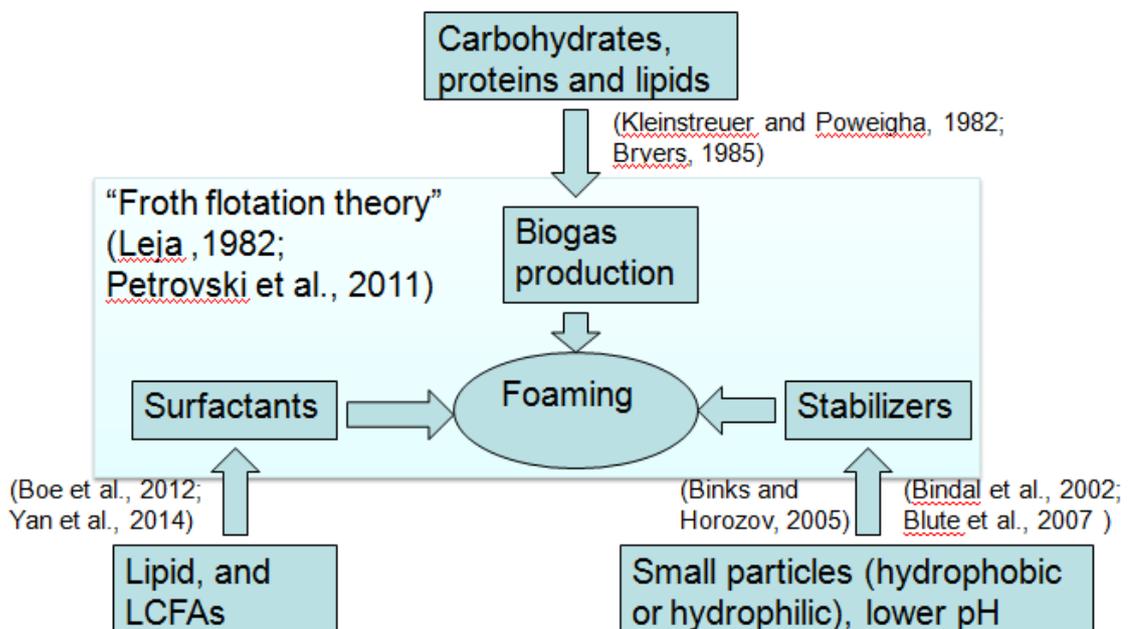


Figure 1. Hypothesis of manure pit foaming

Biogas Production

Carbohydrates, proteins, and lipids can be degraded by different bacteria in the absence of oxygen (O_2 ; (Kleinstreuer and Poweigha, 1982; Bryers, 1985). Biogas is produced during anaerobic digestion (AD), mainly containing CH_4 , CO_2 , and trace amounts of other gases, including NH_3 and H_2S (Kleinstreuer and Poweigha, 1982; Bryers, 1985; Figure 1). The AD process can be divided into 4 phases, including hydrolysis, acidogenesis, acetogenesis and methanogenesis (Kleinstreuer and Poweigha, 1982; Bryers, 1985). In the hydrolysis phase, carbohydrates, proteins and lipids are degraded into simple sugars, amino acids (AAs), and fatty acids, respectively, producing hydrogen (H_2), acetate, and other short-chain fatty acids (SCFAs) as by-products. In the acidogenesis phase, the substrates are further degraded into SCFAs, and produce CO_2 , NH_3 , and H_2S as by-products. Then, in the acetogenesis phase, the SCFAs obtained from the first 2 phases are degraded to acetic acid, H_2 and CO_2 . Finally, in the methanogenesis phase, CH_4 and CO_2 are produced by methanogens. In order to maintain biogas production rate, the pH level of manure must be between 5.5 and 8.5 with the temperature between 30 and 60°C (Kleinstreuer and Poweigha, 1982; Bryers, 1985). Anaerobic deep pits in environmentally controlled swine buildings can provide a stable manure temperature during long-term indoor manure storage compared with other outdoor manure management systems (National Pork Board, 2012), resulting in a relatively stable biogas production.

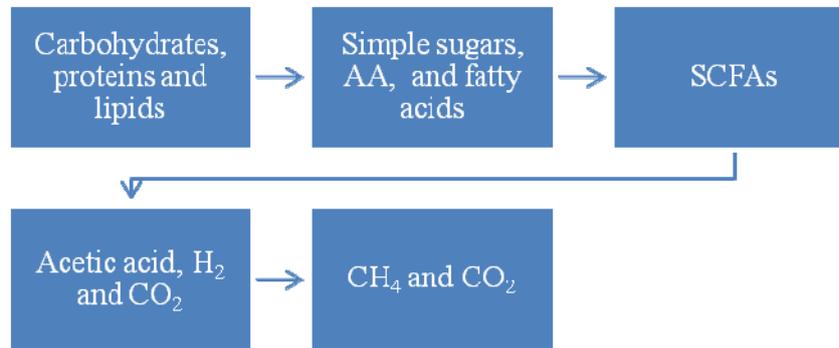


Figure 2. Process stages of anaerobic digestion, modified from Kleinstreuer and Poweigha (1982) and Bryers (1985)

Surfactants and Bio-surfactants

Some studies (Boe et al., 2012; Yan et al., 2014) have suggested that lipids and long-chain fatty acids (LCFAs) may serve as surfactants during the second phase of foam formation. Boe et al. (2012) found that a higher level of sodium-oleate was associated with higher foaming potential in a manure AD system. Yan et al. (2014) reported that the addition of corn oil (mainly linoleic acid) to the swine manure induced foam formation only after 1 week of storage. Moreover, swine manure collected from foaming pits had greater palmitoleic acid, stearic acid, oleic acid, linoleic acid and total LCFAs concentrations than manure collected from non-foaming pits (Yan et al., 2014). This difference was especially significant in the air-liquid interface of foaming manure (Yan et al., 2014).

Bio-surfactants are substances produced by yeast or bacteria during metabolic microbial activity, which can also decrease the surface tension of liquid similar to surfactants (Lin, 1996). Heard et al. (2008) found that substances produced by filamentous *Gordonia* spp. bacteria can serve as bio-surfactants for foam formation in

wastewater. In addition, filamentous *Gordonia* spp. bacteria can serve as stabilizers to prevent foam to collapse. Westlund et al. (1998) found that *Microthrix parvicella* sp. had a similar gas trapping effect to *Gordonia* spp. bacteria.

Stabilizers

Several research groups (Bindal et al., 2002; Binks and Horozov, 2005; Blute et al., 2007) have investigated the effectiveness of small particles (both hydrophobic and hydrophilic) as foam stabilizers during the third phase of foam formation. Binks and Horozov (2005) suggested that small hydrophobic particles can aggregate and increase the viscosity of liquids, resulting in slower liquid flow from the foam layer, and lower incidence of foam collapse and coalescence. Bindal et al. (2002) found that foam formation was increased with a greater concentration of smaller hydrophilic particles (increasing particle size from 8 to 100 nm decreased the final foam height from 550 to 15%). It has been proposed that the self-layering characteristics of small hydrophilic particles inside the foam layer provides a structural barrier against foam coalescence (Bindal et al., 2002). This study also found that large particles have a negative impact on foaming that was stabilized by smaller hydrophilic particles. The addition of a small amount of large particles into foaming wastewater can lead to a large reduction of foam formation (Bindal et al., 2002). In addition, Blute et al. (2007) found that liquid with reduced particle size (from 40 to 6 nm), and lower pH (from 10.0 to 3.4) had higher potential of foam formation after providing a constant air flow.

TEMPORARY SOLUTIONS TO MINIMIZE OR PREVENT MANURE FOAMING

Three studies (Kougias et al., 2013b; Kougias et al., 2014a; Kougias et al., 2015) have reported that the addition of natural oils (rapeseed and sunflower oil) and fatty acids (oleic, octanoic and derivative of natural fatty acids) immediately suppressed foam in AD systems. The exact mechanism of foaming suppression using natural oils and fatty acids was not clear, and studies did not investigate the long-term effect of adding oils and fatty acids to foaming. Yan et al. (2014) observed a similar phenomenon by adding corn oil, which immediately removed the foaming bubbles in foaming manure. However, after only 1 week of storage, corn oil induced foam formation rather than suppressing it (Yan et al., 2014). As a result, the temporary effect of foam suppression by adding oil is not practical. According to (Yan et al., 2014), treating manure foaming by adding oil may cause worse foaming due to a long period of manure storage in deep pits.

Recently, Clanton et al. (2012) reported that adding an ionophore (monensin) into anaerobic manure pits (5 lbs per 100,000 gallons) may help reduce pit foaming incidence and treat actively foaming pits. Ionophores, have been used extensively as feed additives for ruminants to decrease CH₄ production (Thornton and Owens, 1981), increase the ratio of propionic to acetic acid production (Dinius et al., 1976; Richardson et al., 1976), and decrease protein degradation to NH₃ in the rumen (Yang and Russell, 1993). Ionophores are classified as carboxylic polyether antibiotics, and they can disrupt the concentration gradient of ions (H⁺, Na⁺, K⁺, Ca²⁺) among microorganisms, which disrupts energy production, causing cell death (Pressman, 1976). However, using monensin in anaerobic

manure pits is not approved by label specifications from the U.S. Environmental Protection Agency (USEPA) or the Minnesota Department of Agriculture (MDA). Monensin can be lethal to pigs causing dyspnea, ataxia, diarrhea and skeletal muscle necrosis if ingested (Van Vleet et al., 1983; Miskimins and Neiger, 1996). Monensin has been detected in the soil, water and sediment environments near the dairy and broiler farms where it has been fed (Watanabe et al., 2008; Hansen et al., 2009) , suggesting a potential role in the development of antimicrobial resistance bacteria. However, the significance of antimicrobial resistance caused by ionophores is not well established because ionophores have never been used as antimicrobials for humans. In addition, there are no studies suggesting that ionophores have a similar mode of action with therapeutic antibiotics, which is likely due to the high degree of specificity of ionophore resistance mechanisms (Callaway et al., 2003). In addition, an study conducted by Edrington et al. (2003) found that the antimicrobial susceptibility of *E. coli* O157:H7 or *Salmonella typhimurium* was not affected by ionophore treatment.

ROLE OF FEED INGREDIENTS AND PARTICLE SIZE ON MANURE PIT FOAMING

Results from studies suggest that undigested nutrients in manure may serve as surfactants (lipids and LCFAs; Boe et al., 2012; Yan et al., 2014) and stabilizers (small particles; Bindal et al., 2002; Binks and Horozov, 2005; Blute et al., 2007) as well as substrates to produce biogas (fiber and protein; Kleinstreuer and Poweigha, 1982; Smith et al., 1988) during foam formation. Evidence indicates that adding corn distillers dried

grains with solubles (DDGS) in swine diets increased overall manure output due to higher levels of indigestible fiber, resulting in more concentrated dry matter (DM) and nutrients in the manure (Jacobson et al., 2013). However, a comparison of manure from farms feeding diets with and without DDGS did not show significant differences in manure foaming (Yan et al., 2014). Therefore, a better understanding of the underlying mechanism of manure foaming is critical for developing long-term manure foaming mitigation strategies.

Methods to maximize nutrient digestibility in swine diets and minimize nutrient excretion are essential to reduce manure pit foaming. The most common feed ingredients used in areas where manure foaming has been reported are corn, soybean meal (SBM), DDGS, and soybean hulls (SBH; Akdeniz et al., 2013).

Corn-soybean Meal Diets (CSB)

Corn-soybean meal diets are commonly fed in the U.S. pork industry. Corn and SBM have complementary nutrient composition, and when properly combined in a complete diet, can meet all of the energy and digestible AAs requirements of pigs from weaning to finishing (Chiba, 2001). Traditionally, the pork industry in the Midwestern U.S. has relied on CSB diets as the primary energy and nutrient sources, but with increasing feed ingredient prices in recent years, there has been high demand for more cost effective alternative feed ingredients such as DDGS, to reduce overall feed cost (National Pork Board, 2012; Woyengo et al., 2014).

Corn Distillers Dried Grains with Solubles

The use of DDGS in swine feeding programs in the U.S. increased rapidly since 1999 due to the rapid increase in ethanol production, and consequently, DDGS availability and relatively low cost compared with corn and SBM (Renewable Fuels Association, 2015). Distillers dried grains with solubles are a co-product of ethanol production for beverages and bio-fuels (Cromwell et al., 1993). This co-product is an excellent source of energy, AAs, and digestible P in swine diets, and the inclusion of DDGS to CSB diets has generally led to a decrease in diet cost (USGC, 2012).

In general, DDGS has 3 times more neutral detergent fiber (NDF) content than corn (NRC, 2012). However, composition in total dietary fiber (TDF; 18.6 to 37.8%), crude fiber (6.1 to 7.4%), NDF (28.8 to 44.4%) and acid detergent fiber (9.0 to 14.0%; ADF; Urriola et al., 2010; Kerr et al., 2013) is highly variable among DDGS sources. The apparent ileal digestibility (37.5 to 52.1%; AID) and the apparent total tract digestibility (44.5 to 65.8%; ATTD) of NDF also varies among DDGS sources (Urriola et al., 2010; Kerr et al., 2013). As a result, feeding diets containing DDGS may increase fiber excretion due to its higher fiber content compare to CSB diets.

Corn distillers dried grains with solubles has 3 times more crude protein (CP) content compared with corn, and its content is variable among sources (24.6% to 32.9%; Urriola et al., 2010; Kerr et al., 2013). The relative proportions of AAs in DDGS are similar to those in corn because it is derived from corn (NRC, 2012). Corn distillers dried grains with solubles can be used as a protein source because the concentrations of each AA are about 3 fold greater than in corn (Han and Liu, 2010). However, the lysine content of DDGS is relatively low, and therefore, its protein quality is considered poor

compared with conventional protein sources such as SBM (NRC, 2012). In addition, digestibility of AAs in DDGS is about 10% less than corn due to Maillard reaction caused by heating during the production process (Cromwell et al., 1993; Almeida et al., 2013). Moreover, lysine is particularly susceptible to Maillard reactions due to its free amino group (Pahm et al., 2008; Almeida et al., 2013). Therefore, feeding diets containing DDGS may increase N excretion due to its unbalanced AAs profile and reduced digestibility relative to the digestible AAs requirements of pigs.

In recent years, 90% of U.S. ethanol plants began to partially extract oil from thin stillage prior to manufacturing DDGS during ethanol production (Kerr et al., 2013). Extracted corn oil may increase profits of ethanol plants resulting from marketing distillers corn oil to the biodiesel and feed industry (Kerr et al., 2013). As a result, DDGS source vary in oil content from 5 to 12% (Kerr et al., 2013). In addition to changes in energy value, digestibility of other nutrients can be affected by extracting oil prior to manufacturing DDGS. Two studies (Curry et al., 2014; Gutierrez et al., 2014) found that reduced-oil DDGS had lower standard ileal digestibility (SID) of AAs compared with conventional DDGS, which could not be overcome by adding corn oil or soybean oil to the diets. As a result, feeding diets containing reduced-oil DDGS may increase N excretion due to lower SID of AAs compared with conventional DDGS sources.

In addition to nutrient excretion, several studies (Powers et al., 2008; Carter et al., 2012; Spiels et al., 2012; Trabue and Kerr, 2014) have analyzed manure gas emissions of pigs fed diets containing DDGS. With relatively higher CP, S, and fiber content in DDGS compared with CSB (NRC, 2012), greater emissions of NH₃, H₂S and CH₄ from manure

from DDGS fed pigs was expected. Carter et al. (2012) reported that emissions of CH₄, NH₃ and H₂S were increased more than 200% for pigs fed diets containing 40% DDGS compared with those without DDGS supplementation. In addition, Powers et al. (2008) found that emissions of NH₃ and H₂S were also increased in the manure of pigs fed diets containing DDGS compared with CSB diets. However, Trabue and Kerr (2014) found that emissions of NH₃ and H₂S were decreased in the manure of pigs fed diets containing 35% DDGS compared with pigs fed CSB. Also, there was no difference among dietary treatment for CH₄ gas emission (Trabue and Kerr, 2014). These researchers indicated that the most likely mechanism for the reduced gas emission rates was a result of the increased surface crusting in the manure of pigs fed diets containing DDGS. In fact, bio-covers (e.g. polymeric materials, wheat straw, and aged yard waste) have been shown to effectively lower NH₃ emissions from lagoons by over 50% (Zahn et al., 2001). In addition, feeding diets containing DDGS was found to have no effect on H₂S or NH₃ gas emission (Spiehs et al., 2012), and reduced CH₄ gas emission (Powers et al., 2008). Therefore, the effect of feeding DDGS diets on swine manure gas emissions may depend on the formation of crusting on the surface of manure.

In summary, feeding diets containing DDGS may increase DM, fiber, CP and lipid excretion, and potentially result in greater surfactant and stabilizer concentrations in manure compared with manure from pigs fed conventional CSB diets. However, feeding diets containing DDGS may reduce overall biogas production of manure due to the formation of crusting on the surface of manure. Further research into the effect of feeding DDGS on manure foaming is needed.

Soybean Hulls

Soybean hulls are the by-product of the processing of raw soybeans for oil and meal (Smith, 1977). Dehulled SBM contains 48.5 to 49% CP (Smith, 1977), but the hulls can be added back into meal after toasting, grinding, and blending to produce 44 to 45% CP SBM which is also commonly used in commercial swine diets (Smith, 1977).

Soybean hulls have a higher content of dietary fiber (56% NDF) and lower content of CP (12%) than a CSB diet (average 7 % NDF and 15% CP; NRC, 2012). Feeding diets containing SBH will also increase DM and fiber excretion compared with feeding conventional CSB diets (Kornegay, 1978; Kornegay, 1981; DeCamp et al., 2001), which may potentially result in greater surfactant and stabilizer concentration in the manure.

Soybean hulls are a source of both soluble (15%, DM basis) and insoluble (65%, DM basis) fiber for swine (Schweizer and Würsch, 1979), while DDGS contains mainly insoluble fiber (86% to 94% of total fiber; Urriola et al., 2010). The difference between SBH and DDGS in fiber solubility may contribute to different hydrophobicity or hydrophilicity in the manure (Domb et al., 1996). As discussed previously, several studies (Bindal et al., 2002; Binks and Horozov, 2005; Blute et al., 2007) demonstrated that both small hydrophobic or hydrophilic particles can serve as stabilizers for foam formation by different mechanisms. Therefore, feeding diets containing SBH may potentially increase foam stability in the foaming manure by different mechanism compared with feeding DDGS.

In addition to fiber solubility, SBH have higher fiber fermentability in the hindgut of pigs compared with DDGS because of its relatively lower ratio of non-fermentable fiber

to fermentable fiber (Schweizer et al., 1983; Urriola et al., 2010). Consequently, Velthof et al. (2005) reported that the emission of CH₄ increased in the manure containing higher content of fermentable fiber. This study suggested that the manure from pigs fed diets may potentially increase CH₄ emission compared with the manure from pigs fed DDGS diets due to relatively higher ratio of fermentable fiber. However, several studies (Canh et al., 1998; Kreuzer et al., 1998; DeCamp et al., 2001) demonstrated that pigs fed diets containing SBH decreased manure odor emissions due to relatively lower CP and S content compared with DDGS. Canh et al. (1998) found that NH₃ emission decreased by 38% when pigs were fed diets containing SBH compared with control diets containing corn starch. DeCamp et al. (2001) reported a 32% reduction in manure H₂S emission and a 20 % reduction in manure NH₃ emission from pigs fed diets containing 10% soybean hulls and 3.4% supplemental fat. Kreuzer et al. (1998) also reported a similar reduction in NH₃ emission when pigs fed diet containing SBH. Overall, feeding pigs with diets containing SBH may increase manure CH₄ emission, but will reduce NH₃ and H₂S emission. Therefore, the effect of feeding SBH diets on manure biogas production is unknown.

As discussed previously, the pH of manure is another important factor which may affect manure gas production and foam stability. Several studies (Canh et al., 1998; Kreuzer et al., 1998; Kerr et al., 2006; Le et al., 2008) found that the pH of manure decreased from average 9.0 to 7.0 when pigs fed diets containing higher content of fermentable fiber (e.g. soybean hull, sugar beet pulp), which is in favor of both gas production (range from 5.5 to 8.5; Kleinstreuer and Poweigha, 1982; Bryers, 1985) and

foam stabilization (increased foam stabilization when pH drop from 10.0 to 3.4; Blute et al., 2007). Therefore, the differences in fiber characteristics, solubility and fermentability among feed ingredients may be a nutritional connection to cause manure foaming.

Particle Size

Studies have shown that a 1.3% improvement in feed conversion for growing pigs was achieved for each 100 μm reduction in mean particle size from 1000 to 400 μm (Wondra et al., 1995a). In fact, the U.S. pork industry has recently adopted the practice of feeding diets with further reductions in average particle size from 900 μm to 400 μm (National Pork Board, 2012). In nursery pigs, average daily gain (ADG) and gain to feed ratio (G:F) were increased linearly as particle size of corn was reduced from 900 to 300 μm (Healy et al., 1994). In growing pigs, inclusion of 30% DDGS with an average particle size of 308 μm improved dietary ATTD of DM (from 82.8 to 84.3%), compared with 818 μm DDGS diet (Liu et al., 2012). Also in growing pigs, grinding of DDGS from 517 μm to 383 μm increased the ATTD of NDF from 40.2 to 45.3% (Yáñez et al., 2011). In growing-finishing pigs, ATTD of essential AAs was increased from 83.5 to 84.9% as particle size of SBM was reduced from 900 to 150 μm (Fastinger and Mahan, 2003), and DM and N excretion were reduced by 20 and 24%, respectively, when particle size of corn was reduced from 1000 to 700 μm (Wondra et al., 1995a). In lactating sows, DM and N excretion were decreased by 21 and 31%, respectively, when particle size of corn was reduced from 1200 to 400 μm (Wondra et al., 1995b; Wondra et al., 1995c). As a result, pigs fed diets with smaller particle size may result in less DM content, and lower concentration of surfactants and stabilizers in the manure than pigs fed diets with larger

particle size. However, small particles may serve as foam stabilizers to increase manure foam stability (Bindal et al., 2002; Binks and Horozov, 2005; Blute et al., 2007), which may increase manure foaming. Therefore, further studies need to be conducted to determine the overall effect of diet particle size on manure pit foaming based on both nutrients excretion and foam stability.

SUMMARY

Manure pit foaming has caused significant concern related to the health and safety of barn workers and pigs in the pork industry. As a result, a better understanding of the underlying mechanism of manure foaming is critical for developing long-term mitigation strategies. Based on the theory of “froth flotation”, foam formation requires 3 contributing factors, including biogas production, surfactants, and stabilizers, which may come from undigested nutrients in the manure. Therefore, evaluating and comparing nutrient digestibility and excretion of swine diets containing commonly used ingredients is essential for potentially identifying contributing nutritional factors related to manure pit foaming.

Diets containing an increased amount of high fiber ingredients (e.g. DDGS and SBH) and finer particle size have become commonly used in the U.S. pork industry in recent years. The increased dietary fiber content has a negative impact on digestibility of other nutrients such as proteins and lipids. Meanwhile, reducing diet particle size increases nutrient digestibility. Small particles may serve as stabilizers in the foaming manure. However, the interaction effects of fiber source and particle size on nutrient digestibility

and manure foaming have not been studied.

Most U.S. ethanol plants are extracting oil from thin stillage prior to manufacturing DDGS and consequently, variability in lipid and nutrient content among sources has increased. Digestibility of other nutrients (e.g. AAs and protein) can be affected by extracting oil prior to manufacturing DDGS. Also, the effect of fermentation process of manure on manure foaming capability (MFC) have not been evaluated.

Therefore, the hypotheses of two studies conducted in this thesis are:

1) Differences in digestibility in dietary fiber, lipid, and protein using complete feed containing common feed ingredients, including corn, SBM, DDGS, and SBH, with different particle size, would affect MFC.

2) Oil content in DDGS affects nutrient digestibility and may affect manure foaming of fresh feces.

Chapter 2

Effect of diet composition and particle size on nutrient excretion of growing pigs and the propensity to cause manure pit foaming

SUMMARY

Excess accumulation of methane and hydrogen sulfide gas in manure foam is a significant health and safety hazard for both humans and pigs. We hypothesized that dietary characteristics related to fiber and lipid digestibility may alter manure chemical composition and result in a greater risk of manure foaming in anaerobic manure pits. The objective of this experiment was to measure nutrient excretion and manure foaming capability (MFC) of pigs fed 3 diets differing in the source and amount of NDF and ether extract (EE) when ground to 2 particle sizes. Two groups of 24 growing gilts (initial BW = 119.5 ± 8.9 kg) were placed into metabolism crates and allotted randomly to 1 of 6 diets (4 replicates/treatment/group). Dietary treatments consisted of: 1) corn-soybean meal (7.2% NDF, 4.6% EE; CSB), 2) corn + 35% corn distiller's dried grains with solubles (13.7% NDF, 6.2% EE; DDGS), and 3) CSB + 21% soybean hulls (20.0% NDF, 6.8% EE; SBH). Diets were ground to a geometrical mean particle size of 374 ± 29 μm (fine) or 631 ± 35 μm (coarse) and fed for 7 wk. Except for d-21 to d-24, all feces and urine were collected, mixed daily, and stored in simulated deep pit storage tanks. Excretion of DM, NDF, and EE were measured after total feces and urine were collected from d-21 to d-24. The MFC of each manure sample was measured in duplicate in the laboratory using a column and injecting N_2 to simulate foam production. Data were analyzed using the

MIXED procedure of SAS, with individual pig as a random effect and diet composition, diet particle size, and their interaction as fixed effects. There was a diet composition × particle size interaction for MFC ($P < 0.05$), where greater MFC was observed in manure from pigs fed coarse SBH compared with fine CSB and SBH, but not for fine or coarse DDGS. Excretion of DM and NDF were greater ($P < 0.05$) for pigs fed DDGS and SBH than for pigs fed CSB, and excretion of EE was greater ($P < 0.01$) for pigs fed DDGS than those fed CSB or SBH. Dry matter, NDF, and EE excretion was greater ($P < 0.05$) for coarse diets compared to fine diets regardless of diet composition. These results indicate that fiber composition in SBH had a greater impact on MFC than the fiber and lipid composition in DDGS, and larger diet particle size reduces DM, NDF, and EE digestibility causing an increase in excretion in manure and MFC.

Key words: diet particle size, fiber, growing pigs, lipid, manure foaming, nutrient excretion

INTRODUCTION

Manure foam accumulation on the surface of anaerobic deep pits on commercial swine farms has been a significant problem in recent years (Theisen, 2009; Vansickle, 2010). Hydrogen sulfide (H_2S) and methane (CH_4) are produced during fermentative processes in manure, and when these gases are released into the air, H_2S can cause asphyxiation and death in both humans and pigs (OSHA, 2010), and high concentrations of CH_4 can cause explosions in swine buildings (Theisen, 2009; Vansickle, 2010). This problem has caused significant concern related to the health and safety of barn workers

and pigs housed in these facilities. Understanding the underlying causes of manure pit foaming is critical for developing effective long-term mitigation strategies.

Causes of manure foaming have been reported to be related to changes in manure composition (Petrovski et al., 2011; Jacobson et al., 2013). Diets containing an increased amount of high fiber ingredients and processed to a smaller particle size have become commonly used in the U.S. pork industry in recent years (National Pork Board, 2012; Woyengo et al., 2014). Increasing the dietary fiber content reduces the digestibility of other nutrients such as proteins and lipids (Kass et al., 1980; Bach Knudsen and Hansen, 1991; Chen et al., 2013). Therefore, we hypothesized that differences in digestibility of fiber, lipids, and protein in diets containing common feed ingredients such as corn, soybean meal, corn distiller's dried grains with solubles, and soybean hulls, with different particle size, affects manure foaming capability (**MFC**). To test this hypothesis, the objectives of this study were to determine DM and nutrient digestibility, excretion, and MFC from pigs fed diets differing in the source and amount of fiber, lipid, and protein when ground to two particle sizes.

MATERIALS AND METHODS

Animal Management

The experimental protocol used in this study were reviewed and approved by the Institutional Animal Care and Use Committee at Iowa State University (Ames, IA). This experiment was conducted over two 49-d periods at the Iowa State University Swine Nutrition Farm (Ames, IA). Two groups of 24 gilts ($n = 48$; initial BW = 119.5 ± 8.9 kg),

which were offspring from PIC Camborough (Pig Improvement Company, Hendersonville, TN), were moved into an environmentally controlled metabolism room and individually placed in metabolism crates (1.2 × 2.4 m) that allowed for separate collection of feces and urine. Ambient room temperature was maintained at about 18.4 °C, and lighting was provided continuously. Crates were equipped with a stainless steel feeder and a nipple drinker to provide pigs with *ad libitum* access to water.

Diets

Gilts were allotted randomly to 1 of 6 diets, resulting in 4 replications per treatment per group. Dietary treatments consisted of: 1) corn-soybean meal (**CSB**), 2) corn + 35% corn distiller's dried grains with solubles (**DDGS**), and 3) CSB + 21% soybean hulls + 3% soybean oil (**SBH**). All diets were formulated to meet or exceed NRC requirements (NRC, 2012; Table 2.1). After ingredient weighing and mixing of diets, half of each diet was ground using a hammer mill to achieve the desired particle size of 300 µm. The distribution of the size of particles were analyzed using a 13 sieve stack with automatic shaker (Tyler RoTap, Mentor, OH) as described by Baker and Herrman, (2002). The actual geometrical mean particle size for each diet was 374 ± 29 µm (**fine**) and 631 ± 35 µm (**coarse**; Table 2.1), and all diets were fed in meal form. Pigs were fed twice daily a total amount of feed equivalent to 3.0% of their initial BW for 7-wk, which approximated *ad libitum* feed intake for pigs at this BW. Actual feed disappearance was calculated as the difference between the amounts of feed added minus the amount of feed not consumed.

Sample Collection

Samples of diets were collected and ground through a 1-mm screen (Wiley No. 4 Laboratory Mill, Arthur H Thomas Co., Philadelphia, PA) before energy and nutrient analyses. During the d-21 to d-24 total collection period, stainless steel wire screens and stainless steel buckets containing 30 mL of 6 N HCl were placed under each metabolism crate to allow total quantitative collection of all feces and urine from each pig. Feces and urine were collected twice daily before each meal, weighed, and stored at -20 °C until the end of the experiment. Feces from each pig were pooled, dried in a forced air oven at 60° C, weighed, ground to pass through a 1 mm screen (Wiley No. 4 Laboratory Mill, Arthur H Thomas Co., Philadelphia, PA), and mixed to obtain a representative sample for analysis. Energy and nutrient digestibility and excretion were calculated by using this portion of the feces. Likewise, urine samples were thawed, weighed, and pooled within pig to collect a representative sample for analysis. Energy and nutrient balance was calculated by using this portion of the urine.

Except for d-21 to d-24, fecal and urine (without added HCl) output was collected twice daily from each pig and added to 24 individual stainless steel manure storage tanks for the manure composition analysis portion of the experiment. The manure storage system used in this research was designed to simulate manure storage conditions in typical swine facilities (Trabue and Kerr, 2014). Each manure tank was designed to provide a similar surface area (0.75 m²/pig) and a similar air flow (100 L/min during sample collection) over the manure surface to that commonly found in conventional confinement growing-finishing barns with anaerobic deep pit manure storage systems used in the U.S. swine industry.

At the end of each group feeding period (d-49), manure was diluted with water to achieve the same volume for all tanks. Manure samples used for chemical analysis were collected after mixing and stirring each tank with a plastic paddle. The pH of each manure sample was measured using a pH meter (Corning Model 530 with Corning probe #476436, Corning Inc., Corning, NY; Table 2.2). After manure samples were obtained, each manure tank was drained to a level where 7.5 cm of residual manure remained in the tank. This residual manure served as inocula for manure collected and fermented from the second group of pigs fed the same respective dietary treatments.

Chemical Analysis and Calculations

Diet, fecal, and urine samples were analyzed at USDA-ARS, Ames, IA (Table 2.2). To determine DE and ME content of diets, the GE content of the diets, feces, and urine samples were determined using an isoperibol bomb calorimeter (Model 1282, Parr Instrument Company, Moline, IL) using benzoic acid as a standard. The GE content of urine was analyzed following the procedure described by Kerr et al. (2013). Briefly, 1 mL of urine was added to 0.5 g of dried cellulose, subsequently dried at 50° C for 24-h, and repeated 3 times over a 72-h period to provide a total of 3 mL of filtered urine for GE determination. The GE content of the cellulose contained 3 mL of urine was determined using an isoperibol bomb calorimeter (Model 1282, Parr Instrument Company, Moline, IL) using benzoic acid as a standard. Urinary GE was determined by subtracting the amount of energy contained in cellulose from the GE of the combined urine plus cellulose. All energy values are reported on a DM basis. The DM content in diets was analyzed following the AOAC (2005) official method 934.01. The concentration of ether

extract (**EE**) in diets and feces was analyzed using petroleum ether following AOAC (2005) official method 920.39 A, while the concentration of NDF in diets and feces was analyzed using ANKOM Tech. method 13. The concentration of N and S in diets, feces, urine and manure were analyzed using a VarioMAX CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) following the AOAC (2005) official method 972.43. Subsequently, apparent total tract digestibility (**ATTD**) of GE, DM, EE, NDF, N, and S of each diet were calculated using the difference procedure described by Adeola (2001).

The concentration of ammonium (**NH₄⁺**) in manure was analyzed using a NH₄⁺ probe (Table 2.2; Thermo Orion Meter 290A+, probe #9512, Thermo Fisher Scientific Inc., Minneapolis, MN) that was calibrated using a standard curve. Next, using the procedure described by Trabue and Kerr (2014), 3 g of manure was weighed into a 100 mL beaker and 99 mL of nanopure water was added to the sample. Then, 2 mL of ionic strength adjuster solution (Orion 951211, Thermo Fisher Scientific Inc. Minneapolis, MN) were added and mixed. The mixture was maintained at minimum pH 11 in order to accurately measure NH₄⁺ using the probe. The calibrated probe was inserted into the beaker and the concentration was recorded. The control solution and blank were analyzed after each sample to check accuracy of probe operation. All results are reported as NH₄⁺, μmol/g on an as-is basis.

The concentration of sulfide (**S²⁻**) in manure was analyzed using a S²⁻ probe (Table 2.2; Thermo Orion Meter 290A+, probe #9616, Thermo Fisher Scientific Inc. Minneapolis, MN) that was calibrated using a standard curve. The concentrated sodium

sulfide solution was titrated immediately before use to determine its concentration due to the instability of the sodium sulfide crystals. Using the procedure described by Trabue and Kerr (2014), 2 g of each manure sample was weighed into a 100 mL beaker and 38 mL of de-aerated nanopure water along with 40 mL of sulfide antioxidant buffer solution (Orion 941609, Thermo Fisher Scientific, Inc. Minneapolis, MN) were added. The mixture was maintained at pH 12 in order to accurately measure S^{2-} using the probe. The calibrated probe was inserted into the beaker solution and the S^{2-} concentration was recorded. The control solution and blank were analyzed after each sample to check accuracy of probe operation. All results are reported as S^{2-} , $\mu\text{mol/g}$ on an as-is basis.

Manure Foaming Index

All samples of manure were analyzed for MFC index based on the method adapted from Yan et al. (2014), which utilized an air stone to create fine sized and stable air bubbles (Table 2.2). Briefly, a 25 mL manure sample was placed in a graduated cylinder (inner diameter = 2.54 cm), and nitrogen gas (N_2) was pumped into the cylinder bottom through an air stone at a constant flow rate of 100 mL/min to produce air bubbles until a steady state height was reached. Manure foaming capability index was calculated as the height of foam produced divided by the initial foam level. This index was used to indicate the maximum foaming potential of manure samples. Once aeration ceased, the recession time was recorded when the final height of foam returned to its initial level. This measurement was used to indicate the stability of manure samples without further gas production. Each manure sample was measured in duplicate.

Statistical Analysis

The experiment was designed as a 3×2 factorial arrangement of treatments within a randomized complete block design, with the individual pig or manure storage container serving as the experimental unit. With dietary 6 treatments and a total of 24 pigs utilized for each group, there were 4 replicates per treatment per group, resulting in a total of 8 replicates per treatment. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), with individual pig as a random effect, and group, diet composition, particle size, and their interaction as fixed effects. Results are reported as least squares means, and comparisons among treatments were performed using the PDIFF option of SAS with the Tukey-Kramer adjustment for multiple comparisons. Treatment effects were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

Group Effect

Pigs in group 1 were housed at a similar effective environmental temperature (18.2 °C with 23.3% relative humidity) compared with pigs in group 2 (18.6 °C with 21.3% relative humidity). Pigs in group 1 had greater initial and final BW than pigs in group 2. However, there were no differences in nutrient excretion and manure foaming index between the two groups. Moreover, there was no interaction between group and diet composition or diet particle size for nutrient excretion and manure foaming index. As a result, only the main effects of diet composition, particle size, and their interaction will be discussed.

Nutrient Digestibility and Excretion

Dry Matter

Adding DDGS or SBH to diets reduced DM digestibility ($P < 0.01$), which was due to similar DM intake ($P = 0.07$) and increased DM excretion ($P < 0.01$; Table 2.3). Other studies have also reported that feeding diets containing DDGS (Spiehs et al., 2012; Trabue and Kerr, 2014) or SBH (Kornegay, 1978; Kornegay, 1981; DeCamp et al., 2001) increased DM excretion compared with feeding CSB diets. Served studies (Pagilla et al., 1997; Brown and Sale, 2002; Kougias et al., 2013a) reported that increased organic matter loading in anaerobic digestion (AD) systems can cause foaming because excess of DM cannot be fully degraded by the bacteria during fermentation. In the present study, the increased DM excretion caused by feeding SBH-coarse diets increased MFC while feeding DDGS diets did not. Therefore, the difference in specific nutrient excretion should be considered rather than just considering overall DM excretion.

Neutral Detergent Fiber

Pigs fed DDGS or SBH had greater NDF excretion ($P < 0.01$) compared with pigs fed CSB, which was a result of greater NDF intake (Table 2.3). Soybean hulls are a source of both soluble (15%, DM basis) and insoluble (65%, DM basis) fiber for swine (Schweizer and Wüsch, 1979), while DDGS contains mainly insoluble fiber (86% to 94% of total dietary fiber; Urriola et al., 2010). The difference between DDGS and SBH in fiber solubility may contribute to different hydrophobicity or hydrophilicity in the manure (Domb et al., 1996). Studies (Bindal et al., 2002; Binks and Horozov, 2005) have shown that hydrophobic particles had very different mode of action compared to hydrophilic particles when serve as foam stabilizers. Therefore, the difference between

DDGS and SBH in fiber composition may be a nutritional connection related to manure foaming incidence.

Ether Extract

There was an interaction ($P = 0.01$) between diet composition and particle size on EE digestibility (Table 2.3). Diet composition did not affect EE digestibility among pigs fed fine diets, but pigs fed SBH had greater EE digestibility than pigs fed CSB ($P = 0.01$) among pigs fed coarse diets. Overall, pigs fed fine diets had greater EE digestibility than pigs fed coarse diets ($P < 0.01$). This is expected due to soybean oil supplementation in SBH diets (Table 2.1), which is consistent with results reported by Kim et al. (2013) who found that ATTD of intact oil from corn and DDGS is less than in extracted soybean oil. In addition, the digestibility of added soybean oil would not be expected to be improved from diet particle size reduction. Therefore, the similar EE digestibility observed among pigs fed fine diets may be explained by the increased EE digestibility of intact oil in CSB diets during particle size reduction. In the present study, pigs fed DDGS diets had the highest EE excretion ($P < 0.01$) due to relatively high diet EE content and similar EE digestibility compared with pigs fed CSB and SBH. Studies (Boe et al., 2012; Yan et al., 2014) have suggested that lipids and long-chain fatty acids (LCFAs) may serve as foam surfactants. As a result, excess lipid excretion may contribute to manure foaming.

Nitrogen

Pigs fed DDGS had similar N utilization compared to pigs fed CSB or DDGS diets (Table 2.4). However, pigs fed DDGS had greater N excretion ($P < 0.01$) compared with pigs fed SBH or DDGS, which was due to greater CP content in DDGS and greater N

intake ($P < 0.01$) for pigs fed DDGS diets compared with CSB or SBH diets (Table 2.1 and Table 2.4). In addition, AA digestibility in DDGS may also be about 10% less than for corn due to the production of indigestible Maillard products caused by high temperatures used during the ethanol and DDGS production processes (Cromwell et al., 1993; Almeida et al., 2013), and lysine is particularly susceptible to Maillard reactions due to its free amino group (Pahm et al., 2008; Almeida et al., 2013). As a result, formulating swine diets containing DDGS on a digestible AA basis results in greater CP and N content than in CSB diets, and excess dietary N is excreted in manure. In fact, studies (Spiehs et al., 2012; Trabue and Kerr, 2014) have reported increased N excretion and manure NH_4^+ concentration when pigs fed diets containing DDGS. As expected; greater manure N and NH_4^+ concentrations were observed from pigs fed DDGS diets compared with those fed CSB or SBH diets ($P < 0.01$; Table 2.5). Studies (Powers et al., 2008; Carter et al., 2012) reported that emissions of ammonia (NH_3) were largely increased for pigs fed diets containing DDGS compared with those without DDGS supplementation. In contrast, other studies (Spiehs et al., 2012; Trabue and Kerr, 2014) reported that feeding DDGS diets had no effect on manure NH_3 emission or even reduced manure NH_3 emission, regardless of increased manure NH_4^+ concentration. Trabue and Kerr (2014) indicated that the most likely mechanism for the reduced gas emission rates is the increased surface crusting in the manure from pigs fed DDGS diets. In fact, bio-covers have been shown to effectively reduced NH_3 emissions from lagoons by over 50% (Zahn et al., 2001). In the present study, we observed manure surface crusting from pigs fed DDGS diets. Therefore, a lower manure NH_3 emission from pigs fed DDGS diets

would be expected regardless of increased manure NH_4^+ concentration.

Sulfur

Pigs fed DDGS diets had reduced S utilization compared to pigs fed CSB or SBH ($P < 0.01$), which was due to greater S content in DDGS diets, increased S intake ($P < 0.01$), and increased S excretion ($P < 0.01$) in pigs fed DDGS diets (Table 2.1 and Table 2.4). Corn DDGS contains relatively high concentrations of sulfur-containing AAs (Met, Cys, and Tau) and total S (Song et al., 2013; Hanson et al., 2015). Song et al. (2013) found similar results when pigs were fed DDGS diets, which resulted in reduced S utilization and increased S excretion, but increased S retention. As a result, greater S and S^{2-} concentration was observed in the manure from pigs fed DDGS in the present study ($P < 0.01$; Table 2.5). Similar to the manure NH_3 emission, feeding DDGS diets had resulted in inconsistent responses H_2S emission, regardless of increased manure S and S^{2-} concentration (Powers et al., 2008; Carter et al., 2012; Spiels et al., 2012; Trabue and Kerr, 2014). Consequently, the most likely mechanism for the significant difference in emission rates is the increased surface crusting in the DDGS manures restricting H_2S emissions (Trabue and Kerr, 2014). In the present study, we also observed large manure surface crusting from pigs fed DDGS diets. Therefore, a lower manure H_2S emission from pigs fed DDGS diets would be expected regardless of increased manure S^{2-} concentration.

Particle Size

Pigs fed the finely ground diets had greater DM ($P < 0.01$), EE ($P < 0.01$), N ($P = 0.04$), and NDF digestibility ($P = 0.04$) compared with pigs fed coarse diets, which was

a result of reduced fecal DM ($P < 0.01$), EE, ($P < 0.01$), N ($P < 0.01$), and NDF excretion ($P < 0.01$; Table 2.3 and Table 2.4). Wondra et al. (1995a) reported that DM and N excretion were reduced by 20% and 24%, respectively, as particle size of corn was reduced from 1000 μm to 700 μm . In addition, grinding of DDGS from 517 μm to 383 μm increased the ATTD of NDF from 40.2% to 45.3% (Yáñez et al., 2011). Therefore, greater N and NH_4^+ concentration were observed in manure from pigs fed coarse diets compared with pigs fed fine diets ($P < 0.01$; Table 2.5). However, particle size did not affect S utilization and excretion (Table 2.4), and S and S^{2-} concentrations (Table 2.5) were similar among manure samples from pigs fed diets with different particle sizes.

Manure Foaming Characteristics

Greater MFC ($P = 0.01$) and foam recession time ($P < 0.01$) were observed for pigs fed the coarse SBH diet compared with pigs fed the fine SBH and fine CSB diets, but not for manure from pigs fed the fine or coarse DDGS (Table 2.5). The theory of “froth flotation” was first introduced by Leja (1982) and suggested that foam formation in wastewater generally requires three components: gas, surfactants, and stabilizers. First, biogases are produced as a result of the decomposition of organic matter by microbial activity in the manure or manually injected. Then, gas bubbles reach the air-liquid interface, where surfactants can decrease the surface tension of manure. Finally, gases are trapped in foam at the interface, which is stabilized by small particles. If all three phases occur simultaneously, a stable foaming status can be reached. In the present study, N_2 was provided during the first phase of foam formation and MFC was recorded after a stable foaming status was reached.

Studies (Boe et al., 2012; Yan et al., 2014) have suggested that lipids and long-chain fatty acids (LCFAs) may serve as surfactants during the second phase of foam formation. Boe et al. (2012) found that a higher level of sodium-oleate was associated with higher foaming potential in a manure AD system. Yan et al. (2014) reported that the addition of corn oil (mainly linoleic acid) to the swine manure induced foam formation only after 1 week of storage. Moreover, swine manure collected from foaming pits had greater palmitoleic acid, stearic acid, oleic acid, linoleic acid and total LCFAs concentrations than manure collected from non-foaming pits (Yan et al., 2014). This difference was especially significant in the air-liquid interface of foaming manure (Yan et al., 2014). Davis et al. (2015) found that pigs fed diets containing 30% DDGS had similar linoleic acid digestibility compared with a CSB control diet (97.6 vs. 98.2%, respectively). However, the linoleic acid content in the DDGS diets is greater than the linoleic acid content in CSB diets (2.78 vs. 1.69%, respectively; Davis et al., 2015), which may result in greater linoleic acid excretion into manure, allowing it to serve as a surfactant. However, increased lipid excretion from pigs fed DDGS diets did not increase MFC in the present study. This may have been due to inadequate stabilizers in manure. Petrovski et al. (2011) reported that unstable foam (rapidly collapse) was only generated in the absence of foam stabilizers.

Several research groups (Bindal et al., 2002; Binks and Horozov, 2005; Blute et al., 2007) have investigated the effectiveness of small particles (both hydrophobic and hydrophilic) as foam stabilizers during the third phase of foam formation. Binks and Horozov (2005) suggested that small hydrophobic particles can aggregate and increase

the viscosity of liquids, resulting in slower liquid flow from the foam layer and lower incidence of foam collapse and coalescence. Bindal et al. (2002) found that foam formation was increased with a greater concentration of smaller hydrophilic particles (increasing particle size from 8 to 100 nm decreased the final foam height from 550 to 15%). It has been proposed that the self-layering characteristics of small hydrophilic particles inside the foam layer provides a structural barrier against the foam coalescence (Bindal et al., 2002). In addition, Blute et al. (2007) found that liquid with reduced particle size (from 40 to 6 nm) had higher potential of foam formation after providing a constant air flow. Van Weelden et al. (2015) measured the particle size of manure samples from the present study and reported that the average particle sizes of manure was only significantly impacted by the particle size of the diet. As a result, the manure from pigs fed finely ground diets had greater small particles to large particles ratio compared with those fed coarsely ground diets. However, pigs fed coarsely ground diets had greater DM and nutrients excretion in manure with those fed finely ground diets (Table 2.3 and Table 2.4), resulting in overall greater concentration of particles in the manure. Based on the result of MFC, the reduced DM and nutrients excretion caused by feeding coarsely ground diets had a greater impact on MFC even with greater average particle size in the manure.

In the present study, lower manure pH was observed when pigs were fed SBH compared with other diets ($P < 0.01$), and when they were fed coarse diets compared with fine diets ($P = 0.03$; Table 2.5). Other studies (Canh et al., 1998; Kerr et al., 2006; Le et al., 2008) have shown that the pH of manure decreased from an average of 9.0 to 7.0

when pigs were fed diets containing greater fermentable fiber content (e.g. soybean hull, sugar beet pulp). Blute et al. (2007) reported that wastewater with lower pH (from 10.0 to 3.4) had greater potential for foam formation because foam stabilizers are more likely preventing foam from collapsing in lower pH. Therefore, the relatively low pH of manure caused by feeding SBH-coarse diet may be one of the potential contributing factors to manure foaming.

In conclusion, MFC was impacted by diet particle size and fiber source. Greater MFC and recession time were only observed for pigs fed the SBH-coarse diet compared with the other dietary treatments, suggesting that fiber composition in SBH has a greater effect on MFC than fiber in DDGS. In addition, DM excretion and manure pH were two significant factors that contributed to MFC, but there was no clear association between EE, N, S excretion and MFC. These results suggest that diet formulation strategies to maximize dry matter digestibility (e.g. reducing diet particle size) and reduce DM excretion, along with minimizing the amount of coarsely ground SBH in diets will decrease manure foaming incidence in anaerobic manure pit in swine barns.

Table 2.1. Ingredient composition, calculated and analyzed chemical concentration (DM basis), and geometrical mean particle size of experimental diets containing corn distillers dried grains with solubles (DDGS) or soybean hulls

Item	Dietary treatment ¹			
	CSB	DDGS	SBH	
Ingredient, as-fed basis, %				
Corn	79.72	62.50	57.34	
Soybean hulls	-	-	20.75	
Soybean meal (48% CP)	18.00	-	16.80	
Soybean oil	0.30	-	3.32	
DDGS	-	35.10	-	
Limestone	0.87	1.15	0.60	
Monocalcium phosphate (21% P)	0.41	0.10	0.49	
Sodium chloride	0.35	0.35	0.35	
Vitamin mix ²	0.20	0.20	0.20	
Trace mineral mix ³	0.15	0.15	0.15	
L-Lys-HCl	-	0.39	-	
L-Thr	-	0.03	-	
L-Trp	-	0.03	-	
Calculated chemical composition				
ME, kcal/kg	3,750	3,742	3,734	
NDF, %	9.86	21.35	21.35	
N, %	2.74	2.73	2.72	
Lys, %	0.83	0.87	0.87	
SID Lys, %	0.70	0.69	0.71	
SID Met+Cys, %	0.51	0.52	0.46	
SID Thr	0.51	0.46	0.50	
SID Trp	0.17	0.12	0.16	
Analyzed chemical composition				
GE, kcal/kg	4,283	4,511	4,411	
Ether extract, %	4.63	6.21	6.79	
NDF, %	7.16	13.74	19.97	
N, %	2.61	2.76	2.62	
S, %	0.21	0.24	0.21	
Geometrical mean particle size, μm	364	615	352	603 408 675

¹CSB = corn - soybean meal diet, DDGS = corn - distiller's dried grains with solubles diet, SBH = corn - soybean meal - soybean hulls diet.

²The vitamin premix provided the following quantities of vitamins per kg of complete diet: vitamin A, 3,062.5 IU; vitamin D3, 350 IU; vitamin E, 25 IU; vitamin K, 1.5 mg; vitamin B12, 0.025 mg; riboflavin, 1.5 mg; niacin, 28 mg; and pantothenic acid, 13.5 mg.

³The trace mineral premix provided the following quantities of micro-minerals per kg of complete diet: Cu (as CuSO_4), 1.1%; Fe (as FeSO_4), 11.0%; I (as $\text{Ca}(\text{IO}_3)_2$), 200 ppm; Mn (as MnSO_4), 2.6%; Zn (as ZnSO_4), 11.0%; and Se (as Na_2SeO_3), 200 ppm.

Table 2.2. Reference procedures for analyses of diets, feces, urine, and manure

Measurement	Method
GE ¹	Isoperibol bomb calorimeter (Model 1281; Parr Instrument Co., Moline, IL)
DM ¹	AOAC (2005) official method 934.01
Ether extract ¹	AOAC (2005) official method 920.39 (A), petroleum ether
NDF ¹	ANKOM Tech. method 13
N ¹	VarioMAX CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany), AOAC (2005) official method 972.43
S ¹	VarioMAX CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany), AOAC (2005) official method 972.43
Diet particle size ¹	13 half-height sieve shaker (Tyler RoTap, Mentor, OH), Baker and Herrman, (2002)
Manure NH ₄ ⁺ ¹	Trabue and Kerr (2014), NH ₄ ⁺ probe (Thermo Orion Meter 290A+, probe #9512, Thermo Fisher Scientific Inc. Minneapolis, MN)
Manure S ²⁻¹	Trabue and Kerr (2014), S ² probe (Thermo Orion Meter 290A+, probe #9616, Thermo Fisher Scientific Inc. Minneapolis, MN)
Manure pH ¹	pH meter (Corning Model 530 with Corning probe #476436, Corning Inc., Corning, NY)
Manure foaming characteristics ²	Yan et al. (2014)

¹Analyzed by USDA-ARS, Ames, IA.

²Analyzed by University of Minnesota, Saint Paul, MN.

Table 2.3. Apparent total tract digestibility and energy balance (DM basis) of pigs fed diets containing corn and soybean meal (CSB), corn and distillers dried grains with solubles (DDGS), and CSB with soybean hulls (SBH) that were ground to fine ($374 \pm 29 \mu\text{m}$) or coarse ($631 \pm 35 \mu\text{m}$) particle size

Dietary treatment ¹	Diet composition × particle size						Diet composition			Particle size		SEM	P-value		
	CSB -fine	DDGS -fine	SBH -fine	CSB -coarse	DDGS -coarse	SBH -coarse	CSB	DDGS	SBH	Fine	Coarse	Pooled	DC×PS ²	DC	PS
No. pigs	8	8	8	8	8	8	16	16	16	24	24	-	-	-	-
Energy															
GE, kcal/kg	4,278	4,502	4,395	4,287	4,519	4,427	4,255	4,507	4,441	4,395	4,407	26	0.59	0.32	<0.01
DE, kcal/kg	3,949	3,993	3,864	3,796	3,835	3,798	3,848	3,912	3,857	3,939 ^a	3,807 ^b	32	0.69	0.53	<0.01
ME, kcal/kg	3,824	3,853	3,760	3,672	3,703	3,688	3,724	3,776	3,750	3,816 ^a	3,684 ^b				
DM															
Intake, g/d	2,640	2,538	2,388	2,441	2,631	2,372	2,540	2,584	2,380	2,522	2,481	58	0.28	0.07	0.58
Fecal excretion, g/d	210	275	278	251	375	312	230 ^a	325 ^b	295 ^b	254 ^a	313 ^b	16	0.34	<0.01	<0.01
Digestibility, %	92	89	88	90	86	87	91 ^a	87 ^b	87 ^b	90 ^a	87 ^b	1.19	0.65	<0.01	<0.01
NDF															
Intake, g/d	187	344	466	176	365	484	182 ^a	355 ^b	475 ^c	333	342	10	0.54	<0.01	0.49
Fecal excretion, g/d	57	93	87	66	135	92	62 ^a	11 ^b	89 ^b	79 ^a	98 ^b	6.61	0.16	<0.01	0.03
Digestibility, %	70	73	81	62	63	81	66 ^a	68 ^a	81 ^b	74 ^a	69 ^b	5.90	0.30	<0.01	0.04
Ether extract															
Intake, g/d	124	158	164	111	163	159	118 ^a	160 ^b	161 ^b	149	144	3.68	0.32	<0.01	0.37
Fecal excretion, g/d	30	48	35	55	70	55	43 ^a	59 ^b	45 ^a	38 ^a	60 ^b	2.19	0.74	<0.01	<0.01
Digestibility, %	76 ^a	70 ^{ad}	78 ^a	50 ^b	57 ^{bc}	65 ^{cd}	63 ^a	63 ^a	71 ^b	74 ^a	57 ^b	2.29	0.01	<0.01	<0.01

¹CSB = corn - soybean meal diet, DDGS = corn - distiller's dried grains with solubles diet, SBH = corn - soybean meal - soybean hulls diet.

²DC × PS = interaction effect between diet composition (DC) and particle size (PS).

^{abcd} Values within a row of diets composition × particle size, treatments, or diets with different superscripts are different ($P < 0.05$).

Table 2.4. Nitrogen and sulfur balance (DM basis) of pigs fed diets containing corn and soybean meal (CSB), corn and distillers dried grains with solubles (DDGS), and CSB with soybean hulls (SBH) that were ground to fine ($374 \pm 29 \mu\text{m}$) or coarse ($631 \pm 35 \mu\text{m}$) particle size

Dietary treatment ¹	Diet composition × particle size						Diet composition			Particle size		SEM	P-value		
	CSB -fine	DDGS -fine	SBH -fine	CSB -coarse	DDGS -coarse	SBH -coarse	CSB	DDGS	SBH	Fine	Coarse	Pooled	DC×PS ²	DC	PS
No. pigs	8	8	8	8	8	8	16	16	16	24	24	-	-	-	-
N															
Intake, g/d	67.96	69.39	61.54	64.67	73.51	63.16	66.32 ^a	71.45 ^b	62.35 ^a	66.30	67.11	0.01	0.30	<0.01	0.68
Fecal excretion, g/d	6.65	8.83	11.06	8.27	12.01	12.21	7.46 ^a	10.42 ^b	11.64 ^b	8.85 ^a	10.83 ^b	0.17	0.50	<0.01	<0.01
Digestibility, %	90.21	87.12	81.71	87.23	83.71	80.69	88.72 ^a	85.42 ^a	81.20 ^b	86.35 ^a	83.87 ^b	0.88	0.67	<0.01	0.04
Urine excretion, g/d	29.34	29.62	18.20	27.60	29.46	21.00	28.47 ^a	29.54 ^a	19.60 ^b	25.72	26.02	0.36	0.32	<0.01	0.81
Net utilization ³ , %	47.03	44.42	52.00	44.74	43.71	46.34	45.89	44.07	49.17	47.82	44.93	0.36	0.65	0.18	0.20
S															
Intake, g/d	5.38	5.73	4.88	5.17	6.26	5.29	5.28 ^a	5.99 ^b	5.09 ^a	5.33	5.57	0.01	0.13	<0.01	0.13
Fecal excretion, g/d	1.03	1.19	1.38	1.13	1.48	1.45	1.08 ^a	1.34 ^b	1.42 ^b	1.20	1.35	0.10	0.47	<0.01	0.07
Digestibility, %	80.82	79.06	71.19	78.28	76.38	72.43	79.55 ^a	77.72 ^a	71.81 ^b	77.02	75.69	0.95	0.52	<0.01	0.41
Urine excretion, g/d	1.95 ^{ad}	2.52 ^b	1.02 ^c	1.54 ^{ae}	2.29 ^{bd}	1.34 ^{ce}	1.75 ^a	2.41 ^b	1.18 ^c	1.83	1.73	0.91	<0.01	<0.01	0.24
Net utilization ³ , %	67.96	69.39	61.54	64.67	73.51	63.16	46.34 ^a	37.64 ^b	47.93 ^a	42.49	45.45	0.57	0.86	<0.01	0.22

¹CSB = corn - soybean meal diet, DDGS = corn - distiller's dried grains with solubles diet, SBH = corn - soybean meal - soybean hulls diet.

²DC × PS = interaction effect between diet composition (DC) and particle size (PS).

³Net nutrient utilization, % = (intake - feces excretion - urine excretion) / intake × 100%.

^{abcde} Values within a row of diets composition × particle size, treatments, or diets with different superscripts are different ($P < 0.05$).

Table 2.5. Characteristics (as-is basis) of manure from pigs fed diets containing corn and soybean meal (CSB), corn and distillers dried grains with solubles (DDGS), and CSB with soybean hulls (SBH) that were ground to fine ($374 \pm 29 \mu\text{m}$) or coarse ($631 \pm 35 \mu\text{m}$) particle size

Dietary treatment ¹	Diets composition \times particle size						Diets composition			Particle size		SEM	<i>P</i> -value		
	CSB -fine	DDGS -fine	SBH -fine	CSB -coarse	DDGS -coarse	SBH -coarse	CSB	DDGS	SBH	Fine	Coarse	Pooled	DC \times PS ²	DC	PS
No. pigs	8	8	8	8	8	8	16	16	16	24	24	-	-	-	-
Chemical composition,															
N, %	0.41	0.55	0.48	0.44	0.68	0.57	0.43 ^a	0.62 ^b	0.53 ^c	0.48 ^a	0.56 ^b	0.02	0.14	<0.01	<0.01
S, %	0.092	0.089	0.074	0.093	0.099	0.076	0.092 ^a	0.094 ^a	0.075 ^b	0.085	0.089	0.003	0.53	<0.01	0.18
NH ₄ ⁺ , $\mu\text{mol/g}$	346	412	312	367	475	377	357	443 ^b	344 ^a	357 ^a	406 ^b	13	0.37	<0.01	<0.01
S ²⁻ , $\mu\text{mol/g}$	0.32	0.48	0.32	0.33	0.5	0.39	0.33 ^a	0.49 ^b	0.35 ^a	0.37	0.41	0.02	0.64	<0.01	0.25
pH	8.15	8.17	7.83	8.15	7.83	7.62	8.15 ^a	8.00 ^a	7.72 ^b	8.05 ^a	7.87 ^b	0.07	0.24	<0.01	0.03
Foaming index															
MFC ³	44 ^a	130 ^{abc}	91 ^{ab}	154 ^{bc}	124 ^{abc}	186 ^c	99	127	139	88 ^a	155 ^b	18	0.01	0.14	<0.01
Recession time, s ⁴	6 ^a	27 ^{abc}	19 ^{ab}	37 ^{bc}	24 ^{abc}	41 ^c	21	26	30	17 ^a	34 ^b	5	<0.01	0.23	<0.01

¹CSB = corn - soybean meal diet, DDGS = corn - distiller's dried grains with solubles diet, SBH = corn - soybean meal - soybean hulls diet.

²DC \times PS = interaction effect between diet composition (DC) and particle size (PS).

³The sample was aerated through a graduated cylindrical air stone at a constant flow rate of 100 mL/min to produce air bubbles until a steady state height was reached.

Manure foaming capability index was calculated as the height of foam produced divided by the initial foam level.

⁴Once aeration ceased, the recession time was recorded when the final height of foam returned to its initial level.

^{abc} Values within a column with different superscripts are different ($P < 0.05$).

Chapter 3

Effect of oil content of distillers dried grains with solubles on nutrient digestibility, metabolizable energy, and manure foaming capability of growing pigs

SUMMARY

Extraction of oil content in corn distillers dried grains with solubles (DDGS) may affect nutrient excretion and accumulation of foam in manure deep pits as well as decrease ME content for pigs. The objective of this experiment was to measure nutrient digestibility, ME, and manure foaming capability (MFC) of pigs fed 3 diets mixed with DDGS differing in amount of acid hydrolyzed ether extract (AEE). One group of 32 growing gilts (initial BW = 107.7 ± 9.0 kg) were placed into metabolism crates and allotted randomly to 1 of 4 diets (8 replicates/treatment). Dietary treatments were fed for 18-d and consisted of 1) corn-soybean meal based diet (3.4% AEE; CON), 2) CON + 40% low oil DDGS (6.2% AEE), 3) CON + 40% medium oil DDGS (6.5% AEE), and 4) CON + 40% high oil DDGS (7.7% AEE). Diets were fed for 10-d adaptation period and 4-d collection period, where feces and urine were collected and mixed fresh and injected with N₂ in a column to measure MFC. A subset of feces and urine samples were mixed with HCl to estimate apparent total tract digestibility (ATTD) of CP, NDF, and AEE, as well as DE and ME. Data were analyzed using the MIXED procedure of SAS, with individual pig as a random effect and diet composition as fixed effects. Intake of AEE of pigs fed diets with DDGS was greater ($P < 0.01$) than CON, but there were no differences ($P > 0.10$) in excretion of AEE. Excretion of NDF in feces of pigs fed the low (259 g/d), medium (227 g/d), and high (217 g/d) oil DDGS diets were greater ($P < 0.01$) than CON

(121 g/d). Excretion of CP in low (124 g/d), medium (117 g/d), and high (145 g/d) oil DDGS diets was greater ($P < 0.01$) than CON (84). In spite of differences in excretion of NDF and CP when diets contained DDGS, there were no differences in MFC in fresh feces from pigs fed these diets. The DE:GE tended to be greater ($P < 0.09$) in high oil DDGS (69%) than in low oil DDGS (63%), but there were no differences in DE or ME content among low, medium, or high oil DDGS sources. In conclusion, excretion of NDF and CP, but not AEE increased in feces of pigs fed diets with DDGS compared with CON. Greater excretion of CP and NDF does not increase the likelihood of foaming in fresh manure when pigs are fed diets with DDGS.

Key words: corn distillers dried grains with solubles, growing pigs, lipid, manure foaming, nutrient excretion.

INTRODUCTION

Use of alternative feed ingredients decreases diet cost and improves nutritional efficiency of swine feeding programs (Woyengo et al., 2014). However, feeding alternative ingredients often results in manure containing increased concentrations of indigestible nutrients, and variability in nutrient and energy content and digestibility often limits the dietary inclusion levels of alternative feed ingredients. Foam accumulation on manure at the surface of anaerobic deep pits in commercial swine farms has been a significant problem in recent years (Theisen, 2009; Vansickle, 2010).

The causes are unknown, but some have suggested that nutritional modifications may alter nutrient excretion and increase the risk of manure foaming (Jacobson et al., 2013; Van Weelden et al., 2015). Corn distillers dried grains with solubles (DDGS) has 3 times more NDF content compared with corn (NRC, 2012) which has a negative impact on digestibility of other nutrients such as proteins and lipids (Kass et al., 1980; Bach Knudsen and Hansen, 1991; Chen et al., 2013). As a result, more nutrients are excreted into manure which may increase the likelihood of manure pit foaming.

Ethanol plants are extracting oil from thin stillage prior to manufacturing DDGS and consequently variability in nutrient and ME content among sources increased (Kerr et al., 2013). This variable ME content affects growth performance of growing pigs (Wu et al., 2016). We hypothesized that oil content in DDGS may alter manure chemical composition and result in a greater risk of manure foaming. We also hypothesized that less oil content in corn DDGS may decrease ME content in DDGS. Therefore, the

objective of this study was to determine if nutrient excretion, manure foaming capability (**MFC**), and ME content differs among pigs fed DDGS diets with increasing lipid content.

MATERIALS AND METHODS

Animal Management

The experimental protocol used in this study were reviewed and approved by the Institutional Animal Care and Use Committee at University of Minnesota (St. Paul, MN). This experiment was conducted over two 18-d periods at University of Minnesota - Research and Outreach Center (Waseca, MN). One group of 32 gilts (initial BW = 107.7 ± 9.0 kg), which were the offspring of sows (Landrace × Yorkshire; Genetically Advanced Pigs, Winnipeg, Manitoba) mated to Duroc boars (Compart's Boar Store, Nicollet, MN), were moved into an environmentally controlled room and placed individually in metabolism crates (1.2 × 2.4 m) that allowed for separate collection of feces and urine. Ambient room temperature was maintained at about 18.3 °C, and lighting was provided from 700 to 1800. Crates were equipped with a stainless steel feeder and a nipple drinker to provide pigs with *ad libitum* access to water.

Feed Ingredients and Diets

Gilts were allotted randomly to 1 of 4 diets, resulting in 8 replicates for pigs per treatment. Three sources of DDGS with different oil content, including: 1) low oil DDGS (5.7 % ether extract; **EE**, DM basis), 2) medium oil DDGS (8.2% EE), and 3) high oil DDGS (12.4 % EE), were selected for this study (Table 3.6). Dietary treatments consisted of 1) corn-soybean meal based control diet (**CON**), 2) CON + 40% low oil DDGS (**LO**),

3) CON + 40% medium oil DDGS (**MO**), and 4) CON + 40% high oil DDGS (**HO**) and were formulated to meet NRC requirements (Table 3.7). All diets were fed in meal form and contained 0.5% TiO₂ as a digestibility marker used to calculate apparent total tract digestibility (**ATTD**) of nutrients. The distribution and standard deviation of the size of feed particles were analyzed using ANSI/ASAE Standards (2008) method S319.4. The actual geometrical mean particle size for each diet was 660 ± 3.9 µm (CON), 600 ± 3.0 µm (CO), 530 ± 3.25 µm (MO), and 890 ± 3.2 µm (HO; Table 3.7). Pigs were fed twice daily (0800 and 1600) a total amount of feed equivalent to 3.0% of their initial BW for 18 days, which approximated *ad libitum* feed intake for pigs at this BW. Actual feed disappearance was calculated as the difference between the amounts of feed added minus the amount of feed not consumed.

Sample Collection

During the d-15 to d-18 collection period, stainless steel wire screens and stainless steel buckets containing 30 mL of 6 N HCl were placed under each metabolism crate allowing collection of urine from each pig. Fresh feces were collected at the time of excretion and placed into plastic bags. Feces and 5% of urine were collected twice daily during each meal, weighed, and stored at -20 °C until the end of the experiment. Feces from each pig were pooled, dried in forced air oven at 60° C, weighed, ground to pass through a 1 mm screen (Wiley No. 4 Laboratory Mill, Arthur H Thomas Co., Philadelphia, PA), and mixed to obtain a representative sample for analysis. Energy and nutrient digestibility and excretion were calculated by using this portion of the feces. Likewise, urine samples were thawed, weighed, and pooled within pig to collect a

representative sample for analysis. Energy and nutrient balance was calculated by using this portion of the urine.

On the d-14, a small part of fresh fecal and urine output (without adding HCl) were collected from each pig, added to 32 individual plastic bottles, manually mixed to simulate fresh manure and stored at -20 °C until the end of all collections. The ratio of feces to urine was 0.20 for the control diet and 0.26 for DDGS diets, and was calculated based on daily fecal and urine output observed in pigs with fed similar dietary treatments by Spiehs et al. (2012). Manure foaming index was measured by using this part of manure samples.

Chemical Analysis and Calculations

Feed ingredient, diet, fecal, and urine samples were analyzed at Midwest Laboratories, Inc., Omaha, NE (Table 3.8). To determine DE and ME content, GE of feed ingredient, diet, fecal, and urine samples were determined following standard method D5865-13 (ASTM, 2013). Feed ingredients, diets, feces and urine were analyzed following official methods of AOAC (2005) for DM (method 930.15), N (method 990.03), EE (method 945.16), and acid hydrolyzed ether extract (**AEE**; method 945.02). The concentration of NDF in feed ingredients, diets and feces was analyzed using ANKOM Tech. method 13. The ash content of samples were determined by AOAC (2005) official method 942.05. The concentration of titanium in diets and feces was analyzed by Wavelength Dispersive X-ray Fluorescence (Jenkins, 2006).

Apparent total tract digestibility of energy, DM, N, AEE, and NDF of each diet was calculated using the index method described by Adeola, (2001). Energy and nutrient

digestibility of diets were calculated using equations:

$$\text{Energy digestibility, \%} = 100 - 100 \times \left(\frac{\% \text{ index compound in feed} \times \text{GE of feces}}{\% \text{ index compound in feces} \times \text{GE of feed}} \right)$$

$$\text{Nutrient digestibility, \%} = 100 - 100 \times \left(\frac{\% \text{ index compound in feed} \times \% \text{ nutrient in feces}}{\% \text{ index compound in feces} \times \% \text{ nutrient in feed}} \right)$$

Apparent total tract digestibility of each DDGS source was calculated using the procedure described by Adeola (2001). Energy and nutrient digestibility of feed ingredients were calculated using equation:

$$\text{Energy or nutrient digestibility, \%} = 100 \times \left[\frac{(T \times t) - (B \times b)}{a} \right];$$

where T is the digestibility of total diet, t is the amount of the component in the test diet consumed, B is the digestibility of the component in control diet, b is the amount of component in the control diet consumed, and a is the amount of the component in the test feed ingredient added to the control diet $t = b + a$. The digestibility of the control diet, B, and the test diet, T, were determined by the procedure of index method described above.

Manure Foaming Index

All samples of fresh manure were analyzed for MFC index based on the method adapted from Yan et al. (2014), which utilized an air stone to create fine sized and stable air bubbles (Table 3.8). Briefly, a 25 mL manure sample was placed in a graduated cylinder (inner diameter = 2.54 cm), and nitrogen gas (N₂) was pumped into the cylinder bottom through an air stone at a constant flow rate of 100 mL/min to produce air bubbles until a steady state height was reached. Manure foaming capability index was calculated as the height of foam produced divided by the initial foam level. This index was used to indicate the maximum foaming potential of manure samples. Once aeration ceased, the

recession time was recorded when the final height of foam returned to its initial level. This measurement was used to indicate the stability of manure samples without further gas production. Each manure sample was measured in duplicate.

Statistical Analysis

The experiment was designed as a completely randomized design, with the individual pig or manure container serving as the experimental unit. With 4 dietary treatments and 32 pigs, there were for 8 replicates per treatment. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), with individual pig as a random effect and diet composition as fixed effects. Results were reported as least squares means. Comparisons among treatments were performed using the PDIFF option of SAS with the Tukey-Kramer adjustment for multiple comparisons. Treatment effects were considered significant if $P < 0.05$, and trends are reported if $0.05 < P < 0.10$.

RESULTS AND DISCUSSION

Nutrient Composition

All pigs remained healthy during the study and consumed their respective daily ration. Thus, no data were removed from the experiment. The chemical composition of DDGS varied as expected for concentration of AEE, but also for other nutrients. The CV for the concentration of CP (3.8%) among sources of DDGS was less than EE (41.5%) and NDF (19.7%). The mean particle size of high oil DDGS (1,300 μm) was greater than medium oil (370 μm) and low oil (390 μm) DDGS. In agreement with the content of oil in DDGS, LO diet contained less EE (3.77%) than the MO (5.01%) and HO (7.75%)

diets, but the analyzed concentration of NDF was greater in the HO diet (19.20%) than in the MO (17.50%) and LO (17.80%) diets (Table 3.7).

The concentration of nutrients is an important first step toward correct characterization of the nutritional value of feed ingredients. Variability in nutrient concentration among sources of a feed ingredient is caused by multiple factors. Analytical differences among laboratories, differences in analytical procedures, and differences in sampling methods all contribute to differences in reported nutrient content (Kerr et al., 2013). The nutrient composition of corn and soybean meal used in this experiment are similar to those reported in NRC (2012). The concentration GE in low, medium, and high oil DDGS was within the range reported in Anderson et al. (2012), NRC (2012), and Kerr et al. (2013). However, the low oil DDGS in the current experiment had greater concentration of GE than the low oil DDGS evaluated by Anderson et al. (2012; 6%) and Kerr et al. (2013; 12%). The concentration N in low, medium, and high oil DDGS was within the range reported in Anderson et al. (2012), NRC (2012), and Kerr et al. (2013). The concentration of N and EE analyzed in the current experiment and for the experiment of Wu et al. (2016) were different because these samples were analyzed in 2 different laboratories, and may have also resulted from differences in sampling.

Intake, Apparent Total Tract Digestibility, and Excretion of Nutrients

Due to the controlled similar feed intake, there were no differences in DM intake among treatments. The ATTD of DM in CON was greater ($P < 0.05$) than all diets containing DDGS sources, and there were no differences ($P > 0.10$) among LO, MO, or

HO diets (Table 3.9). As a result, pigs fed the 3 DDGS diets had similar fecal DM excretion.

Nutrient intake, ATTD, and subsequent excretion of nutrients were different among the 4 diets (Table 3.9). Intake of N of pigs fed CON was less ($P < 0.01$) than LO, MO, and HO, while intake of N in HO was greater ($P < 0.01$) than LO and MO. The ATTD of N in CON, LO, and MO diets was not different, but the ATTD of N in HO was less ($P < 0.05$) than all other diets. Therefore, the fecal excretion of N was less ($P < 0.05$) in CON than all DDGS diets. There were no differences in fecal excretion of N in LO and MO, but as result of less ATTD of N, the fecal excretion of N in HO was greater ($P < 0.05$) than all other diets. The excretion of N in urine in pigs fed CON was less ($P < 0.05$) than all DDGS diets, and there were no differences among DDGS diets.

The ATTD of AEE was less in CON than all 3 diets containing DDGS (Table 3.9). Apparent total tract digestibility of AEE of the HO diet was greater ($P < 0.01$) than the MO diet, but not different from the LO diet. Despite differences in ATTD of AEE, excretion of AEE was not different among pigs fed diets with DDGS of variable concentration of AEE.

As expected, the intake of NDF of pigs fed the LO, MO, HO diets was greater ($P < 0.05$) than CON, while the intake of NDF of pigs fed HO diet was greater ($P < 0.05$) than MO diet, but not different from LO diet (Table 3.9). The ATTD of NDF was less ($P < 0.05$) in CON than HO, but not different from LO and MO. Therefore, fecal excretion of NDF was less in CON than LO, MO, and HO and there were no differences ($P > 0.10$) among LO, MO, or HO.

The differences in intake, ATTD, and excretion of nutrients among LO, MO, and HO diets was the result of differences in nutrient content among sources of DDGS. There were no differences in calculated ATTD of DM among low, medium, or high oil DDGS (Table 3.10). Similar to the ATTD of nutrients in diets, the ATTD of N in high oil DDGS was less ($P < 0.01$) than all other DDGS. The ATTD of AEE was less ($P < 0.05$) in medium oil DDGS than high oil DDGS, and there were no differences between low and high oil DDGS. The ATTD of AEE was less ($P < 0.01$) in high oil DDGS than low and medium oil DDGS and there were no differences between low and medium oil DDGS. There were no differences in calculated ATTD of NDF among low, medium, or high oil DDGS.

There are multiple modes of action attributed to fiber decreasing ATTD of protein, including increasing endogenous N losses and reducing protein digestion (Schulze et al., 1994). Studies from Kass et al. (1980), Sauer et al. (1991) and Chen et al. (2013) reported that diets containing increased fiber content reduce ATTD of protein. Microorganisms in the gut of pigs retain more N with increasing fiber intake (Cummings et al., 1976; Sauer et al., 1980). When fiber intake increases microbes in the large intestine of growing pigs will utilize fiber a source of microbial energy, if fiber intake is low, microbes will utilize amino acids as energy producing ammonia. This ammonia can be absorbed in the large intestine and decrease calculated ATTD of N. Therefore, it is possible that diets with greater concentration of NDF can have less ATTD of N. Kerr et al. (2013) reported the NDF content and the ATTD of N from 15 DDGS sources. We calculated the correlation between the concentration of NDF and ATTD of N. We observed that the ATTD of N

decreased ($r = -0.51$) as NDF content of DDGS increased among all 15 DDGS sources. Similar result was found in the present study, the ATTD of N in the high oil DDGS, which had the highest NDF content, was less than other DDGS sources. In addition, the variation in ATTD of N may be caused by the difference in nutrient content of corn sources used to produce DDGS as well as differenced in the drying processes of ethanol plants which may increase the production of Maillard by-products (Cromwell et al., 1993; Almeida et al., 2013).

We observed no differences in ATTD of AEE between low and high oil DDGS. The reason is not clear, but data in the literature suggest 2 factors. First, this experiment measure ATTD of AEE, while ATTD is a direct measurement of digestibility of nutrients it doesn't take into account the effect that endogenous losses of fat have on calculated digestibility values. A true digestibility system that corrects for endogenous fat losses will decrease the impact of endogenous losses. When feeding multiple levels and sources of fat, Kil et al. (2010) observed that ATTD of AEE was greater as fat inclusion on the diet increased, but true total tract digestibility was not different. Therefore, we suspect that true digestibility of the high oil DDGS could be less than observed in our experiment. Also, the high oil DDGS source obtained in this experiment contained greater concentration of NDF than low and medium oil DDGS. Similar to the ATTD of N, we estimated that the ATTD of EE decreased as NDF content of DDGS increased ($r = -0.46$) after correlation analysis using data from Kerr et al. (2013). Therefore, the concentration of NDF and fat affects the final ATTD of AEE in our experiment in two opposite ways. This combination effect result in similar ATTD of AEE between low oil and high oil

DDGS.

Manure Foaming Capability

Despite of changes in nutrient digestibility and excretion (amount and composition), we did not observe any changes in MFC in manure from pigs fed the CON, LO, MO, or HO diets (Table 3.11). No foam accumulated during air injection and subsequently, the values of manure foaming capability and recession time were below the detection range indicating 0% change in values.

The absence of manure foaming, either foaming capability or recession time, is an unexpected observation and it is contrary to our hypothesis. The microbial ecology and chemical composition changes during the anaerobic fermentation process of manure, producing compounds that increase manure foaming. In commercial pork production facilities, feces and urine are continually added to deep pits and fermentation is a continuous process over many months. The samples of manure in the current experiment were a mixture of fresh urine and feces collected daily during 3 days, but were frozen immediately after collection and minimal fermentation occurred. The absence of fermentation products may have resulted in no detectable differences in MFC. Bio-surfactants are substances produced by yeast or bacteria during metabolic microbial activity in manure, which can also decrease the surface tension of liquid as surfactants do (Lin, 1996). Heard et al. (2008) found that substances produced by filamentous *Gordonia* spp. can serve as bio-surfactants for foam formation in wastewater. In addition, filamentous *Gordonia* spp. itself can serve as stabilizers to prevent foam from collapsing. Westlund et al. (1998) found that *Microthrix parvicella* sp. had similar gas trapping effect

which can also stabilize the foam. In addition, Kougiyas et al. (2014) analyzed microbial ecology changes in dairy manure anaerobic digestion system before and after foaming appearance in different conditions, including protein-rich, lipid-rich, and carbohydrate-rich substrates. After foaming appearance, a significant increase in the plate counts of microbes was observed for *Streptococcus* sp. (1134%), *Thermoactinomyces* sp. (347%), and *Paenibacillus* sp. (331%) in protein-rich substrate, *Thermoactinomyces* sp. (totally absent before foaming appearance), *Pseudomonas* sp. (51%), *Dialister* sp. (36%), and *Thermotoga* sp. (29%) in lipid-rich substrate, and *Micrococcus* sp. (1838%), *Thermotoga* sp. (150%), *Lactobacillus* sp. (65%), *Bacillus* sp. (78%) and *Pseudonocardia* sp. (26%) in carbohydrate-rich substrate. For swine manure, significant differences in average microbial community richness between foaming and non-foaming in swine deep manure pits has been observed (Pepple et al., 2012; Gates, 2013). Van Weelden et al. (2015) also found that the difference of microbial community structure was caused by difference in fiber composition within the swine manure. However, no published data were available for specific microbial species changes in swine deep manure pits. These results indicate that the absence of MFC in the present study was due to a lack of production of products from long-term anaerobic fermentation which are necessary for manure foam production and accumulation.

Energy Balance

There were no differences in the concentration of DE or ME among low, medium, and high oil DDGS in the present study (Table 3.10). However, low oil DDGS tended ($P < 0.10$) to have less DE/GE than medium and high oil DDGS, likewise, the ME/GE

tended to be less ($P = 0.10$) in low oil DDGS than medium and high oil DDGS. These observations are in agreement with Anderson et al. (2012), NRC (2012), and Kerr et al. (2013). The difference in ME concentration between low oil and high oil DDGS was reported less than 50 kcal/kg in NRC (2012). In addition, Anderson et al. (2012) reported ME values of 7 DDGS sources with oil content from 3.15 to 11.98% EE, DM basis. The sample with least concentration of oil (3.15%) had ME concentration of 3,650 kcal/kg DM and this value was not different than other sources of DDGS with greater oil content. Kerr et al. (2013) also reported ME values of 15 sources of DDGS with oil content ranging from 4.88 to 11.83% EE, DM basis. The sample with least concentration of oil (4.88%) had a ME concentration of 3,289 kcal/kg DM and this value was greater than ME in other sources of DDGS with greater content of oil. Therefore, Kerr et al. (2013) concluded that the difference in ME values did not correspond to the EE concentrations of DDGS sources. However, we observed a numerically lower concentration of ME in the low oil DDGS, and this lower concentration of ME resulted in a slightly reduced gain to feed ratio of growing pigs fed the same low oil DDGS source compared with feeding the high oil DDGS source (Wu et al., 2016). It is possible that low oil DDGS in the current experiment had less ME than high oil DDGS, but the precision of detecting this difference requires more replication due to the relatively large standard error of measurement.

The reason that greater oil content in DDGS may not provide greater ME concentration compared with ME content of low oil DDGS may be a result of differences in the content, digestibility, and utilization of other nutrients such as N and NDF. In the

present study, the ME provided by extra oil content may be decreased by the lowest N utilization in pigs fed HO, or the highest NDF content in high oil DDGS source, or both (Table 3.9). This was expected because Kerr et al. (2013) suggested that dietary fiber is the most important variable in determining the DE or ME content of DDGS with different EE content in growing pigs. Urriola et al., (2010) also suggested that the difference in fiber content and digestibility may contribute to differences in the digestibility of energy in DDGS. Particle size is another important variable to effect DE or ME content in DDGS (Kerr et al., 2013). In the present study, the geometrical mean particle size of high oil DDGS ($1300 \pm 1.9 \mu\text{m}$) was more than 2 times greater compared with low oil ($390 \pm 2.0 \mu\text{m}$) and medium oil DDGS ($370 \pm 2.2 \mu\text{m}$). Liu et al. (2012) reported that the ME contribution of DDGS to the diet is increased by 13.6 kcal/kg DM for each 25 μm decrease in DDGS particle size from 818 to 308 μm . Based on this prediction, the effect of particle size on ME contribution of DDGS would be more than 400 kcal/kg DM. Therefore, the reduced ME due to large particle size may decreased the ME content despite the greater oil content.

In conclusion, fermentation of excreted nutrients in deep manure pits has greater importance in the manure foaming process than the amount or type of nutrients excreted. Changes in diet formulation have minimal effect of manure foaming in swine barns without the effect of manure fermentation. Oil content in DDGS is not the only factor that affects the concentration of DE and ME. Therefore, the differences in particle size and ATTD of other nutrients must be considered in order to accurately predict the DE or ME concentration in DDGS.

Table 3.6. Analyzed chemical concentration (DM basis) and geometrical mean particle size of corn, soybean meal, and corn distillers dried grains with solubles (DDGS) of low, medium, and high oil content analyzed for the present study and analyzed for Wu et al. (2016)¹

Item	Feed ingredients ¹				
	Corn	Soybean meal	Low oil DDGS	Medium oil DDGS	High oil DDGS
The present study					
Analyzed chemical composition					
GE, kcal/kg	4,400	4,700	5,410	4,950	5,250
N, %	1.4	8.6	5.4	5.3	5.0
Ether extract, %	3.8	1.3	5.7	8.2	12.4
Acid hydrolyzed ether extract, %	4.7	3.3	9.6	10.5	14.4
NDF, %	8.0	8.1	28.1	29.0	37.9
Ash, %	1.7	7.8	5.0	4.8	5.1
Geometrical mean and SD of particle size, μm	700 \pm 4.2	1000 \pm 1.2	390 \pm 2.0	370 \pm 2.2	1300 \pm 1.9
Wu et al. (2016)					
Analyzed chemical composition					
N, %			5.5	5.3	5.1
Ether extract, %			6.6	11.0	15.8
NDF, %			31.7	33.3	44.8
Geometrical mean particle size, μm			410	350	900

¹The same samples of DDGS were used in the present study and in Wu et al. (2016).

Table 3.7. Ingredient composition, calculated and analyzed chemical concentration (DM basis), and geometrical mean particle size of experimental diets mixed with corn distillers dried grains with solubles (DDGS) of variable oil content

Item	Dietary treatment ¹			
	CON	LO	MO	HO
Ingredient composition, as-fed basis, %				
Corn	79.79	47.17	47.17	47.17
Soybean meal	17.86	10.56	10.56	10.56
Low oil DDGS	-	40.00	-	-
Medium oil DDGS	-	-	40.00	-
High oil DDGS	-	-	-	40.00
Titanium dioxide	0.05	0.05	0.05	0.05
Dicalcium phosphate	0.65	-	-	-
Limestone	0.92	1.57	1.57	1.57
Sodium chloride	0.30	0.30	0.30	0.30
Vitamin and trace mineral premix ²	0.25	0.25	0.25	0.25
L-Lys HCl	0.18	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00
Calculated chemical composition				
ME, kcal/kg	3,763	3,750	3,749	3,737
N, %	2.62	3.74	3.68	3.58
Lys, %	0.95	1.02	1.03	0.99
SID Lys, %	0.83	0.77	0.79	0.75
SID Met+Cys, %	0.45	0.66	0.65	0.66
SID Thr	0.48	0.65	0.64	0.63
SID Trp	0.16	0.17	0.17	0.16
Ether extract, %	3.44	4.65	6.44	8.37
NDF, %	9.85	18.52	19.15	23.88
Analyzed chemical composition				
GE, kcal/kg	4,340	4,620	4,590	4,740
N, %	2.82	3.97	3.50	3.62
Ether extract, %	2.66	3.77	5.01	7.75
Acid hydrolyzed ether extract, %	3.41	6.19	6.54	7.68
NDF, %	8.50	17.80	17.50	19.20
Geometrical mean particle size, μm	660 \pm 3.9	600 \pm 3.0	530 \pm 3.3	890 \pm 3.2

¹CON = corn - soybean meal diet. LO = CON + 40% low oil DDGS. MO = CON + 40% medium oil DDGS. HO = CON + 40% high oil DDGS.

²The premix supplied the following nutrients per kilogram of diet: 11,023 IU of vitamin A as retinyl acetate; 2,756 IU of vitamin D₃; 22 IU of vitamin E as dl-alpha tocopheryl acetate; 4.41 mg of vitamin K as menadione dimethylpyrimidinol bisulfite;

9.92 mg of riboflavin; 55.11 mg of niacin; 33.07 mg of pantothenic acid (as D-calcium pantothenate); 992 mg of choline (as choline chloride); 0.06 mg of vitamin B₁₂; 14.3 mg of pyridoxine; 1.65 mg of folic acid; 2.20 mg of thiamine; 0.33 mg of biotin; 2.20 mg of I (as ethylenediamine dihydroiodide); 0.30 mg of Se (as Na₂SeO₃); 299 mg of Zn (as ZnSO₄); 299 mg of Fe (as FeSO₄); 19.8 mg of Cu (as CuSO₄); and 17.6 mg of Mn (as MnO).

Table 3.8. Reference procedures for analyses of ingredients, diets, feces, and urine

Measurement	Method
GE ¹	ASTM (2013) standard method D5865-13
DM ¹	AOAC (2005) official method 930.15
N ¹	AOAC (2005) official method 990.03
Ether extract ¹	AOAC (2005) official method 945.16
Acid hydrolysis ether extract ¹	AOAC (2005) official method 945.02
NDF ¹	ANKOM Tech. method No. 13
Ash ¹	AOAC (2005) official method 942.05
Titanium ¹	Wavelength Dispersive X-ray Fluorescence (Jenkins, 2006)
Particle size ¹	ANSI/ASAE Standards (2008) S319.4
Foaming characteristics ²	Yan et al. (2014)

¹Analyzed by Midwest Laboratories, Inc., Omaha, NE.

²Analyzed by University of Minnesota, Saint Paul, MN.

Table 3.9. Apparent total tract digestibility and energy balance (DM basis) of pigs fed corn and soybean meal diets supplemented with 40% corn distillers dried grains with solubles (DDGS) with different oil content

Item	Diet composition ¹				SEM	P
	CON	LO	MO	HO		
No. pigs	8	8	8	8	-	-
DM						
Intake, g/d	2,398	2,526	2,424	2,383	52	0.23
Feces excretion, g/d	135 ^a	217 ^b	186 ^b	195 ^b	10	< 0.01
Digestibility, %	94 ^a	91 ^b	92 ^b	92 ^b	0.42	< 0.01
N						
Intake, g/d	68 ^a	100 ^b	85 ^c	86 ^c	1.60	< 0.01
Feces excretion, g/d	13 ^a	20 ^b	19 ^b	23 ^c	0.78	< 0.01
Digestibility, %	80 ^a	80 ^a	78 ^a	73 ^b	0.73	< 0.01
Urine excretion, g/d	24 ^a	39 ^b	37 ^b	38 ^b	2.24	< 0.01
Net utilization ² , %	45 ^a	42 ^a	35 ^{ab}	29 ^b	2.63	< 0.01
Acid hydrolysis ether extract						
Intake, g/d	82 ^a	156 ^b	159 ^b	183 ^c	2.88	< 0.01
Feces excretion, g/d	55	56	61	58	2.43	0.33
Digestibility, %	32 ^a	64 ^{bc}	61 ^b	68 ^c	1.19	< 0.01
NDF						
Intake, g/d	203 ^a	450 ^{bc}	424 ^b	458 ^c	7.35	< 0.01
Feces excretion, g/d	121 ^a	259 ^b	227 ^b	217 ^b	14	< 0.01
Digestibility, %	40 ^x	42 ^{xy}	46 ^{xy}	53 ^y	3.40	0.08
Energy						
GE, kcal/kg	4,340	4,620	4,590	4,740	-	-
DE, kcal/kg	3,515	3,442	3,418	3,521	35	0.11
ME, kcal/kg	3,422 ^x	3,278 ^y	3,287 ^y	3,367 ^{xy}	40	0.05
DE/GE, %	81 ^a	75 ^b	74 ^b	74 ^b	0.76	< 0.01
ME/DE, %	97.3 ^a	95.5 ^b	96.0 ^b	95.6 ^b	0.30	< 0.01
ME/GE, %	79 ^a	71 ^b	72 ^b	71 ^b	0.82	< 0.01

¹CON = corn - soybean meal diet. LO = CON + 40% low oil DDGS. MO = CON + 40% medium oil DDGS. HO = CON + 40% high oil DDGS.

²Net utilization = (intake - feces excretion - urine excretion) / intake.

^{abc}Values within a row with different superscripts are different (P < 0.05).

^{xy}Values within a row with different superscripts are considered to have a trend (0.05 < P < 0.10).

Table 3.10. Apparent total tract digestibility and energy content (DM basis) of corn distillers dried grains with solubles (DDGS) with different oil content.

Item	DDGS sources			SEM	P
	Low oil	Medium oil	High oil		
No. pigs	8	8	8	-	-
DM					
Digestibility, %	87	89	88	1.14	0.43
N					
Digestibility, %	80 ^a	76 ^a	67 ^b	1.45	< 0.01
Net utilization ¹ , %	40 ^a	25 ^{ab}	16 ^b	5.96	0.03
Acid hydrolysis ether extract					
Digestibility, %	80 ^{ab}	74 ^a	81 ^b	1.61	0.02
NDF					
Digestibility, %	43	49	57	4.97	0.17
Energy					
GE, kcal/kg	5,410	4,950	5,250	-	-
DE, kcal/kg	3,410	3,349	3,606	96	0.16
ME, kcal/kg	3,140	3,161	3,360	112	0.30
DE/GE, %	63 ^x	68 ^{xy}	69 ^y	1.84	0.09
ME/DE, %	93	94	93	0.90	0.59
ME/GE, %	58 ^x	64 ^y	64 ^y	2.15	0.10

¹Net utilization = (intake - feces excretion - urine excretion) / intake.

^{ab}Values within a row with different superscripts are different (P < 0.05).

^{xy}Values within a row with different superscripts are considered to have a trend (0.05 < P < 0.10).

Table 3.11. Characteristics (as-is basis) of manure from pigs fed corn and soybean meal diets supplemented with corn distillers dried grains with solubles (DDGS) with different oil content.

Item	Diet composition ¹				SEM	<i>P</i>
	CON	LO	MO	HO		
No. pigs	8	8	8	8	-	-
Manure foaming capability, % ²	0	0	0	0	-	-
Recession time, s ³	0	0	0	0	-	-

¹CON = corn - soybean meal diet. LO = CON + 40% low oil DDGS. MO = CON + 40% medium oil DDGS. HO = CON + 40% high oil DDGS.

²The sample was aerated through a graduated cylindrical air stone at a constant flow rate of 100 mL/min to produce air bubbles until a steady state height was reached. Manure foaming capability index was calculated as the height of foam produced divided by the initial foam level.

³Once aeration ceased, the recession time was recorded when the final height of foam returned to its initial level.

Overall summary

In the U.S. pork industry, feed represents the largest proportion of the total production cost. Therefore, pork producers are continually trying to improve feed efficiency and reduce feed cost. This has become more important because the prices of conventional feed ingredients, including corn and soybean meal, has increased dramatically in recent years. As a result, the addition of increased amount of high fiber ingredients such as corn distillers dried grains with solubles (DDGS) and soybean hulls (SBH) to swine diets, and processing diets to achieve a smaller particle size have become common practices. However, manure foam accumulation on the surface of anaerobic deep pits on commercial swine farms has been a coincidental problem in recent years, and the potential cause has been associated with changes in diet composition. As a result, a better understanding of the connection between swine diet composition and nutrient excretion and manure foaming is critical for developing long-term mitigation strategies.

Based on the theory of “froth flotation”, foam formation requires 3 contributing factors, including biogas production, surfactants, and stabilizers, which may come from undigested nutrients in the manure. Results from studies have suggested that specific undigested nutrients in the manure may serve as surfactants (lipids and long-chain fatty acids; LCFAs) and stabilizers (small particles) as well as substrates to produce biogas (fiber and protein) in foam formation. This evidence suggest that adding DDGS and SBH in swine diets may increase overall manure output due to higher levels of indigestible fiber, resulting in more dry matter (DM) and nutrients in manure. In addition, smaller particle size of diets may result in a greater concentration of stabilizers in the manure. As

a result, methods to maximize feed digestibility and minimize nutrient excretion appear to be essential for reducing manure pit foaming. The research described in this thesis addressed the effects of feeding alternative feed ingredients (DDGS with variable oil content and SBH) with different particle size on nutrient excretion and manure characteristics of growing pigs.

Results in Chapter 2 suggested that manure foaming capability (MFC) was impacted by diet particle size and fiber source. Greater MFC were only observed for pigs fed coarsely ground SBH diet. The relatively high concentration of soluble fiber in the soybean hulls diet compared with DDGS, which contains a high concentration of insoluble fiber, appears to have a greater effect on MFC. In addition, increased DM excretion and changes in manure pH resulting from diet composition were 2 significant factors that contributed to MFC.

Results in Chapter 3 suggested that manure foaming does not occur when evaluating fresh manure samples, indicating that microbial fermentation is essential for manure foam formation. Further studies are encouraged to compare the microbial ecology between samples of foaming manure and non-foaming manure.

In conclusion, when diets are coarsely ground, manure from pigs fed diets containing significant amounts of soluble fiber source tend to have greater MFC after fermentation. These results suggest that diet formulation strategies to maximize DM digestibility and reduce DM excretion by reducing diet particle size, along with minimizing the amount coarsely ground SBH in diets will decrease manure foaming incidence in anaerobic manure pits.

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