ABSTRACT

WEIFANG ZHANG. Effects of Dietary Fiber and Other Nutrients on Swine Manure Odorants. (Under the direction of Dr. Eric van Heugten).

Three trials were designed to measure the effects of dietary fiber on protein and gross energy (GE) digestion as well as excretion of odor compounds. In trial 1, guar gum was chosen as a water-soluble non-starch polysaccharide (NSP) rich ingredient and was incorporated in the diet at 0% (control), 2%, 4%, and 8%. The results showed that dietary guar gum supplementation impaired growth performance of pigs and decreased nutrients digestibility. Based on the increased fecal short-chain fatty acids (SCFA) concentration and increased dimethyldisulfide and dimethyltrifide emissions from aged manure samples, dietary guar gum supplementation may increase manure odor. The results of odor intensity and manure hedonic score evaluation by a professional panel also indicated that dietary guar gum aggravated the manure odor problem.

The second trial was designed to measure the effects of diet and genotype on nutrient digestion and manure odor. The result indicated that a diet formulated to 2005 standards improved nutrient digestibility compared to a diet formulated to 1980 standards, while the effects of genotype (old vs. modern) were not significant. An excess of nutrients in the 2005 diet tended to increase N excretion through urine and increased manure odor intensity.

The third trial tested the effects of low fiber and soy hulls on nutrient digestion and odor compounds in the air. The data showed that the low fiber diet decreased body weight gain but fecal digestibility data showed that the CP and GE

digestibility of low fiber treatments were improved. Diets with added soy hulls induced the lowest CP and GE digestibilities and decreased ammonia emissions during the first 2 days of an in vitro ammonia assay. Total reduced sulfur (TRS) and short-chain fatty acid emissions were increased in the 10% soy hulls treatment. The odor detection threshold (ODT) evaluation indicated that adding fiber increased the odor intensity.

Overall, our data showed that dietary fiber will aggravated the odor problem in swine production though ammonia emission may be decreased.

Effects of Dietary Fiber and Other Nutrients on Swine Manure Odorants

by

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A thesis submitted to the Graduate Faculty of

North Carolina State University

In partial fulfillment of the requirements for the

degree of Master of Science

Animal Science

Raleigh

2007

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BIOGRAPHY

Weifang Zhang was born in Shangyu, Zhejiang, China, where she received her education before college. In 1994, Weifang was enrolled in the Department of Animal Science, China Agricultural University in Beijing, majoring in animal nutrition and feed technology. In 1998, upon graduation with a Bachelor of Science, Weifang worked briefly at a feed mill of Dabeinong LLC on product quality control. In 1999, Weifang was admitted to the Department of Animal Science, China Agricultural University in Beijing as a master candidate majoring in the swine nutrition. In 2002, upon graduation with a master's degree, Weifang worked briefly at Luke Biological Technology Institution, Beijing, where she worked as a nutritionist in the feed industry. In 2003, Weifang had a chance to study in animal nutrition under the direction of Dr. Theo van Kempen and Dr. Eric van Heugten at North Carolina State University, USA.

ACKNOWLEDGMENTS

I would first like to thank Dr. Eric van Heugten and Dr. Theo van Kempen for granting me the opportunity to investigate an area of swine nutrition & odor research. I have always been interested in odorants analysis during the past few years. You have given me the structured freedom that has allowed me to develop as a person and researcher. I am grateful to North Carolina State University for admitting me as a graduate student. It is with deep gratitude that I acknowledge Dr. van Heugten, Dr. Jerry Spears, Dr. Jack Odle, Dr. Vivek Fellner, and Dr. Theo van Kempen for their seemingly endless patience and invaluable guidance throughout my training, and for making this project possible and challenging. Especially, I would like to thank Dr. van Heugten for providing funding for this work. I am grateful to Dr. Jill Barnes, College of Veterinary Medicine, for serving as the graduate school representative on my committee.

I want to express my deep appreciation to Dr. Sanjay Shah, Department of Biological and Agricultural Engineering, for his wonderful lecture and his support on both of technology and facility sides. I am indebted to Dr. Jack Odle, Dr. Matthew Poore, and Dr. Vivek Fellner for their help with sample analysis in their labs. Also, many thanks to Dr. Lin Xi for his help in setting up methods on the GC/MS. A special thank-you to Dr. Peter Kai for his hard work on the building of the odor chambers.

For the help with sample collection I would like to thank: Lori Gatlin, Chris Lassiter, Jon Holt, Nitin Muley, Katie Sullivan, Christina Smith, Justin Fix, and Kyle Lovercamp. Many thanks to Elizabeth Harris, Preston Burnette, and Oulayvahn Phillips for all their technical assistance in the laboratory. And many thanks to SEU staff for their help during the animal trials.

Thanks to every faculty member, staff, and graduate student in the Department of Animal Science of North Carolina State University for making my study and life enjoyable. At last, thanks to NCPC for the financial support of my research project.

Special thanks to my parents Gaolin Zhang and Shuiqing Xie, for understanding and supporting my studying abroad, and my husband Rongsheng Qiu for his company and cheering my days up.

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Abstract:

It is well known that dietary fiber will impair the growth performance of monogastric animals. Compared to poultry, pigs have a higher capability of large gut fermentation, which is not significant if the level of non-starch polysaccharides (NSP) supplementation is not high. Recently, some researchers suggested that adding more NSP would decrease ammonia emission and possibly, attenuate odor problems. Also, quite a few trials showed no negative effect on growth performance even though NSP rich diets have a relatively lower digestibility.

Few studies measured the effects of NSP on other odorants besides ammonia, which may be because methodologies for odor evaluation are still being developed. Also, related research, including the mechanism of odorants production and emission both in the animal hindgut and the manure storage site, are almost non-existent. Therefore, more fundamental research still needs to be conducted before nutritional recommendations to reduce odor can be made to swine producers.

Introduction:

Manure odor is one of the main negative factors in swine production, especially for intensive pig production units. Livestock odors cause health problem directly or by producing stress and altering moods (Thu, 1997; Schiffman, 1998; Wing and Wolf, 2000), especially for those people working in waste handling. The main odorants could be listed as volatile fatty acids (VFAs), phenol, p-cresol, indole, skatole, diacetyl, and ammonia

(Williams, 1984; O'Neill and Phillips, 1992). Table 1 lists the characteristics of several odorants related to swine housing or lagoons (Zahn et al., 2001).

Diet modification is a potential strategy to reduce odor-causing agents in manure because feed is the original substrate for all odor compounds. On the source of nitrogen, it is accepted widely that reducing the dietary CP level and adding crystalline amino acids works well. But in regard to fiber, evaluations have been limited (van Kempen, 2001).

Fiber is resistant to the digestion by endogenous enzymes from mammalian hosts and it will decrease the digestibility of other nutrients in the feed (Ravindran et al., 1984; Lenis et al., 1996; Schrama et al., 1998) and increase endogenous protein and fat losses (De Lange et al., 1989; Noblet and Perez, 1993). Hotwood et al. (2004) reported the negative impact on ileal starch digestibility by dietary fiber addition. Dietary fiber, particularly soluble NSP, has high water binding capacity (Antoniou and Marquard, 1981; Armstrong et al., 1992; Jensen and Jørgensen, 1994; Bach Knudsen, 2001). Soluble NSP may hold more water in the colon, which stimulates microbial activity by increasing the surface area for the microbes (Noblet and Le Goff, 2001; Bach Knudsen, 2001) and extends the hindgut fermentation time. The apparent digestibility of nitrogen was significantly reduced by dietary inclusion of NSP rich ingredients (Just et al., 1983; Graham et al., 1986; Chabeauti et al., 1991; Mroz et al., 2000; Moeser and van Kempen, 2002). Further, NSP fermentation produces short chain fatty acids (SCFA) and will contribute to unpleasant odors (van Kempen, 2001). In addition, increased fecal bulk will be another negative effect induced by NSP rich ingredients (Moeser et al., 2002). Therefore, supplementation with fiber-rich ingredients will increase the burden for feed production and swine waste management. Our hypothesis is that reducing dietary NSP will decrease the odorant levels.

Dietary fiber in swine feed:

Cereal grains are the main ingredients in swine feed and these grains are predominantly composed of starch and NSP. NSP is the principal part of dietary fiber as it comprises 700 to 900 g/kg of the plant cell wall (Bach Knudsen, 1996) and it includes a mixture of substances such as cellulose, pectin, and hemicellulose. They are a diverse group of molecules with varying characteristics in water solubility, size and structure, which may influence feed digestion and GI tract microbial activity. Certain levels of dietary fiber (around 4%) seem essential to prevent GI tract health problems such as gastric ulceration and could alleviate diarrhea in weaning pigs (Lee and Close, 1987). Malathi et al. (2001) measured the NSP levels of the main feed ingredients in animal production. Compared with the 9.32% of total NSP in corn, the NSP level of soybean meal was as high as 29.02%. The NSP concentration in normal corn-soybean meal diets for growing pigs will be above 10%. Cereal by-products, which are mainly composed of plant cell wall, contain a much higher level of NSP compared with grain diets (Schrama et al., 1998; Bach Knudsen, 2001).

It was expected that almost all of the starch would be completely digested in the small intestine (Sambrook, 1979; Graham et al., 1986; Bach Knudsen and Hansen, 1991). However, some research also showed that around 10% of dietary corn starch reached the hindgut and was fermented there (Keys and DeBarthe, 1974; Gargallo and Zimmerman, 1981). Using an in vitro experiment of colonic fermentation showed that the cornstarch itself is a good substrate for butyrate production (Weaver et al., 1992). Physical form of the feed also is an important factor. Bird et al. (2000) fed brown rice or rice with rice bran diets to growing pigs and showed that excretion of feces of the brown rice group was significantly higher than the rice with rice bran group, though both of diets contained 3.3% of fiber. The possible reason is

the starch was physically covered by rice bran and escaped digestion though it is actually digestible.

Fiber fermentation:

Fiber will be extensively degraded in the hindgut because the microflora in the large intestine of pigs contains all of the predominant ruminal cellulose degrading bacteria (Varel and Yen, 1997). The degradability of the fiber components is quite different with lignin being totally undegradable (Glitsø, et al., 1999). Most of NSP will be degraded to SCFA and gases (H_2, CO_2, and CH_4) by microbes under anaerobic conditions (Cummings and Macfarlane, 1991). The major fermentation products are acetate, propionate, and butyrate, normally accounting for 90 to 95% of total fatty acids (Christensen et al., 1999). Different sources of fiber have different fermentation effects in the large intestine. Insoluble NSP will be less fermentable in the gut lumen, while soluble NSP including guar gum and pectin, will be much more fermentable because of their different rheological properties and chemical activity (Robertson and Eastwood, 1981). For example, soluble NSP will increase the viscosity of stomach contents (Johansen et al., 1996). Most water soluble NSP have been shown to be rapidly fermented in the cecum and proximal colon (Lu et al., 1995; Topping et al., 1997; Bach Knudsen, 2001; 1990). The net energy derived from SCFA contributed about 15 to 20% of the maintenance energy requirement in growing and finishing pigs (Dierick et al., 1989; Yen et al., 1991).

Some fermentative activity can occur in the small intestine though most fermentation occurs in the large bowel. The main fermentation site of beta-glucans (soluble NSP) may be before the cecum because it was reported that its recovery at the end of the ileum was as low as 25-36 % (Bach Knudsen and Hansen, 1991). The bacterial density is low in the stomach

and the upper half of the small intestine (Fuller et al., 1960). It increases rapidly to 10^7 to10^9 cfu/g in the distal part of the small intestine (Savage, 1977). Heijnen and Beynen (1997) reported that about 45 to 70% of retrograded starch (a type of resistant starch) was recovered in the ileal digesta. The result of their trial also indicated that digestion of resistant starch (RS) in the small intestine increased with age.

On the other hand, water insoluble fiber tends to have a greater influence on the distal part of the large colon than soluble fermentable fiber does. McIntyre et al. (1991) reported that wheat bran (contains high level of insoluble NSP) maintained a higher concentration of SCFA in the distal colon in rats than oat bran or guar gum. It also has been reported that wheat bran expedited the transit of digesta through the large intestine (Payler et al., 1975). Feeding insoluble NSP also can increase starch excretion in the feces (Key and Mathers, 1993; Young et al., 1996).

Is NSP a prebiotic?

Another reason to use fiber is that it may be beneficial to the animals' health, by acting as "functional oligosaccharides". People tend to assume such a function of dietary fiber from beneficial effects of dietary fiber to human heath especially in industrialized countries where people eat refined food with low NSP (Topping et al., 1993). A prebiotic is a non-digestible food ingredient that may benefit the host by selectively stimulating the growth and activity of one or limited number of bacteria in the colon, resulting in improved host health (Gibson and Roberfroid, 1995). The fermentation of dietary NSP by GI tract microflora is non-specific (Vervaeke et al., 1991). Dietary guar gum or cellulose supplementation increased all bacteria that were cultured including Lactobacilli, Enterococci, Clostridia etc. (Owusu-Asiedu et al., 2006). Berghouse et al. (1984) cultured the flora of terminal ileum content from ileostomists

who ate either refined or unrefined carbohydrate breakfasts. The result showed all the numbers of main genera of bacteria were increased. A trial with rats showed 3% or 6% fructo-oligosaccharides addition in the diet increased salmonella infection (Ten Bruggencate et al., 2003). On the other hand, a trial showed that fiber supplementation tended to increase beneficial bacteria such as Lactobacillus (Bikker et al., 2006). McDonald et al. (2001) showed that dietary fiber, especially high-viscosity fiber, significantly stimulated the growth of pathogenic E. coli. Gut morphology data showed pigs fed insoluble fiber rich diets were healthier than pigs fed soluble fiber rich diets (Hedemann et al., 2006). Data evaluating parasitic infection showed that pigs fed diets with insoluble NSP higher O. dentatum infection than the pigs fed soluble NSP (Petkevicius et al., 2001).

It seems that there are more than 400 different species of bacteria in the gut (Moore and Holdeman, 1974). The most abundant bacteria in the human hindgut are anaerobic bacteria such as Bacteroides, Eubacteriun, Peptostreptococcus, Enterococcus, Propionibacterium, Bifidobacterium, Lactobacillus, etc. (Bergman, 1990). On the other hand, there are some aerobic and facultative bacteria such as Escherichia coli and Clostridium perfringens which contain endotoxin. These different species display symbiotic and antagonistic relationships during their growth and metabolic processes. For example, Bacteroides thetaiotaomicron (a predominant member of the hindgut microflora) can control Ang4 expression that can kill other bacteria (Hooper et. al., 2003). It is reasonable that the end products of polysaccharide degradation by the out-membrane enzymes from Bacteroides thetaiotaomicron (Sonnenburg et al., 2005) also can be used by other bacteria even pathogens.

In humans, it is recommended to consume more fiber to prevent colon cancer, or some other related diseases. Jensen and Jorgensen (1994) found dietary fiber decreased the pH

value in the stomach and in the colon. The literature contains conflicting results about the relationship between the lower pH value and tumor development. It is widely accepted that lower pH will inhibit the activity of proteolytic bacteria, and reduce toxins, which are involved in carcinogenesis (Govers et al., 1999; Wenk, 2001). For example, the activity of coliform bacteria was inhibited at low pH (Wang et al., 2004). However, Jacobs and Lupton (1986) showed that an acidic luminal environment was actually associated with higher tumor development in the colon. They also found that especially dietary soluble fibers enhanced proximal colon carcinogenesis, though normally the distal colon is the site where cancer tends to develop. Therefore, the complex relationship between colon fermentation and microflora needs to be further researched for colon cancer protection (Wasan and Goodlad, 1996). Soluble fiber such as pectin and guar gum as well as RS have been reported to enhance tumor development and insoluble fiber such as wheat bran was protective against chemically induced carcinogenesis (Harris and Ferguson, 1999; Lu et al., 2000). Thus, the negative effect of soluble NSP may counteract the beneficial effects of insoluble NSP, which may be the reason why dietary fiber, consisting of both soluble and insoluble fiber, had no effect on colorectal cancer (Park et al., 2005).

For weaning pigs, Pluske et al. (1998) conducted a trial with guar gum supplementation and found that this rapidly fermentable carbohydrate actually increased the clinical expression of swine dysentery (SD) when the pigs were experimentally infected with Serpulina hyodysenteriae. Hopwood et al. (2004) compared the beta-haemolytic enterotoxigenic E. coli (ETEC) strains of intestinal swabs in weaning piglets and found that a high fiber diet significantly increased the proportion of ETEC. On the other hand, Mathew et

al. (1993) challenged weanling pigs with K88+ E. coli and found that including 1% galactan in the diet decreased the ratio of K88+ E. coli to total E. coli.

Two other trials also demonstrated that low soluble NSP diet could decrease expression of swine dysentery (Pluske et al., 1996; Siba et al., 1996). For growing pigs, typical feed contains enough fiber already to prevent "starving" of colon microbes, while in people the intake of insoluble dietary fiber may be extremely low. In Britain, a normal person excretes 100 to 200 g feces daily which equals 25 to 50 g dry matter output (Stephen and Cummings, 1980). While for a normal growing pig, around 75 kg body weight, daily fecal output may be as high as 600 to 1200 g, or 200 to 400 g of dry matter (Zhang et al., unpublished data). Corn and soybean meal based diets contain sufficient NSP (Bach Knudsen, 1996) plus non-digestible oligosaccharides (e.g. raffinose, stachyose), which are good substrate for SCFA production. In addition, starch residue at the terminal ileum is the main source of fermentable carbohydrate (Mason, 1984; Cumming and Englyst, 1987). The available substrate for GI tract bacteria will be sufficient under normal conditions, as there are even ample substrates for fermentation in the feces (Zhu et al., 1999), which actually contain lower levels of nutrients than colon digesta. Miller and Varel (2003) added protein, starch or cellulose to the manure from growing pigs fed corn-soybean meal diet, and showed that SCFA production of the control was not less than the other three groups.

Dietary fiber in swine nutrition:

NSP have long been known as antinutritional factors for monogastric animals, though some energy can be extracted by microbial fermentation of NSP (Dierick et al., 1989; Yen et al., 1991). Partridge et al. (1982) fed diets supplemented with 15% pure cellulose to growing

pigs and found that the increased feed intake in pigs fed cellulose did not improve growth performance, but decreased dressing percentage.

Soluble NSP induced more endogenous ileal protein loss than insoluble NSP (Libao-Mercado et al., 2006). For starter pigs, diets containing low levels of insoluble fiber showed higher digestibility and better growth performance than diets with low levels of soluble fiber (Högberg and Lindberg, 2006). Besides the urea trapping effect of dietary fermentation, the reduced ileal digestibility of CP itself also contributed to the decrease of urinary nitrogen excretion and increase of fecal nitrogen excretion (Mroz et al., 2000).

Moeser et al. (2002) did a series of experiments using low fiber diets. They replaced corn grain (NDF 9.6%) by degermed, dehulled corn (NDF 3.7%) and found the latter reduced fecal excretion significantly though the two treatments had the same feed intake. In that experiment, another treatment was a high fiber diet formulated by the addition of soybean hulls, and the extra fiber reduced nitrogen digestibility significantly.

For sows, dietary NSP is recommended, as it will decrease the rate of gastric emptying, and then lead to a prolonged feeling of satiety (Robert et al., 1993; Wang et al., 2004). So it will contribute to reduced hunger and improve welfare for sows, especially gestating sows (Robert et al., 1993; Martin and Edwards, 1994; Brouns et al., 1997). Unlike sows, growing pigs have no hunger problem by restricted feeding and energy intake is important for their optimum growth. Growing pigs are fed ad libitum which means the satiety feeling by fiber is not beneficial to them, and the digestibility of nutrients is significantly lower than sows (Noblet and Shi, 1992). Sows easily fermented almost all of the dietary sugar beet pulp, but to growing pig, the digestibility was only 60% of that for sows (Shi and Noblet, 1993). The possible reason is the hindgut fermentation capability is finite. Bacteria in the gut, as a whole,

will not increase unlimitedly. They will compete for substrate when the supply is short. However, when there is excessive substrate, the main conflict may shift to a water and space contest, product feedback, etc. It is reasonable to speculate that the situation for the microbe in distal part of colon is the latter. It can be referred that 20% of sugar-beet pulp exceeds the fermentation ability of the hindgut, because the weight of digesta in the distal part of colon for the 20% sugar-beet pulp (SBP) group was much higher than that of the control group (Jensen et al., 1995). The increased colon digesta weight may be because of the fermentation of some insoluble NSP was interfered with by the easily fermented SBP (mostly pectin), though part of the increased weight may come from the increased bacterial mass. SCFA absorption may be another limiting step of NSP fermentation. SCFA will accumulate in the hindgut as a result of an imbalance between absorption and production. This may be a greater issue in weaning pigs as more diet residue will reach the large intestine because their digestive system is not mature. It was shown that 5% guar gum supplementation significantly accumulated SCFA in the cecum and colon of weaning pigs (Pluske et al., 1998).

Normal levels of NSP will decrease the digesta transit time in the small intestine though the time which digesta stays in the large intestine is increased (Wenk, 2001). Therefore, NSP actually has a lower DE level as the small intestine is the main section where nutrients are absorbed. The soluble part of NSP tends to increase digesta viscosity and extents its transit time in the small intestine. It also decreases nutrient digestibility because the viscous properties inhibit nutrients from crossing the unstirred layer of digesta for absorption (Eastwood, 1992). It was reported that the conversion ratio of fermentable carbohydrate to digestible energy is approximately 70% (Livesey, 1990). Correspondingly, heat increment (Rijnen et al., 2003) and gaseous energy loss increased with increased microbial activity.

Also the increase in microbial mass and fecal excretion itself reduced net energy for the host (Livesey, 1992).

Furthermore, NSP fermentation will increase the energy cost of the GI tract itself. A good example is germfree rats, which had no NSP fermentation in the GI tract, had a lower metabolic load as the blood supply to the liver and intestine was lower than that of conventional rats (Wostmann et al., 1983). It can be referred that substrate for the hind gut microflora of growing pigs is much higher than that for sows. This implies that a high fiber diet would increase the GI tract burden because the digesta amount was increased (Jensen and Jørgensen, 1994). It is well known that dietary fiber tends to increase gut wall thickness. Because the high water binding capacity of NSP (Johansen et al, 1996), more volume of digesta would pass through the GI tract and the higher water activity is beneficial for bacterial growth (Jensen and Jørgensen, 1994). Increased lumen viable bacterial numbers and fermentation level will stimulate gut epithelia cells. Pell et al. (1995) found fermentable fiber stimulated hindgut wall proliferation of conventional rats, but not in the germ free group. Addition of 5% pectin increased small intestine length from 75.1 cm (fiber-free group) to 90.0 cm and the mucosal weight increased from 3.1 g to 4.1 g (Farness and Schneeman, 1982). Guar gum also increased the weight of the hindgut (Pluske et al., 1998). The energy cost of the increased GI tract mass could be high because its high turnover rate (Bakker et al., 1998).

NSP addition will impact the absorption of other nutrients (Mosenthin et al., 1994). For example, it was reported that guar gum supplementation decreased glucose absorption (Blackburn and Johnson, 1981; Johnson et al., 1984; Nunes and MalmlÖf, 1991). The main impact on protein utilization by dietary fiber is the increase in endogenous nitrogen losses.

During the past couple of decades, many investigations to research the effect of fiber on endogenous nitrogen losses have been reported (Souffrant, 2001).

Taken together, the energy efficiency from NSP fermentation is considerably lower than that from starch and is negligible for growing pigs (Noblet and Le Goff, 2001).

Effects of dietary fiber on ammonia emissions:

Although bacterial fermentation in pigs certainly contributes to the digestion of dietary fiber and endogenous materials, such microbial activity also produces odorants including ammonia, VFA, indoles, and phenols (Cummings and Englyst, 1987; Mackie et al., 1998; Le et al., 2005).

Most studies investigating fiber level and swine manure odor focused on ammonia only. The main principle of those studies is that dietary NSP could shift nitrogen from urine to feces in the form of bacterial protein (Mosentin et al., 1992; Younes et al., 1997; Canh et al., 1998; Kendall et al., 1999; Sutton et al., 1999; Zervas and Zijlstra, 2002) and the latter one is relatively more stable. For microflora in the large intestine, ammonia is a better nitrogen (N) source than amino acids for protein synthesis. They also need a carbon (C) source to match their requirement for energy and metabolic functions. NSP can act as an energy source for colon microbes as most of the digestible carbohydrates are absorbed in the upper small intestine. Produced SCFA will decrease the pH of the colon content, therefore, almost all of the ammonia in the colon will be in the ammonium state. Ammonia absorption into blood will subsequently be reduced and so does urea excretion in urine. A increased net flux of urea N from the blood to the intestine was reported to be enhanced by potato RS (Van der Meulen, 1997). Interestingly, the main source of endogenous urea excretion was not in the hindgut but in the upper GI tract (Mosenthin et al., 1992b). The possible reason is that the pH value of

the layer close to the hindgut wall is controlled strictly, though the lumen pH is more variable (Genz et al., 1999). Also, the main lysine synthesis site, which has nutritional effects for the host, is the small intestine (Torrallardona et al., 2003). The main site of endogenous urea N trapping by bacteria mass synthesis does not appear to match the main site of dietary fiber fermentation. According to the ileal dry matter flow data (Schulze et al., 1995) in growing pigs, almost all of the dietary NSP was passed through the small intestine because 20% of purified NDF supplementation induced ileal dry matter flow that was increased by 23.36 g/kg of DMI. Mosenthin et al. (1992a) infused [^{15}N] urea into the jugular vein of pigs which were fed either a low fiber diet (starch-SBM diet) or a high fiber diet (beet pulp-SBM diet). The results showed that ^{15}N excretion in urine was higher in the high fiber group (79.4% for starch vs 87.5% for beet pulp diet) though fecal ^{15}N excretion was also a slightly higher for the high fiber group (0.13% for starch diet vs 0.21 % for beet pulp diet). The possible reason for the lower ^{15}N recovery in the low fiber group (82.34 % for starch diet vs 90.81 % for beet pulp diet) within 144 hours could be that more infused [^{15}N] urea was retained in body tissues. These data indicated that plasma urea trapped by the NSP fermentation in the large intestine is a very small portion and the increased total fecal N excretion in high fiber diets may be caused by lower CP digestibility.

Higher dietary NSP fermentation will increase fecal SCFA and thereby reduce feces and manure pH (Canh et al., 1998). Rideout et al. (2004) added 5% of chicory inulin in a normal growing pig diet and found that fecal ammonia excretion was not changed although total fecal N increased. They also found that the fecal pH of the inulin group (6.89) was lower than that of the control group (7.08). Mroz et al. (2000) reported that 21% sugar beet pulp or 16% soy hulls increased fecal N, but without a reduction in urinary N. Shriver et al. (2003)

found that 10% soybean hulls or sugar-beet pulp addition did not change fecal or urinary N excretion in low CP, amino acid supplemented diets. High NSP diets induced higher distal colon digesta and water content (Eastwood, 1992; Jensen and Jørgensen, 1994) and are expected to produce more urease in the fresh feces. As high as 97% of urinary N is in the form of urea (McCrory and Hobbs, 2001) and fecal urease is the enzyme source contributing to urea hydrolysis. The increased urease from fresh feces may accelerate urea hydrolysis in swine manure slurry. Urease inhibitors work in reducing ammonia emissions (McCrory and Hobbs, 2001), therefore, enhanced urease by dietary NSP will actually increase ammonia emissions.

Effects of dietary fiber on manure odorants:

Certain important questions remain. Is lower ammonia emission equal to low odorants? Fiber is resistant to endogenous enzymes and can only be fermented by bacteria which produce various odorants. Kerr et al. (2006) reported that dietary cellulose increased manure DM, manure carbon, concentrations of propionic acid, butyric acid, and p-cresol though the concentration of ammonium in the manure was decreased. Although the digestibility of some NSP is as high as 50% (Noblet and Perez, 1993), the undigested proportion is large too (at least 50%). In addition, the higher fermentation means higher bacterial mass and their metabolites, which will increase fecal water content. High NSP diets may induce much more sticky manure and much dirtier pens (van Kempen, 2001). So, under field conditions, odor issues with high NSP diets is troublesome, as more fermentation will accrue when the feces has higher water content and the temperature is relatively high.

Studies suggest that the organic acid from fiber fermentation inhibits the growth of protein degrading bacteria (Wenk, 2001). If so, fermentable NSP will decrease the odorants

from protein fermentation. But, the metabolism of the hindgut microflora is not so simple. Even Lactobacillus and Bifidobacterium, usually were assumed as probiotics, also take part in indole and phenol production (Yokoyama and Carlson, 1979; Honeyfield and Carlson, 1990; Mackie et al., 1998). Thus, the gut microbes work together to degrade substrates and produce odor compounds. However, a study showed that the fecal isobutyric acid, isovaleric acid as well as other SCFAs was significantly increased by pea fiber and pectin supplementation (Jørgensen et al., 1996). Since the branched-chain fatty acids such as isobutyric acid and isovaleric acid are products from branched-chain amino acids, this indicates protein fermentation is increased but not inhibited. Biogenic amines production will also be stimulated by enhanced microbial activity (Veldman et al., 1993). Research on bacteroides fragilis and clostridium perfringens showed that production of some amines were increased under acidic conditions (Alission and Macfarlane, 1989), indicating that protein fermentation was increased, but not decreased, by NSP supplementation. Protein fermentation produces ammonia, amines, branched-chain fatty acids, indole, and phenol that smell worse than straight-chain SCFA by NSP fermentation. Increasing fiber fermentation in the large intestine can produce more toxic products, which will be absorbed and degraded in the liver and then excreted in urine (Spoelstra, 1977; Just et al., 1983).

Ammonia is not the main component that contributes to odor (Williams, 1984; Hobbs et al., 2000). Especially for pig farms that use the manure flush and lagoon treatment system, the ammonia issue is much less because its solubility in water is very high. Fermentable NSP addition to swine diets increased SCFA concentrations in swine manure (Canh et al., 1998; Sutton et al., 1999; Mroz et al., 2000; Shriver et al., 2003). Although such a SCFA increase will decrease ammonia problems, the SCFA per se are important odorants in swine manure.

Zahn et al (1997) showed airborne concentrations of SCFA were highest among other VOCs and strongly contribute to swine odor. However, as several SCFA with different smells are involved, the final contribution from SCFA to odor is variable. Generally, the longer chain fatty acids, e.g. butyric acid, smell worse than acetic acid, but the proportion of acetic acid is much higher in the profile. NSP fermentation will produce extra acetic acid, and beside SCFA, more lactic acid will be produced. These two acids actually have a somewhat pleasant smell. The result of a comprehensive trial showed that manure from pigs fed a low fiber diet tended to have a lower score for odor irritation, but the high fiber group was rated more pleasant than the other treatments (Johnson and van Kempen, unpublished data). Moeser et al. (2003) showed that compared to a corn-soybean meal diet and a high fiber diet, a purified diet (corn starch & casein) reduced irritation and intensity of manure odor. Manure odor pleasantness, was similar for the purified diet and the high fiber diet, while corn-soybean meal showed the worst result. A study evaluating low dietary CP level, which also means higher fiber, showed that the low CP diet decreased manure ammonia emission, while no differences in manure odor offensiveness were found (Otto et al., 2003).

SCFA will be further degraded or used as energy source by some bacteria to produce other odorants such as H_2S, indole, skatole etc. SCFA, indole, skatole, and p-cresol are important components contributing to swine manure odor (Spoelstra, 1977, 1980; Williams, 1984; Zhu et al., 1999; Jensen and Hansen, 2006). Skatole probably is a significant manure odor contributor as it is water insoluble. However, Bastyr and Powers (2002) reported that 4-methylphenol and dimethyl disulfide were the compounds most correlated to odor score.

Practically, increasing dietary fiber will increase the fraction of low quality protein. Plant cell wall of the fiber rich material blocks the digestion of the inner protein. The

apparent digestibility of nitrogen was significantly reduced by added fiber (Chabeauti et al., 1991; Moeser and van Kempen, 2002). Compared to the ileal and fecal CP digestibility of the basal diet, which were 68.6% and 78.3%, supplementing 33% beet pulp in the basal diet decreased ileal and fecal CP digestibility to 57.1% and 74.4% respectively (Graham et al., 1986). Just et al. (1983) reported that the CP digestibility of a low fiber diet was as high as 93% while that of a high fiber diet was 77%. Increased substrate for the hindgut flora will increase the metabolic rate of bacteria and increase unpleasant odor production. Dietary tryptophan may not be the main substrate for skatole formation in the hindgut as most of this amino acid is absorbed in the small intestine (Claus et al., 2003). On the other hand, the increased biomass of hindgut bacteria can serve as an extra source of tryptophan (Wells and Russell, 1996), which is the precursor of indole and skatole. As the digesta remains in the hindgut of pigs for more than 30 hours, it equal to 60 life cycles for some fast growing bacteria (e. g. E. coli.). If the lower pH will inhibit the turnover of AA that will convert to branched-chain fatty acids, then the turnover of AA such as tryptophan and tyrosine probably will be relatively lower under the acidic environment. Then, the accumulated tryptophan synthesis and breakdown is higher which may induce increased production of indole and skatole. Lundström et al. (1988) researched the effect of dietary fiber on fat skatole concentration. In their trial, a high fiber diet increased fat skatole concentration from 0.06 ppm to 0.09 ppm. Unfortunately, no data on fecal concentrations of indole and skatole were provided in that paper, but it is reasonable to deduce that the higher fermentation level formed more skatole in the gut. Another trial showed the concentration of indoles in feces was increased significantly by a high fiber diet (Hawe et al., 1992). Although fecal skatole concentration was decreased slightly, the total excretion was increased. However, the result

of Claus et al. (2003) showed dietary potato starch (contains high level of resistant starch) significantly decreased fecal skatole concentration. They explained that as resistant starch increased large gut butyrate formation, apoptosis of colon mucosa cells was inhibited and subsequently less substrate was available for skatole information. Indole and skatole produced in the gut are absorbed and transformed to glucuronides by liver of the pig and excreted through the urine. When urine is mixed with feces, many constituents are transformed by microbial activity of the fecal bacteria. Such conversions include the hydrolysis of urea to ammonia and CO_2, reduction of sulphate to hydrogen sulphide, and hydrolyses of glucuronides (Spoelstra, 1977), hippuric acid. These enzymatic hydrolyses are comparatively fast processes compared to anaerobic breakdown of plant fibre and protein, which have higher molecular weight and more complex structure (Spoelstra, 1980).

Fiber supplementation tends to increase odor since fiber level is in inverse proportion to nutrients digestibility and microbe will have more fermentable substrates available. Keys and Wood (2001) also pointed out that fiber diets interfere with the use of other nutrients by the pigs. Fiber fermentation may induce other more odorants beside ammonia. Canh et al. (1998) replaced cornstarch with fibrous ingredients in the diets for growing pigs and the result showed that fecal SCFA concentration increased significantly.

On the other hand, aged pig slurry contains much higher malodorous compounds with the anaerobic storage (Spoelstra, 1977; O'Neill and Phillips, 1991). The higher fecal moisture and bacterial activity are the main disadvantages of high fiber diets because such conditions are favorable for manure anaerobic fermentation in the pig buildings. In the lagoon, however, all the organic matter will be degraded mostly by anaerobic fermentation. Some researchers reported that dietary fiber increased fecal excretion by about 80% (Hawe et

al., 1992). As the energy from SCFA has lower efficiency than that from glucose, growth performance will be impacted somewhat. So fiber will increase days to market and aggravate the manure burden for pig houses as well as lagoons. The increased fecal volume by dietary fiber may increase the lagoon odorants emission as the nutrients intensity for the bacteria increased.

Possibility of reducing odorants by changing C:N ratio for hindgut microbe:

From the standpoint of odor reduction, the most ideal status is to balance the C:N ratio, decrease the total biomass, and decrease the total reaction time. Some researchers pointed out that odor problems can be reduced significantly if an optimum balance of dietary and fermentable carbohydrates can be achieved (Le et al., 2005). Information on the C:N ratio for the gut microflora is scarce. Sutton et al. (1999) compared the effects of different dietary protein levels on nitrogen excretion and odor. They found that a medium protein diet produced the highest total fatty acids level in the cecal digesta and in the fresh manure. The authors explained this interesting results by the imbalance in the C:N ratio, which impaired the optimum microbial decomposition. The possibility of C-excess in the pig hindgut is low, as the lactic acid concentration was low, while the VFA level was high (Argenzio and Southworth, 1975). Bacteria reduce acetate to lactate when C sources are excessive (Macfarlane and Macfarlane, 2003). However, dietary fiber supplementation will not automatically increase the C:N ratio as fiber will affect the digestion of other nutrients and increases endogenous protein and fat losses (De Lange et al., 1989; Dierick et al., 1989; Noblet and Perez, 1993). These proteins are more soluble than undigested dietary proteins as the latter ones tend to be water insoluble. Similar to the NSP fermentation, indicating that NSP degradation is the rate-limiting step in their conversion to SCFA (Cummings and

Englyst, 1987; Hopkins et al., 2003). The rate-limiting step in this process of putrefactive protein fermentation is degradation of protein itself (Cummings and Englyst, 1987). Since no data are available, the optimal C:N ratio for the hindgut microflora is very unpredictable.

How about low fiber diet?

We can conclude that higher fiber fermentation will increase hindgut microbial activity and have a higher potential to aggravate odor problems in the swine industry. Indirect evidence to support the relationship between odor intensity and hindgut fermentation is dietary antibiotic supplementation, which is expected to inhibit microbial metabolism. Hawe et al. (1992) found that antibiotic supplementation (200 mg/kg tylosin phosphate) decreased fecal skatole concentration significantly. Similarly, high dietary copper ($CuSO_4$) improved swine manure odor quality, although more sulfur is involved at the same time, and the effect of higher initial manure sulfate concentration is to contribute to higher H_2S release (Arogo et al., 2000). Such an effect may be due to the antibiotic-like function of Cu (Armstrong et al., 2000).

Low fiber diets may be economically unacceptable because prices for most of the higher fiber feed ingredients are typically lower. However, from an environmental standpoint, using fiber rich ingredients will aggravate the odor issues on the limited land around intensive pig farms. So, we need to further evaluate the optimum dietary NSP profile, including the level of soluble and insoluble NSP, and the ratio between them. Theoretically, such an effort will help to achieve an optimum diet that will allow a balance between feed cost and odor (and other air pollutant) issues.

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Table 1 Odor characteristics, olfactory thresholds, and recommended exposure limits for volatile organic compounds identified from air samples at swine production facilities.

organic compound	Average air conc.(mg /m^3)	Odor characteristic	Odor threshold(mg /m^3)
Hydrogen sulfide	0.090	rotten eggs	0.140
Ammonia	3.70	sharp, pungent	0.027–2.2
Dimethyl disulfide	0.017	putrid, decayed vegetables	0.0011–0.61
Dimethyl trisulfide	0.013	nauseating	0.0072–0.023
Acetic acid	0.270	pungent	0.1–2.5
Propionic acid	0.130	fecal	0.0025
Isobutyric acid	0.110	fecal	0.00072
Butyric acid	0.590	fecal, stench	0.00025
Isovaleric acid	0.098	fecal	0.00017
n-Valeric acid	0.360	fecal	0.00026
Phenol	0.025	aromatic	0.23–0.38
4-Methyl phenol	0.090	fecal	0.0021–0.009
4-Ethyl phenol	0.004	pungent	0.0035–0.010
Indole	0.002	fecal	0.0019
3-Methyl indole	0.002	fecal, nauseating	0.00000052-0.0064

(Modified from Zahn et al., 2001)

Chapter II. Effects of guar gum supplementation on gastrointestinal tract and manure odorants in growing pigs

ABSTRACT: This study was designed to measure the effects of water-soluble non-starch polysaccharides (NSP) on protein and GE digestion as well as odor compounds in feces. Our hypothesis was that soluble NSP would impact CP digestion and more odor compounds would be produced in swine manure as a result of an increase in nitrogen and energy available for microbes both in the hindgut and manure. Four diets were formulated with 0% (control), 2%, 4%, and 8% guar gum in a low fiber basal diet. Growing pigs (n = 28) with an initial average BW of 26.8 ± 1.40 kg were allotted randomly into four groups and were fed one of the experimental diets. Pigs were housed at the swine educational unit for an adjustment period of 21 days before a metabolism trial was conducted. After 7 days of another adjustment period in metabolism crates, total feces and urine were collected for 3 days. At the end, all pigs were killed and ileum, cecum, and distal colon digesta were sampled. Results showed that guar gum supplementation impaired growth performance ($P < 0.0001$), especially during the first 3 weeks. The apparent fecal CP and GE digestibility as well the ileal CP digestibility were decreased by guar gum supplementation. Dietary N intake and N retention were negatively impacted by dietary guar gum level. Ammonia emission from fresh manure samples was higher in the high guar gum treatments during the first 12 and 24 hour of the in vitro assay. For the aged manure samples, the ammonia emission was lower in high guar gum treatments during the first 12 hours. There was no significant difference of cecal SCFA concentration among the four treatments while the fecal SCFA concentration was increased by dietary guar gum supplementation. No significant dietary

effect was found on other main odorants from either fresh manure or aged manure samples. However, the emissions of dimethyldisulfide and dimethyltrifide from aged manure were increased by dietary guar gum supplementation. Odor panel evaluation results of aged manure samples showed that dietary guar gum aggravated odor intensity and odor quality though no significant differences were found for fresh manure samples.

INTRODUCTION

Manure odor is one of the main concerns of swine production especially for intensive pig production units. Livestock odors have been implicated to contribute to health problems directly or by producing stress and altering mood (Thu, 1997; Schiffman, 1998; Wing and Wolf, 2000). Diet modification is a potential strategy to reduce odor-causing agents in manure because feed is the original substrate for odor compounds. It is accepted widely that reducing dietary CP level and adding crystal amino acids to maintain amino acid balance is effective in reducing ammonia emission (Hobbs et al., 1996; Sutton et al., 1999; Otto et al., 2003; Shriver et al., 2003). It was also reported that dietary fiber can reduce ammonia emission because fermentable carbohydrates can shift nitrogen from urine to feces in the form of bacterial protein (Canh et al., 1998; Sutton et al., 1999; Zervas and Zijlstra, 2002) and the latter one is relatively more stable. Therefore, manure ammonia production will decrease. A few trials also reported that fermentable carbohydrate supplementation tended to decrease emission of some other odorants (Farnworth, et al., 1995; Willig et al., 2005; Jensen and Hansen, 2006), especially when dietary CP was high (Le, 2006). However, all of the basal diets used in the previous trials contain substantial levels of fiber already. Fiber is resistant to digestion by endogenous enzymes from the mammalian host. It will decrease the

digestibility of nutrients in the feed (Schrama et al., 1998) and increase endogenous protein and fat losses (Noblet and Perez, 1993; De Lange et al., 1989). Schulze et al. (1995) found that dietary wheat bran induced higher ileal endogenous N flow than the purified NSP from wheat bran. The possible reason is that wheat bran contains some soluble NSP and the isolation procedure removed the soluble part of NSP. Dietary fiber, particularly soluble non-starch polysaccharides (NSP), has high water binding capacity (Bach Knudsen, 2001; Antoniou and Marquard, 1981; Armstrong et al., 1992; Jensen and Jørgensen, 1994). Therefore, soluble NSP may hold more water (Johansen et al., 1996) in the colon and cause swelling, which increases the surface area for microbes (Noblet and Le Goff, 2001; Bach Knudsen, 2001) and extends hindgut fermentation time. Armstrong et al. (1993) used 2,6-diaminopimelic (DAPA) as a bacterial mass marker and found that fecal bacteria were higher for the pectin supplemented group compared to control group, while the wheat bran supplemented group contained less bacteria than the control group.

The apparent digestibility of nitrogen was reported to be reduced by dietary inclusion of NSP rich ingredients (Just et al. 1983; Graham et al., 1986; Chabeauti et al., 1991; Mroz et al., 2000; Moeser and van Kempen, 2002). In addition, NSP fermentation produced short chain fatty acids (SCFA), which will contribute to unpleasant odors (Yu et al., 1991; Zhu et al., 1999). Increased fecal bulk was another negative effect induced by NSP rich ingredients (Moeser et al., 2002).

The effects of fiber from the basal diet and its interactive effect with supplemental fiber may contribute to odorants emission. Thus, the effect of certain dietary fiber on swine manure odorants needs to be further studied under conditions where low fiber basal diets are fed. In the present trial we used very low fiber ingredients such as degermed dehulled

(DGDH) corn and soy protein isolate for the basal diet and used guar gum as a soluble NSP (sNSP) source.

The objective of the present study was to determine the effects of guar gum supplementation as a dietary sNSP on manure odorants. We hypothesized that increased dietary soluble fiber would increase levels of odorants and decrease ammonia.

MATERIALS AND METHODS

Diets and animals: This study was conducted using two separate groups of pigs. In batch 1, 16 growing pigs with an initial average BW of 27.2 ± 1.43 kg were used. Pigs were assigned into four groups randomly and each group contained 4 pigs. Four diets with different levels (0, 2%, 4%, or 8%) of guar gum (Jaguar® 4500F, Rhodia, Cranbury, NJ) were fed to 4 pigs within each group. The diets were fed in mash form and formulated to meet or exceed NRC (1998) requirements for growing pigs (Table 1).

In batch 2, 12 growing pigs with an initial BW of 26.2 (± 1.20 kg) were used. Again, pigs were assigned into four groups randomly corresponding to the four diets and each group contained 3 pigs.

Pigs were housed at the swine educational unit (NCSU, Raleigh) for an adjustment period of 21 days. A minimal adjustment period of three weeks was considered necessary to allow for stabilization of the microflora in the large intestine (Longland et al., 1993). Pigs were then transferred to Grinnells laboratory (Raleigh, NC) where they were housed in metabolism cages (0.6 x 1.5 m) individually and given ad libitum access to feed and water.

After a 7-day adaptation period in metabolism cages, a 3-day collection period for feces and urine was conducted. Feces were collected quantitatively on wire screens and were frozen at -20°C until further chemical analysis was conducted. Urine was collected

quantitatively on slope-shaped stainless steel trays in plastic containers placed in ice to minimize gaseous losses of nitrogen. Quantity of feces and urine was recorded and frozen at -20°C as soon as it was collected, twice daily.

On day 11, pigs were anesthetized by i.m. injection of a combination of ketamine (15 mg/kg BW, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (2 mg/kg BW, Phoenix Scientific Inc., St. Joseph, MO). Blood samples were taken from the jugular vein for blood urea nitrogen (BUN) analysis. Then, pigs were killed by anesthetic overdose with sodium pentobarbital (200mg/kg BW, Vortech Pharmaceutical, LTD, Dearborn, MI) administered i.v. Immediately after sacrifice, the gastrointestinal tract was removed and digesta of the end of the small intestine (4 m from the ileo-cecal junction), cecum, and distal colon were sampled and frozen at -20°C for further analysis.

Chemical analyses: Frozen feces and urine were thawed. Then, feces and urine were mixed together at the rates they were produced when their temperatures were close to 0°C and homogenized within respective animal. A portion of this manure was used to determine fresh manure ammonia emission and the remaining manure were stored in 1-L plastic containers and allowed to sit at room temperature for 21 days of anaerobic aging. Both 10 ml of fresh or aged manure were sampled and send to West Texas A&M University for odor hedonic tone and intensity evaluation by a professional panel. The panelists were asked to smell each sample individually and assign a designation of degree of pleasantness or unpleasantness according to a -10 to +10 hedonic tone scale, with 0 being neutral. They also were asked to assign a score for strength of odor by smelling the sample and comparing to a series of standards. The intensity standards were prepared per ASTM E 544-99 with n-butanol, the n-butanol concentrations for the 1-5 scale (very faint, faint, moderate, strong,

and very strong) standards were 250, 750, 2250, 6750, and 20250 ppm, respectively (Guo et al., 2001). The panelists smelled the sample and compared them to the standards for strength of odor and assigned a standard number (1, 2, 3, 4, or 5) that matched the strength of the sample. If a sample fell between 2 standards, a designation of 0.5 was used (0.5 if < 1, 1.5, 2.5, 3.5, 4.5, or 5.5 if > 5).

Frozen fresh or aged manure samples were thawed in a water bath at approximately 20°C and 3 ml of manure samples were transferred to 15 ml test tubes immediately. The pH values were measured by using a pH meter (Accumet® pH model 610 A, Fisher pH, Ambler, PA, USA). Odor compounds from headspace air were adsorbed by Solid Phase Microextraction (SPME) fibers (Carboxen™/Polydimethylsiloxane fiber, Supelco SPME Portable Field Sampler, Supelco, Bellefonte, Pa.) for 30 mins and were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS, GC HP 6890; MS HP 5973) immediately. Compounds were separated on a 30 m × 0.32 mm diameter × 0.25µm film thickness Innowax PEG column (Agilent Technologies, Palo Alto, CA). Injector temperature was maintained at 245°C and detector temperature was 250°C. The column was programmed as follows: flow rate 0.5 ml/min, initial temperature 40°C, initial time 3 min, the temperature ramp 12°C/min to 220°C then hold 10 min. The identities of odor compounds were determined by comparison to the retention times of known standards, and were further confirmed by comparing the mass spectra.

Ammonia emission of the manure samples was determined (Figure 1) by placing 400 ml of the manure mixture in a rectangular (28 L × 9.5 W × 6 H cm) container (Super Oval 1, Tupperware Co., Orlando, FL). Air was drawn through a flow meter (Cole Palmer, Vernon Hills, IL) at a rate of 1.4 L/min, the container with manure, and then through a gas dispersion

tube (Fisher Scientific, Pittsburg, PA) placed in a 500 ml Erlenmeyer flask containing 400 ml dilute sulfuric acid (0.10 N) in order to trap the ammonia released from the manure. This sulfuric acid solution was sampled (6 ml) at 12, 24, 36, 48, 72, and 96 h and was analyzed for ammonia using the procedure of Willis et al. (1996).

Blood urea nitrogen was determined by using a commercial kit (Sigma-Aldrich, St. Louis, MO) according to manufacturer's instructions. Short-chain fatty acid (SCFA) concentrations of the cecal and fecal samples were conducted using a GC (model 3380, Varian Instruments, Walnut Creek, CA) equipped with a FID detector. Ten g of cecal digesta was centrifuged at 2,390 g for 10 minutes. For feces samples, 4 ml of dd water was added to 2 g of feces and centrifuged at 2,390 g for 10 minutes. One ml of supernatant was further centrifuged at 21,000 g for 15 minutes. Meta-phosphoric acid (0.2 ml) containing 2-ethylbutyric acid as the internal standard was added to the supernatant. A Nukol fused silica capillary column (Supelco Inc., Bellefonte, PA) was used to elute the SCFA. Calibrated SCFA standards were used to identify and quantify SCFAs in unknown samples. Branch-chain proportion (BCP) was used as protein fermentation indicator (Awati et al., 2006) and was calculated as follows:

$$\text{BCP}(\%)=\frac{(\text{Isobutyric}+\text{Isovaleric})}{\text{Total VFA}}\times 100\%.$$

Fecal and ileal digesta samples as well as urine samples were dried using a freeze dryer (Heto PowerDry LL3000, ATR, Laurel, MD). Subsequently, feces, ileal digesta samples and feed samples were ground through a 1 mm screen prior to chemical analysis. Dry matter content of 4 feed samples, 28 ileum digesta samples, 28 fecal samples were measured by AOAC (1990) procedures. GE values of feed samples and all freeze dried samples (including

dried urine samples) were determined by an adiabatic bomb calorimeter (model C5000, IKA, Wilmington, NC). All samples were then submitted to the Experimental Station Chemical Laboratories (University of Missouri-Columbia, MO 65211) for chromium and N analyses (urine samples were measured for N only). Chromium was measured by atomic absorption spectrometry after digestion with perchloric acid. Nitrogen content was measured by the Kjedahl method (AOAC, 1990).

The apparent ileal CP digestibility was calculated according to the following equation:

Apparent ileal CP digestibility % = 100 - $[(M_d \times CP_I)/ (CP_d \times M_I)] \times 100$

Where M_d = chromium concentration in the diet (mg/kg), CP_I =CP concentration in ileal digesta (g/kg), CP_d=CP concentration in the diet (g/kg), and M_I = chromium concentration in the ileal digesta (mg/kg).

The equations used for ileal GE calculation and fecal nutrients digestibility calculation were similar to the above equation but used the corresponding index.

Statistical analyses: Data were analyzed by two-way ANOVA using the GLM procedure of SAS (SAS Institute, Cary, NC). Least squares means among diet treatments were evaluated by PDIFF and STDERR and batch was included as blocking factor. Orthogonal contrast comparisons were conducted to determine linear and quadratic effects of guar gum supplementation.

RESULTS AND DISCUSSION:

Body weight gain decreased linearly ($P < 0.001$) with increasing levels of guar gum during the 3 week adjustment period (Table 2). Similarly, weight gain and feed intake were decreased linearly ($P < 0.05$) during the time pigs were on metabolism crates, although feed efficiency was not affected. This is consistent with results of incorporation of soluble NSP

rich ingredient in weaned piglet diets, which resulted in decreased body weight gain (Hopwood et al., 2004).

As shown in table 3, pigs fed 2, 4% and 8% guar gum had higher ($P < 0.05$) wet feces weight than pigs fed the control diet. A trial by Wang et al. (2004) showed sugar- beet pulp and wheat bran inclusion in growing pig diets significantly increased the mass of fecal excretion. However, our trial showed there was no difference among the groups in total fecal dry matter production. This indicates high fermentability of soluble NSP in the large intestine. The pigs consuming the control diet in general had drier feces ($P < 0.05$) than those fed the guar gum supplemented diets; in fact, increasing levels of guar gum linearly decreased ($P < 0.001$) fecal DM. This may have occurred because dietary guar gum stimulated bacteria reproduction and their activity, the limited capability of the hindgut to reabsorb water from the digesta or the extended microbial activity itself induced more bacteria mass or lower molecule metabolite to hold more water. Similarly, the dry matter fraction of the ileal digesta was decreased linearly ($P<0.0001$). At this point, unfermented guar gum itself may contribute to water holding.

No significant differences between treatments were found in daily urine weight. There was a slight increasing trend ($P < 0.10$) in dried urine GE concentration with increasing dietary guar gum. The non-urea GE concentration of dried urine samples was quadratically related with dietary guar gum level ($P<0.10$).

Dietary guar gum decreased ($P < 0.01$) ileal digestibility of CP and fecal digestibility of CP and GE linearly (Table 4), which is consistent with the results of Owusu-Asiedu et al. (2006). Increased SCFAs will stimulate passage rate of the digesta through the gut and decrease nutrient digestibility (Veldman et al., 1993). As fermentable carbohydrates, dietary

NSP serve as an energy source for the microflora in the large intestine of pigs. At the same time, the N sources for fermentation were increased by dietary NSP. It was reported that 4% or 6% guar gum addition extended the half-time of gastric digesta and total N emptying (Rainbird and Low, 1986). But, there were no significant differences in the half-time of gastric dry matter and glucose emptying. The possible reason is that guar gum might lead to an increase in endogenous N secretion (Low and Rainbird, 1984). The same authors also reported that N secretion from small intestinal loops was increased by guar gum addition in the ringer solution.

No significant differences were found for urinary N excretion. A tendency for a quadratic response ($P < 0.10$) to guar gum was observed for BUN, with the greatest concentration of BUN at 4% guar gum inclusion. This seems to conflict with the hypotheses that ammonia will be trapped by higher NSP fermentation in the large intestine. Mosenthin et al. (1992a) infused [^{15}N] urea into the jugular vein of pigs which were fed either a low fiber diet (starch-SBM diet) or a high fiber diet (beet pulp-SBM diet). The result showed that ^{15}N excretion through urine was higher in the high fiber group (79.4% for the starch-SBM diet vs 87.5% for the beet pulp-SBM diet) and that fecal ^{15}N excretion was also slightly higher for the high fiber group (0.13% for starch-SBM diet vs 0.21% for beet pulp-SBM diet). The possible reason for the lower ^{15}N recovery in the low fiber group (82.3% for starch-SBM diet vs 90.8% for beet pulp-SBM diet) within 144 hours could be that more infused [^{15}N] urea was retained in the body tissues. This indicated that ammonia trapped by NSP fermentation in the large intestine appeared to be a very small portion and the increased total fecal N excretion in high fiber diets may be mainly caused by lower CP digestibility.

Beside the ammonia trapping effect of dietary NSP fermentation, the reduced CP ileal

digestibility itself also contributed to the decreased urinary nitrogen excretion and increased fecal nitrogen excretion (Mroz et al., 2000). Nitrogen retention (g/day) linearly decreased ($P<0.05$) with increasing guar gum inclusion which may be explained by higher N intake and also higher ileal or fecal CP digestibility of the control treatment. A higher energy supply of fermentable carbohydrate and protein to the microbes of the large intestine induces high ammonia incorporation via microbial protein synthesis. This will trap more ammonia, but the pathogen challenge may increase due to extensive bacteria growth in the hindgut. It was reported that 10% guar gum supplementation increased proliferation of enterotoxigenic E. coli in the small intestine though increased butyrate production, which is assumed to have a beneficial effect on gut wall heath (McDonald et al., 1999).

The cumulative ammonia emission from the fresh or aged manure samples is shown in Table 5. The only significant differences for the fresh manure samples were the ammonia emissions during the first 12 and 24 hours. The amount of ammonia emission was linearly increased ($P < 0.05$) during these times by guar gum addition. In contrast, our original expectation was that ammonia emission would be decreased by dietary fermentable carbohydrate supplementation. The possible reason is increasing the amounts of NSP induced more substrate for the microbes of the manure. If bacteria in manure were increased by NSP, then that may have caused a higher level of urease activity, and a more rapid release of ammonia. For the later stages, ammonia emission from the control group was increased, although there were no differences among the treatments. Perhaps an accumulation of SCFA and lowering of the pH of the manure could have reduced the ammonia emission. On the contrary to the result of fresh manure, the amount of ammonia emissions from aged control samples during first 12 hours was significantly higher ($P<0.0001$) than guar gum groups and

such an effect became less distinct during later stages. After 21 days of anaerobic fermentation, the SCFA concentrations of the aged manure would be expected to be higher in the guar gum groups, which could decrease ammonia emission.

Table 6 lists the results of main odorants emission from fresh or aged manure samples. Guar gum addition had no significant effects on phenol, p-cresol, indole or skatole emissions. These results are inconsistent with the data of inulin supplementation (Farnworth, et al., 1995; Jensen and Hansen, 2006). It was reported that the main odorants emission such as p-cresol, indole, and skatole were significantly decreased by Jerusalem artichoke (a inulin rich ingredient) feeding (Jensen and Hansen, 2006). No data for dietary guar gum on odorant emission was found by the authors, however, the results of inulin feeding trials may serve as the fitful alternative reference because both guar gum and inulin are soluble NSP rich ingredients. Interestingly, in the present study, emission of dimethyldisulfide and dimethyltrifide from the aged manure was increased by dietary guar gum supplementation which may because of the higher volatility under lower pH condition.

Studies focusing on fecal odorant concentrations showed that guar gum addition decreased fecal p-cresol, and skatole levels (Knarreborg et al., 2002). The metabolism of guar gum by carbohydrate-fermenting bacteria may have decreased the activity of proteolytic bacteria, and reduced the breakdown of tyrosine into p-cresol, and tryptophan into indole and skatole (Jensen et al., 1995). In addition, carbohydrate-fermenting bacteria produce more butyric acid, which will decrease apoptosis of the colon wall (Claus et al., 2003). Since cell debris, but not residual dietary protein is the main source of tryptophan for hindgut bacteria, decreased apoptosis may have decreased tryptophan availability for fermentation. Le et al. (2007) reported that crystalline Trp, Tyr, and Phe supplementation did not affect odor

emission from pig manure. However, crystalline S-containing AA supplementation caused increased odor.

Table 7 and 8 showed the SCFAs concentrations of cecal contents and feces, respectively. There were no significant differences in the cecal content of SCFA levels between the control and the guar gum-supplemented groups. The BCP of the cecal SCFA profile was not affected by guar gum supplementation, indicating that the protein fermentation was not inhibited by carbohydrate fermentation. This does not agree with the results of Awati et al. (2006) that fermentable fiber reduced protein fermentation along the GIT. Cecal SCFA concentrations are affected by the rate of their absorption and intestinal bulk. Since the NSP level of the basal diet was much lower than a standard corn-soybean diet, SCFA production would probably be lower than the maximum absorptive capability of the intestinal wall.

In general, SCFA concentrations of feces linearly increased ($P < 0.05$) when the level of guar gum supplementation increased. Compared to the SCFA levels of the cecal contents, the differences in fecal SCFA levels implicated that the main fermentation was accrued after cecum passage, probably at the colon. Fecal BCP was linearly decreased ($P < 0.001$) by increasing dietary guar gum addition, which indicated relatively less protein fermentation in the high NSP treatments. This agrees with the results of previous researchers (Sauer et al., 1991; Wang et al., 2004; Bikker et al., 2006). However, the concentration of isobutyric acid and isovaleric acid did not differ among treatments; the decreased BCP was mainly caused by the higher concentrations of acetic acid ($P<0.01$) and propionic acid ($P<0.01$) for the guar gum groups.

Table 9 lists the pH values of ileal, cecal, and colonic digesta as well as the fresh or

aged manure samples. There were no significant differences among the treatments for ileal or cecum content. Colonic pH values (Figure 2) and pH of aged manure samples linearly decreased ($P < 0.001$) with increasing guar gum. However, the pH values of the fresh manure samples did not display this trend.

Table 10 lists the odor intensity and odor hedonic score of headspace air samples from fresh or aged manure samples. There were no differences among treatments, except for a tendency ($P < 0.10$) for odor intensity of aged manure samples to linearly increase with increasing levels of guar gum.

Overall, growth performance and nutrient digestibility was impaired by dietary guar gum supplementation. Guar gum supplementation increased SCFA in feces and may have inhibited protein fermentation in the colon. The fermentable NSP from guar gum supplementation and degradable SCFA decreased ammonia emission in aged manure samples, but not fresh manure samples. Emission of acidic odorants such as dimethyldisulfide and dimethyltrifide from aged manure were increased by guar gum supplementation and so was the odor intensity of aged manure.

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Table 1. Formulation of the experimental diets (as fed basis)

Ingredients (%)	Control	2% guar gum	4% guar gum	8% guar gum
DGDH corn [1]	77.00	75.00	73.00	69.00
Guar gum	-	2.00	4.00	8.00
Soybean protein isolate[2]	16.00	16.00	16.00	16.00
Corn oil	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.00	1.00	1.00	1.00
Limestone	1.20	1.20	1.20	1.20
Salt	0.40	0.40	0.40	0.40
Vitamin/Mineral mixture[3]	0.30	0.30	0.30	0.30
Cr_2O_3	0.10	0.10	0.10	0.10
Celite[4]	1.00	1.00	1.00	1.00
Calculated nutrient composition				
DE (Mcal/kg)	3.67	3.64	3.62	3.57
CP (%)	18.81	18.68	18.55	18.28
Ca (%)	0.66	0.66	0.66	0.66
Available P (%)	0.29	0.29	0.28	0.28
Lysine (%)	0.99	0.98	0.98	0.97

[1]The nutrient values of DGDH (degermed, dehulled) corn were the same as those published previously (Moeser et al., 2002).

[2]Cargill, Minneapolis, MN

[3]Provided the following per kilogram of complete diet: vitamin A, 6,358 IU; vitamin D_3, 636 IU; vitamin E, 50 IU; vitamin K, 1.91 mg; riboflavin, 4.81 mg; niacin, 14.41 mg; d-pantothenic acid, 14.41 mg; vitamin B_{12}, 21.195 μg; Zn, 115 mg; Fe, 230 mg; Mn, 19.2 mg; Cu, 9.6 mg; I, 0.29 mg; and Se, 0.29 mg.

[4]A diatomite product, Celite Corporation, Lompoc, California.

Table 2. Growth performance of growing pigs fed low NSP diets containing different levels of guar gum[1]

Treatments	Control	2% guar gum	4% guar gum	8% guar gum	SEM	P Value	Linear	Quadratic
Initial body weight (kg)	26.62	26.59	26.81	26.92	0.578	0.973	0.668	0.992
Adjustment period prior to the metabolism crate portion (0-3 wk)								
3 wk body weight (kg)	38.47	35.84	36.04	32.30	0.908	0.0012	0.0002	0.939
3 wk BW gain (kg)	11.85	9.26	9.22	5.38	0.568	<0.0001	<0.0001	0.894
Metabolism crate portion of the trial (0-10 day)								
End body weight (kg)	48.10	44.73	43.60	39.94	1.19	0.0015	0.0002	0.564
10 day BW gain (kg)	9.63	8.89	7.56	7.73	0.603	0.603	0.032	0.197
10 day Feed intake (kg)	18.65	17.60	15.95	15.31	1.07	0.137	0.029	0.513
10 day G/F	0.518	0.505	0.483	0.505	0.0220	0.708	0.661	0.333
Whole trial (3wk+10 day)								
Total BW gain (kg)	21.48	18.14	16.79	13.18	0.963	0.0001	<0.0001	0.441

[1]Least squares means

Table 3. Effect of different levels of guar gum supplementation on waste excretion and feces and ileal sample dry matter[1].

Index	Control	2% guar gum	4% guar gum	8% guar gum	SEM	P Value	Linear	Quadratic
Feces weight per day (kg)	0.220	0.336	0.417	0.379	0.0396	0.0129	0.0129	0.0202
Feces dry matter fraction (%)	48.9	39.0	30.0	32.2	2.20	<0.0001	<0.0001	0.0016
Feces dry matter per day (kg)	0.108	0.128	0.127	0.116	0.0110	0.548	0.807	0.181
Ileal content dry matter fraction (%)	13.5	11.7	7.3	7.7	0.82	<0.0001	<0.0001	0.012
Urine weight per day (kg)	2.73	3.12	2.98	2.80	0.330	0.834	0.947	0.438
Freeze dried urine GE (cal/g)	2527	2613	2713	2668	51.15	0.0936	0.062	0.091
non-urea GE[2] in dried urine sample(cal/g)	920	1122	1204	1108	85.28	0.150	0.191	0.057

[1]Least squares means

[2]Caculated as GE (per gram) of freeze dried urine minus GE (per gram) of urea

Table 4. Effects of different levels of guar gum on nutrient digestibility and N retention[1].

Index	Control	2% guar gum	4% guar gum	8% guar gum	SEM	P Value	Linear	Quadratic
Apparent ileal CP digestibility	0.692	0.663	0.501	0.510	0.0456	0.0133	0.0047	0.219
Apparent ileal GE digestibility	0.667	0.628	0.587	0.612	0.0422	0.637	0.368	0.343
Apparent fecal CP digestibility	0.899	0.853	0.816	0.784	0.00927	<0.0001	<0.0001	0.0297
Apparent fecal GE digestibility	0.933	0.905	0.880	0.870	0.00657	<0.0001	<0.0001	0.0130
N disappearance in hindgut (g/day)	12.15	10.16	16.33	12.97	2.48	0.381	0.559	0.506
N intake (g/day)	59.2	55.5	49.9	48.3	3.36	0.115	0.025	0.420
N excretion from feces (g/day)	4.99	6.63	7.03	6.95	0.655	0.129	0.072	0.123
N excretion from urine (g/day)	16.4	13. 8	14.0	12.5	2.33	0.706	0.298	0.741
N retention (g/day)	37.9	35.0	28.9	28.8	3.00	0.113	0.033	0.324
BUN (mM/dl)	8.83	10.15	10.45	10.07	0.550	0.202	0.190	0.094

[1]Least squares means

Table 5. Effects of different levels of guar gum on ammonia emissions of 400 ml fresh or aged manure samples[1].

Index	Control	2% guar gum	4% guar gum	8% guar gum	SEM	P Value Linear		Quadratic
			Fresh manure[2]					
12 hour (mmol)	1.65	2.27	2.79	3.40	0.555	0.200	0.0423	0.673
24 hour (mmol)	6.50	12.1	13.9	21.9	4.26	0.125	0.0201	0.929
36 hour (mmol)	37.6	31.7	29.7	37.1	5.95	0.731	0.933	0.268
48 hour (mmol)	47.7	47.3	47.6	45.4	3.03	0.943	0.593	0.802
72 hour (mmol)	49.3	47.2	46.4	47.3	1.25	0.415	0.343	0.170
96 hour (mmol)	46.4	45.7	42.9	45.6	1.54	0.412	0.661	0.174
			Aged manure[3]					
12 hour (mmol)	44.9	34.3	26.9	24.0	2.81	0.0002	<0.0001	0.0292
24 hour (mmol)	56. 4	50.0	43.2	41.0	2.55	0.0016	0.0003	0.104
36 hour (mmol)	55.0	53.7	48.2	49.3	1.66	0.0222	0.0121	0.143
48 hour (mmol)	55.2	49.5	48.6	48.8	1.44	0.0127	0.0134	0.0249
72 hour (mmol)	52.5	49.3	46.7	46.8	1.60	0.0589	0.0207	0.136
96 hour (mmol)	48.2	45.8	44.7	44.8	1.06	0.0591	0.0394	0.137

[1]Least squares means

[2]Manure samples were mixed from individual feces and urine samples at the ratios they were produced.

[3] Manure samples after anaerobic aging at room temperature for 21 days

Table 6. Effects of different levels of guar gum on feces p-cresol and main indolic compounds concentrations [1].

Index	Control	2% guar gum	4% guar gum	8% guar gum	SEM	P Value	Linear	Quadratic
Fresh manure[2] headspace odorants peak area (log_{10}) by SPME								
Phenol (log_{10})	6.14	6.49	6.22	6.45	0.237	0.665	0.504	0.881
p- Cresol (log_{10})	7.72	7.84	7.82	7.92	0.0975	0.531	0.179	0.815
4-ethylphenol (log_{10})	4.34	6.09	5.41	6.14	0.769	0.340	0.202	0.504
Indole (log_{10})	6.20	6.24	6.21	6.34	0.264	0.963	0.646	0.859
Skatole (log_{10})	6.84	6.79	6.61	6.69	0.180	0.821	0.515	0.582
Aged manure[3] headspace odorants peak area by SPME								
Dimethyldisulfide (log_{10})	7.96	8.32	8.57	8.55	0.158	0.0464	0.0166	0.104
Dimethyltrisulfide (log_{10})	7.03	7.45	7.76	7.92	0.174	0.0095	0.0017	0.162
Phenol (log_{10})	7.45	7.67	7.81	7.80	0.110	0.0953	0.041	0.140
p- Cresol (log_{10})	8.23	8.20	8.32	8.25	0.0776	0.741	0.693	0.586
Indole (log_{10})	6.67	6.72	6.91	6.65	0.166	0.680	0.946	0.293
Skatole (log_{10})	6.78	6.80	6.79	6.73	0.115	0.982	0.752	0.799

[1]Least squares means

[2]Manure samples were mixed from individual feces and urine samples at the ratios they were produced.

[3] Manure samples after anaerobic aging at room temperature for 21 days

Table 7. Effects of different levels of guar gum on SCFAs of cecal content[1].

Index	Control	2% guar gum	4% guar gum	8% guar gum	SEM	P Value	Linear	Quadratic
Acetic acid (mM)	46.3	40.7	41.0	48.9	4.99	0.585	0.557	0.221
Propionic acid (mM)	32.8	30.5	35.9	39.2	5.36	0.685	0.296	0.813
Isobutyric acid (mM)	0.646	0.863	0.850	0.873	0.197	0.822	0.501	0.582
Butyric acid (mM)	18.0	11.6	10.9	15.0	2.90	0.306	0.661	0.0751
Isovaleric acid (mM)	0.901	1.34	1.29	1.25	0.324	0.771	0.515	0.442
Valeric acid (mM)	7.35	5.40	6.05	8.31	1.92	0.711	0.569	0.344
Total SCFAs (mM)	106	90.4	96.0	114	12.5	0.573	0.483	0.259
Acetic acid/ propionic acid	1.52	1.49	1.16	1.32	0.156	0.350	0.269	0.315
BCP[2]	1.58	3.42	2.09	2.04	0.798	0.421	0.926	0.368

[1]Least squares means

[2] Branched-chain proportions were calculated as: $\mathrm{BCP}(\%)=\frac{(\text{Isobutyric+Isovaleric})}{\text{Total VFA}}\times 100\%$.

Table 8. Effects of different levels of guar gum on fecal SCFAs[1].

Index	Control	2% guar gum	4% guar gum	8% guar gum	SEM	P Value	Linear	Quadratic
Acetic acid (mmol/kg)	61.9	131	110	142	13.5	0.0022	0.002	0.137
Propionic acid (mmol/kg)	21.4	44.3	48.3	50.2	6.04	0.0106	0.0065	0.0419
Isobutyric acid (mmol/kg)	4.06	5.57	3.54	5.55	0.588	0.0489	0.246	0.452
Butyric acid (mmol/kg)	8.84	23.3	25.0	29.7	3.75	0.0053	0.0018	0.0901
Isovaleric acid (mmol/kg)	8.42	10.6	6.08	9.94	1.25	0.0798	0.751	0.272
Valeric acid (mmol/kg)	5.48	9.30	7.53	10.4	1.39	0.0954	0.0446	0.692
Total SCFAs (mmol/kg)	110	224	200	248	23.9	0.0032	0.0022	0.115
Acetic acid/ propionic acid	10.1	9.62	7.33	7.42	0.835	0.0550	0.0182	0.318
BCP[2]	11.4	7.93	5.25	6.19	0.866	0.0003	0.0005	0.0024

[1]Least squares means

[2] Branched-chain proportions were calculated as: $\text{BCP}(\%)=\frac{(\text{Isobutyric}+\text{Isovaleric})}{\text{Total VFA}}\times 100\%$.

Table 9. Effects of different levels of guar gum on pH values of ileal, cecum, and colon contents and fresh or aged manure[1].

Index	Control	2% guar gum	4% guar gum	8% guar gum	SEM	P Value	Linear	Quadratic
Ileum	6.43	6.69	6.38	6.68	0.253	0.752	0.632	0.851
Cecum	6.19	6.40	6.22	6.14	0.187	0.773	0.650	0.576
Colon	6.71	6.48	6.25	6.23	0.0856	0.0021	0.0006	0.0499
Fresh manure[2]	8.46	8.80	8.56	8.57	0.0892	0.0852	0.912	0.159
Aged manure[3]	9.19	8.95	8.78	8.78	0.0665	0.0008	0.0004	0.0168

[1]Least squares means

[2]Manure samples were mixed from individual feces and urine samples at the ratios they were produced.

[3]Manure samples after anaerobic aging at room temperature for 21 days

Table 10. Effects of different levels of guar gum on manure odor intensity and hedonic score[1].

Index	Control	2% guar gum	4% guar gum	8% guar gum	SEM	P Value	Linear	Quadratic
Fresh manure[2] odor intensity[3]	1.88	1.77	1.98	1.89	0.121	0.689	0.727	0.857
Aged manure[4] odor intensity	2.96	3.27	3.40	3.35	0.142	0.150	0.0833	0.124
Fresh manure hedonic score[5]	-3.05	-2.73	-2.76	-2.89	0.236	0.775	0.799	0.357
Aged manure hedonic score	-5.00	-4.57	-4.84	-5.56	0.310	0.180	0.108	0.152

[1]Least squares means

Manure samples were mixed from individual feces and urine samples at the ratios they were produced.

[3]Odor intensity was evaluated by comparing the odor intensity of the headspace air to the odor intensities of a series of concentrations of n-butanol.

[4]Manure samples after anaerobic aging at room temperature for 21 days

[5]Hedonic score, degree of pleasantness or unpleasantness according to a -10 to +10 hedonic tone scale

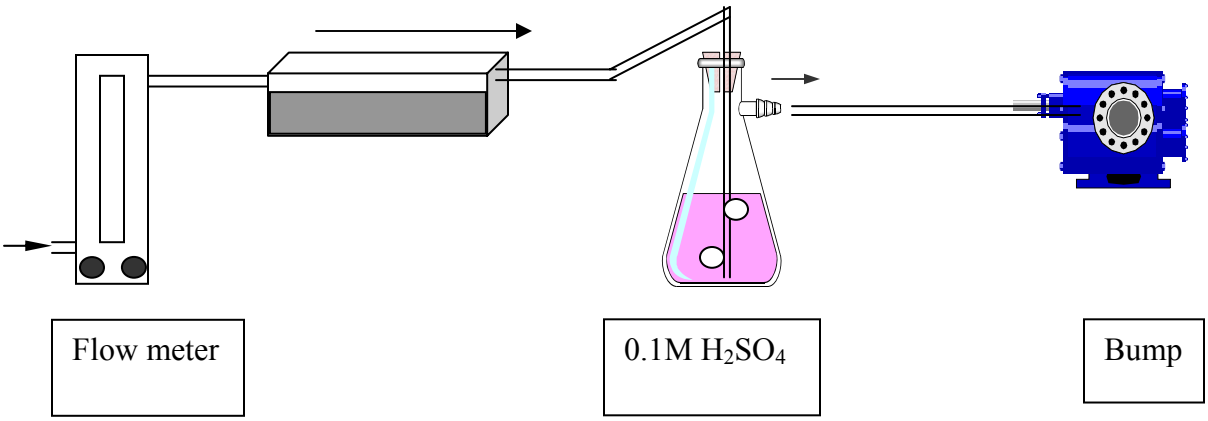


Figure 1 Diagram of the ammonia set-up

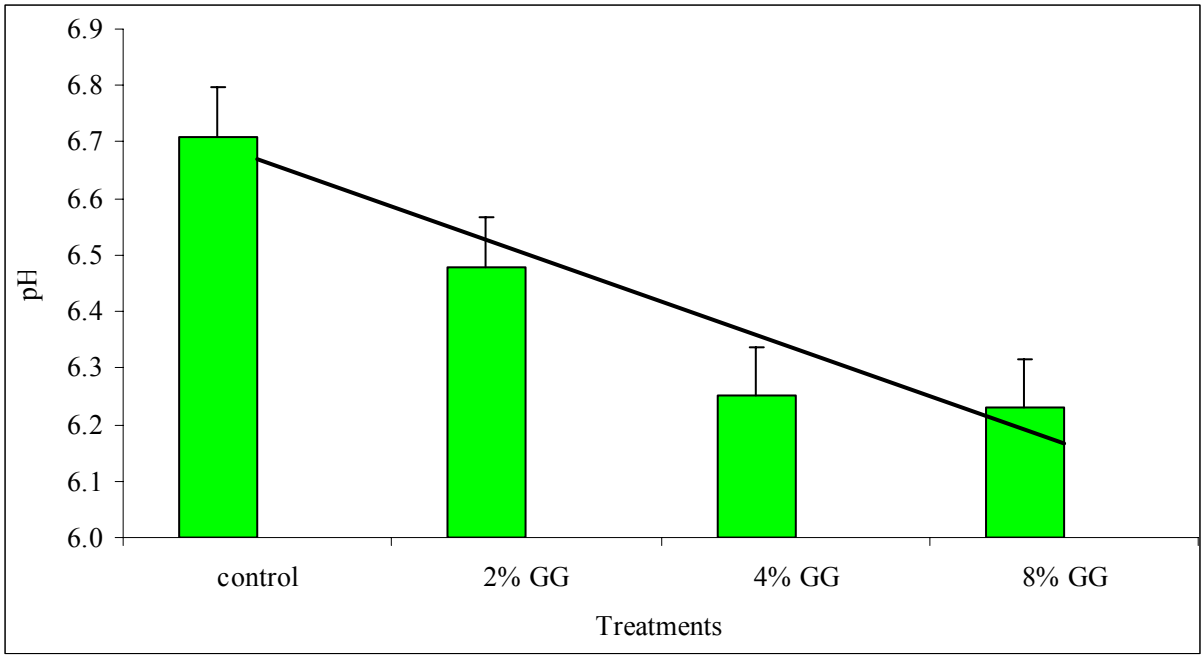


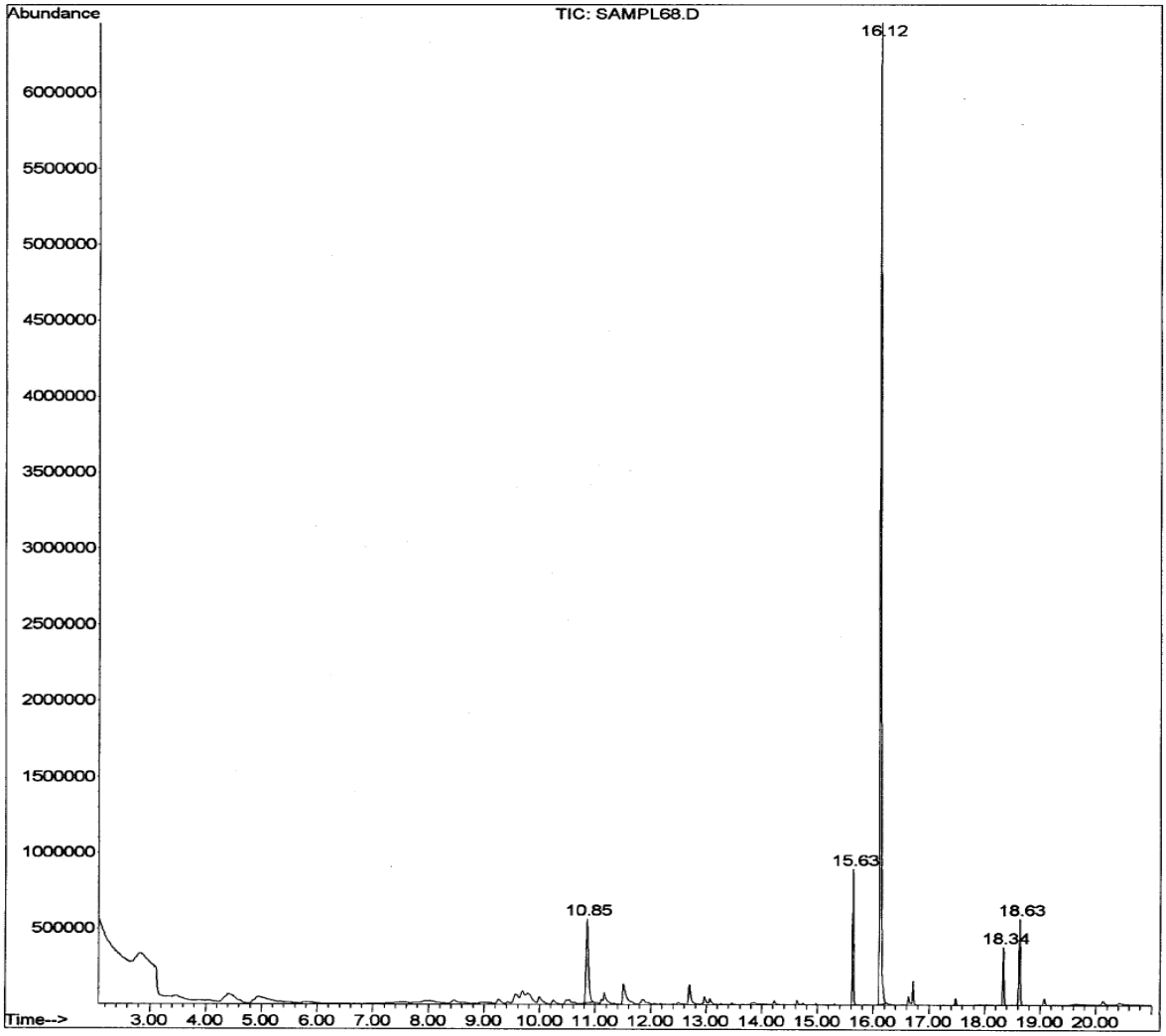
Figure 2 pH values of colon contents as influenced by guar gum (GG) supplementation

Area Percent Report

Data File : D:\DATA\ZHANG\SAMPL68.D
Acq On : 19 Oct 2006 13:16
Sample : fresh manure fiber1(3)
Misc : spme 5 min desorption

Vial: 1
Operator: wz
Inst : Instrum
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e
Method : C:\MSDCHEM\1\METHODS\WFANG100920062.M (Chemstation Integrat
Title :



SAMPL68.D WFANG100920062.M Thu Oct 19 13:37:56 2006 Page 2

Figure 3. Area percent report of manure headspace odorants analysis by GC/MS

Chapter III. Effects of genotype and diet on nutrients digestibility and manure odorants in growing-finishing pigs

ABSTRACT: This study was designed to measure the effects of a simple diet (1980 diet) or a modern diet (2005 diet) and an old genotype (1980) or a modern genotype (2005) on GE, CP, P, and NSP digestion as well as fecal odor compounds. Our hypothesis was that lower nutrient digestibility and surplus nutrients would produce more odor compounds in swine manure. Twenty-seven growing-finishing pigs with an initial average BW of 66.98 kg (± 10.26) were allotted into 4 treatment of a 2 × 2 factorial arrangement. After a 7 day adjustment period in metabolism crates, total feces and urine were collected for 3 days. The genotype did not significantly affect apparent CP and GE digestibility, although the 1980 genotype pigs tended to consume less feed. The 1980 genotype pigs fed the 2005 diet had the lowest feed intake and body weight gain, but their N excretion through urine was the highest. The ammonia emission from fresh manure samples was not significantly different, except for the data of 72 hours, which showed the manure of 2005 genotype pigs fed the 2005 diet emitted more ammonia. For aged manure samples, ammonia emissions also tended to be higher from manure of pigs fed the 2005 diet. The odor scores evaluated by a panel indicated that there was a slight trend that the aged manure of 2005 genotype pigs had higher odor intensity.

INTRODUCTION

Both genetics and dietary improvements have contributed significantly to increased growth performance of pigs over the last 25 years. Higher lean mass growth allows more

nutrients to be deposited (Campbell and Taverner, 1988). Pigs with high lean tissue gain potential had been reported to have higher growth performance and higher protein deposition than pigs with medium lean gain potential (Friesen et al., 1994).

Many changes have taken place in the design of diets for pigs compared to 25 years ago. These include the use of enzymes, crystalline amino acid, improved nutrient balance, improved feed processing (eg, pelleting) and more complicated phase feeding. More balanced diets and the use of various feed additives have improved the digestibility of feed. Growth performance was improved by enzyme supplementation and most studies showed improved digestibility by dietary enzyme supplementation (Pettey et al., 2002). Harper et al. (1997) reported that phytase supplementation in low-phosphorus diets improved phytate P bioavailability and improved growth performance in growing-finishing pigs. Effect of phytase on reducing P excretion by improving P bioavailability has been clearly demonstrated (Sands et al., 2001; Traylor et al., 2001; Nyachoti et al., 2006; Radcliffe et al., 2006). Smith et al. (2004) reported that dietary phytase contributed to decreased manure pH value and decreased ammonia emission.

The efficacy of low CP diets with supplemented crystalline amino acids on reducing manure ammonia emissions has been demonstrated (Sutton et al., 1999; Panetta et al., 2006). Higher feed utilization also contributes to reducing odor-causing agents in manure because there would be less substrate for bacterial activity, which produce odor compounds. Yokoyama et al. (1982) found that weaning pigs on a chlortetracycline-sulfamethazine-penicillin diet tended to have better growth performance and tended to have decreased fecal and urinary p-cresol. Addition of lincomycin sulfate in the diet had a similar tendency but

with a lower range. Pelleting of pig diets increased DM digestibility by 3% and N digestibility by 10% compared to meal diets (Wondra et al., 1995).

The objectives of our study were to compare the contribution of genotype or diet on nutrient digestion and retention in growing-finishing pigs, and determine if genotype and diet affect swine odor.

MATERIALS AND METHODS

First parity white line females were obtained from an unselected commercial population formed in 1980 that had been maintained at NCSU since 1989. These sows were mated using frozen semen of Hampshire or Duroc boars of 1980. Pigs representative of 2005 genotype of similar age were obtained from a NC swine production company.

All pigs were reared at the North Carolina Swine Evaluation Station in Clayton, NC. After farrowing, 28 piglets (14 pigs of the 1980 genotype and 14 pigs of the 2005 genotype) at approximately 7 kg BW were selected and allotted to a 2 x 2 factorial design (factors included genotype representative of 1980 and 2005, and feeding program representative of 1980 and 2005). A simple diet was fed to Group 1 (1980 genotype, 1980 diet) and Group 2 (2005 genotype, 1980 diet) and a modern diet was fed to Group 3 (1980 genotype, 2005 diet) and Group 4 (2005 genotype, 2005 diet). There were 7 replicates per treatment. The characteristics of the feeding programs are shown in table 1. Major differences in feeding programs included diet formulation (no-antibiotics versus antibiotics, no crystalline amino acids versus crystalline amino acids, no-phytase versus phytase), feed processing (meal diets versus pellets), and feeding phases (simple versus phased feeding program). Table 2 lists the 1980 feeding program which consisted of four meal diets. The nutrient levels (eg. lysine from 1.05 to 0.62% and ME from 3262 to 3317 Kcal/kg) were based on formulations from the

1978 Pork Industry Handbook (PIH). The 2005 feeding program consisted of a seven phase program consisting of pelleted diets with improved nutrient levels (eg, lysine from 1.51 to 0.73% and ME from 3428 to 3651 Kcal/kg), and the diet formulation were similar to that used by NC producers (Table 3).

The pigs were allowed ad libitum access to feed and water. After the pigs' body weight reached to approximately 65 kg, 16 pigs with an initial average BW of 65.2 kg were transferred to Grinnells laboratory where the pigs were housed individually in metabolism cages (0.6 × 1.5 m) and given ad libitum access to feed and water. The feed formulations for finishing diets are listed in table 4. After 6 days of adaptation, each pig was fed 6 g of Cr_2O_3 mixed with 300 g of feed on day seven and this marked diet was fed again on day 10. The amount of normal feed intake between the above two meals were recorded. Then total feces and urine was collected for approximately 3 days between the appearances of green feces.

Feces were collected quantitatively on wire screens and were frozen at -20°C until further chemical analysis was conducted. Urine was collected quantitatively on slope-shaped stainless steel trays in plastic containers placed in ice to minimize gaseous losses of nitrogen. Quantity of feces and urine was recorded and frozen at -20°C as soon as it was collected, twice daily.

Two weeks later, 11 pigs (three pigs per treatment, but only two pigs for the 2005 genotype and 1980 diet treatment) with an initial BW of 69.6 kg were used and all the procedures were same as described previously. All of the animal trial procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University.

After the animal trial, frozen feces and urine were thawed and mixed together and

homogenized within respective animal (at the rates they were produced). A portion of this manure was used to determine fresh manure ammonia emission and the remaining manure were stored in a 1-L plastic container and allowed to sit at room temperature for 21 days of anaerobic aging. Both fresh and aged manure were sampled for odor evaluation by a professional odor panel. The panelists were asked to smell each sample individually and assign a designation of degree of pleasantness or unpleasantness according to a -10 to +10 hedonic tone scale, with 0 being neutral. They also were asked to assign a score for strength of odor by smelling the sample and comparing it to standards. The intensity standards were prepared per ASTM E 544-99 with n-butanol. The n-butanol concentrations for the 1-5 scale (very faint, faint, moderate, strong, and very strong) were 250, 750, 2250, 6750, and 20250 ppm, respectively (Guo et al., 2001). The panelists smelled the sample and compared it to the standards for strength of odor and assigned a standard number (1, 2, 3, 4, or 5) that matched the strength of the sample. If a sample fell between 2 standards, a designation of 0.5 was used (0.5 if < 1, 1.5, 2.5, 3.5, 4.5, or 5.5 if > 5).

Ammonia emission of the manure samples was determined by placing 400 ml of the manure mixture in a rectangular (28 L × 9.5 W × 6 H cm) container (Super Oval 1, Tupperware Co., Orlando, Fl). Air was drawn through a flow meter (Cole Palmer, Vernon Hills, IL) at a rate of 1.4 L/min, the container with manure, and then through a gas dispersion tube (Fisher, Pittsburg, PA) placed in a 500 ml Erlenmeyer flask containing 400 ml dilute sulfuric acid (N/10) in order to trap the ammonia released from the manure. This sulfuric acid solution was sampled (6 ml) at 12, 24, 36, 48, 72, and 96 h and analyzed for ammonia using the procedure of Willis et al. (1996).

Fecal samples were dried using a freeze dryer (Heto PowerDry LL3000, ATR, Laurel,

MD), and all the samples including 2 feed samples were ground through a 1 mm screen for chemical analysis. Dry matter content of feed and fecal samples was measured by AOAC method (AOAC, 1990). GE was determined using an adiabatic bomb calorimeter (model C5000, IKA, Wilmington, NC). Subsequently, feed samples (2) and fecal samples (27) were sent to the Experimental Station Chemical Laboratories (University of Missouri-Columbia, MO 65211) for N and P analysis (urine samples were measured for N only). N content was measured by the Kjedahl method (AOAC, 1990). Total P was measured by the AOAC method AOAC 985.01 (A, B, D) for ICP-OES.

The apparent fecal CP digestibility was calculated according to the following equation:

Apparent fecal CP digestibility % = 100 - $[(FE \times CP_f)/ (CP_d \times FI)] \times 100$

Where FE = feces excretion mass during the 3 days (kg), CP_f=CP concentration in feces (g/kg), CP_d=CP concentration in the diet (g/kg), and FI= feed intake during the 3 days (kg).

The equations used for apparent fecal GE and P digestibility calculation were similar to the above equation but used the corresponding index.

Statistical analyses: To compare differences in measured variables among treatments, data were analyzed by two-way ANOVA using the GLM procedures of SAS (SAS Institute, Cary, NC) as a 2 × 2 factorial arrangement. The statistical model included main effects for genotype and diet and the genotype x diet interaction.

RESULTS AND DISCUSSION:

The growth performance data during the metabolism study are summarized in Table 5. In agreement to a previous study (Friesen et al., 1994), our data indicated that both modern genotype and modern diet improved body weight gain of pigs and the contribution from diet

was much higher than genotype. However, the feed intake of the 1980 genotype pigs fed the 2005 diet was lower ($P<0.05$) than the other groups. The low feed intake resulted in a reduction in ADG and worsening feed/gain ratio. The possible reason may be that the nutrient density of the 2005 diet exceeded the lean tissue potential of the 1980 genotype pigs.

The contributions of genotype and diet to improvements in broilers' growth performance were evaluated by Havenstein et al. (1994; 2003). The results showed that both modern genotypes and modern diets improved growth performance significantly (using 1991 diet increased BW of the 1957 genotype birds by 22% and increased BW of the 1957 genotype birds by 14%).

Total fecal mass excretion of 1980 genotype pigs fed the 2005 diet was lower ($P<0.05$) than other treatments (table 6). Interestingly, the lowest ($P<0.05$) GE of non-urea components in dried urine was observed for the 1980 genotype 2005 diet pigs while there were no effects on total GE of urine. This means more energy in the urine sample of this group of pigs originated from urea. The concentrations of amino acid in the 2005 diet were likely higher than the requirements of the 1980 genotype pigs.

Similarly, in table 7, the N excretion from urine of the 1980 genotype fed the 2005 diet was higher ($P<0.05$) than that of other groups, while N excretion from feces was lower ($P<0.05$) than other groups. The lower fecal N excretion was consistent with its higher ($P<0.05$) apparent CP and GE digestibility. Diet had extremely significant effects on apparent CP and GE digestibility. As for the N intake and N retention, the 1980 genotype pigs fed the 2005 diet had significantly higher ($P<0.05$) values than those of other treatments and no significant difference was noticed among the other three treatments. No significant difference was observed among the P digestibilities.

Table 8 lists the ammonia emissions from fresh or aged manure samples. There were no significant differences among any of the treatments for fresh manure samples, except for the data at 72 hours, which showed that the manure of 2005 genotype pigs fed the 2005 diet emitted more ($P<0.05$) ammonia. For the aged manure samples, ammonia emissions from manure from pigs fed the 2005 diet were higher ($P<0.05$) at all time points. The possible reason is that the 1980 diet contained relatively higher levels of dietary fiber and the acidic fermentation products of fiber trapped more ammonium, so less ammonia was converted from urea and emitted.

Table 9 showed the result of odor scores evaluated by a professional panel. There was a slight trend that the aged manure of 2005 genotype pigs had higher ($P<0.05$) odor intensity. The only significant difference ($P<0.05$) was between the 1980 genotype pigs fed the 1980 diet treatment and the 2005 genotype pigs fed the 1980 diet treatment (highest number).

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Table 1. Comparison of characteristics of 1980 vs 2005 feeding programs

1980 Feeding Program	2005 Feeding Program
Diet formulation common to 1980	Diet formulation common to 2005
No antibiotics	Antibiotics
No synthetic amino acids	Synthetic amino acids
Simple nursery diets	Complex nursery diets
Simple feeding program	Phased feeding program
No enzymes	Phytase
Meal feed	Pellet diet

Table 2. 1980 Feeding Program

	Prestarter	Starter	Grower	Finisher
Crude Protein, %	18.3	17.9	15.0	13.3
Metabolizable Energy, kcal/kg	3262	3299	3315	3317
Calcium, %	0.87	0.78	0.67	0.67
Phosphorus, %	0.74	0.70	0.60	0.56
Lysine, %	1.05	0.95	0.75	0.62
Amount budgeted per pig, kg	11.3	15.9	90.7	to market

Table 3. 2005 Feeding Program

	Prestarter	Starter 1	Starter 2	Grower 1	Grower 2	Finisher 1	Finisher 2
Crude Protein, %	22.6	22.3	22.1	17.9	16.9	14.7	12.0
Metabolizable Energy, kcal/kg	3428	3405	3438	3630	3643	3655	3651
Calcium, %	0.84	0.79	0.72	0.52	0.48	0.43	0.39
Phosphorus, %	0.72	0.68	0.64	0.55	0.47	0.41	0.37
Lysine, %	1.51	1.43	1.36	1.22	1.13	0.94	0.73
CTC, g/ton	400	400	400	400	-	-	-
Denaguard, g/ton	35	35	35	-	-	-	-
Tylan, g/ton	-	-	-	-	20	-	-
Stafac, g/ton	-	-	-	-	-	10	5
Amount budgeted per pig, kg	4.54	9.07	13.61	18.1	45.4	56.7	to market

Table 4. Formulations of 1980 and 2005 diets used in the metabolism study.

	1980	2005
Ingredients		
Corn	83.90	72.25
Soybean meal (CP 48%)	13.25	18.75
Lard	-	6.97
Monocalcium phosphate	-	0.49
Dicalcium phosphate	1.25	-
Limestone	0.85	0.70
Salt	0.50	0.34
Selenium premix (0.06%)	-	0.05
[a]Vitamin-minerals premix	0.25	0.10
Lysine (78.8%)	-	0.24
DL- Methionine	-	0.03
L-threonine (98.5%)	-	0.06
Ronozyme	-	0.02
Calculated nutrient composition		
Crude Protein, %	13.3	14.7
Metabolizable Energy, kcal/kg	3317	3655
Calcium, %	0.67	0.43
Phosphorus, %	0.56	0.41
Lysine, %	0.62	0.94
Stafac, g/t	-	11.03

[a] Vitamin and mineral premix of 1980 diet supplied the following per kg of complete diet - 5,540 IU of vitamin A as retinyl acetate, 1,108 IU of vitamin D_3, 22 IU of vitamin E as dl-a-tocopherol acetate, 1.98 mg of vitamin K as menadione dimethylpyrimidinol bisulfite, 165 mg of choline as choline chloride, 22 mg of niacin as niacinamide, 17.6 mg of d-pantothenic acid as dl-calcium pantothenate, 4.4 mg of riboflavin, 1.1 mg of pyridoxine as pyridoxine·HCl, 0.55 mg thiamine as thiamine mononitrate, 0.022 mg of vitamin B_{12} , 0.33 mg of folic acid, 0.04 mg of d-biotin, 110 mg Zn as $ZnSO_4$, 110 mg Fe as $FeSO_4$, 22 mg Cu as $CuSO_4$, 55 mg Mn as MnO, 0.28 mg I as ethylenediamine dihydriodide, and 0.30 mg Se as $NaSeO_3$

Table 5. Effect of genotype and diet on growth performance[1].

Genotype	1980		2005		pooled	P value		
Diet	1980	2005	1980	2005	SEM	genotype	diet	genotype×diet
Start BW(kg)	58.89[a]	68.04[b]	61.75[ab]	78.08[c]	2.88	0.0352	0.0002	0.225
End BW (kg)	66.60[a]	73.55[a]	69.40[a]	86.38[b]	3.02	0.0168	0.0006	0.111
ADG (kg)	0.771[a]	0.551[b]	0.765[a]	0.829[a]	0.0741	0.0802	0.306	0.0677
ADFI (kg)	2.29[a]	2.00[b]	2.39[a]	2.39[a]	0.0890	0.0021	0.0524	0.0547
G/F	0.327[ab]	0.267[a]	0.308[ab]	0.358[b]	0.0317	0.268	0.882	0.100

[1]Least squares means

[abc] Means within a row with different superscripts differ ($P < 0.05$).

Table 6. Effect of genotype and diet program on waste excretion and feces sample dry matter[1].

Genotype	1980		2005		pooled	P value		
Diet	1980	2005	1980	2005	SEM	genotype	diet	genotype×diet
Feces weight per day (kg)	0.807[a]	0.591[b]	0.835[a]	0.832[a]	0.0655	0.055	0.112	0.122
Urine weight per day (kg)	1.978	1.848	1.930	1.684	0.191	0.588	0.340	0.766
Freeze dried urine GE (cal/g)	2387[a]	2370[a]	2396[a]	2525[b]	35.59	0.034	0.138	0.054
non-urea GE[2] in dried urine	1035[a]	658[b]	1053[a]	1135[a]	69.53	0.0022	0.049	0.0039
Feces dry matter ratio	0.324	0.341	0.323	0.320	0.0105	0.315	0.516	0.362
Feces dry matter per day (kg)	0.261[a]	0.198[b]	0.270[a]	0.261[a]	0.0183	0.069	0.067	0.160

[1]Least squares means

[2]Caculated as GE (per gram) of freeze dried urine minus GE (per gram) of urea

[ab] Means within a row with different superscripts differ ($P < 0.05$).

Table 7. Effect of genotype and diet program on nutrients digestibility

Genotype	1980		2005		pooled	P value		
Diet	1980	2005	1980	2005	SEM	genotype	diet	genotype×diet
Apparent fecal CP digestibility	0.797[a]	0.862[b]	0.809[a]	0.843[b]	0.0101	0.742	<0.0001	0.113
Apparent fecal GE digestibility	0.848[a]	0.894[c]	0.859[a]	0.884[bc]	0.0067	0.957	<0.0001	0.124
Apparent fecal P digestibility	0.331	0.416	0.405	0.415	0.0364	0.332	0.202	0.308
N intake (g/ day)	39.2[a]	48.9[b]	44.2[ab]	58.6[c]	3.238	0.0367	0.0015	0.484
N excretion from feces (g/ day)	7.67[ab]	6.71[a]	8.26[ab]	9.05[b]	0.695	0.0474	0.905	0.222
N excretion from urine (g/ day)	6.23[a]	16.01[c]	10.44[b]	12.68[bc]	1.468	0.774	0.0018	0.0265
N retention (g/ day)	25.08[a]	27.05[a]	25.48[a]	37.20[b]	3.047	0.101	0.0377	0.128

[1]Least squares means

[abc] Means within a row with different superscripts differ ($P < 0.05$).

Table 8. Effects of genotype and diet program on ammonia emissions of 400 ml fresh or aged manure samples[1].

Genotype	1980		2005		pooled	P value		
Diet	1980	2005	1980	2005	SEM	genotype	diet	genotype×diet
				Fresh manure				
12 hour (mmol)	0.54	0.84	0.94	1.53	0.339	0.127	0.204	0.672
24 hour (mmol)	2.75	2.74	4.45	6.08	1.23	0.052	0.518	0.512
36 hour (mmol)	8.96	7.22	7.01	12.42	2.16	0.460	0.404	0.112
48 hour (mmol)	18.76	14.34	13.04	21.05	3.85	0.899	0.645	0.12
72 hour (mmol)	35.77^{ab}	26.81^{a}	28.05^{a}	41.34^{b}	4.89	0.494	0.663	0.033
96 hour (mmol)	40.14	36.42	39.05	44.03	3.37	0.345	0.855	0.211
				Aged manure				
12 hour (mmol)	17.99^{a}	36.31^{b}	21.91^{a}	37.22^{b}	4.01	0.556	0.0005	0.713
24 hour (mmol)	30.24^{a}	46.88^{b}	36.64^{a}	55.90^{c}	4.76	0.124	0.0024	0.787
36 hour (mmol)	40.86^{a}	53.48^{ab}	46.28^{a}	59.89^{b}	4.52	0.209	0.010	0.915
48 hour (mmol)	44.46^{a}	50.04^{ab}	48.23^{a}	60.76^{b}	3.92	0.082	0.033	0.389
72 hour (mmol)	46.83^{ab}	48.01^{ab}	46.25^{a}	55.19^{b}	2.79	0.254	0.087	0.183
96 hour (mmol)	43.66^{a}	45.32^{a}	41.93^{a}	51.22^{b}	2.04	0.323	0.015	0.079

[1]Least squares means

[abc] Means within a row with different superscripts differ ($P < 0.05$).

Table 9. Effects of genotype and diet program on manure odor intensity and hedonic score[1].

Genotype	1980		2005		pooled	P value		
Diet	1980	2005	1980	2005	SEM	genotype	diet	genotype×diet
Fresh manure[2] odor intensity[3]	2.35	2.35	2.44	2.34	0.183	0.815	0.781	0.798
Aged manure[4] odor intensity	3.19[a]	3.65[ab]	3.83[b]	3.75[ab]	0.206	0.0866	0.367	0.214
Fresh manure hedonic score[5]	-3.96	-2.88	-2.95	-3.08	0.261	0.713	0.944	0.690
Aged manure hedonic score	-4.63	-4.83	-4.73	-5.03	0.297	0.616	0.409	0.874

[1]Least squares means

[2] Manure samples mixed by individual feces and urine according to their nature ratios

[3] Odor intensity was evaluated by comparing the odor intensity of the headspace air to the odor intensities of a series of concentrations of n-butanol.

[4] Manure samples after anaerobic aging at room temperature for 21 days

[5] hedonic score, degree of pleasantness or unpleasantness according to a -10 to +10 hedonic tone scale

[ab] Means within a row with different superscripts differ ($P < 0.05$).

Chapter IV. Effects of dietary NSP level on fecal digestibility in growing swine and odor concentrations in air

ABSTRACT: This study was designed to measure the effects of dietary non-starch polysaccharides (NSP) on protein and GE digestion as well as excretion of odor compounds. Diets were formulated as a low fiber diet (degermed dehulled corn and soybean protein isolate), a semi-low fiber diet (corn + soybean protein isolate), a commercial control diet (corn + soybean meal), and a high fiber diet (corn + soybean meal + 10% soybean hulls). This trial was divided into four identical batches. For each batch, 40 growing pigs with an initial average BW of 20 kg were allotted randomly into four groups and were fed one of the experimental diets. Pigs were housed at the swine educational unit for an adjustment period of 14 days. Pigs were then transferred into chambers for odorant emissions analysis. Data of the two week adjustment period showed that body weight gain was increased by dietary fiber supplementation, while the fecal digestibility data showed that CP and GE digestibility were reduced by dietary fiber. Dietary fiber decreased ammonia emissions during the first 3 days of collection. Total reduced sulfur (TRS) and short-chain fatty acids (SCFA) emissions were increased in the 10% soy hulls treatment. The odor detection threshold (ODT) evaluation results indicated that fiber increased odor intensity.

INTRODUCTION

Odor from swine production is a public concern, especially for swine in confined animal feeding operations (CAFOs). Dietary manipulation may provide an effective means of reducing odors from swine operations.

Supplementation of diets with non-starch polysaccharides (NSP) has been effective in reducing ammonia emission from swine manure (Canh et al., 1998; Sutto et al., 1999; Mroz et al., 2000). The high availability and low price of soy hulls make it attractive as a source of dietary NSP. Ammonia emission could be decreased because dietary soy hulls can shift urinary N to fecal N (Zervas and Zijlstra, 2002) and, the latter is relatively more stable. Another reason is that the metabolites of dietary fiber will decrease the pH of the manure (Canh et al., 1997), hence will minimize ammonia volatilization. On the other hand, the emissions of acidic odorants such as total reduced sulfur (TRS) and short-chain fatty acids (SCFA) will be increased by dietary fiber. In addition, dietary fiber is resistant to the digestion by endogenous enzymes from mammalian hosts and it will decrease the digestibility of nutrients in the feed (Ravindran et al., 1984; Lenis et al., 1996; Schrama et al., 1998). The effects of bacterial decomposition products of those feed residues as well as fecal bacteria mass itself has not been studied systematically.

This trial was designed to investigate the effects of dietary fiber levels on emission of the main odorants from swine housing and nutrient digestion. Degermed, dehulled (DGDH) corn and soybean protein isolate (SPI) were used as low fiber ingredients and soy hulls were used as a fiber rich ingredient. The objectives were to evaluate the effects of fiber levels, below and above a standard corn-soybean meal diet, on the emission of odorants.

MATERIALS AND METHODS

This experiment was divided into four identical trials. In each trial, 40 growing pigs with an initial BW of 20.4 kg were used. Pigs were blocked by weight and randomly assigned within block to four dietary treatments (10 pigs per treatment). Diets were formulated as a low fiber diet (DGDH corn and soybean protein isolate), a semi-low fiber diet (corn + soybean protein isolate), a commercial control (corn + soybean meal), and a high fiber diet (corn + soybean meal + 10% soybean hulls). The diets were fed in mash form (Table 1).

Pigs were housed at the Swine Educational Unit at North Carolina State University and fed the experimental diets for two weeks to allow for adjustment to the diets. All pigs were fed ad libitum and all conditions were as similar as possible to those in a commercial facility. After the adaptation period, pigs were moved to Grinnells laboratory and 10 pigs within each treatment group were housed in one of four identical air controlled odor chambers. These chambers (3.0 m L × 2.4 m W × 2.0 m H) were designed to control and measure airflow and allow for collection of air from the chambers for ammonia and odor analysis. The chambers were equipped with fully slatted floors and shallow pits with pit recharge. Water (575 L) was pre-charged in each of the pits and resulted in a water depth of approximately 10 cm. The airflow through each chamber was measured by Dwyer DS-300 flow sensors (Dwyer instruments, Inc., Michigan City, IN) and was kept low, at approximately 316 m^3/h, for odorant accumulation. The temperature in the chambers was maintained at 25 °C.

On day 6, ammonia emission was determined by drawing air from each chamber through a flow meter (Cole Palmer, Vernon Hills, IL) at a rate of 1.4 L/min, and then through a gas dispersion tube (Fisher Scientific, Pittsburg, PA) placed in a 500 ml Erlenmeyer flask containing 400 ml dilute sulfuric acid (0.10 N) in order to trap the ammonia. This sulfuric

acid solution was sampled (1.5 ml) at 24, 48, 72, and 96 h and analyzed for ammonia using the procedure of Willis et al. (1996). Pigs were moved out of the chambers on day 8 and ammonia collection was continued for an additional 2 days.

On day 7, TRS concentration of the chambers' exhaust air was measured using a Jerome meter (Arizona Instrument LLC., Tempe, Arizona). In addition, air samples were collected in 10 L Tedlar bags (SKC Gulf Coast Inc., Houston, TX) using a Vac-U-Chamber (Supelco, Bellefonte, Pa.) and were shipped overnight to Iowa State University Olfactometry and Air Quality Laboratory. Odor detection threshold (OTD) was evaluated by a professional panel with 8 panelists using an Ac'Scent International Olfactometer (St. Croix Sensory, Inc., Stillwater, MN). The evaluation was based on a forced-choice ascending concentration series method (E679-04 standard practice) and values are reported as averages (geometric means) of the 8 individual responses.

Odor compounds were adsorbed by solid phase microextraction (SPME) fibers (Carboxen^{TM}/Polydimethylsiloxane fiber, Upelco SPME portable field sampler, Supelco, Bellefonte, Pa.). The fibers were exposured to the exhaust air stream with an air flow of approximately 4.81 m/s for 30 min and were analyzed by a GC/MS (GC HP 6890; MS HP 5973) immediately. Compounds were separated on a 30 m x 0.32 mm diameter x 0.25µm film thickness Innowax PEG column (Agilent Technologies, Palo Alto, CA). Injector temperature was maintained at 245°C and detector temperature was 250°C. The column was programmed as follows: flow rate 0.5 ml/min, initial temp 40°C, initial time 3 min, the temperature ramp 12°C /min to 220°C then hold 10 min. The identities of odor compounds were determined by comparison to the retention times of known standards, and were further confirmed by comparing the mass spectra.

On day 8, fresh feces from the concrete slats of each chamber were sampled and frozen at -20°C for further analysis. Subsequently, the first batch of 40 pigs was removed. The slurry of each chamber were sampled and the pH values were measured by using a pH meter (Accumet® pH model 610 A, Fisher pH, Ambler, PA, USA). After 2 days of continual trapping of ammonia without pigs, the chambers were thoroughly cleaned. This was then repeated another three times to achieve a total of 4 replicates per treatment. Treatments were assigned to each of the chambers such that each dietary treatment occurred in each of the chambers one time to avoid confounding between treatment and chamber.

Fecal samples were dried in a freeze dryer (Heto PowerDry LL3000, ATR, Laurel, MD), then all samples as well as 4 feed samples were ground through a 1 mm screen for dry matter analysis and further chemical analysis. Dry matter content was measured by AOAC (1990) method. GE was determined by an adiabatic bomb calorimeter (model C5000, IKA, Wilmington, NC). Subsequently, feed samples (4) and fecal samples (16) were sent to the Experimental Station Chemical Laboratories (University of Missouri-Columbia, MO 65211) for chromium, N, and NDF analyses. Chromium was measured by atomic absorption spectrometry after digestion of the samples with perchloric acid. Nitrogen and NDF content were measured by AOAC (1990) methods.

The apparent fecal CP digestibility was calculated according to the equation as follow:

Apparent fecal CP digestibility % = 100 - $[(M_d \times CP_f)/ (CP_d \times M_f)] \times 100$

Where M_d = chromium concentration in the diet (mg/kg), CP_f =CP concentration in feces (g/kg), CP_d=CP concentration in the diet (g/kg), and M_f = chromium concentration in the feces (mg/kg).

The equations used for apparent fecal GE, NDF, and ADF digestibility calculation were

similar to the above equation but used the corresponding index.

Statistical analyses: Data were analyzed by two-way ANOVA using the GLM procedure of SAS (SAS Institute, Cary, NC). Least squares means among diet treatments were evaluated by PDIFF and STDERR and batch was included as blocking factor.

RESULTS AND DISCUSSION:

Table 2 lists the growth performance of pigs during the two week adjustment period. On the contrary to the concept that a higher fiber diet reduces pig body weight because of its low energy density, the pig body weight gain of the corn-soybean meal diet and the soy hull diet were significantly higher ($P<0.0001$) than the low fiber diets. Previous studies indicated that the intestinal weight of pigs fed a 5% soy hulls diet was around 50% higher than that of a control diet (Whitney et al., 2006), and the increase in stomach weight was around 25%. According to the result of Moeser et al. (2002), replacing corn by DGDH corn did not impair body weight gain and the GI tract weight of pigs fed DGDH diet was not significantly different from that of pigs fed a corn grain diet. The major difference between the diets of the current trial and that of Moeser et al. (2002) was that in the current trial we used soy protein isolate to replace the soybean meal. These fiber-free ingredients may effectively decrease dietary fiber, especially soluble fiber level. Studies with rats showed the gut wall weight was decreased (Goodlad and Wright, 1983) when an elemental diet was fed. They found that a fiber free diet induced mucosal atrophy, which could be prevented by dietary cellulose addition but Kaolin (used as inert bulk) addition did not have this effect. McCullough et al. (1998) compared the effects of dietary fiber between germ free and conventional rats. The results showed that fiber fermentation by bacteria stimulated crypt branching while fiber itself can increase the gut mass directly.

The sudden change of a common corn-soybean meal based diet fed prior to this study to the very low fiber diet (DGDH diet and SPI diet) may also contribute to the low body weight gain.

Table 3 shows the apparent nutrient digestibility of the 4 diets. Fecal CP and GE digestibility was decreased as dietary fiber level increased ($P<0.0001$). Sohn et al. (1994a) reported that the apparent fecal DM and CP digestibility of soy protein isolate was significantly higher than that of soybean meal. Similarly, the ileal digestibility of amino acids from soy protein isolate was significantly higher than that of soybean meal (Sohn et al., 1994b). Bakker et al. (1995) reported soybean hull supplemented diets significantly decreased apparent crude protein digestibility.

In the present study, the fecal NDF and ADF digestibilities of the DGDH diet were significant higher ($P<0.05$) than the other diets. Dietary soy hulls addition had no significant effect ($P>0.05$) on fecal NDF and ADF digestibility compared to the semi-low fiber and normal corn-soybean meal treatments. The fermentability of NDF and ADF in soy hulls has been reported to be higher ($P<0.05$) than other common plant cell materials such as wheat bran and sugar beet pulp (Chabeauti et al., 1991). Dietary ME decreased ($P<0.05$) linearly with soy hull addition (Kornegay, 1981). A trial in rats showed NSP fermentability of corn bran was around 0.16 (Livesey, et al., 1995) which was much lower than that of soybeans (around 0.87). Dietary fiber has been showed to hold more water in feces. This agrees with the data of beet pulp addition to dog diet which showed lower ($P<0.05$) fecal dry matter (Fahey et al., 1992).

Table 4 lists the ammonia emissions from chambers housing pigs fed different levels of fiber (Figure 2). Day 1 and day 2 was the time that the pigs were housed in the chambers and

the day 3 and day 4 was the period without pigs but with the manure. The ammonia concentrations tended to decrease as dietary fiber level increased during first two days. This result agrees with the result of previous research (Sohn et al., 1994a; Mroz et al., 2000). However, the data collected on day 3 showed that only the ammonia emission of the SPI treatment was significantly ($P < 0.05$) lower than the DGDH treatment. No significant differences were observed among treatments on day 4. The situation in lagoons or manure in regards to ammonia emission may be different from observations using fresh samples from feces or feces and urine. Both fecal N and fecal ammonium concentrations were increased by dietary fiber addition (Canh et al., 1998), and those potential ammonia sources will volatilize when the pH of slurry increases.

In this trial, the highest number for ammonia concentration of the exhausted air was 2.12 ppm. Such a low concentration of ammonia could probably not be detected by the human nose as the odor threshold value (OTV) of ammonia is estimated to be 4.7 ppm (Tamminga, 1992).

Table 5 lists the data of TRS concentrations (Figure 2) and slurry pH values. TRS is defined as all of the gaseous unoxidized sulfur compounds, and the major compound is H_2S (Clanton and Schmidt. 2000). TRS emission was increased with increasing dietary fiber level ($P< 0.05$). Compared to ammonia, H_2S may contribute more to the overall odor as its OTV is 0.5 ppb (Tamminga, 1992) and the range of TRS data of current trial was 4.21 - 8.50 ppb. The result of Hobbs et al. (2000) indicated that H_2S, p-cresol, and acetic acid were the three major odorants correlated with olfactory data.

Slurry pH value of DGDH group was higher ($P < 0.05$) than other groups. The numbers were slightly lower than those Shriver et al. (2003) reported, but have the same trend. This may explain, in part, the lower ammonia emission from pigs fed the higher fiber treatments.

Table 4 lists the results of main odorant peaks analyzed by GCMS. The data indicated that the SCFA emissions were increased ($P<0.05$) with increased dietary fiber level. The amount of odorants that are volatilized from manure not only depends on their concentrations in the manure, but also depends on the manure pH (Conn et al., 2007). The possible reason for a more significant difference in H_2S emission is that the pKa of H_2S is 7.1, which is very close to the pH values of slurry. The pKa of NH_3 is 9.3 and this may explain why NH_3 emission was not as sensitive to changes in pH of the manure. As for the SCFAs, the impact of decreasing pH with increasing fiber may not be as significant as that on H_2S emission because the pKa values for VFAs are around 4.8 to 5 (Perrin and Dempsey, 1974; Mikkelsen et al., 2004).

The phenol emission of pigs fed the soy hulls diet was higher ($P < 0.05$) than that of the DGDH and SPI treatments, but there were no differences in p-cresol ($P=0.398$), indole ($P=0.347$), and skatole ($P=0.512$) concentrations. These results contradict those reported by Jensen and Hansen (2006) with chicory roots (inulin) which showed that SCFA emissions were not different between control and a high fiber diet, but p-cresol, indole, and skatole concentrations tended to be decreased by dietary inulin addition. In the current trial, the higher ($P<0.05$) phenol level in the high fiber diet group may be the result of increased degradation of tyrosine, while the values of p-cresol, another metabolite from tyrosine (Otto et al., 2003), were not different among treatments. The difficulty of predicting protein fermentation by the emissions of the above compounds is that phenol (pKa=9.95), p-cresol

(pKa=10.27), indole (pKa= 16.2), and skatole are organic bases. But as the pKa value of phenol is lower than that of p-cresol, the phenol emission would be more sensitive to slurry pH. Phenol emission may be more representative to the total phenol concentration in the slurry. Therefore, the current data indicated that dietary fiber increased protein fermentation.

It was reported that the major odorants in animal housing were carried by dust (Hartung and Rokicki, 1984; Oehrl et al., 2001). That may explain why the odorants adsorbed on the SPME fibers were much lower ($P<0.05$) after pigs were removed, rather then when they were present (Kaspers, 2002), while the panel evaluation data based on over-night shipped air sampled in Tedlar^{TM} bags were unaffected by the presence or absence of pigs (Kai et al., 2006).

The result of ODT evaluation showed dietary fiber increased ($P<0.05$) odor intensity, which agrees with the data of SCFA, TRS, and phenol emissions. In general, the results of the odorant profile agree with the data of Kerr et al. (2006) which showed that dietary cellulose decreased manure ammonium but increased the concentration of SCFA. Dietary fiber did not affect the emission of other odorants except phenol which may indicate that protein fermentation was stimulated rather than inhibited by dietary fiber.

IMPLICATIONS:

Our results are consistent with the fact that dietary fiber decreases nutrients digestibility. Dietary fiber tends to increase odor intensity. Further study is warranted to assess the effects of dietary fiber on airborne microorganisms and air PM profile.

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Table 1. Formulation of the experimental diets (as fed basis)

Ingredients (%)	Low fiber diet	Semi-low fiber diet	Corn soybean	10% Soy hulls
DGDH corn[1]	80.24	_	_	_
Corn	0	82.91	69.42	62.55
Soybean meal	_	_	26.20	23.10
Soybean protein isolate[2]	15.1	12.40	_	_
Soy hulls	_	_	_	10.00
Corn oil	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.08	1.13	0.82	0.94
Limestone	0.88	0.86	0.86	0.71
Salt	0.35	0.35	0.35	0.35
Vitamin/Mineral mixture[3]	0.25	0.25	0.25	0.25
Cr_2O_3	0.10	0.10	0.10	0.10
Celite[4]	1.00	1.00	1.00	1.00
Calculated nutrients composition				
ME (Mcal/kg)	3.44	3.36	3.34	3.18
CP (%)	18.25	17.52	18.21	17.26
Calcium (%)	0.62	0.62	0.62	0.62
P (%)	0.52	0.52	0.53	0.53
Lys (%)	1.15	1.04	0.97	0.92
NSP (%)	0	8.04	12.42	16.19

[1]The nutrient values of DGDH (degermed, dehulled) corn were taken from Moeser et al., 2002.

[2]Cargill, Minneapolis, MN.

[3]Provided the following per kilogram of diet: vitamin A, 6,358 IU; vitamin D_3, 636 IU; vitamin E, 50 IU; vitamin K, 1.91 mg; riboflavin, 4.81 mg; niacin, 14.41 mg; d-pantothenic acid, 14.41 mg; vitamin B_{12}, 21.195 μg; Zn, 115 mg; Fe, 230 mg; Mn, 19.2 mg; Cu, 9.6 mg; I, 0.29 mg; and Se, 0.29 mg.

[4]A diatomite product, Celite Corporation, Lompoc, California.

Table 2. Growth performance of pigs fed diets with differing fiber content during a 2 week adaptation period[1].

Treatments	DGDH[2]	SPI[3]	SBM[4]	Soy hulls	SEM	P Value
Initial body weight (kg)	20.45	20.31	20.29	20.31	0.0777	0.466
Final body weight (kg)	25.98[a]	25.94[a]	27.42[b]	27.37[b]	0.331	0.0139
ADG (kg/d)	0.395[a]	0.402[a]	0.509[b]	0.504[b]	0.0204	0.0039
ADFI (kg/d)	1.10[a]	1.11[ab]	1.26[ab]	1.27[b]	0.0295	0.0029
G/F	0.355[a]	0.360[ab]	0.403[b]	0.395[b]	0.0123	0.0507

[1]Least squares means

[2]Degermed, dehulled corn and soybean protein isolate diet

[3] Corn and soybean protein isolate diet

[4]Corn and soybean meal diet

[ab] Means within a row with different superscripts differ ($P < 0.05$).

Table 3. Apparent nutrient digestibility and dry matter of fecal samples[1].

Treatments	DGDH[2]	SPI[3]	SBM[4]	Soy hulls	SEM	P Value
Apparent fecal GE digestibility (%)	92.9[a]	80.4[b]	78.8[bc]	72.9[d]	0.816	<0.0001
Apparent fecal CP digestibility (%)	86.4[a]	76.8[b]	76.1[bc]	69.0[d]	0.996	<0.0001
Apparent fecal NDF digestibility (%)	75.9[a]	47.3[b]	42.1[b]	45.7[b]	2.29	<0.0001
Apparent fecal ADF digestibility (%)	81.2[a]	56.6[b]	36.2[b]	44.4[b]	6.63	0.005
Fecal dry matter (%)	35.2[a]	33.4[a]	31.7[ab]	28.8[b]	1.2	0.0233

[1]Least squares means

[2]Degermed, dehulled corn and soybean protein isolate diet

[3] Corn and soybean protein isolate diet

[4]Corn and soybean meal diet

[abcd] Means within a row with different superscripts differ ($P < 0.05$).

Table 4. Ammonia concentrations in the exhaust air from chambers[1].

Index	DGDH[2]	SPI[3]	SBM[4]	Soy hulls	SEM	P Value
Day 1 (ppm)	1.35[a]	0.63[bc]	0.78[b]	0.49[c]	0.075	0.0001
Day 2 (ppm)	2.12[a]	1.50[b]	1.08[c]	0.97[c]	0.129	0.0006
Day 3 (ppm)[5]	2.10[a]	1.09[b]	1.14[ab]	1.20[ab]	0.308	0.135
Day 4 (ppm)[5]	0.81	1.43	1.45	1.16	0.256	0.319

[1]Least squares means

[2]Degermed, dehulled corn and soybean protein isolate diet

[3] Corn and soybean protein isolate diet

[4]Corn and soybean meal diet

[5]Days without the pigs

[abc] Means within a row with different superscripts differ ($P < 0.05$).

Table 5. Total reduced sulfur concentrations in the exhaust air from chambers and slurry pH values[1].

Treatments	DGDH[2]	SPI[3]	SBM[4]	Soy hulls	SEM	P Value
TRS[5] (ppb)	4.21[a]	4.88[ab]	7.92[c]	8.50[cd]	0.24	<0.0001
Slurry pH value	7.97[a]	7.25[b]	7.24[b]	6.93[b]	0.139	0.003

[1]Least squares means

[2]Degermed, dehulled corn and soybean protein isolate diet

[3] Corn and soybean protein isolate diet

[4]Corn and soybean meal diet

[5] Total reduced sulfur

[abcd] Means within a row with different superscripts differ ($P < 0.05$).

Table 6. Peak area of main odorants by SPME and results of ODT[1] by an odor panel[2].

Treatments	DGDH[3]	SPI[4]	SBM[5]	Soy hulls	SEM	P Value
Acetic acid	6.42[a]	6.61[a]	6.78[ab]	7.26[b]	0.197	0.0717
Propionic acid	6.49[a]	6.85[a]	7.23[b]	7.39[b]	0.122	0.0023
Butyric acid	6.74[a]	6.89[a]	7.54[b]	7.69[b]	0.111	0.0004
phenol	5.78[a]	5.96[a]	6.14[ab]	6.65[b]	0.215	0.0881
p-Cresol	6.90	6.86	6.98	7.21	0.149	0.398
Indole	4.49	6.17	5.94	6.13	0.215	0.347
Skatole	6.06	6.21	6.01	6.36	0.172	0.512
			ODT data by a professional panel			
ODT	144[a]	174[ab]	208[ab]	251[b]	30.49	0.149

[1]Odor detection threshold

[2]Least squares means

[3]Degermed, dehulled corn and soybean protein isolate diet

[4] Corn and soybean protein isolate diet

[5]Corn and soybean meal diet

[ab] Means within a row with different superscripts differ ($P < 0.05$).

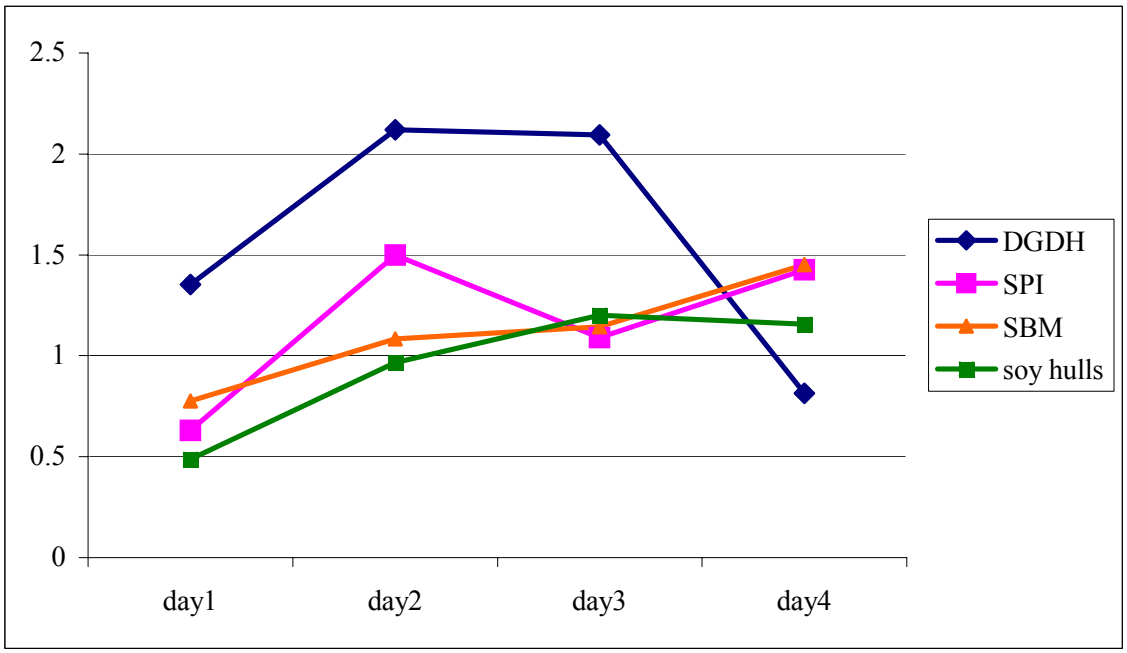

Figure 1. Ammonia concentrations in chamber air

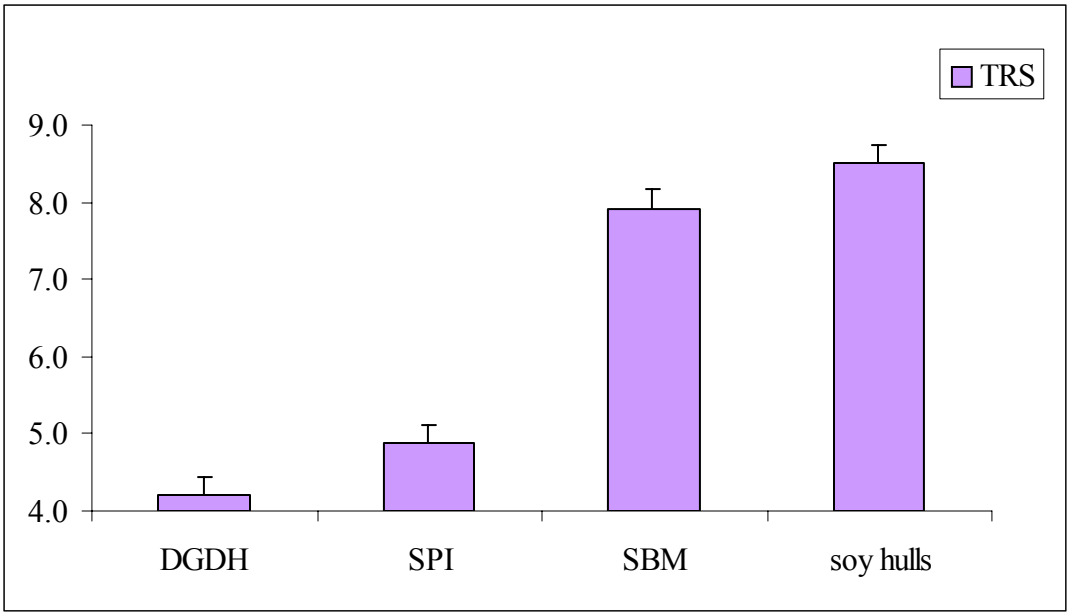

Figure 2. Total reduced sulfur (TRS) concentrations (ppb) in chamber air

APPENDIX

Methods of odor measurements in swine industry—A review

Abstract:

This article reviews the various types of methods in the application of odor measuring in swine production. The operating principles of those methods, as well as their advantages and disadvantages are discussed. The olfactometer evaluation by human nose and chemical compounds determined by GC/MS are the two most reliable methods. But olfactometer evaluation is highly subjective, and the limitation of GC/MS is the complex gas sample collection procedures and the fact that various odorants can not be analyzed by a single run. The common shortcomings of these two methods are that they are labor intensive, expensive and time consuming; therefore, other methods are needed for a fast and reliable analysis. EN and optical instruments (e.g. FTIR and PID) are promising real time methods. They are portable and can be applied to measurements in the field.

Introduction:

Anaerobic fermentation of manure is the primary source of odor generation at swine operations. Various malodorous compounds, including volatile organic compounds (VOCs), hydrogen sulfide, and ammonia are produced by this anaerobic processing (Zahn et al., 2001). More than 168 VOCs have been identified in the air of swine buildings so far (O'Neill and Phillips, 1992; Mackie et al., 1998). Some of them, such as SCFA, indole, skatole, and p-

cresol are important components for swine manure odor (Zhu et al., 1999). The quantification of these trace gases from animal production facilities still is a bottleneck for odor research as the methodologies have not been well established (NRC, 2003). Koziel et al. (2006) used SPME fibers to sample odorants from both of swine and beef cattle operations, and the result showed p-cresol may be the main odorant.

During the past decade, the measurements of airborne odorants from swine production facilities have been extensively researched (O'Neill and Phillips, 1992; Hobbs et al., 1995; Zahn et al., 1997). There are four major classes of methods for the measurement of odor and include dynamic dilution olfactometry (DDO) by human sensory response, chemical analyses by gas chromatography (GC or GC/MS), electronic methods by electronic sensors, and optical methods. The current most popular two methods for odor evaluation are evaluation by an olfactometer, which uses the human nose as a detector, or separation and measuring of individual compounds by GC.

Dynamic dilution olfactometry:

Dynamic dilution olfactometry is a dilution-to-threshold test. The odorous air is diluted a certain number of times with clean air and is sniffed by the panelists. The dilution factor will be highest at first, and then it will be reduced progressively until half of the panelists can detect the odor. The definition of an odor unit (OU) is the mass of odorants in 1 m^3 of air that can be detected by 50% of the human panelists. While the definition of a European odor unit (OUE) is the mass of odorant(s) when evaporated in 1 m^3 of neutral gas at standard conditions results in a physiological response from a panel equivalent to that elicited by 1 European reference odor mass (erom 123 mg n-butanol) evaporated in 1 m^3 of neutral gas at

standard conditions (NRC, 2003).

Many factors interfere with olfactory sensitivity (e.g., age, gender, and occupation). Therefore, individual variability is large, especially for the odor quality, which also introduces the subjectivity of human language (Spoelstra, 1980; Mackie et al., 1998). Though some statistical methods were used to minimize subjectivity in the response of the panelists, the sensitivity of the olfactometer method is low. Clark et al. (2005) added 20% beet pulp in both a high or low protein diet for growing pigs and showed that beet pulp addition decreased manure pH and concentrations of ammonia N. However, odor evaluation by a panel illustrated no change in the odor concentrations or hedonic tone of the slurry.

Another disadvantage of the olfactometer method is some containers such as Tedlar™ bags are used to storage the air samples before the evaluation. Keener et al. (2002) suggested that collection of air samples in Tedlar™ bags may bias olfactory analysis as some gas molecules are adsorbed on the wall of bags and the bag also emits some acetic acid and phenol.

Odorous compounds measured by GC/MS

Proper collection and storage of odor samples prior to analysis by GC/MS is necessary. Headspace solid phase microextraction (SPME) is one of such odorants extraction method. SPME sampler consists of silica fiber coated with carriers such as polydimethylsiloxane, carboxen etc. as a solid adsorbent. VOCs will be adsorbed on the surface of polymer-coated fiber under low temperature. After collection, the SPME samplers will be injected directly into the injection port of the GC/MS (Powers, 2001). Then, the enriched compounds will be released under high temperature and analyzed by a thermal desorption-GC/MS. Beside gas

samples, SPME also can be used in odorant extraction from liquid samples (Rizzuti et al., 1999; Yo, 1999). The advantage of SPME is that it prevents water from entering the GC, which possibly will damage some GC columns. However, as there are many odorants with different physical characters, several carriers may be needed for a signal extraction.

The principle of sorbent tubes is similar to SPME, except the carriers are coated on the surface of tiny beads. Keener et al. (2002) prepared an artificial swine odor by mixing 19 major odorant standard compounds and compared the recoveries of these compounds by direct desorbtion onto a tri-packed sorbent tube or by desorbtion from a Tedlar™ bag onto the tri-packed sorbent tube. The result showed recoveries for directly adsorption method was satisfying.

The "purge and trap" methods are used for already adsorbed odorants. After the compounds on the solid particles were heated and purged by helium gas (Seitz et al., 1999) onto the Tenax trap, which is similar to the sorbent tubes, excess moisture was removed by dry purge and odorants need to be condensed again by liquid nitrogen trap before the thermodesorption step. Data of Razote et al. (2003) showed such a modified method identified more odorants than those of solvent extraction (using dichloromethane) or SPME (CAR/PDMS and PDMS fibers).

Air sampling canisters may be used to collect air samples. Blunden et al. (2005) used 6-Liter SUMMA™ canisters and detected more than 100 VOCs by a GC/MS.

As for the GC/MS itself, not all of key odor components can be measured by a single GC method and the largest limitation is the characteristics of the GC columns (Kai and Schäfer, 2004). These authors recommended to use two or more analytical methods at the same time, for example, use two GC columns with different polarities. Obviously, such a analytical

setup is not perfect because it still would not cover all potential key odorants, and it would cost more time and money.

Another disadvantage is the concentration of individual compounds measured by GC/MS may not correlate well with the actual smell of the samples. Gralapp et al. (2001) reported poor prediction capability of an equation developed from the GC/MS data for the human panelist responses ($R_2 < 0.3$). While the result of Zahn et al. (2001) show that analysis of VOCs by GC/MS can be used to estimate odor intensity.

Odor evaluations by a panel or GC/MS are laborious and expensive measurements. In addition, they are not easily applied to field measurement, and fast odor measurement systems are needed.

Odorants measured by electronic sensors

The other main technology for odor monitoring is by electronic methods, for example, electronic nose (EN). It is a relatively new tool that may be used for real time odor monitoring. They consist of a series of chemical sensors, which can provide a set of measurements to the odorant vapors (Stetter et al., 2000). Resistance of the sensors will change and produce output signals when gas binds on their surface. This binding and resistance change is rapid and temporary. Then a computer organizes all of the signals by "artificial neural network" computer software before the final odor information output (Powers, 2001). The advantage of real time devices is that they can be applied to chemically unstable odor compounds (Hobbs et al., 1995). Because there are many gas sensors and even more of their combinations, the EN can distinguish a complex group of substances very rapidly. But the EN needs to be trained before it can be used successfully. That mean it only

can recognize the component that is similar to what it has learned. Also, its efficiency will be impaired when it is used to detect extremely complex odors.

Another shortcoming of EN is that it can not detect low concentrations of odorants. For example, the OTVs for butyric acid, p-cresol, ethyl mercaptan, and dimethyl sulphide are 1 ppb and for hydrogen sulphide (H_2S) is 0.5 ppb (Tamminga, 1992). Normally, the detection limit of the sensors are 0.1 ppm (Gardner and Bartlett, 1994), so EN may fail to account for the contribution of these trace gases that are important odorants in the entire profile. Hobbs et al. (1995) reported that EN could not discriminate between different odors at low concentrations.

The gold film sensor of the Jerome meter is an exception among the sensors as it is highly selective to hydrogen sulfide (H_2S, sensitive to 1 ppb, Schiffman et al., 2002). The principle of the gold film sensor is also based on a change in electrical resistance by the gas combining on the surface of the film. Ammonia as well as other sulfides (include mercaptan) may potentially interfere (Clanton and Schmidt, 2000), but not SO_2, CO_2, CO and water vapors. It was reported that the Jerome meter (gold film sensor) correlated with odor intensities from an odor panel (Schiffman et al., 2002). But, since more odorants beside H_2S are involved in the odorant profile, normally the result of Jerome meter would not be expected to be a reasonable method to estimate the odor units. The possible reason of the relatively high correlation may be because H_2S concentration is highly correlated with other main odorants.

Optical methods

Fourier transform infrared (FTIR) spectrometry is most useful for identifying chemicals as well as quantitative analyses. Molecules will absorb the IR protons of certain

wavelengths and the frequencies of molecular bonds vibration will be increased accordingly. Such a characteristic absorption by a gas on the path of the light beam causes a reduction of the measured signal intensity detected by a photodiode. Because the strength of the absorption at certain wavelengths is proportional to the gas concentration, FTIR spectrum can be used for calculating gas concentrations. The term Fourier Transform refers to the fact that the collected data will be converted to an interference pattern and the sensitivity of wavelength will be increased.

So far, open-path FTIR (OP-FTIR) was used to measure the concentrations of ammonia and methane (Shores et al., 2005; Childers et al., 2001). The advantage of OP-FTIR is it could be used for some unmeasurable area sources, for example, odorants concentration on the lagoon surface. The disadvantage of dominant atmospheric absorptions are from H_2O and CO_2, so calculations of other minor components will be interfered with as the weight of those peaks are much higher, so their contribution to the system is also higher. A gas filter correlation may eliminate such a problem at a certain level. For the FTIR with a gas cell, the H_2O and CO_2 can be removed before the air sample enters the cell.

The detection limit of FTIR instruments can be as low as 1 ppb (Timmer et al., 2005), therefore allowing their implementation in trace odorants measurements. While Schiffman et al. (2002) reported that their data from the infrared laser detector did not correlate with panel data, the possible reason is most of the low boiling point odorants are adsorbed on airborne particles and they scattered the infrared light to different directions. The odorants molecules are not distributed evenly on the surface of those particles. So such a machine can be used for dust concentration determination, but it will be difficult to separate the information of trace odorants from the entire infrared spectra.

Similar to OP-FTIR, the open-path tunable diode laser absorption spectroscopy (OP-TDLAS) spectrometry also can measure gas concentration, except for the wavelength of OP-TDLAS is in the near-infrared range.

Another potential optical instrument for measuring odor is the photo ionization detector (PID, Mackie et al., 1998). It is a hand-held device used to detect VOCs as well as NH_3 and H_2S. The principle of PID is to ionize the gas molecules by ultraviolet (UV) photons, then collect the produced ions by electrodes, and a current is generated that is proportional to the gas concentration. PID can detect VOCs concentrations as low as a few ppb and is not sensitive to interference by water (Schiffman et al., 2002). The same authors reported that data from the PID correlated with odor intensities from an odor panel. Similarly, Hobbs et al. (1995) reported PID showed a higher sensitivity in odor concentration measurement than EN did.

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