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Effects of feeding distillers dried grains with solubles to finishing swine on animal performance, manure characteristics, and odorous emissions, as analyzed by olfactometry, an electronic nose, and gas chromatography-mass spectrometry

by

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A thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

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This is to certify that the Master’s thesis of

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has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
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INTRODUCTION

The odors associated with the confined production of livestock have become a source of contention. The construction and expansion of animal housing units is often met with resistance due to the nuisance odors frequently arising from the barns, manure storage facilities, and land application of manure. The future of livestock production will depend, in part, on the ability of producers to manage odors. Odor abatement may occur, to some extent, by diet modification. By meeting the nutritional needs of the animal without over-supplementing the diet, the excretion of excess nutrients will decrease. The majority of malodors associated with livestock manure are the result of anaerobic decomposition. If less substrate is available in the feces for bacterial breakdown, the production of odorous compounds will be reduced. Manipulation of the diet to reduce odor has not yet been studied extensively.

A major obstacle to the management of livestock odor is the lack of an objective and reliable means of odor measurement. Currently, olfactometry using trained human panelists is the accepted method for determination of odor concentration (ASTM Standard E679). Olfactometry must be conducted in a controlled laboratory setting and a sufficient number of panelists must be available to conduct the analysis. Gas chromatography coupled to mass spectrometry is frequently used to identify and quantify odorous compounds. However, this does not represent the experience of odor sensation as perceived by a human being. For regulatory purposes, there is a need for a means of accurate odor assessment that is available on-site. Electronic nose analysis with a sensor array continues to be researched as a method of odor evaluation.
The current study was undertaken to examine dietary manipulation as a means to reduce odor. Distillers dried grains with solubles was fed to finishing pigs at varying levels of the diet to determine its effect on swine manure odor. Distillers dried grains with solubles was fed in an attempt to more closely meet the animals’ nutrient requirements. Distillers dried grains with solubles was substituted for both corn and soybean meal and contains an intermediate amount of energy, protein, and amino acids, including the sulfur amino acids, methionine and cysteine. Air samples from the swine feeding facilities were analyzed by olfactometry. Odorous compounds in the air were identified and quantified by gas chromatography-mass spectrometry (GC-MS). The use of an electronic nose to analyze ambient air samples was examined as well. The three methods of odor evaluation were compared and GC-MS response was used to predict olfactometry and electronic nose response.
Distillers Dried Grains with Solubles

Distillers dried grains with solubles (DDGS) is a byproduct from the processing of cereal grains, most often corn, into ethyl alcohol for use as a beverage and a fuel. The sugars and starches are fermented by yeast (the starches are first converted to sugars) and the ethanol is removed by distillation. The stillage that remains is concentrated and dried (NRC, 1981). Since the only fraction that is removed from the original grain is the carbohydrates, the remaining nutrients are concentrated (NRC, 1981). The nutritional value of DDGS depends greatly upon that of the grain from which the ethanol is produced (NRC, 1981). If the ethanol is to be used for beverage production for human consumption, food grade grain must be used and the nutrient quality of the DDGS is assured (NRC, 1981). If less than food grade grain is used to produce fuel alcohol, the nutritional value and perhaps even safety of the byproduct may be unsatisfactory (NRC, 1981).

The quality and other characteristics of DDGS are also affected by the processing it has undergone. Differences in the nutritive value and palatability of different sources of DDGS are in part due to different processing techniques used at different plants. Variables in the process include the temperature of operation, the drying time, and the drying temperature. Since it is a dried product, some sources of DDGS may be heat damaged and have a burnt odor or flavor. Carpenter (1970) noted that the variability among different sources of DDGS is primarily due to differences among processing plants, since the variability of an individual plant’s processing procedures will be reduced. Therefore, the variability in DDGS from any one source will be a result of the variability in the grain used for processing.
Distillers dried grains with solubles is an excellent source of energy, protein, and many vitamins and minerals, and contains approximately 8% fat and 8% fiber. When fed to swine and poultry at high levels of the diet, DDGS can result in diet limitations of the amino acids lysine and tryptophan. Previous research in swine has shown that the maximum level of feeding DDGS with no detrimental effect upon performance approaches twenty percent, although mixed results have been reported (Wahlstrom et al., 1970; Harmon, 1974; Harmon, 1975). The continued feeding of DDGS is important to the corn, ethanol, and livestock industries.

Distillers dried grains with solubles is a variable feedstuff, with differing nutrient content based upon the type of grain processed and processing methods. Cromwell et al. (1993) examined DDGS from different sources: seven beverage and two fuel alcohol producers. The physical, chemical, and nutritional qualities of the feedstuffs were examined. Variations in color and odor as well as nutrient content were observed. These differences may have been due to differences in the processing and drying techniques, in particular, overheating during drying, since lower nutritional quality was observed for those sources of DDGS that were darkest in color and had a burnt smell. The greatest variation was in lysine content, with a 2.1-fold difference between the highest concentration of 0.89% and the lowest concentration of 0.43%. One-day old chicks were fed twelve corn-based diets for 21 days. Distillers dried grains with solubles was included at 20% of the diet. Cromwell et al. (1993) found that the source of DDGS significantly (P < .01) affected chick weight gain, feed intake, and feed efficiency.

Wahlstrom et al. (1970) fed DDGS to grow-finish pigs at levels of 0, 5, 10, and 20% of the diet. A basal corn-soybean meal diet containing 15% protein served as the control.
Distillers dried grains with solubles was substituted for both corn and soybean meal and diets were formulated to be isonitrogenous. Average daily gain was not significantly affected. Feed efficiency dropped significantly (P < .05) with 20% DDGS in the diet as compared to the control. This decrease in efficiency may be due to the reduced apparent digestibility of the diet containing 20% DDGS as determined by fecal analysis with chromic oxide indicator. Also, since the diets were formulated to be isonitrogenous, the 20% DDGS diet contained 0.50% lysine in contrast to 0.65% lysine in the control diet. In a second experiment, fat was supplemented to make the DDGS diets isocaloric with the control. The diets were fed with and without supplemental lysine. In the supplemented diets, additional lysine was included to provide a total of 0.75% lysine. No significant differences in average daily gain, feed intake, or feed efficiency were observed. When fed the 20% DDGS diet with supplemental lysine, pigs ate 12% less feed, gained 8% faster, and were more efficient than those fed 20% DDGS without additional lysine. Wahlstrom et al. (1970) conducted a third experiment, feeding the control diet, the 20% DDGS diet, and the 20% DDGS diet supplemented with either 0.15 or 0.25% lysine providing 0.65 and 0.75% total lysine, respectively. The exclusion of supplemental lysine significantly decreased both average daily gain (P < .01) and feed efficiency (P < .05) with the inclusion of 20% DDGS. When the 20% DDGS diet was supplemented with lysine (either 0.15 or 0.25%), no significant differences in gain or efficiency were observed. The results of the study imply that DDGS can be fed at 5 or 10% of the diet with no negative effects on performance. Inclusion of 20% DDGS reduces performance, which may be due to reduced digestibility and decreased lysine content. Lysine supplementation to a level of at least 0.65% of the diet apparently corrected for the performance losses.
Harmon (1974) conducted a series of experiments with feeding DDGS and examining the feed's lysine and tryptophan availability. In the first experiment, DDGS was fed to 11 kg pigs at 9, 18, and 27% of the diet. A constant ratio of corn and soybean meal was maintained in the diets, along with a constant amount of tryptophan, though the percent tryptophan from DDGS increased with increasing DDGS. The diets were fed for 28 days. The results showed that as DDGS in the diet increased, average daily gain decreased significantly (P < .01). The same diets were also fed with 0.03% supplemental DL-tryptophan and those pigs showed no differences in gain or feed conversion. The conclusion was that the tryptophan in DDGS diets was not limiting.

In the second experiment, DDGS was included in the diet at 0 and 9% with and without supplementation of 0.08% DL-tryptophan. Pigs with an initial body weight of 10 kg were used. The diets were fed for 28 days with no differences in gain or feed conversion observed. Again, this demonstrated that the tryptophan in DDGS is available to weaned pigs.

In the third and fourth experiments, 5 to 11 kg pigs were fed 5, 10, 15, or 20% DDGS, along with a control diet that was a corn and sesame meal mix. A constant lysine level was maintained by decreasing lysine HCl with increasing DDGS. Although the diets were formulated with the DDGS as containing 0.8% lysine, laboratory analysis indicated the DDGS had just 0.62% lysine. So lysine was decreased by 0.009% with each 5% increase in DDGS included in the diet. These diets were fed for 21 days. A significant (P < .01) decrease in gain and feed efficiency was observed with increasing lysine from DDGS. Thus, the lysine from DDGS was limiting for very young pigs.

A fifth experiment was conducted with 58 kg finishing pigs. A 2 x 2 factorial design included treatments of 0 or 8% DDGS and 0 and 0.24% lyamine (50% lysine). Animals were
maintained on the trial for 42 days. No differences in gain or efficiency were evident, leading to the conclusion that the lysine in DDGS is available to finishing pigs.

Harmon (1975) conducted further research with DDGS, examining it as a lysine source. The basal diet was a corn-sesame meal mix to which was added 5, 10.1, 15 and 20% DDGS. There was decreasing supplementation of lysine HCl with increasing DDGS. Pigs with an initial weight of 15 kg were fed for 27 days with no significant differences in average daily gain or feed efficiency. The conclusion was that lysine from the DDGS was not limiting.

In a second experiment, the control was a wheat-soybean meal diet with 0.86% lysine. Pigs weighing 12 kg were given one of three diets: the control diet, a 10% DDGS diet, and a diet supplemented with lysine HCl to provide an amount of lysine equal to that supplied by DDGS. The third experiment was conducted with a wheat-soybean meal control diet to which was added 10 or 20% DDGS. A fourth and fifth diet had supplemental lysine HCl included to supply an amount of lysine equal to that from the two levels of DDGS. Eleven kilogram pigs were used. The combined results of experiments two and three showed no differences in gain or efficiency. Thus, the lysine from the DDGS was as available as the lysine from the lysine HCl.

Cromwell and Stahly (1986) carried out a number of experiments looking at DDGS for grow-finish swine. Unlike the aforementioned studies, Cromwell and Stahly formulated diets on a lysine basis and allowed the crude protein level of the diets to fluctuate.

In experiments one and two, DDGS was included in a corn-soybean meal diet at 0, 5, 10, 20, or 40%. Distillers dried grains with solubles replaced both corn and soybean meal in order to keep the lysine constant at 0.80%. The initial weights of the animals were 29 and 35
kg and final weights were 96 and 94 kg. Incorporating up to 20% DDGS did not decrease gain and efficiency but the inclusion of 40% DDGS did significantly (P < .01) reduce gain by 8% and feed conversion by 12%.

A third experiment examined the effects of DDGS at lower levels of the diet. Distillers dried grains with solubles was added to the diet at 0, 2.5, 5, and 10%. Diet formulation was on a lysine basis and animals were fed from 30 to 99 kg. The inclusion of DDGS at these levels did not affect feed intake, weight gain, or feed efficiency.

In the fourth experiment, pigs were fed 10 and 30% DDGS from two different sources. Diets were formulated to be isolysinic. There were no negative effects of 10% DDGS on growth or efficiency. Distiller dried grains with solubles at 30% decreased both gain and feed conversion. There was also found to be a difference in pig performance with the two different DDGS sources. The pigs given one source gained weight more quickly and with greater efficiency than those receiving the other source, indicating the presence of variations in DDGS sources and the effects these variations can have on animal productivity.

The fifth experiment used diets formulated on a crude protein basis. A basal corn-soybean meal diet was used as the control along with diets containing 0, 15, and 30% DDGS. The DDGS diets were supplemented with crystalline L-lysine to provide a lysine content similar to the control. In addition to the 30% DDGS ration that was formulated on a crude protein basis, Cromwell and Stahly (1986) also included a treatment of 30% DDGS formulated on a lysine basis. All lysine in the diet was supplied by DDGS, corn, and soybean meal; no supplemental lysine was added. These rations were fed from 20 to 95 kg. Those pigs receiving the 15% DDGS diet showed slight reductions in growth and efficiency. Greater performance reductions were seen in animals fed a 30% DDGS treatment that was
formulated on a crude protein basis and then supplemented with crystalline lysine. In contrast, no decrease in weight gain was observed when 30% DDGS was included on a lysine basis. The conclusion was that amino acids other than lysine were limiting.

In summary, Cromwell and Stahly (1986) fed DDGS at levels up to 20% of diet (on a lysine basis) to 620 pigs from 31 to 96 kg and observed a 3-4% reduction in growth and efficiency. This reduction in performance was not significantly different from that of animals receiving control diets. Including 30 to 40% DDGS significantly (P < .001) reduced gain by 6 to 10% and efficiency by 8 to 12%. Cromwell and Stahly (1986) hypothesized that this performance loss may have been due to the fiber content of DDGS.

**Odor and the Swine Industry**

As the swine industry has become more concentrated, with larger numbers of animals confined and raised in smaller areas, and as people with little previous interactions with agriculture have moved from urban to rural areas, complaints about swine odors have increased. Legislation to control odor has been enacted in some states. The future viability of the swine industry will depend, in part, on the ability of producers to manage odor effectively and economically.

**Diet and Odor**

The odor associated with swine and their manure is produced from the anaerobic breakdown of undigested feed components in the manure (Burnett, 1969; Miner, 1977; Ritter, 1989). By increasing nutrient utilization and decreasing nutrient excretion, there should be less substrate available for this anaerobic breakdown and thus less odor production.
Altering the composition of what is entering the pig ought to affect what leaves the animal. Dietary modification may be a less expensive, and potentially more effective, means to change manure characteristics and reduce odor. Also, improving nutrition will not only affect odor production but also improve growth performance, improve efficiency, and decrease feed costs.

The anaerobic breakdown of protein and amino acids in swine waste produces odorous compounds such as volatile fatty acids (Spoelstra, 1980; Zhu et al., 1999). Phenols arising from the decomposition of the amino acid tyrosine, and indoles, generated by the breakdown of tryptophan, have been implicated as important components of agricultural malodors (Spoelstra, 1980).

Hobbs et al. (1996) conducted an experiment with growing and finishing pigs in which a total of six diets were fed, three at each stage of growth. The three diets included a standard commercial ration, fed as the control, and two experimental diets formulated on a least-cost basis; one with all commercial ingredients included and one considering only those ingredients home-grown in the United Kingdom (wheat, barley, peas, field beans). The experimental diets were designed to provide minimally feasible crude protein concentrations and were supplemented with synthetic essential amino acids to provide an ideal protein ratio. The amount of nitrogen in the excreta of those pigs fed the experimental diets was significantly (P < .001) less. The slurry was centrifuged and the supernatant liquid was analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Ten odorants were quantified, including five volatile fatty acids, three phenols, and two indoles. The results indicated that a reduction in dietary crude protein corresponded to a reduction in odorous compound concentrations in swine manure. Five of the odorants were found to be
significantly (P < .05) less in manure from pigs fed grower diets low in protein when compared to manure from pigs fed the standard commercial control diet. Different odorous compounds were reduced in concentration with the two different low protein diets (commercial versus home-grown ingredients). A greater reduction in odorant concentration was seen with the finishing pigs as compared to the growers. Nine odorants were significantly (P < .05) reduced in the manure of finishing pigs fed the least cost diet. The concentration of the odorants was approximately 50% lower. Four of the odorants were significantly (P < .05) less for the finishing diet that included home-grown ingredients. Decreasing excess crude protein in the diet and supplementing with highly digestible synthetic amino acids reduced nitrogen excretion as well as odorant concentrations, and did so with diets formulated on a least cost basis.

Diets differing in protein level were fed by Sutton et al. (1996) to study the effects of diet on odor from swine manure. Grow-finish pigs were used for the experiment. The four com-soybean meal diets included 10% crude protein, 10% crude protein supplemented with four synthetic essential amino acids, 13% crude protein, and 18% (excess) crude protein. Gas chromatography-mass spectrometry analysis of fresh manure revealed a decrease in propionic, butyric, and valeric acids when pigs were fed the diet containing 18% crude protein. These observations are possibly the result of inhibited microbial metabolism due to increased ammonia or pH of the manure.

Sutton et al. (1997) fed four experimental grow-finish swine diets to determine the effect of diet on odor. The corn-soybean meal diets included a 13% crude protein (CP) diet, a 10% CP diet supplemented with four synthetic essential amino acids, a 10% CP diet with four synthetic amino acids and 5% cellulose, and a 10% CP diet with four synthetic amino
acids and 2% sucrose thermal oligosaccharide caramel. Following GC-MS analysis, fresh manure from the diet containing 5% cellulose was found to contain significantly higher levels of acetic and butyric acids as well as total volatile fatty acids (P < .05). In addition, a non-significant increase in propionic acid was observed with the 5% cellulose diet. Increasing the fiber content of the diet increased the odorous compounds arising from the manure.

Fructooligosaccharides (FOS), a source of soluble dietary fiber, was fed to weaned pigs to determine the effect on odor (Bunce et al., 1995). The four dietary treatments included 0, 0.75, and 1.5 g FOS per day and 1.5 g FOS per day plus carbadox (antibiotic). Feeding FOS (1.5 g per day) with or without carbadox reduced fecal levels of 4-methylphenol, indole, and 3-methylindole by three-fold. Researchers concluded that the reduction in odorous compounds that occurred with the feeding of FOS was possibly due to the inhibition of bacteria in the large intestine that produce odorous tyrosine and tryptophan metabolites (Bunce et al., 1995).

Shurson et al. (1999) manipulated the amount of sulfur in the diet of weaned pigs by removing dried whey, replacing copper sulfate with zinc oxide, and using a trace mineral premix that contained no sulfur. The amount of sulfur in Phase I, II, and III nursery pig diets was decreased by 13, 39, and 19%, respectively. Fecal and urinary sulfur excretion was monitored. Urinary sulfur excretion and manure sulfur excretion (urine plus feces) were reduced when animals were fed the low sulfur diet. However, the amount of sulfur excreted in the feces by pigs fed the low sulfur diet actually increased. No differences in animal growth or feed efficiency were observed. The experiment was repeated to measure the effects of reduced dietary sulfur on odor and hydrogen sulfide emissions. Odor was
evaluated by olfactometry (ASTM Standard E679). A tendency for increased odor and hydrogen sulfide emissions with increased dietary sulfur content was observed. Again, no performance differences were noted. Shurson et al. (1999) concluded that dietary sulfur content does significantly affect swine manure odor.

Kellems et al. (1979) fed Holstein heifers different levels (25, 50, 75%) of three grains (corn, milo, barley) to determine the effect of diet on volatilization of hydrogen sulfide. The type of grain fed had no effect on hydrogen sulfide volatilized from the manure. Feeding 25 or 50% grain also did not affect hydrogen sulfide levels (P > .05); however, an increase in hydrogen sulfide with 75% grain in the diet was observed (P < .05). Sagebrush was added to a 25% barley-75% alfalfa diet at 1.0 and 1.5% of dry matter. Peppermint oil was added at 0.25% of the diet dry matter. The inclusion of sagebrush in the diet did not alter the offensiveness of waste as rated on a scale of 0 (not offensive) to 15 (highly offensive) by human panelists (P > .10). Addition of peppermint oil significantly (P < .05) decreased simulated manure (50% feces, 50% urine) offensiveness. When only the feces were evaluated, with water replacing the urine, odor offensiveness was unaffected. Kellems et al. (1979) proposed that the odor of peppermint oil associated with the urine fraction of the manure masked the odor.

Hereford steers were fed different diets to examine dietary effect on odor (Watts and Tucker, 1993). Diets included sorghum and barley, both dry-rolled and steam-flaked. Feces were collected and used to simulate feedlot pads for odor analysis by dynamic dilution forced-choice olfactometry. Feces from cattle fed sorghum diets generated greater odor than that of cattle fed barley diets (P < .05). Type of processing of the sorghum and barley did not
affect odor arising from the feedlot pad. Differences in odor may have been due to the higher starch content and lower pH of the manure from cattle fed dry-rolled sorghum.

The Human Sense of Smell

The human sense of smell is very complex and rather little understood. The complexity of odors and the lack of knowledge about the interactions between odorant molecules and receptors have hindered the understanding of the process of olfaction (Gardner and Bartlett, 1999; Barth 1973). The olfactory system includes the nose, olfactory epithelium, olfactory nerve, the olfactory bulb of the brain, and the olfactory cortex, along with other portions of the cerebral cortex (Gardner and Bartlett, 1999). The first step in the process of odor perception is the inhalation of volatile chemical molecules (Gardner and Bartlett, 1999; Barth, 1973). Following inhalation, these volatile compounds reach the olfactory epithelium high in the nose (Gardner and Bartlett, 1999). The olfactory epithelium is composed of olfactory receptor cells (Gardner and Bartlett, 1999). The receptors are covered by an aqueous mucous layer (Gardner and Bartlett, 1999; Barth, 1973). Odorous compounds must pass through the mucous layer prior to interacting with the receptor proteins (Gardner and Bartlett, 1999). The signals generated upon the interaction of odorant molecules and receptor cells are conducted by the olfactory nerve to the olfactory bulb (Gardner and Bartlett, 1999). Processing of the signals occurs in the olfactory cortex (Gardner and Bartlett, 1999).

Odor is subjective in nature and difficult to measure objectively. Individual responses to odors vary greatly. Sensitivity to odor varies with age, gender, health status, and personal habits, such as smoking (Gardner and Bartlett, 1999). Olfactory sensitivity
peaks after puberty and declines beyond the age of 70 and females tend to respond to odors with greater sensitivity than do males (Gardner and Bartlett, 1999). The sense of smell is highly linked to the limbic system which controls emotions (Schiffman, 1998). Odors can trigger a variety of emotional and psychological responses and can also cue the retrieval of memories. Previous encounters with an odor can affect reactions to subsequent exposures, as can preconceived attitudes or expectations (Dravnieks and Jarke, 1980). The human olfactory system can detect odors at extremely low concentrations (parts per billion, parts per trillion) that are often below the limit of detection for instruments such as the gas chromatograph, mass spectrometer, and electronic nose (Miner, 1977; Bartlett et al., 1997; Mackie et al., 1998). Over one hundred and fifty odorous compounds have been identified in livestock wastes and air surrounding livestock facilities (O’Neill and Phillips, 1992).

**Methods of Odor Measurement**

The human nose is still the best detector available because odor sensation is so complex, is a very individual reaction, and the mechanisms of olfaction are not well understood. The olfactory methods available measure the intensity, but do not address the character, of an odor. Odor can be evaluated in terms of concentration, intensity, and quality. Olfactometry using trained human panelists is the accepted method for determination of odor concentration (ASTM Standard E679). According to the standard, an odor sample is diluted with odorless air at a number of levels and the dilution series is presented in ascending order of odor concentration. From one level to the next, the dilution decreases and the amount of odorous air increases. The first few levels include the sample diluted with a large amount of odorless air so evaluation can begin below the threshold of detection. Preferably, multiple
presentations (two odorless air samples and the diluted odor sample) are made at each level of dilution. When a forced-choice method is used, a panelist must identify the presentation that is different from the others at each level, even if it is a guess. This permits use of all the data. The threshold of detection is the dilution level at which the panelist can determine a difference between the diluted and the odorless samples. After the detection threshold is reached, the panelist continues the evaluation at the next level or two to be certain the identification was not made by chance.

Olfactometry is not necessarily an objective measure and there are some questions as to repeatability both within and between laboratories because of panelist training and subjectiveness (Miner, 1977; Dravnieks and Jarke, 1980; Clanton et al., 1999). According to Hangartner et al. (1989), a panelist’s response from one day to the next can vary by as much as three-fold. Dravnieks et al. (1986) found that the log of a panelist’s detection threshold had a standard deviation ranging from 0.12 to 0.37.

Liquid dilution can also be used for odor analysis (Ritter, 1989). Manure can be diluted with water to a range of concentrations and then evaluated by a panel. Barth et al. (1974) diluted stored dairy manure with water to create five dilution levels. For each level, two blank samples of water and one diluted manure sample were presented in Erlenmeyer flasks that had been painted black to avoid bias based on appearance of the diluted manure. Panelists evaluated the samples in an ascending series; the dilution decreased and odor increased from one level to the next. At each dilution level, panelists identified the flask in each set of three that contained the odorous sample. The threshold level was the dilution level at which half of the panelists successfully identified the odorous sample and half did not. Odor was expressed as a function of total volatile organic acid, hydrogen sulfide, and
ammonia content of the manure ($R^2 = .92, .80, .71$, respectively). Adding hydrogen sulfide to the prediction equation slightly improved the observed relationship between manure constituents and odor dilution ($R^2 = .95$ versus $R^2 = .94$). Reddell and Sweeten (1975) evaluated manure odor using liquid dilution where manure was diluted to various concentrations with water. Panelist variability was analyzed. Reddell and Sweeten (1975) observed that each panel member had a distinct and repeatable odor probability distribution.

One method of odor concentration evaluation that is available on-site is the scentometer. The scentometer (Barneby and Cheney, Columbus, OH) is a plastic box with a number of air inlets and two sniffing ports (Ritter, 1989; Mackie et al., 1998). Two of the air inlets have activated charcoal filters to remove odors and provide clean air (Ritter, 1989). The remaining inlets are of varying diameter to permit a range of dilutions of odorous air to be sampled (Ritter, 1989). An observer begins by opening the port of smallest diameter to start with the largest dilution (lowest concentration) of the odor (Miner, 1977). As successively larger ports are opened, the dilution of the odorous air decreases and the odor concentration increases. When the evaluator can first detect the odor, the odor threshold has been reached. Odor concentrations are expressed as dilutions to threshold (Reddell and Sweeten, 1975). The range of dilutions to threshold possible includes 1.5, 2, 7, 15, 31, 170, and 350 (Sweeten et al., 1977; McFarlane and Metheney, 1988). Scentometer measurements are frequently conducted by an individual observer rather than a panel. Drawbacks to the use of the scentometer include variability in the sensitivity of the individual conducting the evaluation and odor fatigue since sampling is conducted on-site and within the odorous environment (Miner, 1977; Dravnieks et al., 1978; Ritter, 1989). The large difference between the dilution levels is also imprecise (Miner, 1977; Ritter, 1989).
Sweeten et al. (1977) used the scentometer to measure odor at cattle feedlots. The concentrations detected ranged from 1.5 to 170 dilutions to threshold (DT). The average odor concentration at a 4,000 head feedlot was higher near the manure containment facilities (68 DT at the settling basin and 46 DT at the retention pond) than at the feedlot itself (31 DT). Downwind from the feedlot the dilutions to threshold were even lower (1.5 to 2 DT).

Sweeten (1998) also used the scentometer to evaluate odor at swine facilities. A 8,400 sow farrow-to-finish facility was observed to have an average odor concentration of 66 DT. Odor concentration was greatest at the lagoon serving the grow-finish barns (92 DT) and the grow-finish barns themselves (91 DT). The odor decreased as the observer moved to the property line (16 DT) and a neighboring residence (1.8 DT). Odor concentrations were much lower at a facility with 200 sows; 31 DT near the manure storage and 25 DT at the buildings.

Another device used for on-site odor evaluation was a portable olfactometer (Sweeten et al., 1982). This instrument uses different amounts of 1-butanol as a standard to which sample odors (the ambient air) are compared (McFarland et al., 1982; Sweeten et al., 1982; Ritter, 1989). The range of 1-butanol concentrations is from 0 to 80 ppm (McFarland et al., 1982; Ritter, 1989). Sweeten et al. (1982) used the olfactometer at a number of locations including livestock facilities, meat packing plants, and oil refineries, and determined the device to be adequate for use in odor evaluation.

Odor can be evaluated subjectively in terms of intensity (strength) or in terms of quality (i.e., offensiveness) (Miner, 1977; Spoelstra, 1980; Ritter, 1989; Misselbrook et al., 1993). Odor quality is evaluated by describing the odor or comparing the sample odor to familiar odors (Miner, 1977; Ritter, 1989). Evaluation of odor quality is difficult because of
the challenges that come with trying to describe odors (Ritter, 1989; Mackie et al., 1998). Odor can also be evaluated by ranking, a procedure in which an arbitrary scale is used to describe either the intensity or offensiveness of an odor (Ritter, 1989). Typically, a scale of 0 to 10 is used, with 0 indicating no odor or not offensive and 10 representing a very intense or offensive odor (Miner, 1977; Zhu et al., 1997).

The measurement of odor concentration by dilution is more direct and objective than that of odor quality or intensity (Barth, 1973; Miner, 1977; Mackie et al., 1998). However, each of these evaluations requires the use of the human nose as a detector, so none is completely objective. Use of a forced-choice method, such as that used with dynamic olfactometers, in which a panelist must simply identify the presence or absence of an odor is generally a better method than ranking, as the human nose cannot distinguish small differences between levels of intensity (Dravnieks, 1972; Zhu et al., 1997).

Odors that are equal in concentration may not be equal in offensiveness or intensity (Dravnieks, 1972; Barth, 1973; Dravnieks and Jarke, 1980; Misselbrook et al., 1993). Misselbrook et al. (1993) related odor concentration to odor intensity. Odor concentration was determined using two forced-choice dynamic dilution olfactometers. Following odor concentration analysis, odor intensity was evaluated at suprathreshold concentrations. Intensity was ranked on a seven point scale, with a rating of 0 indicating no odor and a rating of 6 indicating an extremely strong odor. There were significant differences in the relationships between concentration and intensity for swine and poultry manure odor. The linear regression for the relationship between swine manure odor intensity and concentration accounted for 68% of the variance, while that for poultry manure accounted for 84% of the
variance. Thus, two odors of equal concentration may be perceived as having different intensities.

Perception of a mixture of odorants, such as those in swine odor, is very different from how each chemical would be perceived independently (Barth, 1973; Mackie et al., 1998; Zhu et al., 1999). The interaction of odorants is not always additive in nature and the concentration of a mixture cannot be calculated by simply adding the concentrations of each component (Barth, 1973; Hangartner et al., 1989; Hobbs et al., 1995; Zhu et al., 1999). Odorants can act as additive agents, counteractants, masking agents, or be synergistic in nature. The combination of two odorants can have an odor equal to that of either one of the components, have an odor less than that of one of the components, have an odor equal to the sum of the components, or even have an odor greater than the sum of the components (Barth, 1973).

Ideally, one or two components in swine manure could be identified as indicators of malodor. However, as O’Neill and Phillips (1992) noted, approximately 168 odorous compounds have been identified in livestock animal manure or in the air surrounding livestock facilities. Odor is very site specific and varies from one farm to the next. No one compound has been found to always be present and indicative of odor intensity. Correlations between the concentrations of compounds in the manure and olfactometry measures are not high (Sutton et al., 1999). Schaefer (1977) correlated olfactometry evaluation of ventilation air from swine buildings with gas chromatography analysis. The greatest correlation was found between olfactometry and 4-methylphenol ($r = .71$).

Burnett (1969) combined gas chromatography with organoleptic evaluation to analyze the odor of poultry manure. As the manure sample was separated and each
compound eluted from the chromatography column, a panelist described the odor. Five odorous substances were identified, including ammonia or an aliphatic amine, acetic acid, propionic acid, isobutyric acid, and butyric acid. A number of odors were detected by the human evaluators though no corresponding peaks were recorded by the gas chromatograph, indicating that compounds were present in amounts great enough to generate an odor, but not great enough to be detected instrumentally. A similar study of swine manure was conducted by Schaefer (1977). After separation by gas chromatography, a panel evaluated the odor of the eluted compounds. The substances identified as being of importance in swine manure odor included six volatile fatty acids (C2 through C6), phenol, 4-methylphenol, indole, and 3-methylindole. Hammond et al. (1989) also used gas chromatography followed by organoleptic evaluation to identify odorous compounds from swine manure. Dimethyl disulfide and dimethyl trisulfide were considered to be the major components of the odor, with indole, 3-methylindole, and 4-methylphenol as less important constituents. Gas chromatography-mass spectrometry was used to identify the odorous compounds. There were unidentifiable compounds that were making major contributions to the odor.

Hartung and Hilliger (1977) used gas chromatography with a flame ionization detector (FID) and a flame photometric detector (FPD) to identify and quantify odorous compounds in the air of swine facilities. Samples from swine facilities were compared to ambient air. A correlation between sensory perception of the odor and chromatogram peak area was observed, with greater peak areas for more odorous samples. The FPD is used to detect sulfur-containing compounds. For the swine odor samples, three to six peaks were identified with the sulfur filter while for the ambient air only zero to three peaks were
identified. The positive correlation between peak area and odor was more pronounced when comparing the sulfur compounds rather than the total peak area from the FID analysis.

One of the problems associated with instrumental analysis is the low concentration of odorous compounds in air. The human nose is capable of detecting these compounds, but often they are present at concentrations below the level of detection for analytical instrumentation such as the gas chromatograph and mass spectrometer (Miner, 1977; Mackie et al., 1998).

**Electronic Nose**

The sensor array of an electronic nose detects the chemicals that humans perceive as odors and records numerical results. The instrument will generate a different pattern of response for different types of samples. Each sensor has an individual characteristic response and some of the sensors overlap and are sensitive to similar chemicals, as are the receptors in the human nose (Gardner and Bartlett, 1999; Qu et al., 1999). A single sensor is partially responsive to a broad range of chemicals and more responsive to a narrow range of compounds (Osmetech, Crewe, UK). An array of sensors is responsive to a great number and many types of chemicals, with certain sensors in the array being moderately to extremely sensitive to specific compounds (Persaud et al., 1996). Electronic noses have frequently been used in the food, beverage, and perfume industries for product development and quality control (Bartlett et al., 1997). The technology is relatively new to the agriculture industry, although the potential for application is certainly great.

Sensors for electronic noses have been made of a number of materials, most commonly conducting polymers and metal oxides (Bartlett et al., 1997; Gardner and Bartlett,
A sensor is composed of a sensitive material that interacts with compounds in the sample; this interaction causes a response that is detected by a transducer and marked by an output signal (Gardner and Bartlett, 1999).

Conducting polymers change electrical resistance when volatile chemicals adhere to them. This change in electrical resistance is reversible; sensors return to baseline resistance when volatile compounds are desorbed (Gardner and Bartlett, 1999; Qu et al., 1999). The polymers primarily used for this type of sensor include pyrroles and anilines (Bartlett et al., 1997). Conducting polymers are operational at room temperature. One drawback to the use of conducting polymers is their sensitivity to humidity which can alter the baseline conditions of the instrument and also the results (Gardner and Bartlett, 1999).

Sensors have also been made from semi-conducting metal oxides. One common example of this type of sensor is the Taguchi-type sensor, composed of a metal oxide, usually tin dioxide (SnO$_2$), which is doped with a small amount of a metal catalyst, such as palladium or platinum (Gardner and Bartlett, 1999). Semi-conductor metal oxide sensors undergo a change in conductivity when volatile compounds interact with them (Gardner and Bartlett, 1999). This type of sensor is operated at a raised temperature (300 to 550°C) which can cause sample destruction (Gardner and Bartlett, 1999). Metal oxide sensors are not well suited for use in detecting sulfur- and nitrogen-containing compounds (Bartlett et al., 1997). Metal oxide semi-conductor field effect transistor (MOSFET) sensors are field effect sensors consisting of a metal, oxide insulator, semi-conductor configuration. The metal part of the sensor is a catalyst such as platinum or palladium. When the surface potential of the semi-conductor is altered by a chemical in the sample, the change in surface potential is detected and a signal is generated (Haugen and Kvaal, 1998; Gardner and Bartlett, 1999).
Grain samples (wheat, barley, oats) that had initially been evaluated for off-odors by two human inspectors were subsequently analyzed by an electronic nose in a study conducted by Borjesson et al. (1996). The electronic nose included both metal oxide semi-conductor field effect transistor (MOSFET) sensors and tin dioxide semi-conductors. Grain inspectors characterized the samples as normal, musty, moldy, acid, sour, burnt, or foreign. Prior to electronic nose analysis, Borjesson et al. (1996) grouped the grain samples into four categories (moldy/musty, acid/sour, burnt, normal) based on the descriptions of the inspectors. Grain samples were later regrouped into just two categories (good and bad) since most of the off-odors were moldy/musty. When using four categories, the electronic nose successfully classified approximately 75% of the grain samples. The success rate of the electronic nose increased to approximately 90% when the number of categories for classification was reduced to two. These results are greater than the agreement between the two human grain inspectors who placed only 54% of the grain samples in the same category.

Blixt and Borch (1999) used an electronic nose to evaluate spoilage of beef samples. The sensors in the electronic nose included ten metal oxide semi-conductor field effect transistors, four tin dioxide Taguchi-type sensors, and one sensor sensitive to carbon dioxide. Data from only eleven of the fifteen sensors was used as four of the sensors failed to respond or became overloaded. The extent of spoilage of the beef samples was analyzed by a trained odor panel as well. Odor was described using a scale from 1 (no odor) to 9 (very strong odor). Panel response was well correlated to response of two of the Taguchi-type sensors ($r = .94$).

Persaud et al. (1996) used GC-MS analysis to identify odorants in fresh swine slurry. They then created an artificial slurry containing six volatile fatty acids, two phenolic
compounds, and two indolic compounds. The artificial slurry was analyzed with an
electronic nose containing a conducting polymer sensor array. The twenty sensors consisted
of substituted pyrroles. The intensity of the sensor response increased with an increase in the
concentration of the artificial slurry. However, the concentrations of artificial slurry used
were much greater than the threshold for human perception.

Hobbs et al. (1995) used an electronic nose with twenty polypyrrole sensors to
analyze samples from both pig and chicken slurry. The sensors included five different
polypyrrole compounds, four sensors of each type. It was found that the electronic nose
could distinguish between the two slurries. The sensor response patterns to the swine and
poultry slurries were different and could be used to identify and distinguish the two slurries.
However, the electronic nose was less sensitive than olfactometry measures. Gas
chromatography-mass spectrometry was used to confirm the compositional differences of the
two slurries. The GC-MS analysis of the pig slurry revealed the primary odorants to be 4-
methylphenol and 3-methylindole. Acetic acid, undecane, and 4-ethylphenol were also
identified. Odorants identified in the chicken slurry included dimethyl disulfide and
dimethyl trisulfide.

Two electronic noses with conducting polymer sensors were used in comparison to
olfactometry for the measurement of odor after cattle slurry was applied to grassland
(Misselbrook et al., 1997). An AromaScan electronic nose with thirty-two polypyrrole
sensors and an Odourmapper with twenty polyindole sensors were used. A single line was
fitted to the data. The linear relationship between odor concentration from olfactometry
measures and the electronic nose response explained 59 and 62% of the variance for the
AromaScan and Odourmapper, respectively. These relationships were significant (P < .01).
In this experiment, the electronic noses were found to respond to manure odors at concentrations lower than those reported earlier. Samples were taken at three times (20, 80, and 140 min) following slurry application. The electronic noses and the olfactometry panelists detected a decrease in odor concentration as the time following manure application increased.

**Gas Chromatography-Mass Spectrometry**

Gas chromatography is a process by which sample components are separated based on their chemical and physical properties. An injection with a syringe is made into the injection port to load the sample into the inlet and onto a column. A carrier gas (mobile phase) moves the substances through the column. The column is coated with a stationary phase material. Different substances in the mixture partition between the carrier gas and the stationary phase coating in different ways. The mixture becomes more separated as it moves through the column. Each compound moves through the column at its own rate and elutes with its own retention time, which can be used for identification. The area under a peak on the chromatogram corresponds to the concentration of an analyte. When the gas chromatograph is coupled to a mass spectrometer, the compounds that are eluted from the column are ionized and then identified based upon their mass spectra which consists of a bar graph showing the relative amounts of each fragment ion (Wolfe, 1984). Each compound has a unique mass spectrum that can be matched to a library and identified. In chemical ionization, a pre-ionized reactant gas, such as methane, transfers a charge in a reaction with the molecules of the sample, which are subsequently ionized (Schlunegger, 1980). An ion moves between electric and magnetic fields and is accelerated and moved into a curved
trajectory based on its mass to electric charge ratio (Frigerio, 1974). The ions land on a collector and create a signal, the intensity of which is proportional to the number of ions. The relative abundance of the ions is recorded (Frigerio, 1974).

Solid Phase Microextraction

Solid phase microextraction (SPME) is a method of sample preparation for gas chromatography that uses no solvents. Other methods of sample preparation include purge and trap or headspace to concentrate volatiles, liquid-liquid extraction, supercritical fluid extraction, and solid phase extraction (Supelco, 1997). Solid phase microextraction uses a 1 cm fused silica fiber coated with a phase material (Supelco, 1997). The phase material is a polymer-based coating to which organic chemicals adsorb. Equilibrium between the sample in the air and the sample on the phase coating material of the fiber is achieved in two to thirty minutes (Supelco, 1997). The chemicals partition between the phase material and the sample. So at equilibrium, the amount of sample on the fiber is proportional to the concentration in the original sample. Solid phase microextraction is a one step extraction and thermal desorption process. The heat of the inlet of the gas chromatograph desorbs chemicals from the fiber (Supelco, 1997). Since there is a direct extraction of the sample from the air onto the fiber there is less sample loss and sample collection can be done on-site. With one step sample extraction and introduction there is again less sample loss and better minimum detection limits. The process is sensitive to levels of parts per trillion for many analytes (Supelco, 1997). Since it uses no solvents, SPME requires no solvent disposal and there are no solvent peaks to cover up sample peaks that elute early in the run. This method is also less expensive since solvents do not have to be purchased and disposed of and the
fibers are reusable. Overall, SPME appears accurate, reproducible, efficient, and not complicated to use. Linear responses with different sample concentrations can be achieved (Supelco, 1997).

Chai and Pawliszyn (1995) determined the precision of SPME followed by gas chromatography-mass spectrometry to have a 1.5-6% relative standard deviation. The average precision was a 3% relative standard deviation. Researchers concluded that SPME is a highly effective and reliable means of sampling volatile organic compounds.

Gorlo et al. (1997) used SPME with GC-MS and found the chromatogram peak area, and thus amount of sample on fiber, to be linearly related to the concentration of a standard solution containing five organic compounds. The correlation coefficients for the linear regressions ranged from 0.988 to 0.999.

Zahn et al. (1997) used diethyl ether, headspace (gas phase) SPME, and liquid phase SPME to extract organic compounds from swine slurry for GC-MS analysis. Not one of the methods was capable of extracting all the compounds, but SPME was found to be the more efficient method.

Rizzuti et al. (1999) employed SPME with GC-MS to evaluate the use of peat for the removal of odor-causing compounds from swine manure. The use of SPME in conjunction with GC-MS was determined to be an inexpensive, precise, and efficient technique for identification and quantification of odorous compounds. The effects of the peat on swine manure odor were also analyzed by an odor panel. Panelists characterized the odor using a number of odor descriptors provided by the researchers. Odor panel responses were correlated to GC-MS results. Manure samples described as having no odor following peat treatment also had the greatest reductions in the compounds quantified by GC-MS, in
comparison to samples described as having a light manure odor that had lesser reductions in peak area from GC-MS analysis.
MATERIALS AND METHODS

Facility Description

The experiment was conducted at the Iowa State University Swine Nutrition and Management Research Center outside of Ames, Iowa, beginning March 15, 1999 and ending August 26, 1999. The experiment was divided into six, 24-d periods. A total of seventy-two crossbred finishing pigs (average initial body weight = 86 kg) were used over the duration of the trial; twelve new pigs fed during each of the six periods. Two environmentally controlled, mechanically ventilated feeding rooms, 3.81 m × 3.96 m, were available for the experiment. Within the room, six finishing pigs were housed in a 2.44 m × 2.44 m pen with woven-wire flooring. For the first two periods, the pen in each room was temporarily constructed of fiberglass gating with a single nipple waterer in each pen. Permanent metal gating was installed after the conclusion of the second period. In addition, a Trojan Water Swing (Trojan Livestock Equipment, Conrad, IA) waterer with two drinking nipples was placed in each room at that time. Prior to the beginning of the fifth period, water sprinklers were installed in each room due to the increasing temperatures as the experiment moved into the summer months. Average room temperature during the course of the experiment was 22.7°C (range: 10.0°C to 34.4°C). Two 0.36 m exhaust fans provided the ventilation for each room. The rooms were lighted continuously. Each feeding room was equipped with a shallow manure storage pit under the pen. The pit sloped from 7.62 cm to 17.78 cm with a 30.48 cm deep, 15.24 cm wide gutter at one end. The pits were emptied by a pull-plug drain. Due to the limited manure storage that was available, the pits were drained and rinsed each Thursday (d 7). The rooms were power-washed between feeding periods.
Nutrition Study

Three diets were fed during the experiment. The three diets were fed in each room on a rotating basis as shown in Table 1. Each diet was fed twice in each room. The control diet was a basal corn-soybean ration and contained no distillers dried grains with solubles (DDGS). The two dietary treatments included either 5 or 10% DDGS. The DDGS replaced both corn and soybean meal (Table 2). Isolysinie diets were formulated using Iowa State University Extension MCS-5 software (Spike et al., 1998). Diets were formulated to contain equal metabolizable energy to lysine ratios. The software was also used for cost analysis of the diets. Two Smidley (Marting Manufacturing of Iowa, Inc., Britt, IA) flip-top single trough stainless steel self-feeders were provided in each room. Pigs were allowed access to feed and water ad libitum. Feed disappearance was recorded. To track performance, animals were individually weighed at the beginning and end of a feeding period. Animals were assigned to treatment groups based on weight and gender, providing a similar animal profile.

<table>
<thead>
<tr>
<th>Period</th>
<th>Feeding Room A</th>
<th>Feeding Room B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0% DDGS$^2$</td>
<td>5% DDGS</td>
</tr>
<tr>
<td>2</td>
<td>5% DDGS</td>
<td>10% DDGS</td>
</tr>
<tr>
<td>3</td>
<td>10% DDGS</td>
<td>0% DDGS</td>
</tr>
<tr>
<td>4</td>
<td>10% DDGS</td>
<td>5% DDGS</td>
</tr>
<tr>
<td>5</td>
<td>0% DDGS</td>
<td>10% DDGS</td>
</tr>
<tr>
<td>6</td>
<td>5% DDGS</td>
<td>0% DDGS</td>
</tr>
</tbody>
</table>

$^1$ diets fed to six finishing pigs (average initial body weight = 86 kg) per room during each 24-d period

$^2$ DDGS = distillers dried grains with solubles, expressed as kg DDGS per kg diet
Table 2. Composition of experimental diets, \(^1\) kg ingredient per kg complete diet (%)

<table>
<thead>
<tr>
<th>Feedstuff</th>
<th>0% DDGS(^2)</th>
<th>5% DDGS</th>
<th>10% DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>86.44</td>
<td>82.20</td>
<td>77.80</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>11.26</td>
<td>10.50</td>
<td>9.90</td>
</tr>
<tr>
<td>DDGS</td>
<td>0.00</td>
<td>5.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Salt</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Vitamin and trace mineral premix(^3)</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.00</strong></td>
<td><strong>100.00</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

\(^1\) diets fed to six finishing pigs (average initial body weight = 86 kg) per room during each 24-d period
\(^2\) DDGS = distillers dried grains with solubles, expressed as kg DDGS per kg diet
\(^3\) supplied per kg of diet: vitamin A, 4,409 IU; vitamin D, 1,102 IU; riboflavin, 6.6 mg; pantothenic acid, 17.6 mg; niacin, 33 mg; vitamin B\(_{12}\), 22 \(\mu\)g; zinc, 75 mg; iron, 87.5 mg; manganese, 30 mg; copper, 8.75 mg; and iodine, 0.1 mg

between rooms within a feeding period. A feed sample was collected for proximate analysis from each batch of feed that was mixed. Feed samples were analyzed for amino acid content by CN Laboratories (Courtland, MN). Analysis for crude protein, fiber, energy, and sulfur content was conducted by the Dairy One Forage Testing Laboratory (Ithaca, NY).

**Odor Analysis**

Air samples were collected during the last three weeks of each four-week period, thus allowing the first week for dietary acclimation. Samples were collected on Mondays and Thursdays (d 4 and 7) and were transported to the Iowa State University campus for analysis. A battery-powered Supelco 10 Liter Air Sampler (Supelco, Bellefonte, PA, Model 1062) was
used to collect air samples in Tedlar bags. An ambient air sample is collected by creating a vacuum outside the sample bag. The vacuum creates negative pressure inside the sample bag, thus causing the ambient air to enter the sample bag. During sample collection, the air sampler was placed on the floor as close to the pit as possible.

Olfactometry

An 11 L air sample was collected from each room for analysis by olfactometry. Room air samples were analyzed using the Ac'scent International Olfactometer (St. Croix Sensory, Stillwater, MN) located in the Olfactometry and Air Quality Laboratory on the Iowa State University campus. The method of dynamic dilution triangular forced-choice olfactometry with an ascending concentration series was used to determine odor concentration. A minimum of eight trained human panelists comprises an odor panel. Panelists undergo training to eliminate any individuals who may be hypo- or hypersensitive and to maintain a standard deviation of 0.5 or less. During a sample evaluation, panelists are exposed to three different stimuli presented at a sniffing port. Panelists are randomly presented with two blanks (clean, odorless air) and one odorous sample. Serial dilutions are made in which the odorous air is diluted with clean air. The dilutions are two-fold. As a panel member progresses through the dilution levels, the amount of clean dilution air decreases and the amount of sample air increases. Thus, the dilution decreases and the concentration of odorous air increases with each level. Panelists identify which presentation smells differently from the other two. Panelists must distinguish one stimulus as being different, guessing if they are unable to detect a difference, so that as much data as possible can be used. Panelists do indicate if their response is a guess or a detection. The detection
threshold is the level of dilution at which a panelist is able to correctly detect a difference between the odorous sample and the two blanks. In order to begin below the anticipated detection threshold, a large amount of clean, odorless air is used to dilute the odorous sample. This creates the lowest concentration of the odor instrumentally achievable. The amount of dilution air is decreased until a panelist reaches their threshold of detection. A panelist is done with the evaluation when they correctly identify the presentation that contains the odorous sample at one level, and then correctly identify the sample at the next level. The panelist must continue to correctly identify the odorous samples at the next level in order to eliminate the possibility that the sample was identified by chance.

Electronic Nose Analysis

Using the battery-powered air sampler, 1 L air samples were collected for analysis by the electronic nose. Air samples were analyzed using an AromaScan A32S electronic nose (Osmetech, Crewe, UK) located in the Iowa State University Food Sciences and Human Nutrition Department. The AromaScan contains thirty-two semi-conducting polypyrrole sensors. Air that is drawn through activated charcoal is used as the baseline reference, and then the odorous air is analyzed. The first step of the five-step sampling protocol involves drawing reference air across the sensors with no data being recorded to allow conditions within the instrument to stabilize. Then reference air is drawn across the sensors with the recording of data to allow for the establishment of a stable baseline. The third step is to draw the actual sample air across the sensors; the sample data, in terms of the change in electrical resistance of each of the sensors, is recorded. In the fourth step, a wash solution is passed across the sensors to remove any volatile chemicals that may still be adhered to them. The
final part of the procedure is to again draw reference air across the sensors to remove any remaining wash solution and to return to the baseline conditions.

**Air Composition Analysis**

A Hewlett Packard G1530A gas chromatograph coupled to a 5973 mass selective detector (GC-MS) was used to identify and quantify odorous compounds in the feeding room air. The mass spectrometer operated by chemical ionization with methane gas and was powered by a turbo pump. Two capillary columns were used, both with an inner diameter of 0.25 mm and a film thickness of 0.25 µm. The first section consisted of 7 m of HP-Wax column that was fused to 30 m of HP5-MS column (Agilent Technologies, Inc., Wilmington, DE). The inlet temperature was 230°C at 7.83 psi and a split injection with a split ratio of 10:1 was made. Helium was used as the carrier gas at a flow rate of 1.1 mL/min. The oven temperature was ramped from 35°C to 235°C as follows: 35°C for one minute, then increased by 10°C/min to 165°C, then increased by 2°C/min to 185°C, and then increased by 10°C/min to the final temperature of 235°C. The total run time was 29 min. Hewlett Packard Chemstation software was used to record and integrate the data. The method was developed by trial and error with changes being made to the temperature ramping, carrier gas flow rate, and split versus splitless injections, until suitable analyte separations were achieved.

Solid phase microextraction (SPME) fibers were used to absorb compounds in the air of the feeding rooms for analysis by mass spectrometry. The Supelco SPME Portable Field Sampler was used (Supelco, Bellefonte, PA). It contained a fused silica fiber coated with a 75µm thick partially crosslinked Carboxen™/polydimethylsiloxane phase material. SPME
fibers were exposed for 30 min to allow the compounds to equilibrate between the ambient air and the phase coating. The SPME field sampler was placed above the gutter end of the manure pit to allow the fiber to be exposed to the headspace above the greatest amount of manure.

A standard swine odor solution was formulated based upon the artificial slurry developed by Persaud et al. (1996). The chemicals used were obtained from Aldrich Chemical Company (Milwaukee, WI). Additional odorous compounds that were consistently present in the initial air samples were identified and added to the standard solution. A total of sixteen non-sulfur protein metabolites present in the ambient air were routinely quantified (Table 3). Standard solutions were prepared at concentrations of 100, 50, 25, 10, and 5% of the stock standard to generate a linear prediction curve. The stock standard swine odor solutions were stirred for approximately 30 min prior to the removal of a 1 mL sample that was placed in a 40 mL glass vial with a Teflon lined septum and hole cap. The SPME fiber was exposed to the headspace above the 1 mL of standard solution in the vial for 30 min.

Manure Analysis

A composite manure sample was collected from the pit in each room, following manual mixing and agitation. All manure analyses were conducted in the Iowa State University Department of Animal Science. To determine the amount of total solids (dry matter), manure samples were oven dried in porcelain crucibles for three to four days at 55°C until a consistent dry weight was obtained (Standard Methods of Water and Wastewater, method 2540B). Samples were ashed in a muffle furnace at 550°C for 5 hrs to determine volatile solids content (organic matter) (Standard Methods of Water and Wastewater, method
Table 3. Composition of synthetic swine odor solution used for quantification by GC-MS of sixteen air analytes from air samples taken in feeding rooms housing finishing pigs

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration (ppm)</th>
<th>Analyte</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>1365.00</td>
<td>3-methylphenol</td>
<td>62.00</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>358.00</td>
<td>4-ethylphenol</td>
<td>3.99</td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>604.00</td>
<td>3-ethylphenol</td>
<td>3.99</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>236.00</td>
<td>2,6-bis(1,1-dimethylethyl)phenol</td>
<td>2.00</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>301.00</td>
<td>Indole</td>
<td>6.00</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>90.00</td>
<td>3-methylindole</td>
<td>4.00</td>
</tr>
<tr>
<td>Phenol</td>
<td>20.00</td>
<td>2-methylindole</td>
<td>4.00</td>
</tr>
<tr>
<td>4-methylphenol</td>
<td>62.00</td>
<td>4-methylindole</td>
<td>4.00</td>
</tr>
</tbody>
</table>

1stock standard prepared in 0.03 M oxalic acid solution
2GC-MS = gas chromatography coupled to a mass spectrometer detector

2540E). The chemical oxygen demand was analyzed using Hach digester tubes according to Hach method 8000 (Hach, Loveland, CO). The colorimetric reaction was measured using a spectrophotometer. Total phosphorus content was analyzed using procedures of the AOAC (Association of Official Analytical Chemists) method 7.123, involving a digestion procedure followed by colorimetric determination using molybdovanadate for color development. Total Kjeldahl nitrogen was determined using digestion with a selenium catalyst followed by distillation (AOAC, method 2.057).

**Statistical Analysis**

Room served as the experimental unit in the incomplete randomized block design. Dietary influence on odor concentration, manure composition, electronic nose response, and
air composition was evaluated statistically using the mixed procedure of SAS version 6.01 (1997). Fixed variables included Room, Diet, Day, and Panelist. Period was included as a random variable. Since only two rooms were available for the experiment, but three dietary treatments were considered, Diet was confounded within the Room × Period interaction. To generate an odor prediction equation from the air analytes, stepwise regression procedures were used with the sixteen air analytes considered as cubic terms. The GC-MS results were used to predict olfactometry panelist response and were also compared to the electronic nose response. Correlation procedures were used to relate human panelist response to manure characteristics and electronic nose response.
RESULTS

Influence of DDGS on Animal Performance, Manure Characteristics, and Odor

The three diets were subsampled with each batch that was mixed. All nineteen samples were sent to a commercial lab for individual analysis. Nutrient analyses were conducted on an as-fed basis (average diet dry matter content = 89.11%). Table 4 depicts dietary treatment compositions, pooled over time. Contrasts were used to identify any differences in nutrient composition among the three diets (Table 4). There was a significantly greater amount of crude protein (P = .02) and acid detergent fiber (P = .01) in the diet containing 10% DDGS as compared to the other two diets. As the amount of DDGS in the diet increased, there was an increase in neutral detergent fiber (P < .0001), as well as a slight decrease in net energy for gain (P < .0001). The diets did not differ significantly (P > .10) in either lysine or tryptophan content; these are the two amino acids in which DDGS tends to be most limiting for swine. The addition of 10% DDGS increased the amount of valine, phenylalanine, and tyrosine above that in the 0 and 5% DDGS diets (P = .02). The amount of leucine increased with increasing dietary DDGS (P = .0003, Table 4). Threonine and isoleucine content was significantly greater in the 10% DDGS diet than in the control (P = .04 and P = .07, respectively; Table 4). The sulfur amino acid content of the 10% DDGS diet was significantly greater than that of both the 0 and 5% DDGS diets (methionine, P = .02; cysteine, P = .01). Elemental sulfur in the diet increased with increasing DDGS (P < .0001, Table 4). No differences in calcium content of the diets were found, but the addition of DDGS to the diet did significantly increase the phosphorus in the diet (P = .005, Table 4).
Table 4. Least squares means of nutrient composition of experimental diets for finishing pigs containing 0, 5, or 10% DDGS

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>0% DDGS</th>
<th>5% DDGS</th>
<th>10% DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP (%)</td>
<td>12.09a</td>
<td>12.51a</td>
<td>13.34b</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>4.29a</td>
<td>4.50a</td>
<td>5.38b</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>8.16a</td>
<td>9.56b</td>
<td>10.66c</td>
</tr>
<tr>
<td>NEG (mcal/lb)</td>
<td>0.58a</td>
<td>0.57b</td>
<td>0.57c</td>
</tr>
<tr>
<td>Lys (%)</td>
<td>0.65</td>
<td>0.63</td>
<td>0.64</td>
</tr>
<tr>
<td>Trp (%)</td>
<td>0.12</td>
<td>0.11</td>
<td>0.12</td>
</tr>
<tr>
<td>Thr (%)</td>
<td>0.45a</td>
<td>0.46a,b</td>
<td>0.48b</td>
</tr>
<tr>
<td>Val (%)</td>
<td>0.58a</td>
<td>0.59a</td>
<td>0.63b</td>
</tr>
<tr>
<td>Leu (%)</td>
<td>1.18a</td>
<td>1.24b</td>
<td>1.34c</td>
</tr>
<tr>
<td>Ile (%)</td>
<td>0.48a</td>
<td>0.49a,b</td>
<td>0.52b</td>
</tr>
<tr>
<td>Phe (%)</td>
<td>0.60a</td>
<td>0.61a</td>
<td>0.65b</td>
</tr>
<tr>
<td>Tyr (%)</td>
<td>0.49a</td>
<td>0.50a</td>
<td>0.54b</td>
</tr>
<tr>
<td>Met (%)</td>
<td>0.24a</td>
<td>0.24a</td>
<td>0.26b</td>
</tr>
<tr>
<td>Cys (%)</td>
<td>0.25a</td>
<td>0.26a</td>
<td>0.27b</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>0.64</td>
<td>0.65</td>
<td>0.63</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.44a</td>
<td>0.46b</td>
<td>0.48b</td>
</tr>
<tr>
<td>S (%)</td>
<td>0.12a</td>
<td>0.13b</td>
<td>0.15c</td>
</tr>
</tbody>
</table>

1DDGS = distillers dried grains with solubles, expressed as kg DDGS per kg diet
2CP = crude protein, ADF = acid detergent fiber, NDF = neutral detergent fiber, NEG = net energy for gain, all analyzed on as-fed basis
a,b,c means within a row with different superscripts differ (P < .10)
Treatment effects were analyzed and dietary inclusion of DDGS resulted in no significant (P > .05, Table 5) differences in animal performance, as measured by average daily gain and feed efficiency. There was greater feed disappearance (P < .10) with 10% DDGS than with both 5 and 0% DDGS. There was no difference (P > .10) in feed disappearance when comparing the 5% DDGS diet to the control.

Manure characteristics are depicted in Table 6. No significant differences due to diet were observed for total solids, volatile solids, chemical oxygen demand, total Kjeldahl nitrogen, or phosphorus content of manure (P > .10, Table 6). Day was a significant (P < .05) model variable for each of these parameters, but this was to be expected as the amount of manure accumulated and the storage time of the manure present increased from one sampling day to the next within each week. As there was limited manure storage available, the manure pits were rinsed and drained on a weekly basis. The first sampling day of each week reflected four days worth of manure storage and accumulation while the second sampling day represented seven. As the storage time of the manure increased, the anaerobic breakdown of the manure would have progressed. The solids would have been broken down and the dilution would have increased as wasted water from the drinkers and the sprinklers entered the pit. Nitrogen would have been lost through ammonia volatilization. The chemical oxygen demand would have decreased with increased storage time and extent of decomposition as well. Also, sampling error in collecting manure from the pits could have contributed to the differences observed, particularly with respect to Day effects on phosphorus content (measured on a dry basis).

The log geometric means of the dilution ratios were determined. This is the more common way of reporting olfactometry results. The log geometric means were compared
Table 5. Least squares means of animal performance measures over 24-d periods, as influenced by dietary inclusion of 0, 5, or 10% DDGS$^1$

<table>
<thead>
<tr>
<th></th>
<th>0% DDGS</th>
<th>5% DDGS</th>
<th>10% DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW (kg)</td>
<td>84.4</td>
<td>85.9</td>
<td>87.0</td>
</tr>
<tr>
<td>Final BW (kg)</td>
<td>103.0</td>
<td>105.3</td>
<td>105.9</td>
</tr>
<tr>
<td>ADG$^3$ (kg)</td>
<td>0.80</td>
<td>0.79</td>
<td>0.78</td>
</tr>
<tr>
<td>ADFD$^4$ (kg)</td>
<td>2.75$^a$</td>
<td>2.73$^a$</td>
<td>2.91$^b$</td>
</tr>
<tr>
<td>F:G$^5$</td>
<td>0.29</td>
<td>0.30</td>
<td>0.26</td>
</tr>
</tbody>
</table>

$^1$DDGS = distillers dried grains with solubles, expressed as kg DDGS per kg diet
$^2$BW = body weight
$^3$ADG = average daily gain
$^4$ADFD = average daily feed disappearance per pig, calculated as total feed into room per period divided by six pigs per room and 24 d per period
$^5$F:G = feed to gain ratio
$a,b$means within a row with different superscripts differ (P < .10)

Table 6. Composition of manure from finishing pigs fed diets containing 0, 5, or 10% DDGS$^1$

<table>
<thead>
<tr>
<th>Parameter$^2$</th>
<th>0% DDGS</th>
<th>5% DDGS</th>
<th>10% DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 4</td>
<td>Day 7</td>
<td>Day 4</td>
</tr>
<tr>
<td>TS (%)</td>
<td>19.36$^a$</td>
<td>14.78$^b$</td>
<td>20.63$^a$</td>
</tr>
<tr>
<td>VS (%)</td>
<td>16.41$^a$</td>
<td>12.17$^b$</td>
<td>17.32$^a$</td>
</tr>
<tr>
<td>P (% TS)</td>
<td>1.96</td>
<td>1.94</td>
<td>2.09$^a$</td>
</tr>
<tr>
<td>TKN (% TS)</td>
<td>2.75</td>
<td>2.71</td>
<td>2.77$^a$</td>
</tr>
<tr>
<td>COD (g/L)</td>
<td>217.54$^a$</td>
<td>163.03$^b$</td>
<td>230.54$^a$</td>
</tr>
</tbody>
</table>

$^1$DDGS = distillers dried grains with solubles, expressed as kg DDGS per kg diet
$^2$TS = total solids, kg solids/kg wet manure; VS = volatile solids, kg solids/kg wet manure; P = phosphorus, dry matter basis; TKN = total Kjeldahl nitrogen, dry matter basis; COD = chemical oxygen demand, as-is basis
$^3$day = day of sampling each week, either d 4 or d 7
$^a,b$superscripts indicate differences between samples collected on d 4 and d 7 (P < .05); no diet differences were observed
using paired t-tests. The probability that the olfactometry data from the air samples taken inooms housing pigs fed 0 and 5% DDGS was not significantly different was $P = .94$. The
paired t-test comparing 0 and 10% DDGS resulted in a $P$ value of .24. The statistical
possibility that odor samples from pigs fed 5 and 10% DDGS were the same was $P = .23$.
The log geometric means were also analyzed using the mixed procedure of SAS. This
allowed for the examination of the effects of Room, Diet, and Day. Table 7 shows the least
squares means of the log geometric means from olfactometry evaluation of the ambient air
samples. There were no significant diet effects on the responses of the panelists ($P > .10$).
Day of sampling (d 4 versus d 7) was significant ($P < .01$).

Each panelist's individual response, in terms of odor dilution threshold, was analyzed
as well. By using the mixed procedure of SAS with Panelist as a fixed variable, a more
powerful test was conducted which allows for greater explanation of the variability in the
results. The log of odor dilution as measured by olfactometry was used to normalize the data
because odor dilution was measured on an exponential scale. Normalizing the data provided
a more uniform distribution of the data without affecting the results (Table 8). There were no
significant treatment effects upon odor dilution threshold ($P > .10$). However, a non-
significant ($P = .16$) trend for increasing odor concentration with increasing dietary content
of DDGS was identified using a linear contrast. Odor dilution was affected by Room ($P < .01$), Day ($P < .01$), and Panelist ($P < .01$). The Room effect was possibly due to differences
in the ventilation systems. Room and Period were confounded; therefore time or season may
have influenced Room effect. Effects arising from the day of sampling and the human
panelists involved in the olfactory evaluation were expected. Since the amount of manure in
the storage pit increased from one sampling day to the next within a week, the odor generated
Table 7. Least squares means of log geometric means of detectable dilution ratios of collected room air samples when finishing pigs were fed 0, 5, and 10% DDGS\textsuperscript{1}

<table>
<thead>
<tr>
<th>Period 1 \textsuperscript{2}</th>
<th>0% DDGS</th>
<th>5% DDGS</th>
<th>10% DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 4</td>
<td>2.17</td>
<td>2.06</td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>2.26</td>
<td></td>
<td>2.18</td>
</tr>
<tr>
<td>Period 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>2.48</td>
<td>2.38</td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>2.49</td>
<td></td>
<td>2.46</td>
</tr>
<tr>
<td>Period 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4 \textsuperscript{a}</td>
<td>2.06</td>
<td>2.26\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>Day 7 \textsuperscript{b}</td>
<td>2.67\textsuperscript{b}</td>
<td>2.62\textsuperscript{b}</td>
<td></td>
</tr>
<tr>
<td>Period 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td></td>
<td>2.29</td>
<td>2.24\textsuperscript{a}</td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
<td>2.37</td>
<td>2.64\textsuperscript{b}</td>
</tr>
<tr>
<td>Period 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>2.30</td>
<td></td>
<td>2.29</td>
</tr>
<tr>
<td>Day 7</td>
<td>2.54</td>
<td></td>
<td>2.49</td>
</tr>
<tr>
<td>Period 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>2.21</td>
<td>2.22\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>2.51</td>
<td></td>
<td>2.61\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{1}DDGS = distillers dried grains with solubles, expressed as kg DDGS per kg diet
\textsuperscript{2}empty cells reflect not all diets fed during each Period due to facility limitations
\textsuperscript{a,b}different superscripts indicate within diet, Day was significant (P < .10)
Table 8. Least squares means of detectable dilution ratios of collected room air samples when finishing pigs were fed diets containing 0, 5, or 10% DDGS

<table>
<thead>
<tr>
<th>Period 1</th>
<th>0% DDGS</th>
<th>5% DDGS</th>
<th>10% DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odor dilution</td>
<td>Log odor dilution</td>
<td>Odor dilution</td>
</tr>
<tr>
<td>Day 4</td>
<td>203.82</td>
<td>5.08</td>
<td>159.12</td>
</tr>
<tr>
<td>Day 7</td>
<td>211.98</td>
<td>5.21</td>
<td>185.42</td>
</tr>
<tr>
<td>Period 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>407.91</td>
<td>5.68</td>
<td>330.90</td>
</tr>
<tr>
<td>Day 7</td>
<td>489.02</td>
<td>5.81</td>
<td>409.94</td>
</tr>
<tr>
<td>Period 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>178.86a</td>
<td>4.70a</td>
<td>287.35a</td>
</tr>
<tr>
<td>Day 7</td>
<td>584.04b</td>
<td>6.13b</td>
<td>555.51b</td>
</tr>
<tr>
<td>Period 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>246.27</td>
<td>5.27</td>
<td>215.90a</td>
</tr>
<tr>
<td>Day 7</td>
<td>331.93</td>
<td>5.40</td>
<td>578.28b</td>
</tr>
<tr>
<td>Period 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>298.66a</td>
<td>5.39a</td>
<td>394.01</td>
</tr>
<tr>
<td>Day 7</td>
<td>505.29b</td>
<td>5.82b</td>
<td>426.97</td>
</tr>
<tr>
<td>Period 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>187.58a</td>
<td>5.11a</td>
<td>205.96a</td>
</tr>
<tr>
<td>Day 7</td>
<td>588.30b</td>
<td>5.78b</td>
<td>640.71b</td>
</tr>
</tbody>
</table>

1DDGS = distillers dried grains with solubles, expressed as kg DDGS per kg diet
2empty cells reflect not all diets fed during each Period due to facility limitations
3different superscripts indicate within diet, Day was significant (P < .10)
from the manure would be expected to increase. As more manure was added to the pit each day, more substrate was available for breakdown by bacteria, thus more odorous compounds would have been generated. Also, the dissolved oxygen available would have decreased, and the resultant shift to anaerobic breakdown would have generated compounds of a more odorous nature, such as branched chain fatty acids.

A panelist effect upon odor dilution threshold would be expected because of the inherent variability among human beings. Sensory perception of an odor is an individual response, differing from one person to the next. The date on which a panelist participated in the panel was also determined to have a significant (P < .01) influence on panelist response.

The proportion of total variability in the odor concentration measurements explained by differences between the panelists over the treatments was calculated to have an $R^2$ of .23. This low number indicates that only a quarter of the total variability in the olfactometry analysis can be accounted for by differences between the panelists. The differences among the panelists are minimized by the olfactometry training process during which individuals are screened to ensure they are neither hypo- nor hypersensitive to odors. The variability among the panelists within the treatments across time had an $R^2$ of .12, suggesting that an individual panelist’s response was consistent over time.

**GC-MS Analysis of Air Samples**

There were no dietary treatment effects on the sixteen air analytes measured by GC-MS ($P > .10$, Table 9). Day effects ($P < .05$) on the majority of the analytes (excluding acetic acid, butyric acid, and 3-ethylphenol) were observed. Room effects were noted for isobutyric acid, 3-methylphenol, 3-ethylphenol, and 2,6-bis(1,1-dimethylpropyl)phenol ($P < .05$) as were
Table 9. Least squares means of air analyte concentrations collected from experimental rooms housing finishing pigs fed diets containing 0, 5, or 10% DDGS\textsuperscript{1}

<table>
<thead>
<tr>
<th>Analyte</th>
<th>0% DDGS</th>
<th></th>
<th>5% DDGS</th>
<th></th>
<th>10% DDGS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 4</td>
<td>Day 7</td>
<td>Day 4</td>
<td>Day 7</td>
<td>Day 4</td>
<td>Day 7</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>176,818</td>
<td>166,336</td>
<td>113,415</td>
<td>143,290</td>
<td>174,579</td>
<td>147,701</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>52,278</td>
<td>67,679</td>
<td>49,667</td>
<td>55,765</td>
<td>63,669</td>
<td>59,840</td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>23,115</td>
<td>55,220</td>
<td>25,779</td>
<td>38,105</td>
<td>24,585</td>
<td>25,811</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>27,850</td>
<td>31,758</td>
<td>27,259</td>
<td>27,901</td>
<td>32,060</td>
<td>29,289</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>17,706\textsuperscript{a}</td>
<td>26,444\textsuperscript{b}</td>
<td>20,221</td>
<td>26,123</td>
<td>21,151</td>
<td>22,371</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>8,289</td>
<td>12,895</td>
<td>9,249</td>
<td>10,849</td>
<td>9,296</td>
<td>11,188</td>
</tr>
<tr>
<td>Phenol</td>
<td>2,601\textsuperscript{a}</td>
<td>6,171\textsuperscript{b}</td>
<td>2,196\textsuperscript{a}</td>
<td>5,483\textsuperscript{b}</td>
<td>2,817\textsuperscript{a}</td>
<td>5,222\textsuperscript{b}</td>
</tr>
<tr>
<td>4-methylphenol</td>
<td>24,075</td>
<td>24,956</td>
<td>20,925</td>
<td>30,033</td>
<td>26,729</td>
<td>28,038</td>
</tr>
<tr>
<td>3-methylphenol</td>
<td>3,659</td>
<td>5,528</td>
<td>2,711</td>
<td>3,446</td>
<td>3,447</td>
<td>4,146</td>
</tr>
<tr>
<td>4-ethylphenol</td>
<td>1,368</td>
<td>3,517</td>
<td>1,266</td>
<td>1,643</td>
<td>1,925</td>
<td>1,665</td>
</tr>
<tr>
<td>3-ethylphenol</td>
<td>491</td>
<td>639</td>
<td>344</td>
<td>338</td>
<td>759</td>
<td>566</td>
</tr>
<tr>
<td>2,6-bis(1,1-dimethylethyl)phenol</td>
<td>247</td>
<td>64</td>
<td>127</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indole</td>
<td>411</td>
<td>477</td>
<td>596\textsuperscript{a}</td>
<td>1,186\textsuperscript{b}</td>
<td>416</td>
<td>668</td>
</tr>
<tr>
<td>3-methylindole</td>
<td>250</td>
<td>120</td>
<td>6,925\textsuperscript{a}</td>
<td>8,689\textsuperscript{b}</td>
<td>1,475</td>
<td>2,460</td>
</tr>
</tbody>
</table>

\textsuperscript{1}DDGS = distillers dried grains with solubles, expressed as kg DDGS per kg diet
\textsuperscript{ab}superscripts indicate differences between samples collected on d 4 and d 7 (P < .10); no diet differences were observed.
Room × Diet interaction effects on acetic acid, propionic acid, butyric acid, valeric acid, 4-methylphenol, 3-methylphenol, 3-ethylphenol, and 2,6-bis(1,1-dimethylethyl)phenol (P < .10).

Air analyte concentrations from this experiment were compared to those of samples taken from eight commercial swine finishers (Table 10). The concentrations of the analytes were generally lower in the ISU feeding rooms than in the commercial finishing barns. This is to be expected as the number of pigs, amount of manure and dust, length of manure storage, and animal residence time in the facility would have been greater for the commercial sites. Also, not all of the compounds identified in the ISU rooms were observed at the commercial sites. This could be attributed to differences in diet, number of molecules competing for absorption onto the phase coating of the SPME fiber, and sampling technique (samples at the commercial sites were taken approximately 0.3 m above the floor whereas ISU samples were collected at floor level).

The sixteen air analytes were individually correlated with the log of the odor concentration using simple correlation procedures, both before and after removing the seven panelists with a standard error greater than .70. A large standard error indicates variability in a panelist’s responses. Panelists with a large standard error were removed in an attempt to improve the correlations. All of the correlations were fairly low and removing the panelists with the greatest standard error did not improve them very much. The analytes best correlated with odor concentration were 3-methylphenol (r = .23), 2,6-bis(1,1-dimethylethyl)phenol (r = .14), 4-methylindole (r = .12), and indole (r = .11) (P < .05).

Much work has been done to attempt to identify chemicals in swine manure odor that could serve as indicator compounds. Schaefer (1977) found that 4-methylphenol was fairly
Table 10. Comparison of air analyte concentrations from experimental rooms housing finishing pigs fed diets containing 0, 5, or 10% DDGS\textsuperscript{1} and eight commercial swine finishing barns

<table>
<thead>
<tr>
<th>Analyte Concentration (ppb)</th>
<th>Commercial Finishing Barn One</th>
<th>Two</th>
<th>Three</th>
<th>Four</th>
<th>Five</th>
<th>Six</th>
<th>Seven</th>
<th>Eight</th>
<th>ISU Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>908,622</td>
<td>1,139,358</td>
<td>503,260</td>
<td>486,516</td>
<td>792,282</td>
<td>587,710</td>
<td>623,088</td>
<td>905,633</td>
<td>140,543</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>373,560</td>
<td>577,286</td>
<td>199,267</td>
<td>229,398</td>
<td>372,323</td>
<td>331,299</td>
<td>227,533</td>
<td>323,164</td>
<td>54,852</td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>29,885</td>
<td>38,888</td>
<td>26,597</td>
<td>30,891</td>
<td>46,346</td>
<td>44,396</td>
<td>38,359</td>
<td>43,687</td>
<td>9,511</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>280,611</td>
<td>367,984</td>
<td>86,070</td>
<td>117,761</td>
<td>336,084</td>
<td>290,247</td>
<td>159,450</td>
<td>242,630</td>
<td>29,095</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>34,902</td>
<td>35,797</td>
<td>17,362</td>
<td>18,551</td>
<td>42,235</td>
<td>39,522</td>
<td>25,575</td>
<td>35,592</td>
<td>19,316</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>75,921</td>
<td>109,700</td>
<td>32,832</td>
<td>40,197</td>
<td>78,131</td>
<td>59,711</td>
<td>30,585</td>
<td>45,117</td>
<td>7,638</td>
</tr>
<tr>
<td>Phenol</td>
<td>11,172</td>
<td>11,605</td>
<td>13,550</td>
<td>15,675</td>
<td>9,379</td>
<td>10,875</td>
<td>6,354</td>
<td>8,050</td>
<td>4,035</td>
</tr>
<tr>
<td>4-methylphenol</td>
<td>28,964</td>
<td>17,532</td>
<td>11,717</td>
<td>17,605</td>
<td>41,853</td>
<td>55,707</td>
<td>31,783</td>
<td>31,862</td>
<td>25,370</td>
</tr>
<tr>
<td>3-methylphenol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1,585</td>
</tr>
<tr>
<td>4-ethylphenol</td>
<td>1,897</td>
<td>1,319</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1,782</td>
</tr>
<tr>
<td>3-ethylphenol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>164</td>
</tr>
<tr>
<td>2,6-bis(1,1-dimethylethyl)phenol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19</td>
</tr>
<tr>
<td>Indole</td>
<td>915</td>
<td>799</td>
<td>256</td>
<td>234</td>
<td>1,341</td>
<td>1,800</td>
<td>607</td>
<td>819</td>
<td>403</td>
</tr>
<tr>
<td>3-methylindole</td>
<td>252</td>
<td></td>
<td></td>
<td>614</td>
<td>1,124</td>
<td>77</td>
<td>847</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-methylindole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>179</td>
<td></td>
</tr>
<tr>
<td>4-methylindole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>113</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1}DDGS = distillers dried grains with solubles, expressed as kg DDGS per kg diet
highly correlated with odor intensity (r = .71). Spoelstra (1980) reasoned that 4-methylphenol and the volatile fatty acids (VFAs) in the manure could serve as indicators. Zhu et al. (1999) pointed out the necessity for analyzing individual VFAs, rather than total VFA content, since short chain VFAs are less odorous than long and branched chain VFAs. Although present at greater concentrations, short chain VFAs may make a lesser contribution to odor (Zhu et al., 1999). Zhu et al. (1997) reported a low correlation (r = .16) between total VFAs and odor dilution threshold. Using GC-MS, Hobbs et al. (1995) analyzed swine slurry and found the major odorants to be 4-methylphenol and 3-methylindole. Other compounds identified included acetic acid, undecane, and 4-ethylphenol. Zahn et al. (1997), using GC-MS technology, identified C₂ through C₉ organic acids as contributing to the odor arising from swine slurry and only rarely detected indole and 3-methylindole in ambient air above swine slurry.

**Electronic Nose Evaluation of Air Samples**

The thirty-two sensors of the electronic nose are sensitive to different groups of chemicals to differing degrees, including amines, carboxylic acids, alcohols, esters, aromatics, and ketones. A great deal of data is generated during the analysis of a sample with the electronic nose, not all of it meaningful. Of the thirty-two sensors, those sensors that are most sensitive to agricultural odors could be selected and the not useful data eliminated. Unfortunately, most of the sensors included in the AromaScan electronic nose that was available for use are not overly responsive to some of the compounds found in swine manure odor, including indoles and sulfur containing compounds (Osmetech, Crewe, UK).
Most of the sensors are moderately sensitive to amines and carboxylic acids, though, and some are responsive to aromatics (Osmetech, Crewe, UK).

Figure 1 illustrates the response of the electronic nose sensors to the three experimental diets. The graph demonstrates that the electronic nose did not detect any differences in odor arising from pigs fed 0, 5, or 10% DDGS. The principal component for the response of the thirty-two electronic nose sensors was generated. Mixed procedures of SAS were used to test for diet effects on the eigenvector value. No significant diet (P > .10) effect was observed. The synthetic swine odor solutions used for GC-MS analysis were also analyzed by the electronic nose. Sensor response to the solutions is shown in Figure 2. The eight sensors that responded linearly to the synthetic swine odor solutions were selected and the test for diet effect on sensor response was repeated. Again, no significant (P > .10) effect of diet on sensor response was observed. Using the AromaScan software, principal component analysis (PCA) maps were made (Figures 3 and 4). Principal component analysis was performed in two and three dimensions. The lack of clustering on the PCA maps again demonstrates that the electronic nose sensors did not differentiate among air samples taken from the rooms housing pigs fed either 0, 5, or 10% DDGS.

Odor Prediction Using Instrumental and Organoleptic Methods

The manure characteristics measured were not highly correlated with odor concentration. Interestingly, all the correlation coefficients were negative: COD, $r = -.24$; TS, $r = -.15$; P, $r = -.15$; N, $r = -.14$; VS, $r = -.13$ (P < .01). The greatest correlation was between chemical oxygen demand and odor ($r = -.24$). Williams (1984) correlated odor offensiveness as measured by human panelists with chemical analyses of swine manure.
Figure 1. Electronic Nose Sensor Responses to Room Air Samples from Pigs Fed 0, 5, or 10% Distillers Dried Grains with Solubles (DDGS, expressed as kg DDGS per kg diet)
Figure 2. Electronic Nose Sensor Responses to a Synthetic Swine Odor Solution at Varying Concentrations
Figure 3. Two Dimensional Principal Component Analysis (PCA) Map of Electronic Nose Response to Air Samples from Rooms Housing Finishing Pigs Fed 0, 5, or 10% Distillers Dried Grains with Solubles (DDGS, expressed as kg DDGS per kg diet)
Figure 4. Three Dimensional Principal Component Analysis (PCA) Map of Electronic Nose Response to Air Samples from Rooms Housing Finishing Pigs Fed 0, 5, or 10% Distillers Dried Grains with Solubles (DDGS, expressed as kg DDGS per kg diet)
Odor offensiveness was rated on a scale of 0 (inoffensive odor) to 5 (very strongly offensive odor). The chemical analyses included total solids, total suspended solids, chemical oxygen demand, five-day biochemical oxygen demand, and nitrogen (ammoniacal, Kjeldahl, and organic). The greatest correlation was between the natural log of BOD and odor offensiveness \((r = .84)\). In contrast to the current experiment, a positive correlation was observed by Williams (1984).

An equation was generated to use the air analytes to predict odor dilution threshold. With all analytes considered as cubic terms an \(R^2\) value of .27 was obtained. Removing the insignificant \((P > .10)\) terms and including them as quadratic or linear variables reduced the \(R^2\) value to .21. The poor prediction capability indicates that additional analytes may require consideration, although the repeated occurrence of other analytes in the air samples was not evident from GC-MS analyses.

The principal component for the response of the thirty-two electronic nose sensors was generated. The eigenvector value was then correlated to the log of odor dilution threshold. The electronic nose evaluation of the air samples was not strongly correlated to the olfactometry measures \((r = .18; P < .01)\). The equation generated from the GC-MS analysis predicted the electronic nose response with an \(R^2\) of .81. Thus, the two instrumental methods of odor analysis were fairly compatible, but the instrumental methods were not highly related to organoleptic analysis.
Animal performance was not significantly (P > .05) affected by the dietary treatments. Feeding DDGS at 5 and 10% of the diet did not affect the average daily gain or feed efficiency of the late finishing pigs. This is in keeping with the results reported by Wahlstrom et al. (1970), Harmon (1974), and Cromwell and Stahly (1986), who fed DDGS at levels up to 10% with no effects on performance. Any differences in performance would have actually been unexpected since in this experiment DDGS was included at low levels and the diets were fed for only 24 days. Inclusion of DDGS in the diet did not significantly affect manure composition. Dietary effects on manure composition would also have been unexpected.

No significant (P > .10) treatment effects on odor dilution threshold were observed but there was a non-significant (P = .16) trend for increasing odor concentration with increasing dietary DDGS. This trend was likely due to the slightly increasing amount of protein, sulfur amino acids (methionine and cysteine), and elemental sulfur in the diets with DDGS. The excess protein in the diets would have been broken down anaerobically to volatile fatty acids, phenols, and indoles, which would have contributed to the odor perceived by the human panelists during olfactometry. The amino acids valine and isoleucine are broken down to the branched chain volatile fatty acids, isobutyric and isovaleric acid, respectively (Spoelstra, 1980). The anaerobic decomposition of tyrosine produces phenol, 4-methylphenol, and 4-ethylphenol (Spoelstra, 1980; Hammond, 1989; Mackie et al., 1998). Phenylalanine is broken down to phenol as well (Hammond, 1989). Cysteine and cystine can be degraded to hydrogen sulfide, carbon disulfide, and diethyl sulfide, while methionine is
converted to methyl mercaptan, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide (Spoelstra, 1980; Hammond, 1989). As reported by Shurson et al. (1999), swine manure odor increases with increasing dietary sulfur content. The amount of dietary fiber also increased with increasing DDGS. As described by Hawe et al. (1992), the addition of dietary fiber significantly increased the excretion of indole (P < .05) and 3-methylindole (P < .01) in the feces of swine. Sutton et al. (1997) also reported an increase in manure volatile fatty acid content when swine were fed diets containing 5% cellulose. Although significant alteration in odor concentration was not observed with the addition of DDGS to the diet, there is evidence for the potential of dietary composition to influence odor. Diet manipulation as a means to reduce odor needs to be investigated further.

Sulfur compounds contribute to swine odor (Spoelstra, 1980; Williams, 1984; O’Neill and Phillips, 1992) but were not directly measured in this experiment. The gas chromatograph-mass spectrometer was not configured to analyze sulfur compounds and the sensors in the electronic nose were not sensitive to sulfur (Osmetech, Crewe, UK). However, sulfur compounds would have contributed to the responses of the human olfactometry panelists. This could account for the fact that the equation generated using the 16 analytes from GC-MS analysis was capable of predicting the electronic nose response (R² = .81), but not olfactometry response (R² = .27).

One challenge associated with instrumental analysis of livestock manure odor is the low concentration of odorous compounds in air. Of 168 compounds that have been identified in livestock waste or in the ambient air, thirty compounds have detection thresholds of 0.001 mg/m³ or less (O’Neill and Phillips, 1992). Of the ten compounds with the lowest detection thresholds, six are sulfur-containing compounds. The human nose is capable of detecting
these compounds, but they are present at concentrations generally below the level of detection for analytical instrumentation such as the gas chromatograph, mass spectrometer, and electronic nose (Miner, 1977; Bartlett et al., 1997; Mackie et al., 1998). Since these compounds can be detected by the human nose in very small amounts, it is likely that they made a contribution to the odor that was perceived by the olfactometry panelists but were undetected by instrumental analyses. Burnett (1969) reported that when using gas chromatography with organoleptic analysis of the eluted compounds, odors were detected by human observers when no peaks were recorded on the chromatogram. These results demonstrate the ability of the human nose to detect odorous compounds that an instrument cannot.

The use of instrumental techniques to predict human response to odor has important implications for monitoring livestock facilities for avoidance of odor nuisance problems with neighbors. Potentially, a portable electronic nose could be used to monitor odor on-site and GC-MS analysis could be used to predict odor as well. If the compounds that make significant contributions to livestock odor could be quantified with GC-MS and an equation used to predict odor, problems associated with odor management could be remedied before they reach great proportions.

As limited research has been done to date with the electronic nose as a means for measuring agricultural odors, this type of work will be important in the future. One area that requires attention is the development of sensor technology. The potential for sensor development specific to application is great. The sensitivity of conducting polymers can be adjusted to the type of samples that will be analyzed by changing functional groups on the polymer or the type of polymer itself (Haugen and Kvaal, 1998). Combining different types
of sensors into one electronic nose can also broaden selectivity and increase sensitivity (Gardner and Bartlett, 1999). The difficulty would be satisfying the different operating conditions required for each sensor type (Haugen and Kvaal, 1998). Overcoming the challenges of humidity effects on sensors and extending sensor life will also be important to the future use of electronic noses (Gardner and Bartlett, 1999).
CONCLUSIONS

Distillers dried grains with solubles is a byproduct from the processing of corn into ethanol and is an abundant resource, particularly in the Midwest; therefore, its inclusion in late finishing swine diets may be economical. There was no difference in feed cost among the three diets. As it had no negative effects on pig performance and it is an economical and available byproduct, the continued feeding of DDGS is justified.

A non-significant trend for increasing odor with increasing DDGS demonstrates the potential for diet formulation to improve odor. This increase in odor was likely due to the increased levels of dietary crude protein, amino acids, and elemental sulfur that were observed with increasing DDGS in the diets.

The two instrumental methods of odor analysis, GC-MS and electronic nose, were fairly comparable. However, neither instrumental method was highly correlated with organoleptic evaluation by olfactometry. Though the equation generated from the GC-MS response was capable of predicting electronic nose response ($R^2 = .81$), it was not very useful for predicting olfactometry response ($R^2 = .23$). The results of this study suggest that human panelist responses may be based on detection of compounds that were not included in GC-MS quantification procedures and were not well detected by the electronic nose. These results are also likely due to the presence of odorous compounds that were below the instrumental limits of detection yet detectable by the human nose.

Sulfur compounds are detected by the human nose at very low concentrations and would have likely contributed to the olfactometry panelists’ perception of the air samples. The GC-MS was not configured to analyze sulfur-containing compounds and the sensors of
the AromaScan electronic nose are not sensitive to sulfur. In the future, GC-MS analysis with different methods and synthetic odor solutions will be conducted. Also, further research into training the electronic nose and selecting those sensors that are best suited to agricultural odor evaluation will be necessary. Eventually, the point may be reached where a prediction equation based on GC-MS analysis or on-site electronic nose evaluation can be used for regulatory purposes.
REFERENCES CITED


