ODOR FROM PIG PRODUCTION:

ITS RELATION TO DIET

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LE DINH PHUNG

ODOR FROM PIG PRODUCTION:

ITS RELATION TO DIET

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ABSTRACT. **Le, D. P. Odor from pig production: Its relation to diet.** *Odor from pig manure creates a serious nuisance for people living near pig farms. Odor is a mixture of various compounds, of which 4 groups may be the major contributors: sulfurous compounds, indolic and phenolic compounds, volatile fatty acids, and ammonia and volatile amines. Odor is evaluated both sensorily (odor concentration, intensity and hedonic tone) and chemically. Odor originates mainly from microbial conversion of protein (CP) and fermentable carbohydrates (FC) in the intestine of pigs and by microbial conversion of urinary and fecal compounds in the manure. There is increasing interest in reducing odor at the source by altering diets. In four different experiments, dietary factors were investigated for effects on odor emission, odor intensity and hedonic tone and ammonia emission from growing and finishing pigs' manure. Lowering dietary CP level from 18 to 12% and supplementing essential amino acids (AA) reduced odor emission by 80%. Supplementing sulfur-containing AA at a level of three times the animal requirement increased odor emission by 723%. Diets with low levels of CP and sulfur-containing AA increased odor hedonic tone (producing less unpleasant odor). Dietary CP and FC had an interactive effect on odor emission. At a high dietary CP level, increased FC level decreased odor emission, while at a low CP level, increased FC level increased odor emission from pig manure. Ammonia emission from pig manure was reduced by a low dietary CP level and supplementing most essential AA and by increasing dietary FC. The correlation between odor emission and ammonia emission was low and deemed non-significant. Dietary approaches which are efficient in reducing ammonia emission may have no or even opposite impacts on odor reduction. From our studies, we can conclude that sulfurous compounds were the most important odorous compounds causing odor nuisance. When minimal diet requirements are met, dietary alterations did not affect animal performance. Altering multiple dietary factors and evaluating their correlations affecting odor production and emission is more efficient in odor nuisance reduction than altering a single dietary factor. Dietary alterations are shown to be very effective in reducing odor nuisance from pig manure.*

Key words. *Odor, Pigs, Diet, Manure, Protein, Amino Acids, Fermentable carbohydrates.*

IN MEMORY OF MY FATHER

IN DEDICATION TO MY MOTHER

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1

General Introduction

Pig production has undergone considerable changes in the last decades in terms of improving production efficiency and production characteristics (animal mortality, animal welfare and product quality). The changes in animal production have resulted in large and intensive pig production systems. These systems generate minerals, odor, ammonia, and dust, which may exceed levels tolerated by the human population (Tamminga, 1992; Jongbloed & Lenis, 1998). Odor from livestock production facilities in general and from pig production facilities in particular may cause nuisance for the residents in the surrounding areas, especially in areas where both many animals and humans are concentrated. In The Netherlands, 11% of the total population experienced annoyance from odor from agricultural activities, while the odor annoyance level was 10% for industrial activities and 7% from traffic (Anonymus, 2001). The Dutch government has set targets to reduce odor nuisance and to eliminate severe nuisance by the year 2010. From now on, additional annoyance should be prevented. Odor regulations are in force to reach these targets. The current regulation for animal housings is based on specified setback distances between new or expanding livestock production facilities and sensitive residential categories. Regulations are specified for the number and type of animals and human population. Odor regulations play an important role in the current reconstruction and zoning schemes for intensive animal production in the rural area, and determine in many cases the maximum scale of livestock operation. At the same time they ensure the sustainability of pig production.

Odor is a complex mixture of various volatile compounds. More than 300 different odorous compounds may contribute to odor nuisance from pig production facilities (Schiffman *et al.*, 2001). Odorous compounds can be classified into four main groups: (1) sulfurous compounds, (2) indolic and phenolic compounds, (3) volatile fatty acids (VFA), and (4) ammonia and volatile ammines (Hobbs *et al.*, 1997; Mackie *et al.*, 1998). Odor is emitted from animal houses, manure storage, and during field application of manure. In The Netherlands, manure must be covered in manure stores allowing no odor emission, and manure must be incorporated into the soil when it is applied to the field. Odor from manure application causes nuisance but occurs for short periods in the year. Therefore, odor from field application is considered much less as a problem than the continuous odor emission from animal houses. Odor from the animal house comes from feed, the animal body, urine, feces and manure in the manure pit. In general, feed and animal body odors are not regarded as offensive, but the odor generated from manure in the manure pit is (Hansen, 2005).

Odor emits from the source of its production into the air where it is dispersed and exposes itself to the human population. Odor stimulates the human olfactory cells located in the nose

cavity. The sensory cells are the interface between the environment and the brain. The sensory perception of odor is a precondition for odor annoyance. Sensory perception is determined by the detectability, the intensity, and the character of the odor stimulus. This information is then processed in the brain, in the cognitive appraisal process. If this appraisal leads to a negative appreciation, the perceived odor is considered a nuisance and may result in complaints (Power & Stafford, 2001).

Odor can be characterized in two ways: by sensory evaluation and by chemical evaluation. The sensory perception of odor has three major dimensions: odor concentration (detectability), odor intensity and odor hedonic tone. The first two dimensions express the odor strength, and the last one is the odor offensiveness or the relative pleasantness or unpleasantness of odor. By chemical evaluation, different compounds in the odorous air can be identified. It is important to note that in case of complex mixtures of odorous compounds it is difficult to link the two evaluation systems. Both are complex and mixtures of chemical compounds as from animal production facilities have not been studied in relation to sensory perception.

Odor is mainly produced by the microbial conversion of feed components in the large intestine of pigs and after excretion by microbial conversion of excreta under anaerobic conditions in manure. Odor precursors are excreted via feces and via urine into manure. Via the fecal pathway, odor precursors may be included in undigested feed components and in endogenous products. Odor precursors may be present in urine as well, and may be included in metabolic end products of excess nutrients after being absorbed in the small intestine and included in detoxicated products absorbed from the large intestine of animals (Fig. 1). Proteins/amino acids (AA) and fermentable carbohydrates (FC) are the main precursors for odor production (Mackie *et al.*, 1998). Sulfurous, indolic and phenolic compounds, VFA, and volatile amines are produced from protein and AA fermentation, while the fermentation of FC produces straight-chain VFA.

Efforts have been made to minimize odor. Attempts were mainly focused on cleaning odorous compounds in the air emitted from pig production facilities. Efforts so far can be characterized as minimizing odor after it has been produced. In other words, they are end-of-pipe solutions. Reducing odor at the source of production by altering dietary composition has a great potential but it is an unexplored field. According to Sutton *et al* (1999) odor production mainly arises from an excess of degradable protein and a lack of specific FC during microbial fermentation; thus the main dietary components that should be altered to reduce odor are protein and FC.

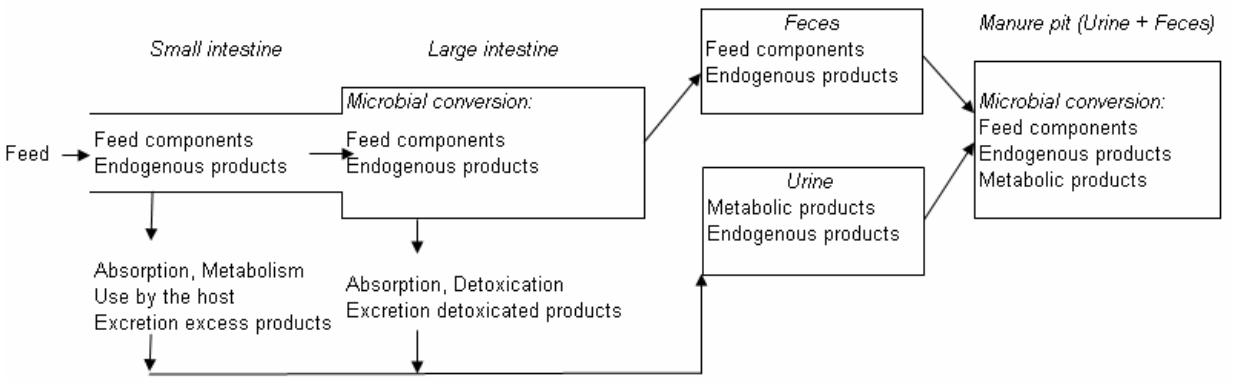

Fig. 1. Sources of odor precursors for odor production in the manure

Studies on pig odor so far have focused on effects of dietary CP and FC on different groups of odorous compounds in the manure or in the air above the manure surface, although the relationships between each odor group on one hand and odor strength and offensiveness on the other hand are not yet clear. Effects of dietary CP level and FC on odor strength and offensiveness of the odorous air emitting from pig manure measured by olfactometry have not been studied well and results were very inconsistent. In addition, the effects of CP and FC were studied independently whereas CP and FC may have an interactive effect on odor production. Interactions are expected because in the large intestine of pigs and in the manure stores, microbiota use protein as a nitrogen source and obtain energy from FC and also from CP for their biomass synthesis.

A division can be made between protein that is broken down in the small intestine to AA and absorbed in the small intestine and protein which escapes the small intestinal digestion, called ileal non-digestible protein or fermentable protein. Fermentable protein may be used by bacteria in the large intestine. It can be broken down to odorous compounds. A change in the level of fermentable protein may alter odor production in the large intestine of animals and in the manure. Effects of fermentable protein level on odor strength and offensiveness from pig manure have not yet been studied. Excess AA absorbed in the small intestine may also be converted to precursors for odor production. In this respect some AA seem to be more important than others. Sulfurous compounds and the aromatic compounds of indoles and phenols are considered most important for odor nuisance from pig production facilities. Tryptophan (Trp), Phenylalanine (Phe) and Tyrosine (Tyr) are main substrates for the synthesis of indolic and phenolic compounds. The S-containing AA, Methionine and Cystine are main substrates for the synthesis of S-compounds (Mackie *et al*., 1998). A change in the concentration of these AA in the diet may alter the level of odorous compounds produced in the manure. The effects of these AA on odor strength and offensiveness have not yet been studied.

The overall goal of the present study is to assess the potential impacts of dietary factors on odor strength and offensiveness from pig manure, focusing on the effects in the ileum, in the large intestine and after excretion in the manure. The specific objectives are to test the hypotheses that:

1. Dietary CP level is an important factor in odor production from pig manure
2. Sulfur-containing AA and Trp, Phe and Tyr are important precursors for odor production from pig manure
3. Apparently ileal non-digestible protein (fermentable protein) fermented in the large intestine of pigs is an important source for odor production from pig manure
4. Dietary CP and FC levels do interact with regard to odor production from pig manure.

Ammonia emission causes serious environmental problems such as acidification and eutrophication of natural ecosystems. Ammonia emission may lead to increased leaching of potassium, magnesium and calcium out of the soil (Likens *et al.*, 1996). Dietary alterations can influence ammonia emission (Canh *et al.*, 1998a; Canh *et al.*, 1998b; Sutton *et al.*, 1999). Odor abatements by dietary alterations are only of interest if they do not increase ammonia emission from pig manure. Therefore, this study also evaluates the effects of these dietary factors on ammonia emission from pig manure and its relation to odor emission. In addition, dietary alterations should maintain normal performance of pigs otherwise they are not feasible under practical conditions.

It has been suggested that environmental factors like air and manure temperature, ventilation rate, manure emitting area, and manure dilution ratio may influence odor production and emission from the manure. However, literature hardly contains any information with regard to the quantitative effects of these environmental factors on odor from pig manure. In order to study specific effects of dietary factors on odor from pig manure, effects of environmental factors should be controlled.

This thesis includes eight chapters. Following the current chapter, Chapter 2 is a literature review describing the current state-of-the-art of knowledge on livestock odor in relation to diet. The central point of this review is the relationship between dietary composition and odor production. From this review, we identified gaps in the knowledge on minimizing odor by altering diets. Based on these gaps, different experiments were devised.

Chapter 3 presents a study on the effects of environmental factors on odor from pig manure. This is a technical study providing knowledge on sampling and measuring odor concentration from pig manure. In addition, it identifies environmental factors having potential

effects on odor production from pig manure. These factors should be controlled in dietary experiments. In this study, a controlled lab experiment according to a face-centered central composite arrangement with blocks was carried out to evaluate the effects of temperature, ventilation rate, emitting area, and dilution ratio on odor from pig manure and manure characteristics.

Chapters 4, 5, 6, and 7 describe different studies to test the hypotheses 1, 2, 3 and 4, respectively. Chapter 4 presents a study on the effects of different dietary CP levels on odor from pig manure. It was hypothesized that odor from pig manure can be minimized by reducing dietary CP and supplementing most essential AA. In Chapter 5, a study on the effects of specific crystalline AA supplementation to the diet on odor from pig manure is described. This is to measure the effect of ileal absorbed AA on odor from pig manure. Two groups of AA were used. The first group was supplemented with sulfur-containing AA, and the other with Trp, and Phe+Tyr. It was hypothesized that a surplus of S-containing AA or Trp, Phe, and Tyr in the diet would result in higher odor production from pig manure. Chapter 6 describes a study on the effects of different fermentable protein levels on odor from pig manure. This is to study the effect of the level of protein broken down in the large intestine on odor from pig manure. It was expected that a high level of protein entering into the large intestine of pigs would result in more breakdown products from protein in the large intestine of pigs. These breakdown products would be excreted in feces or in urine dependent on the amount that is incorporated in the biomass. The three mentioned studies used a randomized complete block arrangement having three treatments in six blocks. In Chapter 7, a study on the effects of CP and FC level and their interaction on odor from pig manure is presented. A 2 x 3 factorial complete block arrangement was used. It was assumed that dietary CP and dietary FC do interact with regard to odor production and emission. Dependent variables in all dietary experiments are odor concentration and emission, odor intensity, odor hedonic tone, ammonia emission from pig manure, and manure characteristics (pH, ammonium, total nitrogen, indolic, phenolic, sulfurous compounds and VFA concentrations).

Chapter 8 is a general discussion. In this chapter, the findings from the five studies are discussed in connection to each other, as well as the implications for odor reduction by dietary approaches. This chapter also discusses the limitations of this study and proposes further studies.

2

ODOR FROM ANIMAL PRODUCTION FACILITIES: ITS RELATION TO DIET

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ABSTRACT. *Though bad odor has always been associated with animal production, it did not attract much research attention until in many countries the odor production and emission from intensified animal production caused serious nuisance and was implicated in the health problems of people living near animal farms. Odor from pig production facilities is generated by the microbial conversion of feed in the large intestine of pigs and by microbial conversion of pig excreta under anaerobic conditions and in manure stores. Assuming that primary odor-causing compounds arise from an excess of degradable protein and a lack of specific fermentable carbohydrates during microbial fermentation, the main dietary components that can be altered to reduce odor are protein and fermentable carbohydrates. In the present paper we aim to give an up-to-date review of studies on the relationship between diet composition and odor production, with the emphasis on protein and fermentable carbohydrates. We hypothesize how odor might be changed and/or reduced by altering the diet of pigs. Research so far has mainly focused on the single effects of different levels of crude protein and fermentable carbohydrates on odor production. However, also important for odor formation are the sources of protein and fermentable carbohydrates. In addition, it is not only the amount and source of these compounds that is important, but also the balance between them. Furthermore, research so far has focused on effects of dietary protein and fermentable carbohydrates on different groups of odorous compounds in the manure or in the air above the manure surface, although the relationships between each odor group with odor strength and offensiveness are not yet clear. On the basis of our review of the literature, we hypothesize that odor nuisance from pig production facilities might be reduced significantly if there is an optimum balance between protein and fermentable carbohydrates in the diet of pigs.*

Key words: *Odor, diet, pig*

INTRODUCTION

Although bad odor has always been associated with animal production, only within recent decades has it attracted increased attention. This is mainly because of the increase in human population and in intensification of animal production in many countries throughout the world. The odor produced and emitted from such intensive animal production can cause serious nuisance to individuals living in the vicinity of livestock farms and was related by some authors to health problems, for example, accelerated decline in pulmonary function, bronchitis, sinusitis, inflamed nasal mucosa, throat irritation and headaches (Schenker *et al.*, 1991; 1998; Donham, 2000; Iverson *et al.*, 2000).

The odor generated in animal production facilities comes from feed, animal bodies, urine, feces and manure. Odor production is influenced by many factors, such as dietary composition and environmental factors (Fig.1). Odor is mainly generated by microbial conversions of non-utilized dietary nutrients and endogenous products secreted in the gastrointestinal tract under anaerobic conditions. There are 4 main groups of odor: sulfurous compounds; phenols and indoles; volatile fatty acids (VFA); ammonia and volatile amines.

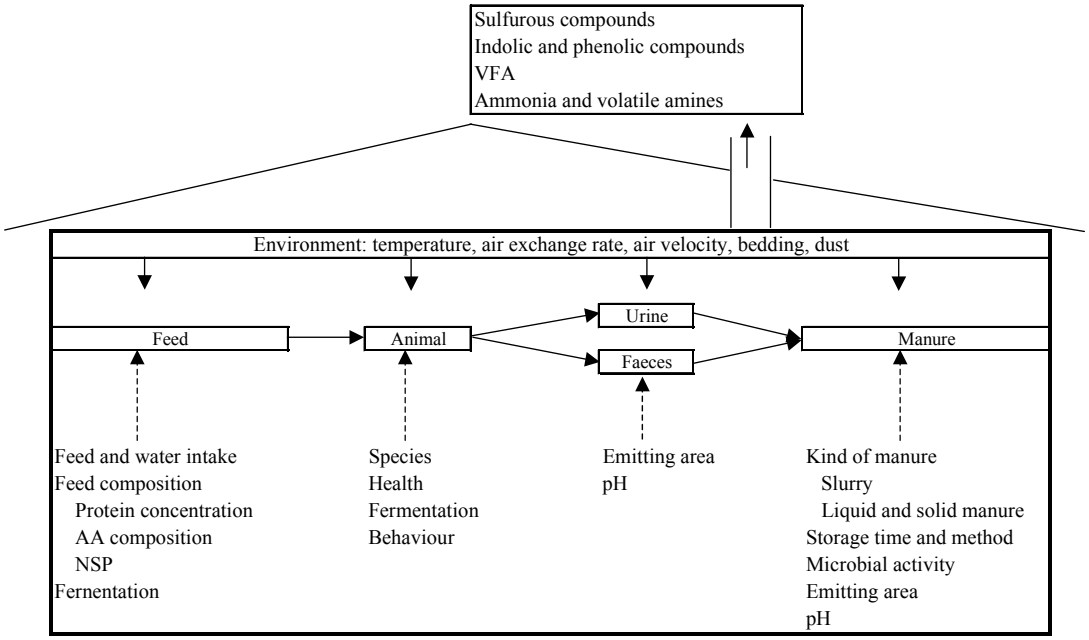


Fig. 1. Sources of odor and factors influencing odor, AA = Amino acids; NSP = Non-starch polysaccharides, VFA = Volatile fatty acids

Various means of reducing odor production and emission have been invented and applied, such as bio-scrubbers (Schirz, 1985), bio-filters (Noren, 1985), chemical and biological additives, masking agents, treatment of wastes, and manure-spreading machinery (Phillips *et al.*, 1990). These remedies have so far mainly focused on preventing odor from being emitted.

These end-of-pipeline interventions are generally costly and/or prone to malfunction. Very few studies so far have focused on reducing the formation of odorous compounds at source, e.g. in the large intestine of the animal or in manure storage. The fermentation and hydrolysis of apparently undigested nutrients in the large intestine produces odor directly or provides precursors for odor formation in the manure.

Measurements of odor emission in different farm locations with similar housing systems have shown large variations, with coefficients of variation ranging from 25 to 140% (Ogink & Groot Koerkamp, 2001). Diet probably contributes greatly to the variation of odor, because its composition is directly related to odor production. Therefore, odor can be altered by changing the amount and source of each component in the diet. Based on the principle that the primary odor-causing compounds evolve from an excess of degradable proteins and lack of specific fermentable carbohydrates during microbial fermentation (Sutton *et al.*, 1999), the main nutrients in the diet that can be altered to reduce odor production and emission are probably proteins and fermentable carbohydrates. In addition, feed additives can be used to improve the digestibility of specific complexes within feed ingredients and/or to alter the pH of manure to a pH less favorable for odor production.

We suspect that odor production and emission from animal production facilities can be altered by dietary composition. However, research still has to be done before it is possible to manage this process. The present review describes the current state-of- the-art of the science of livestock odor in relation to diet. It examines odor compounds from animal production facilities, especially from pig production facilities with most emphasis on within the large intestine, and within manure. We have attempted to pinpoint the nature of smell, the detection threshold and concentration of important odorous compounds. Later, we address the principles of odor formation and the roles of different bacteria in odor formation and describe the standard methods used to characterize the sensory and chemical values of odor. We discuss the relationships between the diet and odor composition and production and describe different dietary approaches to reduce odor. From this, we are able to identify gaps in the knowledge on reducing odor by altering diets, from which research strategies can be derived.

ODOROUS COMPOUNDS FROM ANIMAL PRODUCTION FACILITIES

SOURCES OF ODOR AND THE PRINCIPAL GROUPS OF ODOR

Odor generated in animal production facilities comes from (i) feed, (ii) animal body, (iii) urine and feces or the mixture of both, the manure. The most significant source of odor is from

the excreta: urine, feces and manure, especially their decomposition during collection, handling, storage, and spreading. Odor is emitted into the air from buildings or external manure storage sites or from manure application in the field. There are a great number of odorous compounds present in animal production facilities. O'Neill and Phillips (1992) summarized 168 odorous compounds identified in various studies in animal production facilities. As already mentioned, they can be classified into sulfurous compounds, volatile fatty acids (VFA), phenols and indoles, and ammonia and volatile amines. Thirty out of these 168 compounds have an odor detection threshold of 1$\mu g/m^3$ or less (Table 1). Recently, Schiffman *et al*. (2001) identified a total of 331 different compounds from pig production facilities in North Carolina.

Although a huge number of odorous compounds have been identified from animal production facilities, the sources from which they originate are poorly described. Geypens *et al*. (1997) isolated a total of 120 different volatile organic compounds from human feces, of which 25 remained unidentified. Schaefer *et al*. (1974) detected more than 70 compounds, which they assumed to have originated from particles of feed rather than from animal manure. Drasar & Hill (1974) found indole, 3-methyl indole (skatole), phenol, 4-methylphenol (p-cresol) and 4-ethylphenol in the urine of pigs. These compounds originate from the putrefactive decomposition of bacteria in the large intestine of the animal. They are then detoxified by the liver and excreted via urine. According to Spoelstra (1977) phenol, p-cresol, and 4-ethylphenol are mainly present in urine as glucuronides. Glucuronides are rapidly and easily converted by glucuronidase in feces to the compounds mentioned. Odor from the animal body, such as the cutaneous and oral odor, has not been well described. The main sweat compounds from the animal are thought to be propionic and butyric acid (Jackman, 1982). Volatile sulfur compounds, methylamine, dimethylamine, propanonic acid, butyric acid, indole, 3-methyl indole, and cadaverine are reported to cause oral malodor (Goldberg *et al.*, 1994; Goldberg *et al.*, 1997; Nakano *et al.*, 2002). Previous studies have not described clearly the contribution of different sources to the odor production and concentration in animal production facilities. Further studies are required.

Many authors have attempted to elucidate relationships between different odorous compounds or chemical odor groups and odor strength and offensiveness or have tried to find odor markers. Spoelstra (1980) recommended using p-cresol and VFA as indicators of odor offensiveness from animal production facilities; Williams and Evans (1981) suggested VFA, phenol, p-cresol and 3-methyl indole as the main odor markers, while Barth *et al*. (1974) reported VFA, NH_3 and H_2S as the main odor markers from animal production facilities.

Table 1. Compounds with low odor detection threshold in manure (O'Neill & Phillips, 1992)

Range of detection threshold (C_{od}; µg m^{-3})	Compound	Lowest detection threshold (C_{od}; µg m^{-3})*
$C_{od} \leq 0.01$	Methanethiol	0.0003
	2-propanethiol	0.0025
	2-propene-1-thiol	0.005
	2,3-butanedione	0.007
$0.01 \leq C_{od} \leq 0.05$	Phenylethanoic acid (Phenyl acetic acid)	0.03
	Ethanethiol	0.043
	4-methylphenol (p-cresol)	0.05
$0.05 \leq Cod \leq 0.1$	Hydrogen sulphide	0.1
	1-octene-3-one	0.1
$0.1 \leq C_{od} \leq 0.25$	Benzenethiol	0.14
	2,4-decadienal	0.18
	3-methylbutanoic acid	0.2
	2,6-dimethylphenol	0.2
	3-methylphenol	0.22
	2,4-nonadienal	0.25
	Dacanal	0.25
$0.25 \leq C_{od} \leq 0.5$	Trimethylamine	0.26
	Octanoic acid	0.3
	Nonanal	0.3
	Methylthiomethane	0.3
	Ethyldithioethan	0.3
	2-phenylethanol	0.35
	3-methylindole (skatole)	0.35
	Butyric acid	0.4
	2-methylphenol	0.4
	2-butene-1-thiol	0.43
	2-nonenal	0.5
$0.5 \leq C_{od} \leq 1.0$	Indole	0.6
	Petanoic acid	0.8
	Butanal	0.84

* Lowest odor detection threshold: The lowest concentration that has a 0.5 probability of being detected under the conditions of the test (CEN standard 13725, 2003)

According to Schaefer (1977) the primary malodor compounds from animal production facilities are associated with VFA, phenol, p-cresol, indole, and 3-methyl indole. Williams (1984) and Hobbs *et al*. (1997) produced a list of four major groups of odorants: VFA, indoles, phenols and sulfides. According to Curtis (1993), the odor groups are ammonia and volatile

amines, sulfurous compounds, VFA, indoles and phenols, alcohols and carbonyls. It would be very efficient in terms of odor reduction if a single compound or a group of compounds could be identified as an odor marker in a specific animal production system. However, the mentioned-above studies did not show very consistent results for odor markers. This inconsistency can be explained, because there are a great number of odorous compounds that are produced in different amounts under different circumstances. In addition, not only the individual odor concentration is important, but the way they interact with each other as well. Furthermore, different diets in different areas of the world might play an important role in the production of the different odorous compounds. Although the marker of odor differed between the mentioned-above studies, and one single odor marker can not be expected for all animal production systems, we can see that there are four general odor groups in animal production facilities: VFA, sulfurous compounds, indoles and phenols, and ammonia and volatile amines

VOLATILE FATTY ACIDS

Volatile fatty acids are commonly reported as being major constituents of odor from animal production facilities. About 60% of the total VFA in manure (w/w) are present as acetic acid. The next most dominant acids are propionic, butyric (n-butyric), 2-methylpropionic (*iso*-butyric), 3-methylbutyric *(iso*-valeric), pentanoic (n-valeric), and capric acids (McGill & Jackson, 1977; Cooper & Cornforth, 1978; Spoelstra, 1980). The odorous nature of VFA progresses from the pungent smell of acetic acid to the distinctly unpleasant and offensive smell of valeric and caproic acids (Morrison, 1987 cited by Zhu, 2000). VFA with high carbon numbers have a lower odor detection threshold (Mackie, 1994). A high concentration of VFA in pig manure may not cause very offensive malodor because a large proportion of VFA could be composed of short-chain VFA that are potentially less offensive.

The detection threshold, concentration and odor nature of some important VFA compounds are listed in Table 2; their chemical structures and their potential precursors are listed in Table 3. Although all the researchers used the technique of gas chromatography-mass spectrometry (GC-MS), it is surprising that concentrations of odorous compounds in general, and VFA in particular, vary so widely among different studies and among different kinds of samples. The variation is probably created by different sampling and measuring methods, different sources of samples, etc. The exact source of samples of odorous air compounds is very important, but in many reports it is unclear. In addition, the studies cited in Table 2 were published from 1975 to 1997 and therefore an important reason for the variation of the concentration of odorous compounds could be the changes that have taken place in the last 30

years in animal production systems (for example, in diet, animal breeds, and housing systems). Furthermore, the detection thresholds of odorous compounds also vary widely. This is probably due to the fact that in the past the measuring odor concentration was not standardized. So different protocols were used to determine odor detection threshold. The variation of odor detection threshold can be reduced by standardizing measuring methods.

SULFUROUS COMPOUNDS

Sulfur is present in numerous compounds at various states of oxidation. For example, sulfur has a +6 charge as sulfate anion, a +4 charge as gaseous sulfur dioxide and a sulfite anion, no charge as elemental sulfur, and a –2 charge as a sulfide anion. Several authors have reported that sulfurous compounds are important constituents of odor from livestock manure (Schaefer, 1980; Odam *et al.*, 1986; Ohta & Kuwada, 1998). The sulfur excreted in fresh manure is about 76 and 51g per 1000 kg animal mass per day for pig and dairy cattle, respectively (American Society of Agricultural Engineer, 1998). Sulfur excretion is quantitatively similar in feces and urine. When diets contain higher sulfur levels, the excretion ratio is shifted in favor of urine (Bouchard & Conrad, 1973). According to O'Neill and Phillips (1992) six of the ten compounds with the lowest odor detection threshold contain sulfur. In addition, Table 1 shows that the three compounds with the lowest odor detection threshold all contain sulfur. Furthermore, it has been shown that sulfurous compounds are the most offensive compounds. Table 2 shows that the odorous nature of sulfurous compounds progresses from the putrid smell of dimethyl disulfide and methanethiol to the rotten eggs smell of hydrogen sulfide.

Hydrogen sulfide is considered one of the most dangerous gases; it has been reported to be responsible for many animal and human deaths (Donham *et al.*, 1982 cited by Ji-Qin *et al.*, 2000). However, its concentration is usually low, unless the manure is agitated (Patni & Clarke, 1990). Schaefer *et al*. (1974) have reported that hydrogen sulfide in ventilation air has a concentration of about 4 $\mu g\ m^{-3}$. Hobbs *et al*. (1999) observed that the rate of hydrogen sulfide emission decreased from 100 to 28 $g\ m^{-2}\ d^{-1}$ during a 112 d study of stored pig manure. They also reported that there was no correlation between hydrogen sulfide concentration and odor concentration. Clanton & Schmidt (2001), however, found that the Pearson correlation coefficient between odor concentration and hydrogen sulfide concentration in the air from pig production facilities was 0.731; this is higher than that of 0.20 determined by Jacobson *et al.* (1997), also in air from pig production facilities.

Table 2. Nature of smell; detection threshold and concentration of important odorous compounds from pig production facilities

Groups	Odorous compounds	Nature of smell	Detection threshold ($\mu g\ m^{-3}$)	Authors	Concentration (μgm^{-3})	Source	Authors
Volatile fatty acids	Acetic (Ethanoic) acid	Pungent or vinegar	25-10000	Schaefer *et al.*(1974), Miner *et al.* (1975), Lunn & van de Vyver (1977), Phillips *et al.* (1979), Kowalewsky *et al.* (1980), Spoelstra (1980), Klarenbeek *et al.* (1982), Williams (1984), Yasuhara *et al.* (1984), Van Geelen & Van der Hoek (1985), Hammond *et al.* (1989) and Zahn *et al.* (1997)	0.0015-6700	Ventilation air	Schaefer *et al.* (1974), Miner *et al.* (1975), Kowalewsky *et al.* (1980) and Van Geelen & Van der Hoek (1985)
					1800-4700	Headspace air	Hobbs *et al.* (1997)
					1120-2690	Wet slurry	Hobbs *et al.* (1996)
					2-15.7*	Stored manure	Spoelstra (1979)
					270	Air at 1.5 m above manure basin	Zahn *et al.* (1997)
	Propionic (Propanoic) acid	Fecal	2.5-890	Schaefer *et al.*(1974), Miner *et al.* (1975), Lunn & van de Vyver (1977), Schaefer (1977), Phillips *et al.* (1979), Kowalewsky *et al.* (1980), Spoelstra (1980), Klarenbeek *et al.* (1982), Williams (1984), Yasuhara *et al.* (1984), Van Geelen & Van der Hoek (1985), Hammond *et al.* (1989) and Zahn *et al.* (1997)	0.002-1100	Ventilation air	Schaefer *et al.* (1974), Miner *et al.* (1975), Kowalewsky *et al.* (1980) and Van Geelen & Van der Hoek (1985)
					20-2500	Headspace air	Hobbs *et al.* (1997)
					148-400	Wet slurry	Hobbs *et al.* (1996)
					1.2-6.6*	Stored manure	Spoelstra (1979)
					130	Air at 1.5 m above manure basin	Zahn *et al.* (1997)
	Butyric (Butanoic) acid	Fecal or stench	0.25-42000	Schaefer *et al.*(1974), Miner *et al.* (1975), Lunn & van de Vyver (1977), Schaefer (1977), Phillips *et al.* (1979), Kowalewsky *et al.* (1980), Spoelstra (1980), Klarenbeek *et al.* (1982), Williams (1984), Yasuhara *et al.* (1984), Van Geelen & Van der Hoek (1985) and Hammond *et al.* (1989)	0.001-617	Ventilation air	Schaefer *et al.* (1974), Miner *et al.* (1975), Kowalewsky *et al.* (1980) and Van Geelen & Van der Hoek (1985)
					1100-4000	Headspace air	Hobbs *et al.* (1997)
					250-350	Wet slurry	Hobbs *et al.* (1996)
					0.4-3.1*	Stored manure	Spoelstra (1979)
					590	Air at 1.5 m above manure basin	Zahn *et al.* (1997)

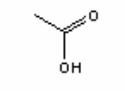
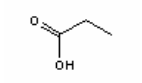
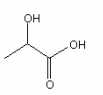
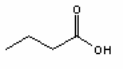
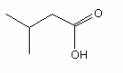
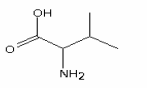
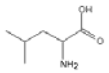
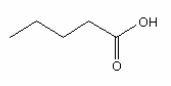
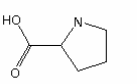
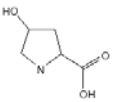
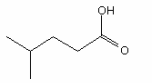
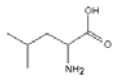
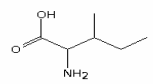
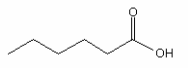
Groups	Odorous compounds	Nature of smell	Detection threshold ($\mu g\ m^{-3}$)	Authors	Concentration (μgm^{-3})	Source	Authors
	3-Methylbutyric acid	Fecal	0.017-6.9	Schaefer *et al.* (1974), Miner *et al.* (1975), Lunn & van de Vyver (1977), Spoelstra (1980) and Williams & Evans (1981)	0.0012-210	Ventilation air	Schaefer *et al.* (1974), Kowalewsky *et al.* (1980) and Van Geelen & Van der Hoek (1985)
					800-1100	Headspace air	Hobbs *et al.* (1997)
					50-200	Wet slurry	Hobbs *et al.* (1996)
					0.2-1*	Stored manure	Spoelstra (1979)
					98	Air at 1.5 m above manure basin	Zahn *et al.* (1997)
	Pentanoic (n-valeric) acid	Fecal	0.26-120	Schaefer *et al.* (1974), Miner *et al.* (1975), Lunn & van de Vyver (1977), Spoelstra (1980) and Williams & Evans (1981)	0.0012-80	Ventilation air	Schaefer *et al.* (1974), Kowalewsky *et al.* (1980) and Van Geelen & Van der Hoek (1985)
					200	Headspace air	Hobbs *et al.* (1997)
					70-90	Wet slurry	Hobbs *et al.* (1996)
					0.1-1*	Stored manure	Spoelstra (1979)
					360	Air at 1.5 m above manure basin	Zahn *et al.* (1997)
	4-Methyl pentanoic acid	-	37	Schaefer *et al.* (1974), Lunn & van de Vyver (1977), Spoelstra (1980) and Yasuhara *et al.* (1984)	0.001-160	Ventilation air	Schaefer *et al.* (1974), Kowalewsky *et al.* (1980) and Van Geelen & Van der Hoek (1985)
					0.2-1*	Stored manure	Spoelstra (1979)
	Hexanoic (n-caproic) acid	Pungent	20-520	Schaefer *et al.* (1974), Lunn & van de Vyver (1977), Spoelstra (1980), Yasuhara *et al.* (1984) and Zahn *et al.* (2001)	10	Ventilation air	Schaefer *et al.* (1974)
					110	Air at 1.5 m above manure basin	Zahn *et al.* (2001)
	Heptanoic (oenanthic) acid	Pungent	2.8-33	Schaefer *et al.* (1974), Miner *et al.* (1975), Lunn & van de Vyver (1977), Spoelstra (1980) and Zahn *et al.* (2001)	3	Ventilation air	Schaefer *et al.* (1974)
					8	Air at 1.5 m above manure basin	Zahn *et al.* (2001)
Ammonia and volatile amines	Ammonia	Sharp or pungent	27-37800	Schaefer *et al.*(1974), Miner *et al.* (1975), Lunn & van de Vyver (1977), Kowalewsky *et al.* (1980), Spoelstra (1980), Klarenbeek *et al.* (1982), Williams (1984) and Zahn *et al.* (1997)	100-18000	Ventilation air	Schaefer *et al.* (1974), Klarenbeek *et al.* (1982),
					3700	Air at 1.5 m above manure basin	Zahn *et al.* (2001)

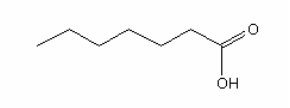
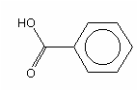
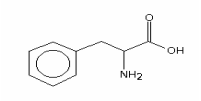
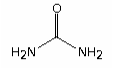
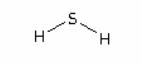
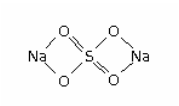
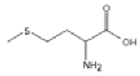
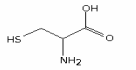
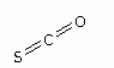
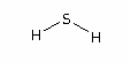
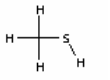
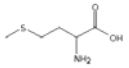
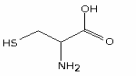
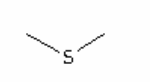
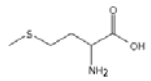
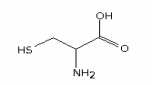
Groups	Odorous compounds	Nature of smell	Detection threshold ($\mu g\ m^{-3}$)	Authors	Concentration (μgm^{-3})	Source	Authors
Sulfurous compounds	Hydrogen sulfide	Rotten eggs	0.1-270	Schaefer *et al.* (1974), Banwart & Bremmer (1975), Lunn & van de Vyver (1977), Schaefer (1977), Phillips *et al.* (1979), Kowalewsky *et al.* (1980), Spoelstra (1980), Hammond *et al.* (1989) and Zahn *et al.* (1997)	4	Ventilation air	Schaefer *et al.* (1974)
					90	Air at 1.5 m above manure basin	Zahn *et al.* (2001)
	Carbonyl sulfide	-	250	Banwart & Bremmer (1975) and Spoelstra (1980)	-		
	Carbon disulfide	-	-		-		
	Methanethiol (Methyl mercaptan)	Garlic or putrid	0.0003-38	Banwart & Bremmer (1975), Lunn & van de Vyver (1977), Schaefer (1977) and Spoelstra (1980)	36000	Headspace air	Hobbs *et al.* (1997)
	Dimethyl sulfide	Stench	0.3-160	Banwart & Bremmer (1975), Lunn & van de Vyver (1977), Schaefer (1977) and Spoelstra (1980)	0.0022	Ventilation air	Miner *et al.* (1975)
					14000	Headspace air	Hobbs *et al.* (1997)
	Dimethyl disulfide	Putrid, decayed vegetable	1.1-610	Banwart & Bremmer (1975), Miner *et al.* (1975), Lunn & van de Vyver (1977), Spoelstra (1980) and Zahn *et al.* (2001)	12000	Headspace air	Hobbs *et al.* (1997)
					17	Air at 1.5 m above manure basin	Zahn *et al.* (2001)
	Dimethyl trisulfide	Nauseating	7.3	Miner *et al.* (1975), Lunn & van de Vyver (1977), Spoelstra (1980) and Yasuhara *et al.* (1984)	5000	Headspace air	Hobbs *et al.* (1997)
	Ethanethiol (Ethyl mercaptan)	-	0.043-0.33	Schaefer (1977), Spoelstra (1980) and Hammond *et al.* (1989)	-		
Indolic and phenolic compounds	Phenol	Aromatic	22-4000	Schaefer *et al.*(1974), Miner *et al.* (1975), Lunn & van de Vyver (1977), Schaefer (1977), Phillips *et al.* (1979), Kowalewsky *et al.* (1980), Spoelstra (1980), Klarenbeek *et al.* (1982), Williams (1984), Yasuhara *et al.* (1984), Van Geelen & Van der Hoek (1985), Hammond *et al.* (1989) and Zahn *et al.* (1997)	0.0025 –5	Ventilation air	Miner *et al.* (1975) and Schaefer *et al.* (1974)
					3700-4800	Headspace air	Hobbs *et al.* (1997)
					16-47	Wet slurry	Hobbs *et al.* (1996)
					0.007-0.055*	Stored manure	Spoelstra (1979)
					10-55	Stored manure	Hobbs *et al.* (1999)
					25	Air at 1.5 m above manure basin	Zahn *et al.* (2001)
	3-Methylphenol (m-cresol)	-	0.22-35	Spoelstra (1980)	4	Ventilation air	Kowalewsky *et al.* (1980)

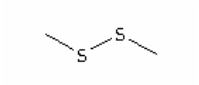
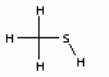
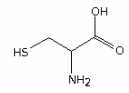
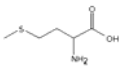
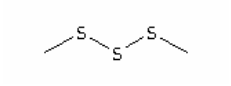
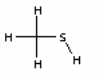
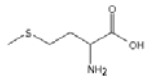
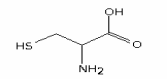
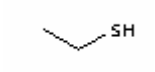
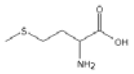
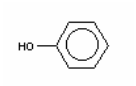
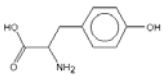
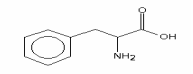
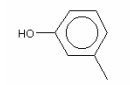
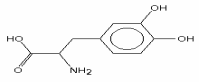
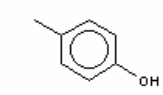
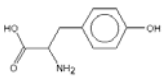
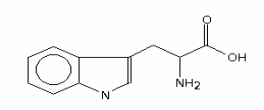
Groups	Odorous compounds	Nature of smell	Detection threshold ($\mu g\ m^{-3}$)	Authors	Concentration (μgm^{-3})	Source	Authors
	4-Methylphenol (p-cresol)	Fecal	0.05-24	Schaefer *et al.*(1974), Miner *et al.* (1975), Lunn & van de Vyver (1977), Schaefer (1977), Phillips *et al.* (1979), Klarenbeek *et al.* (1982), Williams (1984), Yasuhara *et al.* (1984), Hammond *et al.* (1989) and Zahn *et al.* (1997)	4600-7000	Headspace air	Hobbs *et al.* (1997)
					30-60	Wet slurry	Hobbs *et al.* (1996)
					0.14-0.34*	Stored manure	Spoelstra (1979)
					10-55	Stored manure	Hobbs *et al.* (1999)
					90	Air at 1.5 m above manure basin	Zahn *et al.* (2001)
	4-Ethylphenol	Pungent	3.5-10	Zahn *et al.* (1997)	500-4900	Headspace air	Hobbs *et al.* (1997)
					0.3-6.4	Wet slurry	Hobbs *et al.* (1996)
					0.006-0.072*	Stored manure	Spoelstra (1979)
					4	Air at 1.5 m above manure basin	Zahn *et al.* (2001)
	Indole	Fecal/Stench	0.0.6-7.1	Schaefer *et al.* (1974), Spoelstra (1977), Lunn & van de Vyver (1977), Schaefer (1977) and Spoelstra (1980)	3	Ventilation air	Schaefer *et al.*(1974)
					100-500	Headspace air	Hobbs *et al.* (1997)
					4-9.8	Wet slurry	Hobbs *et al.* (1996)
					0-0.001*	Stored manure	Spoelstra (1979)
					2	Air at 1.5 m above manure basin	Zahn *et al.* (2001)
	3-Methyl indole (skatole)	Fecal Nauseating	0.0005-6.4	Schaefer *et al.* (1974), Spoelstra (1977), Lunn & van de Vyver (1977), Schaefer (1977), Spoelstra (1980), Williams & Evans (1981), Williams (1984), Yasuhara *et al.* (1984) and Zahn *et al.* (1997)	3	Ventilation air	Schaefer *et al.*(1974)
					100-400	Headspace air	Hobbs *et al.* (1997)
					1.7-3.6	Wet slurry	Hobbs *et al.* (1996)
					0.009-0.054*	Stored manure	Spoelstra (1979)
					2	Air at 1.5 m above manure basin	Zahn *et al.* (2001)

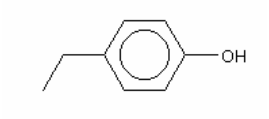
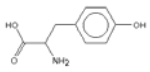
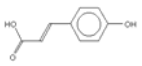
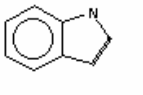
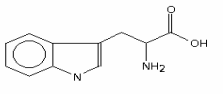
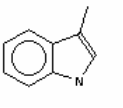
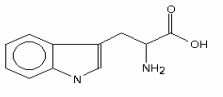
*: g/kg wet weight

Table 3. Origin of odorous compounds

Groups	Odorous compounds	Main origin	Authors
Volatile fatty acids	Acetic (ethanoic) acid 	Dietary fibre, L-glycine, L-alanine, L-cysteine, L-lysine, L-serine, L-threonine, L-hydroxyproline, L-aspartate, L-glutamate, L-histidine	Nisman (1954), Stadtman (1963), Loesche and Gibbons (1968), Elsden and Hilton (1978), Turton, *et al.* (1983), Mortensen *et al.* (1987), Rasmussen *et al.* (1988), Stryer (1995), Sutton *et al.* (1999)
	Propionic (Propanoic) acid 	Dietary fibre, Lactate  L-Alanine, L-threonine, L-alanine + L-threonine, L-aspartate, L-methionine	Nisman (1954), Loesche and Gibbons (1968), Elsden and Hilton (1978), Schlegel (1986), Rasmussen *et al.* (1988), Sutton *et al.* (1999)
	Butyric (Butanoic) acid 	Dietary fibre, L-cysteine, L-hydroxyproline, L-lysine, L-serine, L-threonine, L-aspartate, L-glutamate, L-histidine	Loesche and Gibbons (1968), Elsden and Hilton (1978), Turton *et al.* (1983), Mortensen *et al.* (1987), Rasmussen *et al.* (1988), Hammond *et al.* (1989), Sutton *et al.* (1999)
	3-Methylbutyric acid 	Fibre L-Valine  L-Leucine 	Elsden and Hilton (1978), Britz and Wilkinson (1983), Rasmussen *et al.* (1988), Sutton *et al.* (1999)
	Pentanoic (n-valeric) acid 	Fibre L-Proline  L-Hydroxyproline 	Rasmussen *et al.* (1988), Sutton *et al.* (1999)
	4-Methyl pentanoic acid 	L-Leucine  L-Isoleucine 	Nisman (1954), Elsden and Hilton (1978), Rasmussen *et al.* (1988)
	Hexanoic (n-caproic) acid 	Ethanol, acetate, CO_2	Smith *et al.* (1985), Kenealy *et al.* (1995)

Groups	Odorous compounds	Main origin	Authors
	Heptanoic (enanthic) acid 	Benzoic acid 	Bisaillon *et al.* (1994), Schneider *et al.* (1997), Gummalla and Broadbent (2001)
		L-Phenylalanine 	
Ammonia and volatile amines	Ammonia 	Urea 	Wozny (1977), Suzuki *et al.* (1979), Aarnink *et al.* (1996), Canh *et al.* (1998)
		Deamination of amino acids	
Sulfurous compounds	Hydrogen sulfide 	Sulfate 	Ohkishi *et al.* (1981), Schlegel (1986), Claesson *et al.* (1990), Sutton *et al.* (1999)
		L-Methionine 	
		L-Cysteine 	
	Carbonyl sulfide 	Hydrogen sulfide 	Ren (1999)
	Carbon disulfide	Carbonyl sulfide 	Banwart and Bremmer (1975), Ren (1999)
	Methanethiol (methyl mercaptan) 	L-Methionine 	Segal and Starkey (1969), Kreis and Hession (1973), Ferchichi *et al.* (1985), Inoue *et al.* (1995), Hori *et al.* (1996), Mackie *et al.* (1998), Sutton *et al.* (1999), Yoshimura (2000)
		L-Cysteine 	
	Dimethyl sulfide 	L-Methionine 	Kadota and Ishida (1972), Kelly *et al.* (1994), Sutton *et al.* (1999)
		L-Cysteine 	

Groups	Odorous compounds	Main origin	Authors
	Dimethyl disulfide 	Methanethiol 	Segal and Starkey (1969), Chin and Lindsay (1994), Sutton *et al.* (1999)
		L-Cysteine 	
		L-Methionine 	
	Dimethyl trisulfide 	Methanethiol 	Segal and Starkey (1969), Chin and Lindsay (1994), Sutton *et al.* (1999)
		L-Methionine 	
		L-Cysteine 	
	Ethanethiol (Ethyl mercaptan) 	L-Methionine 	Akobe (1936)
Indolic and phenolic compounds	Phenol 	L-Tyrosine 	Ichihara *et al.* (1956), Brot *et al.* (1965), Bakke (1969), Hammond *et al.* (1989), Sutton *et al.* (1999)
		L-Phenylalanine 	
	3-Methylphenol (m-cresol) 	DOPA 	Drasar and Hill (1974)
	4-Methylphenol (p-cresol) 	L-Tyrosine 	Bakke (1969), Hammond *et al.* (1989), Hengemuhle and Yokoyama (1990), Sutton *et al.* (1999)
		L-Tryptophan 	

Groups	Odorous compounds	Main origin	Authors
	4-Ethylphenol 	L-Tyrosine  p-Coumaric acid 	Drasar and Hill (1974), Spoelstra (1977), Hammond, *et al.* (1989), Hengemuhle and Yokoyama (1990)
	Indole 	L-Tryptophan 	DeMoss R and Moser (1969), Drasar and Hill (1974), Elsden *et al.* (1976), Hammond *et al.* (1989), Sutton *et al.* (1999)
	3-Methyl indole (skatole) 	L-Tryptophan 	Drasar and Hill (1974), Yokoyama and Carlson (1974), Chung *et al.* (1975), Elsden *et al.* (1976), Hammond *et al.* (1989), Hengemuhle and Yokoyama (1990), Honeyfield and Carlson (1990), Jensen and Jørgensen (1994), Sutton *et al.* (1999)

There are several possible reasons for this inconsistency. Sampling and measuring methods, on basic of which odor and hydrogen sulfide concentration were measured, might differ between these studies. The air sample might be taken from different animal types, from different days, from different farms and the animal might be fed different diets. In addition, hydrogen sulfide production and emission seems to be very much influenced by housing system and manure management, for example, regularly flushing of manure or storing the manure for a long time in a manure pit might give larger differences in hydrogen sulfide production and emission.

Hydrogen sulfide and methanethiol (methylmercaptan) are the most commonly reported sulfurous compounds causing odor offensiveness in pig manure (Spoelstra, 1980). According to Bremmer (1975), hydrogen sulfide and methanethiol represented 70 to 97% of the total sulfur volatilized in manure. They also reported that for pigs and poultry, the amount of methanethiol produced exceeded the amount of hydrogen sulfide produced. Beard & Guenzi (1983) stated that most of the sulfur emanated in the form of hydrogen sulfide (39%), methanethiol (34%) and dimethyl sulfide (21%). According to Hobbs *et al*. (1997) the methanethiol concentration in the headspace air is about 36000 $\mu g/m^{-3}$. It is from 947 to 120 $x10^6$ times higher than the detection threshold (Table 2). Therefore, methanethiol may be a very important compound causing odor nuisance.

Apart from hydrogen sulfide and methanethiol, the other sulfurous compounds identified in air from pig production facilities include carbon disulfide, 2-propanethiol, dimethyldisulfide,

dimethyltrisulfide, 2-methylthiopropane, methaethiocyclopentane, 1-methylthiopentane, dimethyltetrasulfide and dimethylhexasulfide (Odam *et al.*, 1986).

The detection threshold, concentration and odor nature of some important sulfurous compounds are listed in Table 2; their chemical structures and their precursors are listed in Table 3. Like VFA, they vary widely among studies and kinds of samples. In general, the concentrations of sulfurous compounds in the air are higher than the concentrations of VFA. In addition, their detection thresholds are lower than VFA. Furthermore, the nature of smell of sulfurous compounds seems to be more offensive. As a result, sulfurous compounds may cause much more odor nuisance than VFA.

PHENOLES AND INDOLES

Phenol, p-cresol, 3-methyl phenol (m-cresol), and 4-ethylphenol are important representatives of phenolic compounds, whereas indole and 3-methyl indole are indolic compounds. These two kinds of compounds are considered as the main compounds responsible for the smell in the ventilation air of pig houses (Schaefer, 1977; Williams & Evans, 1981; O'Neill & Phillips, 1992). The nature of the smell of indole and phenol compounds progresses from the aromatic smell of phenol to the stench of indole and the nauseating smell of 3-methyl indole. Schaefer *et al*. (1974) quoted by O'Neill & Phillips (1992) synthesized the smell of pig manure, in which phenolic compounds were represented in high concentrations (v/v): p-cresol (64%), phenol (26%). Other compounds, for example, n-butyric acid, skatole, and indole were present in lower concentrations. Williams and Evans (1981) reported an increase in concentrations of phenol, p-cresol and 3-methyl indole, and a decrease in the concentration of indole during the accumulation of pig manure in a store. Spoelstra (1980) indicated that the phenol concentration increased during the 150 d measuring period, while indole, p-cresol and 3-methyl indole concentrations increased initially but decreased after 40, 65 and 70 d, respectively.

Despite the great variation among studies, it can be seen from Table 2 that the concentration of p-cresol in headspace air ranges from 4600 to 7000 $\mu g\ m^{-3}$. The concentration of p-cresol in ventilation air, wet slurry and stored manure is higher than that of the other phenol and indole compounds listed in Table 2. In addition, it also has a lower odor detection threshold than the other compounds. Therefore, it seems safe to conclude that p-cresol is an important compound in terms of odor nuisance compared to other indolic and phenolic compounds. The next most important compounds might be indole and 3-methyl indole. Although phenol has a rather high concentration in headspace air (3700-4800 $\mu g\ m^{-3}$) it has a

high detection threshold (22-4000 μg m^{-3}); in addition, the smell of phenol is aromatic, thus phenol may not contribute to odor nuisance in contrast to other indolic and phenolic compounds.

AMMONIA AND VOLATILE AMINES

Ammonia has a sharp and pungent smell. The main source of ammonia is urea (Spoelstra, 1980). The ammonia concentration in air samples taken from animal houses, manure tanks and fields spread with manure has been found to correlate well with odor intensity (r^2 = 0.72) as measured by olfactometry (Kowalewsky *et al.*, 1980). Schulte (1985) and Miner (1995) found a high correlation between ammonia and odor emission from livestock facilities. However, Liu *et al*. (1993), Oldenburg (1989), Verdoes and Ogink (1997), and Williams (1984) found only a low correlation between ammonia and odor emission from pig houses. According to Oldenburg (1989), ammonia does not seem to be an important odorous compound. He also reported that mean ammonia concentrations were below 8 ppm in cattle barns, between 5 and 18 ppm in pig houses and between 5 and 30 ppm in poultry houses. Studies in the USA suggest that if ammonia levels exceed 7 ppm, workers may suffer clinical effects (Donham *et al.*, 1989). Wathes *et al*. (2002) reported that weaner pigs, broiler chickens and adult laying hens were significantly averse to ammonia at concentrations of 20 ppm and higher.

The volatile amines from animal production facilities may include methylamine (putrid smell), ethylamine (fishy smell), trimethylamine (ammoniac-like smell), cadaverine (foul smell), and putrescine (smell of putrefaction). Volatile amines make up a very small part of the volatile nitrogenous compounds. Concentrations of volatile amines from animal production facilities were rarely found in literature.

RÉSUMÉ

A great number of odorous compounds have been identified in animal production facilities. However, the relative contribution of the different sources (for example, animals, feed, feces, urine, and manure) to the formation of odorous compounds has not yet to be determined. In order to be able to propose solutions for odor abatement, it is important to clearly identify the different sources of odorous compounds. Sulfurous compounds, indoles and phenols, and VFA are probably important groups of odorous compounds from animal production facilities. The huge variation among studies in the odor concentration and odor detection threshold of odor compounds largely responsible for odor nuisance (Table 2) might be attributable to the fact that the determined odor concentration is related to many factors (for

example, dietary composition, environmental factors, measuring methods and standards, sources of sample). In addition, the relative importance of different compounds causing odor nuisance has seldom been described. In order to propose feasible and efficient solutions for odor reduction it is important to accurately identify the concentration, detection threshold and main source of each odorous compound, and the relative importance of different odorous compounds from animal production facilities. This requires further studies.

PRODUCTION OF ODOROUS COMPOUNDS FROM ANIMAL PRODUCTION FACILITIES, AND THE BACTERIAL REACTIONS INVOLVED

When feed passes through the digestive tract, food nutrients are hydrolyzed into smaller molecular structures that can be absorbed and used for the growth and development of the animal. The non-utilized nutrients and endogenous compounds in the gastrointestinal tract are excreted via urine and feces. The biological degradation process performed by micro-organisms, which starts in the intestine under anaerobic conditions, continues after excretion. This anaerobic microbial degradation process has been represented in Fig. 2. Different groups of odorous compounds are produced during anaerobic degradation. Most groups are produced from different precursors in different ways, which may in turn interact with the production of others.

VOLATILE FATTY ACIDS

Volatile fatty acids are mainly formed by microbial conversions of plant fiber and protein residues in the large intestine and in manure under anaerobic conditions. During fermentation, energy is obtained from organic compounds that serve as electron donor and acceptor, replacing oxygen in the latter function.

Dietary fiber residues may include cellulose, hemicellulose and lignin. Lignin is very difficult to degrade under anaerobic conditions. Cellulose and hemicellulose are first hydrolyzed by microbial enzymes into oligomers and/or monomers. The latter are subsequently converted by the microbes into VFA such as acetic, propionic and butyric acids. The proportion of acids produced can vary, depending on the type of substrate available, the composition of the anaerobic flora and the prevailing pH. Van Soest (1983) described different pathways of carbohydrate metabolism in general and of dietary fiber in particular in the rumen of cattle (Fig. 3). The same pathways of carbohydrate metabolism are assumed in the large intestine of mono-gastric animals, although the amount and ratio of end products may differ.

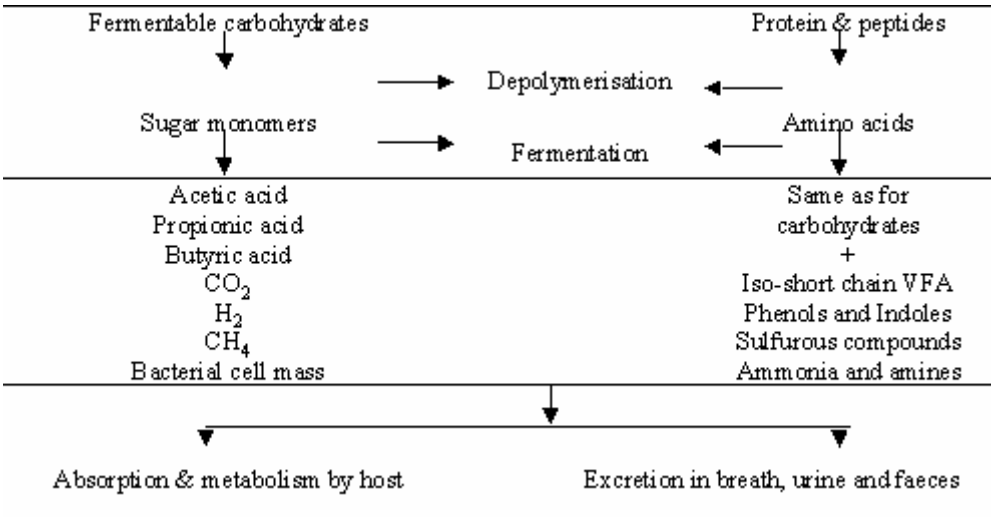


Fig. 2. Major fermentation products formed by the microbiota in the gastrointestinal tract of pigs. VFA, volatile fatty acids (adapted from Jensen & Jørgensen, 1994)

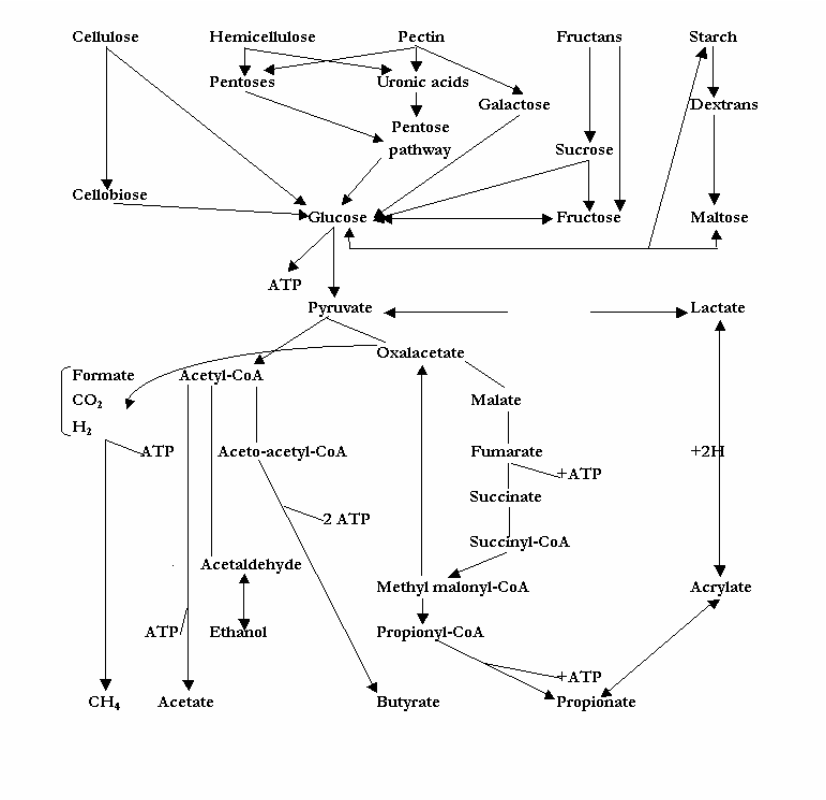


Fig.3. Pathways of carbohydrate metabolism in the rumen (van Soest, 1983)

Apart from being formed from carbohydrates, acetic, propionic and butyric acids are also produced by deamination of amino acids such as L-glutamate, L-lysin, L-alanine. (Tables 4 and 5). Ammonia, CO_2 and [H] are additional end-products of this deamination-decarboxylation. The general mechanism of a deamination-decarboxylation is presented in equation 1.

$$R - \underset{\displaystyle NH_2}{\underset{|}{CH}} - COOH + 2H_2O \rightarrow R - COOH + NH_3 + 4[H] + CO_2 \quad [1]$$

According to Mortensen *et al*. (1987) and Rasmussen *et al*. (1988), carbohydrates are easily converted into acetic acid, propionic acid, and butyric acid in fecal incubation systems, but this has never resulted in the production of branched-chain VFA such as *iso*-valeric acid, *iso*-butyric acid. The latter VFA originate from the breakdown of peptides. Peptolytic bacteria hydrolyze proteins into amino acids. The latter are then deaminated and decarboxylated to branched-chain VFA. Examples are given in equations (2), (3) and (4).

Valine + $2H_2O \rightarrow$ *Iso*-butyric acid+NH_3 + CO_2 [2]

Leucine + $2H_2O \rightarrow$ *Iso*-valeric acid +NH_3 + CO_2 [3]

Iso-leucine + $2H_2O \rightarrow$ 2-methylbutyric acid +NH_3 + CO_2 [4]

Table 4. Deamination reactions by anaerobic bacteria in the gastrointestinal tract and manure (adapted from Mackie *et al.*, 1998)

Amino acid	Corresponding volatile fatty acids produced
Alanine, glycine, serine	Acetic acid
Threonine	Propionic acid
Glutamate, aspartate	Acetic, propionic acid
Valine	*Iso*-Butyric acid
Leucine	*Iso*-Pentanoic acid
Iso-leucine	2-Methylbutyric acid
Phenylalanine	Phenylacetic acid
Tyrosine	*p*-Hydroxylphenylacetic acid
Tryptophan	Indoleacetic acid→3-methyl indole
Tyrosine	Phenylacetic acid, phenylpropionic acid

In the gastrointestinal tract of pigs, micro-organisms can synthesize short-chain VFA (fatty acids with chain lengths of two to six carbon atoms) from unabsorbed nutrients (Giusi-Perier *et al.*, 1989). According to Müller & Kirchgessner (1985) and Engehard (1995), 66 to 99% of the short-chain VFA produced in the large intestine can be absorbed and used as an energy source for the host animal. In addition, short-chain VFA have a high odor detection threshold. Therefore, short-chain VFA produced in the large intestine of animals are probably not a major concern in terms of odor nuisance.

Briefly, VFA are produced from proteins and carbohydrates under anaerobic conditions in the large intestine of animals and in manure storage. Carbohydrates are transformed to straight-

chain VFA only. Proteins are transformed to both straight-chain VFA and branched-chain VFA. Short-chain VFA in the large intestine can be used as an energy source for the host animal and thus are probably not a big problem in terms of odor nuisance. However, when they are in manure storages, VFA may be volatilized and cause malodor.

SULFUROUS COMPOUNDS

There are two main ways of sulfide production: sulfate reduction and the metabolism of sulfurous AA.

Metabolism of sulfurous amino acids. When manure is stored anaerobically, organic sulfurous compounds such as the AA methionine, cysteine and cystine are broken down to release sulfurous compounds. Various anaerobic bacteria perform this process, in which sulfurous AA are used as carbon and energy sources by the microbes. Some intermediates are produced that can volatilize and create odor. An example is the hydrolization of methionine, from which methanethiol (methyl mercaptan) is formed, which can be further degraded to sulfide (American Society of Agricultural Engineers, 1989), equations (5) and (6).

$$CH_3S(CH_2)_2CHNH_2COOH + H_2O \rightarrow CH_3SH \text{ (methanethiol)} + NH_3 + CH_3CH_2COCOOH \quad [5]$$

$$CH_3SH + H_2O \rightarrow CH_3OH + H_2S \quad [6]$$

Methanethiol as a product of L-methionine degradation can be chemically converted to dimethyl disulfide and dimethyl trisulfide in the presence of Cu(II) or ascorbate plus Fe(III), for example (Parliment *et al.*, 1982; Chin & Lindsay, 1994; Bonnarme *et al.*, 2001).

Sulfate reduction. The other main source of sulfide formation is sulfate. In urine, sulfate is the primary form of sulfur excreted. Spoelstra (1980) stated that the primary origin of sulfide in manure is the reduction of sulfate into sulfide. Sulfate reduction proceeds via assimilatory or dissimilatory pathways. In the assimilatory process, bacteria produce enough reduced sulfur for the biosynthesis of cysteine and methionine. This is in contrast to the dissimilatory process, in which sulfate is used as electron acceptor for an anaerobic respiration comparable to the aerobic respiration with oxygen. During respiration with sulfate, copious amounts of malodor are generated. This process has been characterized by Clanton and Schmidt (2001) and Sawyer and McCarty (1978): equation (7). The bacteria that are sulfate-reducers belong to the genera *Desulfovibrio*, *Desulfotomaculum*, *Desulfobacter*, *Desulfococcus*, and *Desulfonema* (Schlegel, 1986).

$$SO_4^{2-} + \text{organic matter} \xrightarrow[bacteria]{anaerobic} S^{2-} + H_2O + CO_2 \quad [7]$$

Hydrogen sulfide might be transformed to carbonyl sulfide and carbon disulfide (Ren, 1999), although these respective reactions have not been described for gut bacteria.

$$H_2S + CO_2 \rightarrow COS + H_2O \quad [8]$$

$$COS + H_2S \rightarrow CS_2 + H_2O \quad [9]$$

According to Spoelstra (1980), sulfate-reducing bacteria also produce trace amounts of COS, CS_2, and methyl, ethyl and propyl mercaptans.

Briefly, sulfurous compounds are produced under anaerobic conditions from two main sources: sulfate in the urine and proteins or amino acids containing sulfur in manure. Various bacteria are involved in the production process.

INDOLES AND PHENOLS

Phenolic compounds e.g. phenol itself, p-cresol and 4-ethylphenol originate from the microbial degradation of L-tyrosine in the intestinal tract of animals and in manure storage (Fig. 4).

L-tyrosine can be deaminated to 4-hydroxy-phenylpropionic acid, which is either decarboxylated to 4-ethylphenol, or oxidized to 4-hydroxyphenylacetic acid. 4-Hydroxyphenylacetic acid is then either decarboxylated to p-cresol or further oxidized to 4-hydroxybenzoic acid. The latter is decarboxylated to phenol (Drasar & Hill, 1974). L-Tyrosine can also be split directly to release ammonia, phenol, and pyruvic acid by *Clostridium tetanomorphum* (Brot *et al.*, 1965) and *E. coli* ("*B. coli* phenologenes"; (Ichihara *et al.*, 1956).

Hammond *et al*. (1989) observed that p-cresol was formed from L-tyrosine and L-tryptophan when bacteria from pig manure were incubated with these amino acids in a synthetic medium. Hengemuehle and Yokoyama (1990) isolated an anaerobic Gram-positive bacterium from the caecal contents of weaning pigs, which produced p-cresol by decarboxylation of 4-hydroxyphenylacetic acid as described in Fig. 4.

Drasar and Hill *et al*. (1974) reported that 3-methylphenol (m-cresol) is one of the metabolites of the degradation of 3,4-dihydroxyphenylalanine (DOPA). DOPA is the precursor of neurotransmitters such as dopamine, norepinephrine, and epinephrine; it is produced by oxidation of L-tyrosine by the O_2-dependent enzyme monophenol monooxygenase (Dorland, 2003). DOPA is an amino acid, but is not in the group of 20 amino acids that are the building blocks of protein. Because only very small amounts of DOPA are expected to be available to intestinal bacteria, the reaction mentioned above cannot generate much 3-methylcresol.

Phenolic compounds are absorbed in the large intestine by the host animal and detoxicated

in the liver by conjugation with glucuronic acid, resulting in glucuronides, or sulfuric acid, resulting in sulfates (Smith & Williams, 1966). However, the sulfate conjugation is of minor importance in pigs (Capel *et al.*, 1974). In manure, urinary glucuronides are hydrolyzed by fecal β-glucuronidase to release phenolic compounds, again as given in Fig. 4.

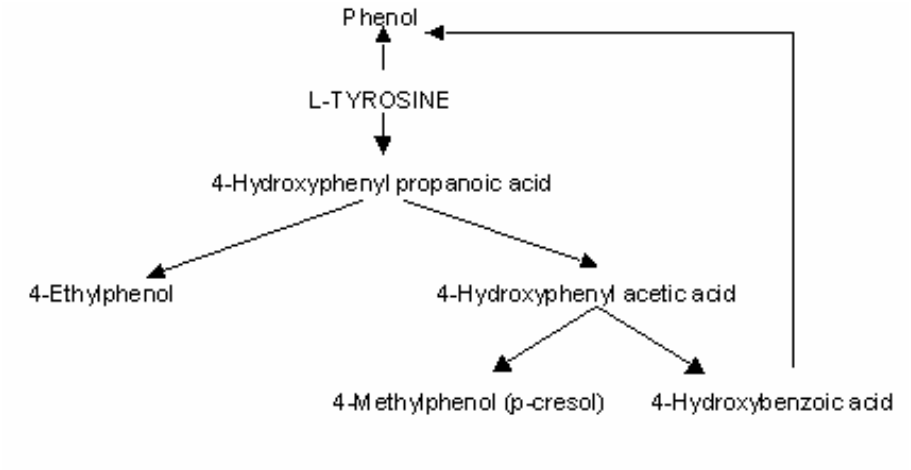


Fig. 4. Breakdown of L-tyrosine in manure stored anaerobically

Indole production is shown in Fig. 5. Indole and skatole are produced in the large intestine of animals and in manure by microbial fermentation of L-tryptophan. Indoles are partly absorbed and detoxicated by the liver to glucuronides, for example, 3-hydroxyindole, hydroxyskatoles and indole-3-carboxylic acid. Then, indolic detoxication products are excreted via the urine. The unabsorbed part of indole and skatole is excreted via feces. Therefore, indole and skatole can be found in fresh feces. Feces contain a high level of β- glucuronidase of bacterial origin. This enzyme hydrolyses glucuronides. Therefore, it is expected that mixing feces with urine causes the amounts of free indolic compounds to rise.

The ability to form indole from tryptophan is a taxonomic feature to distinguish between different enterobacteria. The following bacteria are able to form indole from tryptophane: *E. coli* and *Proteus* (except *Proteus mirabilis*), some *Shigella*, *Aeromonas liquefaciens*, some *Fusobacterium* species, *Bacteroides melaninogenicus,* some *Bacteroides fragilis* subspecies, *Bacteroides coagulans*, *Paracolobactrum coliforme*, *Photobacterium harveyi*, *Bacillus alvei*, some clostridia, *Propionibacterium acnes*, and *Micrococcus aerogenes* .

Tryptophan is converted to indole-3-acetic acid by *E. coli*, *Citrobacter* sp., *Bacteroides fragilis* subsp. *thetaiotamicron*, and *Clostridium* (Chung *et al.*, 1975; Elsden *et al.*, 1976). This conversion occurs by transamination of tryptophan to indolepyruvic acid and subsequent decarboxylation (Chung *et al.*, 1975). *Lactobacillus* strain 11201 and three unidentified isolates from the pig intestine have been shown to be able to degrade indole-3-acetic acid to skatole

(Yokoyama & Carlson, 1974; Yokoyama *et al.*, 1977; Hengemuehle & Yokoyama, 1990; Honeyfield & Carlson, 1990). *Clostridium scatologenes* DSM 757 is capable of generating 3-methyl indole directly from L-tryptophan (Mikkelsen & Jensen, 1996).

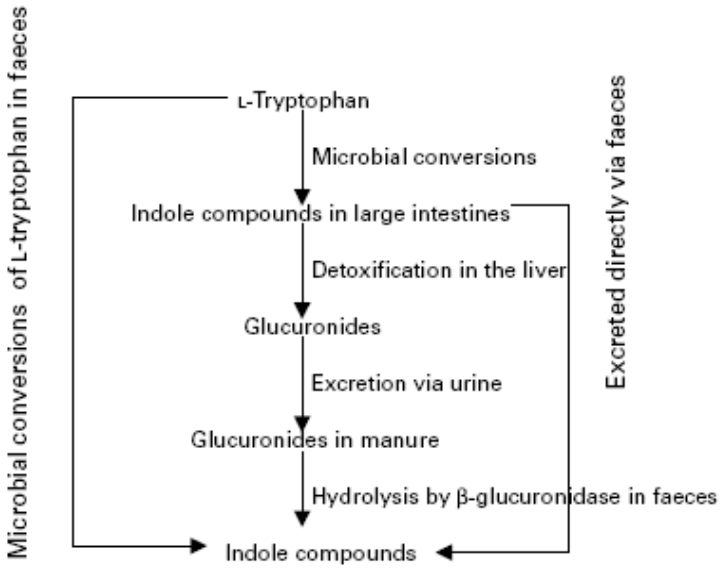


Fig. 5. The production of indole compounds from L- trytophan

From in vitro experiments, Mogens *et al*. (1995) found that the production of indole and skatole is a pH-dependent process: the highest rate of production was observed between pH 6.0 and 7.0, and less than half of the maximum activity was observed at pH 5.0 or 8.0. The pH had dramatic effects on the relative production of indole and 3-methyl indole from tryptophan. High pH values favored the production of indole, while low pH values favored the production of 3-methyl indole.

Briefly, phenol and p-cresol are produced from L-tyrosine; indole and 3-methyl indole are produced from L-tryptophan. There are three sources of indole and phenol compounds in manure:

Degradation of the AA L-tryptophan and L-tyrosin in manure;

Direct excretion from the large intestine of animals via feces after being formed from Tryptophan and Tyrosine;

Released from glucuronides in urine when placed in contact via feces.

AMMONIA AND VOLATILE AMINES

Ammonia and volatile amines are the main nitrogenous compounds produced during manure storage. When proteins and AA are used as an energy source, their deamination releases ammonia. In manure, Lehninger (1975) cited by Hobbs *et al*. (1999), found an enzymatic

gateway used by bacteria to convert AA to L-glutamate and then oxidatively deaminate them into ammonia and the respective fatty acids or residual structures. However, the main source of ammonia is urea (Spoelstra, 1980; Aarnink *et al.*, 1993). Ammonia present in manure largely arises from the breakdown of urea. Urea is formed in the liver as the end-product of the protein-destroying metabolism of the animal and is excreted by the kidneys. Urea is quickly hydrolyzed by urease present in feces and fouled floors and converted into ammonium ions. Urease activity is ubiquitous among intestinal bacteria; it has been observed in strains of many species such as *Bacteroides multiacidus*, *Bacteroides ruminicola*, *Bifidobacterium bifidum*, etc. (Varel *et al.*, 1974; Wozny *et al.*, 1977; Suzuki *et al.*, 1979). Some of the ammonium ions will dissociate to form free ammonia. Ammonia emission into the air is a slow process, controlled by factors such as ammonia concentration, pH and temperature (Aarnink, 1997).

$$CO(NH_2)_2 + 3H_2O \xrightarrow{urease} 2NH_4^+ + 2OH^- + CO_2 \Leftrightarrow 2NH_3{}^{\uparrow} + 2H_2O + CO_2 \qquad [10]$$

In manure, ammonia is in equilibrium with ammonium. The rate of ammonia emission depends on this equilibrium. The pH is one of the most important factors influencing ammonia emission. Ammonia volatilization increases with increasing manure pH (Stevens *et al.*, 1989; Sommer & Husted, 1995; Aarnink, 1997). At a solution pH of 9.24, ammonia occurs equally in the form of NH_4^+ and NH_3(aq). Below a pH of 7, ammonia is almost exclusively present as NH_4^+, thereby reducing volatilization as ammonia gas.

Under anaerobic conditions, volatile amines are often produced from protein-containing products. There are three possible mechanisms of microbial formation of volatile amines.

First, under certain conditions in the gastrointestinal tract and most likely during storage of fresh manure, amino acids undergo decarboxylation (Table 5). This mechanism was proposed by Bast *et al*. (1971) cited by Spoelstra (1980). Bacterial genera with decarboxylase activity include *Bacteroides, Bifidobacterium, Selenomonas, Streptococcus* and the enterobacteria.

Second, Bast (1971) cited by Spoelstra (1980) obtained experimental indication that the formation of hexylamine and ethylamine by *Sarcina* lutea, hexylamine by *Escherichia coli,* and *iso*-butylamine by *Aerobacter aerogenes* came about by amination of the corresponding aldehydes.

Third, another source of amines in manure is urine. For example, the daily excretion of dimethylamine is estimated at 20 mg in humans, of which around 50% originates from choline by the activity of gut flora. Choline is degraded to either ethylamine plus ethanolamine or to trimethylamine which is easily demethylated (Drasar and Hill, 1974).

Table 5. Decarboxylation reactions by anaerobic bacteria in the gastrointestinal tract and manure (Mackie *et al.*, 1998)

Amino acid	Corresponding amine produced
Glycine	Methylamine
Alanine	Ethylamine
α-Aminobutyrate	Propylamine
Orithine	Putrescine → pyrolidine
Arginine	Putrescine → pyrolidine
Norvaline	Butylamine
Lysine	Cadaverine→ pyrolidine
Histidine	Histamine
Tyrosine	Tyramine
Tryptophan	Tryptamine
Phenyl amine	Phenyl ethylamine

Briefly, ammonia is produced from deamination of AA when they are used as energy sources by bacteria, and by hydrolysation of urea in urine when it comes into contact with urease. Urea is the main source of ammonia from animal production facilities. Volatile amines are produced from AA by decarboxylation. In addition, they can be produced by amination of aldehydes and by demethylation of choline.

RÉSUMÉ

Microbial activities are responsible for odor generation in the large intestine of the animal and in manure storage. Odorous compounds are the intermediate or end products of microbial conversions under anaerobic conditions. The precursors of odorous compounds are non-utilized nutrients from the diet and endogenous products. Proteins and fermentable carbohydrates are the most important precursors of odorous compounds. Table 3 summarizes different odorous compounds and their precursors. The odorous compounds included in this table are thought to be the main causes of odor nuisance from pig production facilities.

MEASUREMENTS OF ODOR

Odor is the property of a chemical compound or mixture of compounds which, above a certain concentration activate the sense of smell and thus initiate an odor sensation (Winneke, 1994). A substance can create an odor impression if it meets certain preconditions e.g. volatility, water solubility, fat solubility and polarity.

Odor can be characterized in three different ways:

By sensory evaluation;

By chemical evaluation;

By electronic sensor evaluation.

The sensory perception of odor can be characterized by three major parameters:

Concentration;

Intensity;

Hedonic tone.

OLFACTOMETRY

The three sensory parameters of odor mentioned above are measured by olfactometry. Olfactometry is based on the use of human panels and an olfactometer, which is in essence a dilution device. The principle of the olfactometry is to establish an odor's characteristics in relation to its concentration, intensity and hedonic value.

There are two basic types of olfactometer: static and dynamic. The static olfactometer presents a set volume of diluted sample to the panelist for assessment. The dynamic olfactometer is an apparatus that mixes odorous air from the sample bag with a stream of odor-free air. Because the apparatus produces a continuous stream of different air dilution it is called a dynamic olfactometer. As a result, in dynamic olfactometry a series of known dilutions of the odor sample is offered to a human panel.

Depending on the standard of odor measurement, the minimum numbers of persons on a panel may vary from 4 to 16. For example, the European standard requires at least 4 persons. Each individual of the panel is pre-selected on the basis of ability to detect odorants of known odor threshold such as hydrogen sulfide or n-butanol (C_4H_9OH). The objective of pre-selection of panel members is to reduce the variability in odor perception between panel members. Individuals who exhibit abnormal responses should be excluded.

Olfactometry is considered to be a standard method for measuring odor concentrations in odor units (ou), because dynamic olfactometry has the best potential for high accuracy and repeatability. The accuracy and repeatability of the measurements are improved by selecting panel members with similar odor sensitivity based on a standard odorous gas, for example, n-butanol.

ODOR CONCENTRATION

Odor concentration measured by olfactometry is expressed as ou or odor units per cubic meter (oum^{-3}). One ou is defined as the amount of odor-causing gases which, when diluted in 1 m^3 of air, can just be distinguished from clean air by 50% of the members of an odor panel. The definition of ou is rather complex, because it tries to quantify a physiological response to an odorous gas in which different components may be present.

Odor concentration is the most commonly used parameter for signifying the strength of odor. As the sense of smell is complex, it is not surprising that measuring odor is a complicated process and individual responses to odor vary greatly. Therefore, standards must be followed to ensure accuracy and consistency. In Europe, odor measurements have been made for more than 20 years based on various methods, different panel selections, a variety of olfactometers and different reference substances. Recently a working group from The European Standardization Organization (CEN) completed a new standard method CEN standard 13725 to measure odor concentration by olfactometry (CEN standard 13725, 2003).

The European ou (ou_E) is that amount of odorant(s) which, when evaporated into one cubic meter of neutral gas at standard conditions, elicits a physiological response from a panel equivalent to that elicited by one European Reference Odor Mass (EROM), evaporated in one cubic meter of neutral gas at standard conditions (CEN standard 13725, 2003).

According to European standard (CEN standard 13725, 2003 page 17), *one EROM, evaporated into one cubic meter of neutral gas at standard conditions, is the mass of substance that will elicit the D_{50} physiology response (detection threshold), assessed by an odor human panel in conformity with this standard, and has, by definition, a concentration of 1 $ou_E m^{-3}$. There is one relationship between ou_E for the reference odorant and that for any mixture of odorants. This relationship is defined only at the D_{50} physiological response level, where: 1 EROM ≡ 123 µg n-butanol (CAS-Nr. 71-36-3) ≡ 1 ou_E for the mixture of the odorants. This linkage is the basis of tractability of odor units for any mixture of odorants to that of the reference odorant. It effectively expresses odor concentration in terms of n-butanol mass equivalent.*

The odor concentration is expressed as a multiple of one $ou_E m^{-3}$ of neutral gas. The odor concentration can only be assessed at a presented concentration of 1 ou_E m^{-3}. The odor concentration, in $ou_E\, m^{-3}$, can be used in the same manner as mass concentration (kg m^{-3}).

Odor measurement in compliance with the European standard is described by CEN standard 13725 (CEN standard 13725, 2003). The mixed odorous air and the odor-free air are

randomly assigned to the two air tubes. The panelist has to choose from which tube the odorous air is flowing, and has to indicate his or her certainty (certain, fairly sure, doubtful). In general, the first mixture has a very large volume of the diluent (odorless gas). As a result, the human panel cannot detect odor. In subsequent presentations, the volume of the diluent is reduced by a predetermined factor. The series is ended at the dilution step at which all panel members have with certainty pointed out the correct tube in which the mixture of odorous air is flowing. Odor concentration can be calculated based on the volume of diluent at certain stage and the volume of diluent from the preceding step. The odor concentration in terms of odor units per m^3 of air is calculated as the geometric mean of the measured individual odor threshold of the panel members.

It is important to know that not all odors have the same ability to cause annoyance at a given concentration. It is not easy to account for differences in annoyance potential in quantitative terms. Therefore, most calculations used to predict the impact of odor use odor concentration only, ignoring different characteristics of odor. The odor concentration reduces the question "how strong and unpleasant is this odor?" to a detection threshold. However, measurements of odor concentration alone are insufficient to assess human perception of odor (Misselbrook *et al.*, 1993). The pleasant smell of one odor and the annoying smell of another odor may have the same odor concentration but certainly differ in offensiveness. Some odors judged acceptable or even pleasant at low concentrations could become annoying at higher concentrations (Punter *et al.*, 1986). Thus, odor can be more thoroughly characterized by also assessing the intensity and hedonic tone, as well as the odor concentration.

ODOR INTENSITY

Odor intensity is the second parameter of the sensory perception of odorants. It refers to the magnitude of the odor sensation. The relationship between odor intensity and logarithm of odor concentration is expected to be linear.

There are two main methods of measuring odor intensity: the odor intensity referencing scale (OIRS) and the category estimation technique. A common OIRS method uses n-butanol as a standard reference odorant. The principle of this method is to compare the intensity of an odor to the intensities of different but known concentrations of n-butanol. As described in the previous section, there are two standard procedures for measuring odor intensity using n-butanol reference. These include dynamic-scale and static-scale procedures.

The category estimation technique method can be derived from the standard document of VDI Guideline 3882: 1997, part 1, Determination of Odor Intensity, Düsseldorf, Germany. The

principle of its measurement is to vary the odor concentration and thus vary perceived intensity. At each concentration presented, human panelists are asked to indicate a value of perceived odor intensity from a seven-point scale that ranges from no odor to overwhelming odor. The values of I are then plotted against the logarithm of odor concentration. The regression line characterizes the relation between perceived intensity and odor concentration. By comparing the intercept and slope of the regression lines, different odors can be characterized.

HEDONIC TONE

Hedonic tone is used to evaluate odor offensiveness. The odor offensiveness is a measurement of the unpleasantness or pleasantness of a perceived odor. The perception of hedonic tone varies widely among people and is strongly influenced by individual odor experience, personal odor preference, and the emotional context in which the odor is perceived. A method for measuring hedonic value is based on the standard document of VDI Güideline 3882: 1997, part 2, Determination of Hedonic tone, Dusseldorf, Germany. The principle of measurement is to vary the odor concentration and thus vary hedonic value. At each presentation, human panelists are asked to indicate perceived hedonic value, using a nine-point hedonic scale ranging from very pleasant to offensive. Pain *et al.* (1990) described a six-point scale only. The hedonic value of all panel members at each concentration level is calculated, and plotted against the odor concentration in $ou_E m^{-3}$. There should be a linear relationship between the logarithm of the odor concentration and the hedonic value at that concentration.

CHEMICAL EVALUATION OF ODOR

Odor from animal production facilities is usually comprised of a complex mixture of individual compounds. The mixture can be chemically characterized by determining which compounds are in the mixture of odor and at which concentrations. To analyze the mixture, three successive steps are essential: sampling and pre-concentration of the odor, separation of components, and identification of the separated components. The basic technique for separating odorous compounds is gas chromatography. This technique separates mixtures of gaseous compounds into individual compounds by injecting them onto specific columns that partition these compounds according to vapor pressure and solubility. Because the various compounds of the sample interact with the absorbent to different degrees, compounds will be released from the tube at different and specific times. These elution times are compared to those of known compounds, for identification. In addition, peak areas and heights can be used to quantify the concentration of each odor compounds. The use of specific detectors, such as mass

spectrometry, greatly improves the certainty with which compounds may be identified on the basis of their ionized molecular fragment patterns (Zahn *et al.*, 1997). The most sensitive technique for identifying volatile odorous compounds in combination with gas chromatography is mass spectrometry (Mellon, 1994). This combination of separation and identification is called GC-MS. With this method, volatile compounds can be quantified as well as identified.

ELECTRONIC SENSOR EVALUATION

Although olfactometry is considered the most precise method for quantifying odor at present, using a human nose as a sensor to measure odor concentration is labor intensive, time consuming and presents difficulty if on-site measurements are desired. In addition, sensory evaluation methods have a number of limitations. These include rapid saturation of olfactomtery senses by some odor compounds, individual variation in sensitivity to different odor, fatigue as a result of adaptation, etc. Currently, researchers are investigating the feasibility of an alternative to olfactometry: using an electronic nose to measure odor concentration. An electronic nose is defined as an instrument consisting of an array of electronic chemical sensors with partial specificity and an appropriate pattern-recognition system capable of recognizing odor. When presented with an odor, the electronic nose would initially classify the odor type. Then, using programmed knowledge about the relationship between sensor response and odor concentration for that odor type, the electronic nose would give an integrated response or value for odor concentration. The main application area of this device is quality control, especially in the food-processing industry, but it is still far from implementation in livestock odor.

RÉSUMÉ

Odor is mainly evaluated sensorily, and chemically. Using olfactometry, three parameters of sensory characterization of odor e.g. concentration, intensity and hedonic value can be evaluated. Olfactometry is considered to be a standard method to measure odor concentration in odor units. Using GC-MS, mass concentration of different compounds of odor is quantified. Electronic sensor evaluation seems to be attractive; however, it is still far from implementation in livestock odor. Measuring odor is a complicated process and the measuring results vary greatly. The basic of the problems related to measuring odor is: the huge number of odorous compounds at very low concentration and complicated relations between the mixture of odor compounds and human perception. Therefore, standards must be followed and strictly applied. A new and well-recognized standard of odor measurement is the European standard.

ODOR FROM ANIMAL PRODUCTION FACILITIES RELATED TO DIETS

The availability, type and level of odor precursors in the digestive tract of animals and in manure determine the production of odorous compounds. To alter odor production, one may reduce the availability of precursors for odor formation and/or alter the pH in the digestive tract of animals, in urine and in manure. Altering the level and source of proteins and fermentable carbohydrates may be used as important means to implement these strategies, because proteins and fermentable carbohydrates are the main precursors of odor formation. Other possible ways of altering odor production that have been considered are feed additives and other feeding strategies, for example, feed processing, phase feeding and liquid and dry feeding.

ODOR FROM PIG PRODUCTION FACILITIES RELATED TO PROTEIN AND AMINO ACIDS IN DIETS

Attempts to reduce odor production and emission by altering diets have focused on protein. Research so far has focused on two areas: reducing ammonia emission and reducing the emission of other odorous compounds. Many studies were done on ammonia emission reduction because of its environmental effect, not because of trying to reduce odor emission. Although the relationship between ammonia and odor is still debatable, there is a relationship with protein intake. An excessive protein intake will increase both ammonia emission and odor emission. An excessive intake of protein or of AA, or both has a big effect on fecal and urinary nitrogen excretion and thus on ammonia emission. In addition, excessive protein from the diet is excreted in three forms: (1) urea, glucuronides and sulfate in urine, (2) non-digested proteins in feces, and (3) bacterial proteins in feces. These excreta are major precursors for odor formation. Blair *et al*. (1999) reported that with traditional dietary practices (14% CP), growing-finishing pigs may retain less than 40% of the nitrogen fed. According to Aarnink (1997) nitrogen retention of growing-finishing pig was 30% of the nitrogen in feed (Fig. 6). Therefore, a good base for reducing nitrogen excretion and odor production is by reducing the amount of protein in the diet.

The principle of reducing nitrogen excretion and ammonia emission through protein is to ensure that the amount of protein in a diet matches the protein requirement and to increase the efficiency of the animals' protein utilization. There is abundant literature on the impact of the reduction of dietary protein supply to pigs on the reduction of nitrogen excretion and ammonia emission (Kerr, 1995; Hobbs *et al.*, 1998; Zijlstra *et al.*, 2001; Zervas & Zijlstra, 2002). Nitrogen excretion and ammonia emission can be reduced appreciably by reducing the crude protein content in diets. Diets with reduced protein content are often supplemented with essential AA. Reduced CP diets, supplemented with crystalline AA, have been shown to reduce

fecal nitrogen excretion by 25 to 30% (Cromwell & Coffey, 1993; Jongbloed & Lenis, 1993). According to Sutton *et al*. (1999) and Shriver *et al*. (2003), reduced CP diets supplemented with AA decrease not only nitrogen excretion but also manure pH and thus ammonia emission. Generally, as a guide, for each 1% unit reduction in dietary CP combined with AA supplementation the estimated ammonia losses are reduced by 10% in pigs and poultry (Aarnink *et al.*, 1993; Jacob, 1994; Kay & Lee, 1997; Sutton *et al.*, 1997).

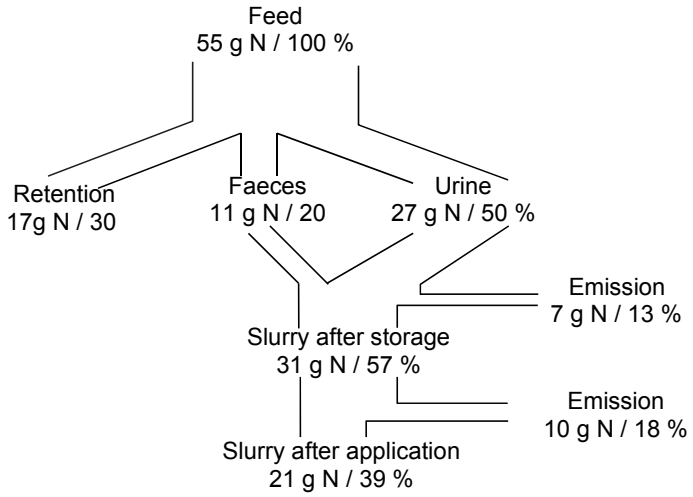


Fig. 6. Nitrogen flow in growing–finishing pigs (Aarnink, 1997)

The impact of feeding a reduced-CP and AA-supplemented diet on reducing odorous compounds is, however, inconsistent. Hobbs *et al*. (1996) showed that five out of 10 odorous compounds in the manure of growing pigs and nine out of 10 odorous compounds in the manure of finishing pigs declined when pigs were fed reduced-CP diets with supplemented AA, compared with pigs fed commercial diets. They also reported reductions of VFA, branched-chain VFA, p-cresol, indole and 3-methyl indole in manure from pigs fed low protein diets (14 and 13% CP for grower and finisher diets, respectively) compared with pigs fed high-protein diets (21 and 19% CP for grower and finisher diets, respectively). Sutton *et al*. (1998) reported a 62% reduction of volatile organic sulfur compounds (dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, dimethyl sulfoxide and carbon disulfide) in 53 kg gilts when their diet of 13% CP diet was compared with a 8% CP and AA supplemented diet. According to Stevens *et al*. (1993) increasing the protein content of diets increased the excretion of sulfurous compounds capable of producing sulfide under anaerobic conditions. In addition, in rats, the amounts of phenol, p-cresol, and 4-ethylphenol in the caecum was found to be reduced when the amount of dietary protein was reduced (Bakke, 1969).

However, Sutton *et al*. (1999) found that the concentration of volatile organic compounds

in the headspace air of manure stored anaerobically did not differ between pigs fed a 10% CP and AA-supplemented diet and pigs fed a standard 13 % CP diet. They also observed no differences in concentration of phenolic or sulfurous compounds in the feces from pigs fed 10, 13 or 18% CP diets. In addition, neither Obrock *et al*. (1997) nor Cromwell *et al*. (1999) found a difference in aerial sulfide concentration after feeding a reduced-CP and AA-supplemented diet compared to a standard one. Furthermore, Obrock *et al*. (1997) reported no difference in odor concentration between pigs fed 13% and 9% CP with AA-supplemented diets.

Moreover, Otto *et al*. (2003) showed an increase in total VFA concentration in the manure and a tendency to increase odor offensiveness from pigs fed reduced CP and AA-supplemented diets. In addition, Cromwell *et al*. (1999) reported higher levels of butyric and valeric acid but lower acetic acid in manure when pigs were fed a reduced-CP and AA-supplemented diet, while Shriver *et al*. (2003) reported lower VFA concentrations in manure from pigs fed the reduced-CP but AA-supplemented diet. The effect of dietary protein levels on odor in above-mentioned studies was inconsistent. There are some possible reasons for this inconsistency. These studies might have used different dietary compositions, e.g. different types of protein and fermentable carbohydrates. The type of diets is expected to play a role in odor production. In addition, animal type, housing system and manure storage system where the odor sample was collected might differ between studies. Furthermore, environmental factors, which influence odor production and concentration (Le *et al.*, 2005a), when and where the studies were done might differ. Moreover, different sampling and measuring methods might partly contribute to the inconsistency of the above-mentioned studies.

Types of protein have effects on odor. According to van Heugten and van Kempen (2002) diets containing fishmeal and a high sulfur content from adding up to 12% feather meal showed a high odor concentration. They also reported that including feather meal at up to 8% increased concentrations of butyric, pentanoic, and *iso*-valeric acids in feces, although concentrations of m-cresol, p-cresol, indole and decane were reduced. Studies on the effect of protein types on odor got little attention until now. Therefore, further studies in this field are required.

A logical concern arising from reducing protein level in diets is the possible effect on animal productivity. Oldenburg and Heinrichs (1996) found no negative effects on performance and leanness of pigs between 50 and 110 kg when protein levels in diets were reduced from 17% to 13.5%. According to Canh *et al*. (1998) lowering dietary CP (16.5, 14.5 and 12.5%) and supplementing AA could reduce ammonia emission up to 50% from manure of growing–finishing pigs while maintaining a normal growth rate. In an experiment in which

dietary protein was reduced from 19% to 15% in starter diets, from 16% to 12% in grower diets and from 14% to 11% in finisher diets, with or without AA supplements, Kerr *et al*. (1995) found that a reduction in pig performance and carcass muscle can be prevented by supplementing with the proper AA. According to Lopez *et al*. (1994) and Hahn *et al*. (1995) pigs fed reduced-CP diets (a reduction of 3.5 to 4%) supplemented with AA had similar carcass characteristics to pigs fed diets with a normal CP.

Briefly, diets generally contain a larger amount of proteins than the animals require. Only a proportion of dietary protein is used for growth or other production activities of the animal. Usually a large part is excreted via urine and feces. Proteins and their metabolites in the excreta are precursors for odor formation. Reducing the amount of proteins in the excreta will decrease the available substrates that microbes can metabolize to odor compounds. It is clear from the literature that ammonia from animal production facilities can be decreased considerably by reducing the amount of protein in the diet. However, in the case of other odorous compounds the situation is not so straightforward. Ammonia is a single compound and the techniques and equipment for measuring it has already been standardized. Total odor, however, is a complex mixture of various compounds, which interact each other. Its measurement techniques and equipment still require standardization. This may have contributed to the inconsistency in the measured effect of reduced-CP and AA-supplemented diets on odor. However, based on basic knowledge, we believe that feeding animals diets with reduced-CP and supplements of AA can decrease odor. To maintain normal growth rate, essential AA should be supplemented.

ODOR FROM PIG PRODUCTION FACILITIES RELATED TO DIETARY FERMENTABLE CARBOHYDRATES

In common with protein, the type and level of fermentable carbohydrates have received much attention in dietary approaches to reduce odor production and emission. Researchers, however, have mainly focused on ammonia; few have examined odor concentrations as measured by olfactometry. The principle of reducing ammonia production and emission through fermentable carbohydrates is to shift nitrogen excretion from urine to feces and to reduce the pH of manure. Increasing the fermentable carbohydrates in diets can result in bacterial proliferation due to an increase in the source of energy for bacteria in both the gastrointestinal tract and in the manure. Bacteria will use ammonia as a source of N for protein synthesis, thus reducing ammonia absorption into blood and urea excretion via urine. Fermentable carbohydrates in the gastrointestinal tract shift urinary nitrogen excretion to fecal nitrogen excretion in the form of bacterial protein (Younes *et al.*, 1997), which is less

susceptible to rapid hydroxylation. Therefore, inclusion of fermentable carbohydrates in diets can reduce ammonia emission. Other researchers who have observed this phenomenon include Morgan & Whittemore (1998) and Cromwell *et al*. (1999).

Generally, the inclusion of fermentable carbohydrates in pig diets will increase VFA concentration in feces and manure storage and thereby will reduce pH and thus ammonia emission (Sutton *et al.*, 1997; Canh *et al.*, 1998d; Kendall *et al.*, 1999). Sources of fermentable carbohydrates have an impact on nitrogen excretion and ammonia emission, because of the different components in these carbohydrates (Bakker, 1996; Canh *et al.*, 1997; 1998d Fig.7; Mroz *et al.*, 2000; Zijlstra *et al.*, 2001; Zervas & Zijlstra, 2002).

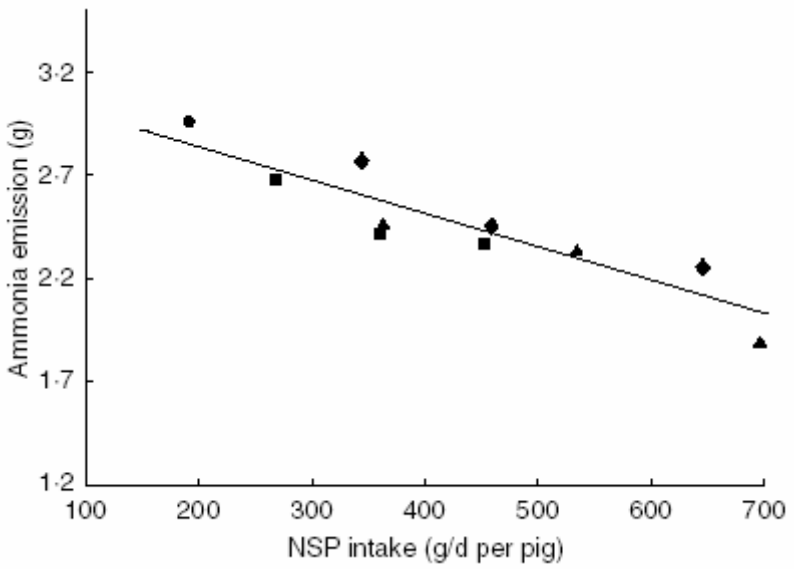


Fig. 7. Ammonia emission from manure during a 16-d storage period related to the daily intake of NSP. (●), control; (■), coconut; (▲), soybean; (◆), sugar beet; (-) fittest line (Adapted from Canh *et al.*, 1998d)

Although increasing fermentable carbohydrates in diets has a reducing impact on ammonia loss, it clearly increases manure VFA concentrations (Canh *et al.*, 1997; 1998b; 1998d; Sutton *et al.*, 1999; Shriver *et al.*, 2003). This increase may impact on manure odor concentration, because VFA are important odorous compounds in manure storage (Schaefer, 1977; Williams, 1984; Chen *et al.*, 1994; Zahn *et al.*, 1997). However, the relationship between the concentration of each odorous compound and odor concentration measured by olfactometry is still unknown. The increase of VFA concentration may increase and/or reduce the concentration of other compounds and odor concentration. Decamp *et al*. (2001) reported a 32% increase of total VFA concentration in 6-week-stored manure of pigs fed 10% soybean hulls when compared with no soybean hulls added. In the headspace gases there was a 20% reduction in aerial ammonia, a 32% reduction in hydrogen sulfide and an 11% reduction in odor

concentration when soybean hulls were added. Goa *et al*. (1999) reported a trend to decrease excretions of p-cresol and 3-methyl indole in fresh feces (Fig. 8) by adding fibers to the basal diet. Moeser *et al*. (2001) fed soybean hulls to pigs not adapted to high fibre diets and noted a decrease in odor. However, Gralapp *et al*. (2002) reported no difference in odor concentration when 10% distillers dried grain was added to the diets of finishing pigs. Moreover, Hawe *et al*. (1992) reported increased excretions of indole and 3-methyl indole in the feces of pigs fed diets containing sugar beet pulp as a fermentable fiber source. Knarreborg *et al*. (2002) observed a significant reduction in the production of indole and 3-methyl indole in the proximal and distal part of the hindgut in pigs fed a diet rich in sugar-beet pulp. They believed that easily fermentable carbohydrates such as sugar-beet pulp stimulate microbial growth and hence the demand for AA for protein synthesis, leaving less tryptophan for conversion to 3-methyl indole.

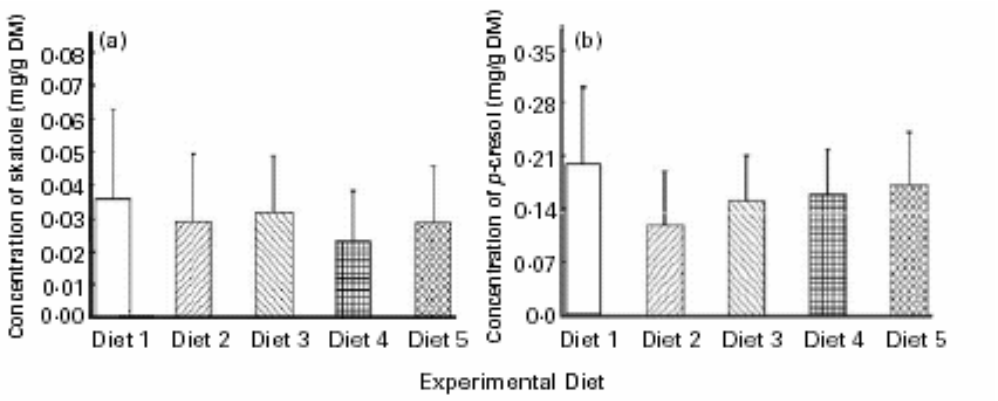


Fig. 8. Adding cellulose and pectin to a maize- and soybean meal-based diet: the effect on 3-methyl indole (skatole) and p-cresol in feces. Diet 1, basal diet; diet 2, basal diet + 4.5% cellulose; diet 3, basal diet + 9.0% cellulose; diet 4, basal diet + 4.5% pection; diet 5, basal diet + 9.0% pectin (Adapted from Goa *et al.*, 1999)

The literature contains very little information on the effect of sources of fermentable carbohydrates on the production and emission of odor compounds other than ammonia. Different sources of fermentable carbohydrates are fermented differently by pigs. Thus, different sources of fermentable carbohydrates provide different precursors for odor formation. The effect of fermentable carbohydrate sources depends on the composition of components. Microbial activity in the large intestine is generally increased when diets contain a high concentration of soluble fiber (Jørgensen & Just, 1998). The enhanced microbial activity in the digestive tract means an increase in the excretion of microbial substances, thus a reduction in the proportion of very volatile compounds such as urea in total excretion.

Apart from their effects on environment, adding fermentable carbohydrates to pig diets has

some controversial disadvantages. They can reduce the apparent ileal and total tract digestibility of protein (Shi & Noblet, 1993; Bakker, 1996), of fat (Dierick *et al.*, 1989), of minerals (Jongbloed, 1987) and of energy. The principles that cause these changes are: a reduced absorption of nutrients, which reduces the true nutrient digestibility; an increased secretion of digestive juices; an increased microbial synthesis of fat and protein, which reduces apparent nutrient digestibility; a reduced retention time of the digesta in the gastrointestinal tract, causing reduced nutrient digestion.

In brief, fermentable carbohydrates have been studied as a means to reduce both ammonia and other odorous compound production and emission from animal production facilities. It is clear from the literature that including fermentable carbohydrates in diets can reduce ammonia emission from animal production facilities considerably. However, the effect on other odorous compounds and odor nuisance is inconsistent and not yet clear. Further studies on the effect of type and level of fermentable carbohydrates on odor production and concentration are required before conclusions can be drawn and the application can be used to reduce odor from animal production facilities.

ODOR FROM PIG PRODUCTION FACILITIES RELATED TO ADDITIVES IN DIETS

Feed additives are one of the biochemical and chemical agents that can reduce odor from animal production facilities (Ritter, 1989). The principles of using feed additives to reduce odor formation and emission are to:

Alter the micro flora in the large intestine of animals and in manure;

Change the pH into one less favorable for odor formation;

Bind odor.

Microbial activities in the large intestine of the animal both produce odorous compounds and provide precursors for odor formation in manure; thus it is expected that altering the microflora and nutrient supply has the potential to change one or more groups of odorous compounds.

Altering the pH of urine and manure has received the most attention in efforts to use feed additives to reduce ammonia emission. At a low pH, ammonia is protonated to ammonium (NH^+_4), which remains in solution due to its charge. Some kinds of acid salts have been added into diets to reduce ammonia emission based on the principle of pH reduction. According to Canh *et al*. (1998a) the addition of calcium salts including $CaSO_4$, $CaCl_2$ and Ca-benzoate to diets decreased urinary pH; as a result, ammonia emission was reduced by 30, 33 and 54%,

respectively.

A change in pH may also change the release of other odorous compounds such as hydrogen sulfide. For example, at a high pH, hydrogen sulfide will be reduced but ammonia release will be enhanced. The literature contained very little further information on the relationship between pH and other odorous compounds from animal production facilities.

Some feed additives are reported to bind ammonia or inhibit urease. Amon *et al*. (1995) reported a 26% reduction in ammonia emission when fattening pigs were fed De-Odorase (a yucca extract). Some other investigations have also observed reduced ammonia emission after adding yucca extracts to pig diets (Cromwell *et al.*, 1999; Colina *et al.*, 2001). However, at present, its inclusion in pig diets to reduce odor is not strongly supported by research. No information on the use of feed additives to bind odor other than ammonia was found in the literature.

In brief, like the two other means of reducing odor (proteins and carbohydrates), the use of feed additives has mainly focused on reducing ammonia emission. Acidifying additives has proved to be effective in reducing ammonia emission. However, its impact on odor has not been evaluated yet.

OTHER FEEDING STRATEGIES

In addition to using proteins, fermentable carbohydrates and feed additives strategically to curtail odor formation, liquid and dry feeding, phase feeding, and feed processing have also been studied in this context. According to Hobbs *et al*. (1997) the odor concentration from the manure of pigs fed a 4:1 (water: dry feed) diet was significantly less than that of pigs fed dry feed and 3:1 diets. H_2S was the major odorant in the 3:1 and dry feed diets. The organic nitrogen in manure declined concomitantly with an increase in the water content of the diets, possibly due to improved digestibility for diluted diets and hence less substrate for odor formation. Nahm (2002) reported that in growing and finishing pigs, phase feeding can reduce N excretion by 10-13% and odor from manure by 49-79%. He also observed that a 27% reduction of N excretion in finishing pigs and a 22-23% reduction of N excretion in piglets could be achieved when pigs are fed with proper ground feed. Van der Peet-Schwering *et al*. (1996) reported that moving from a 2-phase diet system to a multi-phase programme with optimal housing resulted in a 17% reduction in ammonia emission. In general, the mentioned-above feeding strategies, especially a phase feeding regime, showed promising results to reduce odor production. However, these findings were not confirmed by other studies. Therefore, further studies are still required before conclusions can be finally drawn and the application can

be used in practices.

RÉSUMÉ

Dietary composition and odor production and emission have a cause-and-effect relationship. Altering dietary composition, especially the sources and levels of proteins and fermentable carbohydrates seems a promising approach to reduce odor nuisance. The attempts made so far to alter diets to reduce ammonia emission have achieved much; the approach can reduce ammonia emission considerably. One shortcoming of most studies to date is that odorous compounds are considered in isolation, for example, relative changes are measured only in single compounds or in one group of compounds. Only a few studies have used olfactometry to assess the effect of altering dietary composition on odor emission.

CONCLUSIONS, GAPS OF KNOWLEDGE AND FURTHER STUDIES REQUIRED

Odor nuisance from animal production is especially a problem in densely concentrated livestock farming areas, like those in The Netherlands. It results from the intensification of animal production in the vicinity of a dense population. Such intensive animal production can cause serious nuisance and according to some authors may be even related to health problems as a result of odor production and emission.

Livestock odor does not come from an individual compound but from a complex mix of various compounds. Numerous odorous compounds from animal production facilities have been identified in various studies. However, to date, odorous compounds from different sources e.g. feed, animal bodies, urine, feces and manure, have not been well described. The main source of odor from animal production facilities is excreta. The odorous compounds that mostly cause nuisance can be classified into 4 main groups: sulfurous compounds, indoles and phenols, VFA, and ammonia and volatile amines.

Odor production is mainly based on microbial conversions involving many bacteria. Odorous compounds are the intermediate or end products of microbial conversions of nutrients in the diet that are not utilized. The main precursors of odor formation are proteins and fermentable carbohydrates. The different odorous compounds interact with each other: an increase of one compound may cause others to increase or decrease or both.

Odor is evaluated sensorily and chemically. Using apparatus, the sensory characteristics of odor strength and offensiveness can be quantified by human noses. This technique is called olfactometry. The chemical characteristics of odor can be evaluated by using GC-MS equipment to determine the concentrations of different odorous compounds. Electronic sensor

evaluation appears to be promising, but it is still a long way from being applied in research on livestock odor.

Despite inconsistencies between studies, it proved possible to compile a list of around 20 important odorous compounds from animal production facilities. The odor concentrations of these compounds from animal production facilities vary widely, depending on diet, climate factors, housing system, pig breed, sampling and measuring methods, etc.

Studies on altering diets to reduce odor production have tended to have two distinct aims: to reduce ammonia emission and to reduce the emission of other odorous compounds. The main reason of reducing ammonia emission was because of its environmental problem, not because of its odor potential. Though there are many reports on ammonia emission being successfully altered by adjusting diets, reports of the impact of altering diets on the emission of odorous compounds other than ammonia are inconsistent.

It is clear that many odorous compounds are produced from the breakdown of proteins. Therefore, a promising approach towards reducing odor is to reduce the total protein concentration so that less nitrogenous substrate is available to the microbes inside and outside the animal. Up to now, studies have focused on certain specific odorous compounds and have tended to ignore the effect of protein level on odor production measured by olfactometry. Furthermore, there are hardly any published studies on the effects of protein sources on odor production. Moreover, the role of specific AA, which are precursors for important odorous groups, on odor strength and offensiveness was not yet studied.

The role of fermentable carbohydrates in odor production is not straightforward. Depending on the type and amount of fermentable carbohydrates, different populations of bacteria can be favored; some of them may reduce odor, while others may increase odor. In common with studies on protein, studies on the effect of fermentable carbohydrates on odor production have tended to focus on certain groups of odorous compounds, though the relationship between each odor group with odor production measured by olfactometry is not yet clear. The literature contains hardly any reports of the effects of fermentable carbohydrates on odor production measured by olfactometry. Nor has the role of specific sources of fermentable carbohydrates on odor production been evaluated.

It is clear that feed additives can reduce ammonia substantially. It remains speculative, however, whether adding these salts will affect microbial fermentation in the large intestine of animals; additives may have no effect on other odorous compounds than ammonia. Generally, the effects of feed additives should always be studied in a wider context. An additive might

solve one problem but generate another. This hypothesis remains to be tested, however.

Dietary proteins and fermentable carbohydrates offer the means to reduce odor strength and offensiveness at source, because they are main precursors of odor production. Research has so far tended to focus on single effects of different levels of CP or fermentable carbohydrates on odor compounds and more or less on odor nuisance. However, it is not only the amount and source of these compounds that is important but also the balance between them, because microflora in the large intestine and manure storage use fermentable carbohydrates as a source of energy and N for protein synthesis. On the basis of our review of the literature, we hypothesize that odor nuisance from pig production facilities can be reduced significantly by achieving an optimum balance between proteins and fermentable carbohydrates in the diet. However, more research must be done in order to arrive at a general principle for reducing odor.

3

EFFECTS OF ENVIRONMENTAL FACTORS ON ODOR EMISSION FROM PIG MANURE

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ABSTRACT. *Altering environmental factors may change odor emission from pig manure. The main objective of this laboratory-scale study was to examine the effects of temperature, ventilation rate, emitting area, and manure dilution ratio on odor emission from growing pig manure, while also testing their effects on manure characteristics. Manure was placed in vessels with different surfaces (303, 475, and 595 cm^2), and water was added to create different dilution ratios (0%, 50%, and 100%). The vessels were connected to glass capillaries with different ventilation rates through the headspace (0.5, 1.0, and 1.5 L min^{-1}) and were placed in climate-controlled rooms with different temperatures (10°C, 20°C, and 30°C). We used a face-centered central composite design with 54 experimental units in 2 rounds of 3 blocks. Odor samples were taken at the end of experiment (after 7 d). Manure samples were collected at the start and at the end of the experiment. The mean odor emission from the manure vessel was 2326 ou_E h^{-1} m^{-2}. Increased temperature, ventilation rate, and dilution ratio increased odor emission. Emitting area did not influence odor emission, but positively influenced total-N loss. Total-N loss increased as temperature and ventilation rate increased, but decreased as dilution ratio increased. Lowering temperature and ventilation rate can be considered as starting points to reduce odor emission from pig manure in practical conditions. The effects of dilution ratio and emitting area on odor emission could not be fully separated from the effect of headspace volume in this study and should be further studied.*

Keywords. *Environmental factors, Manure, Odor emission, Pigs.*

INTRODUCTION

The odor formed and emitted from intensive pig production systems can be a serious nuisance to people living in the vicinity of pig farms and has been related to health problems by some authors (Schenker *et al.*, 1991; Schenker *et al.*, 1998; Donham, 2000; Iverson *et al.*, 2000). In addition, some authors reported that some odorous compounds can affect both health and production efficiency of the animal (Boer *et al.*, 1991; Tamminga, 1992). Within pig production, growing pigs are the main source of odor. Odor mainly comes from manure (Mackie *et al.*, 1998). As a result, studies on odor from growing pig manure should receive high priority.

Odor is mainly formed from microbial conversion of organic compounds in manure. Odor is emitted into the air from buildings or external manure storage sites. Environmental factors, for example, temperature, ventilation rate, dilution ratio, and emitting area, may influence the odor generation and emission process. Generally, the formation of most odorous compounds increases at high temperatures. High temperatures stimulate the formation of ammonia (Brunsch, 1997), hydrogen sulfide (Ni *et al.*, 2002), 4-methyl phenol (p-cresol), and 3-methyl indole (skatole) (Spoelstra, 1977) in manure. Ventilation rate seems to be important as well, and is required in determining odor emission (Zhu *et al.*, 2000). According to Oldenburg (1989) and Verdoes and Ogink (1997), there is a positive relationship between odor emission from pig houses and ventilation rate. Guingand *et al.* (1997) reported a 29% reduction in odor emissions from a growing-finishing pig house as the ventilation rate was reduced by 50%. According to Mol and Ogink (2003) and Ogink and Groot Koerkamp (2001), reducing the emitting area of the manure pit can decrease odor emission in pig houses. Dilution of manure is thought to have an effect on odor formation and emission because water is a solvent for bacterial conversions. In this solvent, odorous compounds are produced and broken down. However, we could not find any data in the literature on the effect of dilution on odor formation and emission.

The literature contains very little quantitative information on the effects of environmental factors in controlled conditions on odor concentration and emission from manure as measured by olfactometry. Furthermore, existing information on the effects of temperature and ventilation rate on odor emission in practice are difficult to interpret because these factors are generally confounded with each other and with other variables, for example, pig weight. In addition, the effects of the interactions of different environmental factors on odor concentration and emission have not received much attention in previous studies. Altering and controlling environmental factors might reduce odor concentration and emission from pig manure. The objective of this

study was to quantify the effects of temperature, ventilation rate, emitting area, dilution ratio, and their interactions on odor concentration and emission from growing pig manure in combination with quantifying their effects on manure characteristics.

MATERIALS AND METHODS

Controlled lab experiments were conducted to determine how odor concentration and emission from manure and manure characteristics were affected by different environmental factors: temperature, ventilation rate, manure dilution ratio, and emitting area. The study used a laboratory setup with manure vessels whose headspace was ventilated by fresh air. The fresh air came from inside the climate-controlled room. The air in the climate-controlled room came from outside.

Independent factors included:

- Temperature (T) of the manure and air was set at 3 levels: 10°C, 20°C, and 30°C. One of these temperatures was maintained in each of 3 climate-controlled rooms.
- Ventilation rate of the manure vessels (V) was set at 3 levels: 0.5, 1.0, and 1.5 L min^{-1}. It was controlled by critical glass capillaries.
- Emitting area (E) was set at 3 levels: 303, 475, and 595 cm^2. Emitting area was controlled by vessels having different areas.
- Manure dilution rate (D) was set at 3 levels: 0%, 50%, and 100% (w/w). Manure was diluted with 0%, 50%, and 100% water.

Dependent factors included:

- Odor concentration and odor emission from the manure vessels.
- Manure characteristics, which included pH, dry matter, ash, total-N, ammonium, total-N loss, and individual volatile fatty acids: acetic, propanoic, butyric, pentanoic, iso-butyric, iso-pentanoic, and total volatile fatty acids (VFA).

EXPERIMENTAL DESIGN

A face-centered central composite design with blocks was used, according to the scheme of Cochran and Cox (1966). The experiment had 3 blocks. Each block had 9 treatment combinations (Table 1). Block was the effect of day. Within each block, the 9 treatment combinations were started on the same day. We replicated the entire experiment once.

Table 1. Design of the experiment

Block	Dilution (%)	Emitting area (cm^2)	Temperature (°C)	Ventilation rate (L min^{-1})
	0	303	10	0.5
	100	303	10	1.5
	0	595	10	1.5
	100	595	10	0.5
I	0	303	30	1.5
	100	303	30	0.5
	0	595	30	0.5
	100	595	30	1.5
	50	475	20	1
	0	303	10	1,5
	100	303	10	0.5
	0	595	10	0.5
	100	595	10	1.5
II	0	303	30	0.5
	100	303	30	1.5
	0	595	30	1.5
	100	595	30	0.5
	50	475	20	1
	0	475	20	1
	100	475	20	1
	50	303	20	1
	50	595	20	1
III	50	475	10	1
	50	475	30	1
	50	475	20	0.5
	50	475	20	1.5
	50	475	20	1

Fig. 1 is a schematic of the laboratory setup for the experiment. Vessels were placed in climate-controlled rooms. There were 9 manure vessels per room. Manure was placed in the vessel and kept under experimental conditions for 7 days. Each vessel was closed with a lid, with a rubber gasket between the lid and the wall of the vessel to make the vessel airtight. Air entered the vessel via 24 holes of 1 mm diameter, located at the edge of the lid. Air was exhausted from the vessel by a hole of 5 mm diameter in the middle of the lid. From a previous test (unpublished results), we visually found that there was no direct shortcut from the incoming air to the outgoing air in the vessel. Air entering the vessel was from the climate-controlled

room. Air entering the room was outside air. The incoming air in the vessels had the same absolute amount of water vapor (8.42 g m^{-3}); therefore, relative humidities in the climate-controlled rooms were 90%, 49%, and 28% in the 10°C, 20°C, and 30°C rooms, respectively.

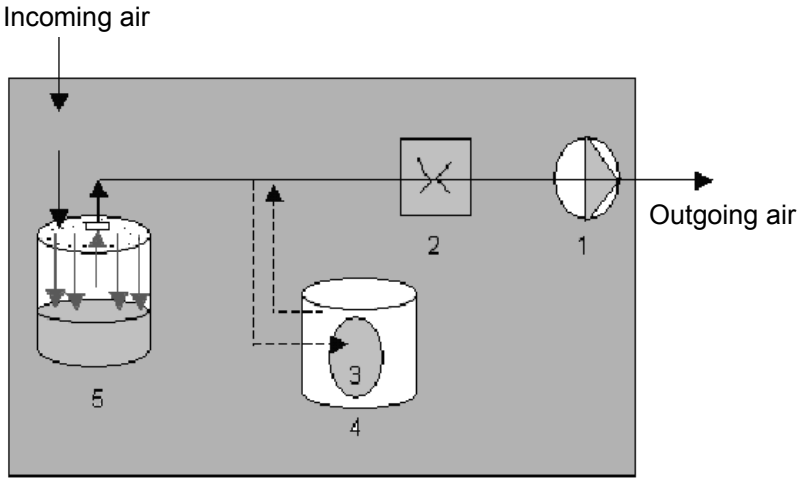

Fig. 1. Schematic view of the laboratory setup of the experiment in a climate-controlled room: 1 = pump, 2 = critical glass capillary, 3 = sample bag, 4 = container, and 5 = manure vessel

The ventilation rate was controlled by critical glass capillaries connected to vacuum pumps that pulled the air from the vessels. The outgoing air from the vessels was released outside (Fig. 1). Odor samples were always collected at a flow rate of 0.5 L min^{-1}, while the vessel ventilating rate remained constant during sampling. At the ventilation rate of 0.5 L min^{-1}, a 0.5 L min^{-1} critical glass capillary was used. At the ventilation rate of 1.0 L min^{-1}, two 0.5 L min^{-1} critical glass capillaries were used, and at the ventilation rate of 1.5 L min^{-1}, one 0.5 L min^{-1}, and one 1.0 L min^{-1} critical glass capillaries were used.

As mentioned above, the vessels had different surfaces (303, 475, and 595 cm^2) but the same height (23.5 cm), so they had different volumes. Net vessel volumes for the three surfaces were 7116, 11164, and 13991 cm^3, respectively. A blank vessel (without manure but with the same amount of clean water) was placed in each climate-controlled room to investigate the background odor concentration, or the odor concentration of the incoming air. The laboratory setup of the blank vessel was exactly the same as that of the manure vessels.

MANURE AND PIGS

Manure was taken from a deep pit below a barn where pigs from 25 to 50 kg were kept. The pigs were housed in partially slatted floor pens. They were fed *ad libitum* a typical commercial diet with 170 g CP, 46.62 g crude fiber, and 9.9 MJ NE per kg of feed. The manure was released at a high velocity to a temporary storage container. The manure was mixed in the

temporary storage container before sampling. A total of 200 kg of manure was collected. The manure pit had not been emptied for two months prior to manure collection.

The collected manure was mixed for 5 min and divided it into 2 parts. One part was used in the first round of the experiment, and the other part was used in the second round. The manure for the second round was stored in a room at 4°C for nine days until it was used. The manure was mixed again for 5 min before each vessel was filled with 2.0 kg of manure. Water was added to each vessel to dilute the manure to either 50% or 100%. Finally, all vessels were stored in a room at 4°C for one to three days depending on the assigned day (block) in the experimental system.

SAMPLES AND SAMPLING

Two kinds of samples were taken: manure samples and odor samples.

Manure Samples

Manure samples were taken at the beginning and end of the experiment. At the beginning of each round of the experiment, at the same time that manure was put into vessels, we took a duplicate sample. At the end of the experiment, after odor samples were collected, the manure in each vessel was mixed for 1 min, and a 1 kg manure sample was then taken from each vessel.

Odor Samples

After running the experiment for 7 days, we collected odor samples from the air leaving the vessels. The sampling method for delayed olfactometry using the "lung principle" was used. A 40 L Nalophaan odor sampling bag was placed in a rigid container (Fig. 1). The sample bag had been flushed with compressed and odorless air 3 times before it was placed in a rigid container for collection of the odor sample. The sample bag was used once for one odor sample, as recommended by CEN Standard 13725 (2003). The air was removed from the container using a vacuum pump, and the vacuum in the container caused the bag to fill with a volume of sample equal to the volume removed from the container. The flow rate of air entering the sample bag was 0.5 L min^{-1}.

The experimental system kept running while the odor sample was collected, and the total ventilating rate was not changed during sampling. One odor sample was taken from each manure vessel. At the same time, an air sample was taken from the blank vessel in each climate-controlled room.

During transport and storage, odor samples were kept at a temperature above the dewpoint

of the sample to avoid condensation. This was achieved by warming the rigid container of the odor bag. The interval between sampling and measuring the odor concentration did not exceed 24 h. This procedure was recommended by CEN Standard 13725 (CEN, 2003).

SAMPLE ANALYSES AND CALCULATIONS

Manure Samples

All manure samples were analyzed in duplicate. Dry matter, ash, and total-N were analyzed according to AOAC methods (AOAC, 1990), and ammonium-N was determined spectrophotometrically according to NEN Standard 6472 (NEN, 1983). Volatile fatty acids, e.g., acetic, propanoic, butyric, pentanoic, iso-butyric, and iso-pentanoic, were measured using a Packard 427 gas chromatograph equipped with a flame ionization detector (Derikx *et al.*, 1994).

The concentrations of each VFA, total VFA, total-N, ammonium-N, and dry matter were calculated per kg of manure and per kg of ash. Total-N loss was calculated by subtracting total-N in the manure after the experiment from that before the experiment. The weight of manure after the experiment was calculated with equation 1:

$$W_E = \frac{Ash_B . W_B}{Ash_E} \quad (1)$$

where W_E = weight of manure after the experiment (kg)

W_B = weight of manure before the experiment (2.0 kg)

Ash_B = ash concentration before the experiment (g/kg)

Ash_E = ash concentration after the experiment (g/kg).

Odor Samples

The European standard (CEN standard 13725, 2003) was used to measure odor concentration by olfactometry. Odor concentration of the examined sample was expressed in European odor units per cubic meter air ($ou_E\ m^{-3}$). One odor unit is defined as the amount of odor-causing gases that, when diluted in 1 m^3 of air, can just be distinguished from clean air by 50% of the members of an odor panel. Six qualified panelists, who were screened to determine their odor sensing ability (20 to 80 ppb n-butanol), provided their responses to two sniffing tubes of a dynamic dilution olfactometer. The odorous and odorless air was randomly presented in one of the two sniffing tubes. At each presentation, each panelist indicated via an electronic keyboard which sniffing tube released the odorous air. They declared whether their selection

was "guess," "not certain," and "certain." A range of at least six dilution steps, each differing from the next by a factor of 2, was presented to the panelists in ascending concentration. Initial sample presentations were below the panelist detection threshold. Odor concentrations were increased until all panelists in 2 consecutive steps certainly indicated the correct sniffing tube with odorous air. The entire range of dilution steps was repeated 3 times.

From the indication of each individual panelist, odor concentration was calculated in three steps:

1. Calculating the individual detection threshold: this is the geometric mean of the last non-detectable (guess or not certain) dilution ratio and the first certain detectable dilution ratio.
2. Calculating the panel detection threshold: this is the geometric mean of the individual detection thresholds of all panelists.
3. Retrospective screening of each panelist threshold: according to CEN Standard 13725 (2003).

Steps 2 and 3 were repeated until there were no outlying individual results; the odor concentration reported is the geometric mean of the individual detection thresholds of the panelists.

Because the incoming air may be odorous, the difference in odor concentration between the outgoing and incoming air should be used to calculate the net odor concentration (Smith & Dalton, 1999; Miller *et al.*, 2001). Net odor concentration ($ou_E\ m^{-3}$) of the manure in the vessel was calculated as the difference between the odor concentration of the odor sample from the manure vessel and that of the blank vessel.

Net odor emission per time unit was defined as the number of odor units emitted from the manure vessel (after correcting for that of the blank sample) per time unit. It was calculated by multiplying the ventilation rate with the corresponding net odor concentration:

$$E_t = C_{odor} \frac{V}{1{,}000} 60 \qquad (2)$$

where

E_t = odor emission per hour ($ou_E\ h^{-1}$)

C_{odor} = odor concentration ($ou_E\ m^{-3}$)

V = ventilation rate ($L\ min^{-1}$)

60 = 60 min h^{-1}

1,000 = 1,000 L m^{-3}.

Net odor emission per surface unit was defined as the number of odor units emitted from the manure vessel per hour per surface unit. It was calculated with equation 3:

$$E_{t,a} = \frac{C_{odor}.V.60.10{,}000}{E.1{,}000} \quad (3)$$

where

$E_{t,a}$ = odor emission per hour per square meter manure area (ou_E h^{-1} m^{-2})

E = emitting area (cm^2)

C_{odor} = odor concentration (ou_E m^{-3})

V = ventilation rate (L min^{-1})

60 = 60 min h^{-1}

1,000 = 1,000 L m^{-3}

10,000 = 10,000 cm^2 m^{-2}.

Equation 3 can be abbreviated as in equation 4.

$$E_{t,a} = \frac{C_{odor}.V.600}{E} \quad (4)$$

Gross odor concentration and odor emission were defined as the concentrations and emission of the exhaust air only (not subtracting the contribution of inlet odors).

DATA ANALYSIS

We used the Genstat statistical package, 7th edition (GenStat VSN International Ltd., 2004) to analyze the effect of environmental factors on odor concentration and emission and manure characteristics by using model 1:

$$Y = \beta_0 + R_i + B_i + \beta_1 T + \beta_2 V + \beta_3 E + \beta_4 D + \beta_5 TV + \beta_6 TD + \beta_7 TE + \beta_8 VD + \beta_9 VE + \beta_{10} ED + error$$

where

Y = dependent factors (odor concentration, odor emission, and manure characteristics)

β_0 to β_{10} = regression coefficients

R_i = effect of round (i = 1 to 2)

B_j = effect of block (the day starting the experiment with certain manure vessels, j = 1 to 3)

T = effect of temperature (°C)

V = effect of ventilation (L min^{-1})

D = effect of manure dilution (%)

E = effect of emitting area (cm^2).

The model contains linear terms and two-way interaction terms (block and round effect). Backward elimination was used to determine the interaction effects when running model 1, i.e., removing the interaction with the highest P-value at each iteration until all remaining interactions were significant ($P < 0.05$) or removed. A natural log (base e) transformation was applied to odor concentration, odor emission, the concentration of total and individual VFA, total-N, ammonium-N, and total-N loss, since they were skewed and non-normally distributed. Additionally, basic descriptive statistics (mean, standard deviation. and range of dependent variables) were estimated.

RESULTS AND DISCUSSION

EFFECTS OF ENVIRONMENTAL FACTORS ON ODOR CONCENTRATION AND EMISSION

The net mean odor concentration, odor emission per hour, and odor emission per hour per square meter of the manure in the vessel were 1663 $ou_E\ m^{-3}$, 93.40 $ou_E\ h^{-1}$, 2326 $ou_E\ h^{-1}\ m^{-2}$, respectively. They ranged from the lower detection limit of 224 to 6562 $ou_E\ m^{-3}$, from 8.6 to 590.6 $ou_E\ h^{-1}$, and from 263 to 19505 $ou_E\ h^{-1}\ m^{-2}$, respectively (Table 2).

Table 2. Descriptive statistics of odor concentration and emission from pig manure (n = 54)

Variables[a]	Mean	SD[b]	Min. - Max.
Net odor concentration ($ou_E\ m^{-3}$)	1663	1337	224 - 6562
ln(net odor concentration)	7.15	0.76	5.41 - 8.79
Net odor emission per hour ($ou_E\ h^{-1}$)	93.4	88.3	8.60 - 590.6
ln(net odor emission per hour)	4.24	0.79	2.15 - 6.38
Net odor emission per hour per square meter ($ou_E\ h^{-1}\ m^{-2}$)	2326	2843	263 - 19505
Odor concentration of blank samples ($ou_E\ m^{-3}$) (n = 6)	115	17	98 - 140
Gross odor concentration ($ou_E\ m^{-3}$)	1779	1335	333 - 6672
Gross odor emission per hour ($ou_E\ h^{-1}$)	100.3	89.2	12.2 - 600.5
Gross odor emission per hour per square meter ($ou_E\ h^{-1}\ m^{-2}$)	2491	2885	318 - 19831

[a] ln = natural logarithm.

[b] SD = standard deviation.

The mean odor concentration of the blank sample was 115 $ou_E\ m^{-3}$. It ranged from 98 to 140 $ou_E\ m^{-3}$. The gross odor concentration, odor emission per hour, and odor emission per hour per square meter were about 7% higher than the net concentration and emission rate. This implied that the incoming air was not totally free of odor. In practical situations, odor in the

incoming air can supply a significant amount of the odor in the outgoing air, for example, in a study by Lim *et al*. (2001), it was about 40%.

Table 3. Effects of environmental factors on odor concentration and emission and manure characteristics (*n* = 54)

Response variables[a]	Estimated regression coefficients[b]									R^{2c}	RSD[d]
	Constant	T (°C)	V (L min^{-1})	E (cm^2)	D (%)	TV	TE	TD	ED		
ln(odor conc., ou_E m^{-3})	6.65***	0.058***	-0.42**	-0.0007	0.004**	--[e]	--	--	--	61.3	0.47
	(0.36)	(0.008)	(0.16)	(0.0005)	(0.002)						
ln(odor emission, ou_E h^{-1})	2.60***	0.058***	0.68***	-0.0007	0.004**	--	--	--	--	63.3	0.48
	(0.37)	(0.008)	(0.16)	(0.0005)	(0.002)						
pH	7.61***	0.018*	-0.11*	0.0005	-0.004***	--	44E-6**	--	--	88.1	0.14
	(0.19)	(0.008)	(0.05)	(0.0004)	(0.0005)		(17E-6)				
ln(total VFA, g/kg ash)	5.31***	0.024	0.43	0.00005	0.001	-0.03**	-0.0001***	--	--	83.5	0.27
	(0.41)	(0.018)	(0.21)	(0.0007)	(0.0009)	(0.01)	(33E-6)				
ln(acetic acid, g/kg ash)	4.89***	0.026	0.41	-0.0003	0.002*	-0.03**	-0.0001**	--	--	74.5	0.31
	(0.47)	(0.021)	(0.24)	(0.0008)	(0.001)	(0.01)	(37E-6)				
ln(propanoic acid, g/kg ash)	4.96***	-0.043	1.01	-0.0028***	-0.006**	-0.09***	-	--	--	79.7	0.65
	(0.68)	(0.025)	(0.57)	(0.0007)	(0.002)	(0.023)					
ln(butyric acid, g/kg ash)	4.06***	-0.075	-0.127	0.0023	-0.008	--	-0.0003**	0.0006*	--	82.3	0.73
	(1.01)	(0.044)	(0.25)	(0.0019)	(0.006)		(89E-6)	(0.0002)			
ln(pentanoic acid, g/kg ash)	1.93**	-0.08***	-0.067	0.0003	-0.008*	--	-0.0002**	0.0006***	--	85.1	0.45
	(0.65)	(0.03)	(0.15)	(0.0012)	(0.004)		(54E-6)	(0.0001)			
ln(iso-butyric acid, g/kg ash)	1.21*	0.054*	0.36	0.003**	0.01***	-0.03**	-0.0002***	-	-23E-6**	81.6	0.35
	(0.56)	(0.023)	(0.27)	(0.001)	(0.003)	(0.01)	(42E-6)		(8E-6)		
ln(iso-pentanoic acid, g/kg ash)	2.46***	0.017	0.39*	0.002**	-0.001	-0.031***	-0.0002***	0.004***	-13E-6*	89.3	0.25
	(0.41)	(0.017)	(0.20)	(0.0007)	(0.003)	(0.009)	(30E-6)	(88E-6)	(6E-6)		
ln(total-N, g/kg ash)	5.33***	-0.005**	0.031	-0.0001**	-0.0001	-0.007***	--	62E-6***	--	88.4	0.04
	(0.04)	(0.001)	(0.030)	(44E-6)	(0.0003)	(0.001)		(13E-6)			
ln(ammonium-N, g/kg ash)	4.99***	-0.01***	0.02	0.0001*	-0.0002	-0.009***	--	0.0001***	--	87.4	0.06
	(0.79)	(0.003)	(0.05)	(72E-6)	(0.0005)	(0.002)		(22E-6)			
ln(total-N loss, g/vessel)	-2.51***	0.06***	0.68***	0.0009**	-0.007***	--	--	--	--	87.7	0.24
	(0.18)	(0.004)	(0.08)	(0.0003)	(0.0008)						

[a] ln = natural logarithm.

[b] * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$; values in parentheses are standard errors.

[c] R^2 = percentage variance accounted for.

[d] RSD = residual standard deviation.

[e] -- = dropped from the model by backward elimination because of its non-significant effect.

The effect of environmental factors on both net and gross odor concentration and odor emission was analyzed. Because the trend and comparable magnitude of the estimated regression coefficient were found to be the same, the effect of environmental factors on net odor concentration and odor emission are presented in this article. All references to odor concentration and odor emission in this article imply the net values unless otherwise stated. The effect of environmental factors on odor emission per square meter was not analyzed because

emitting area was one of the factors in the study. The odor emission in the regression analysis was odor emission per hour.

Temperature, ventilation rate, and manure dilution ratio influenced odor concentration and odor emission of the manure in the vessel ($P < 0.05$) (Table 3), but emitting area and the two-way interaction terms of the four mentioned environmental factors did not ($P > 0.05$). As temperature and manure dilution ratio increased, odor concentration increased, but odor concentration decreased when ventilation rate increased.

Odor emission from the manure in the vessel increased with temperature, ventilation rate, and manure dilution ratio. The models for odor concentration and odor emission with different environmental factors as independent factors accounted for 61.3% and 63.3% of the variation, respectively. The effects of temperature and ventilation rate on odor emission were much larger than those of manure dilution ratio and emitting area. Temperature and ventilation rate alone accounted for about 58.9% of the variance. When adding emitting area and manure dilution ratio separately to the model, only 0.2% and 4.2% extra variance, respectively, was accounted for.

If other independent variables in the model were kept constant, both $\ln(C_{odor})$ and $\ln(E_t)$ increased about 0.058 units as temperature increased by 1°C. When temperature increased from 10°C to 30°C, the estimated odor concentration and odor emission increased by 1726 $ou_E\ m^{-3}$ and 90 $ou_E\ h^{-1}$, respectively. This is equivalent to about 216%. Increasing the manure temperature increases the emissions and the bacterial biogenesis of odorous compounds. Higher temperatures stimulate the formation of ammonia (Brunsch, 1997), hydrogen sulfide (Ni *et al.*, 2002), 4-methyl phenol (p-cresol), and 3-methyl indole (skatole) (Spoelstra, 1977) in manure. Therefore, it was expected that increased temperature was associated with increased odor concentration and emission from the manure in the vessel. This finding was consistent with that of Mol and Ogink (2003), who found that cooling off the upper layer of the manure and the air-boundary layer in a manure pit could reduce odor concentration and emission from animal houses. It should be mentioned, however, that in our experiment the ventilation air and the manure temperature were the same. With manure cooling, only the temperature of the surrounding air and the top layer of the manure in the manure pit is influenced by the cooling system, not the deep layer of the manure in the manure pit and the rest of the air in pig houses. Cooling the upper layer of the manure proved to be an important principle to reduce ammonia emission from pig houses. Our findings show that manure temperature has a big influence on both ammonia and odor emissions. A lower temperature gives lower emissions by slowing odor formation and odor release from the manure. On the other hand, a higher temperature stimulates

the breakdown of odorous compounds to end products such as methane and carbon dioxide. However, as discussed by Pain and Bonazzi (1993), this is a far slower process than the formation process.

If other independent variables were kept constant, $\ln(C_{odor})$ was reduced by 0.42 ($P < 0.05$) as the ventilation rate increased from 0.5 to 1.5 L min^{-1}. However, in that case, $\ln(E_t)$ increased by 0.68. When the ventilation rate increased from 0.5 to 1.5 L min^{-1}, the estimated odor concentration decreased by 600 ou_E m^{-3} (34%) and the estimated odor emission increased by 52 ou_E h^{-1} (97%). An increased ventilation rate provides higher dilution of odorous compounds with fresh air and so reduces odor concentration. Odor emission, however, is increased because of higher partial pressures between odorous compounds in the manure and in the air. The positive relationship between ventilation rate and odor emission found in this study was consistent with that of Oldenburg (1989) and Verdoes and Ogink (1997). In our study, ventilation rate was independent of temperature. This was normally not the case in previous studies, where the effect of ventilation rate on odor emission was more or less confounded with that of temperature and animal mass.

Increased manure dilution ratio was associated with increased odor concentration and odor emission ($P < 0.05$). When the manure dilution ratio increased from 0% to 100%, the estimated odor concentration and odor emission increased by 563 ou_E m^{-3} and 30 ou_E h^{-1}, respectively. This is equivalent to about 50%. The reason is probably that increased manure dilution reduced pH ($P < 0.05$) (Table 3). When the manure dilution ratio increased from 0% to 100%, the estimated pH decreased by 0.4, which might create favorable conditions for the emission of odorous compounds such as VFA. The other reason is that increased manure dilution ratio favored the dilution of odorous compounds from organic materials into liquid. Therefore, odorous compounds were more easily exchanged to the air. In addition, one might expect that the effect of manure dilution ratio on odor concentration and emission was partly confounded with that of headspace volume. Because the vessels had different surfaces but the same height (23.5 cm), they were different in the total volume and thus different in headspace volume. The total volumes of the vessels with 303, 475, and 595 cm^2 surfaces were 7116, 11164, and 13991 cm^3, respectively. Although, no significant effects of the interaction between dilution ratio and headspace volume on odor concentration and emission ($P > 0.05$) were found, and the correlation between dilution ratio and headspace volume was quite low (r = -0.28), the confounding effects of dilution ratio and headspace volume could not be fully excluded.

Emitting area did not significantly influence odor concentration and emission ($P > 0.05$). Our finding was not consistent with those of Mol and Ogink (2003) and Ogink and Groot

Koerkamp (2001). When measuring odor emission from manure pits at a certain point of time, they reported that reducing emitting area could reduce odor emission. There are three possible explanations for this inconsistency. First, most odorous compounds are less soluble in water than ammonia. They are quickly emitted to the air after being produced in the manure. As a result, emitting area was expected to have a significant effect on nitrogen loss (mainly in the form of ammonia) but not on less soluble odorous compounds. This can be confirmed by the significant effect ($P < 0.05$) of emitting area on total-N concentration in manure after the experiment, and on total-N loss (Table 3). Second, in our experiment, odor samples were collected from exactly the same amount of manure in all treatments. In the previous studies, the emitting area was probably confounded with the amount of manure. The system with a small emitting area generally had manure pits with less manure, and the manure was more often removed from the pig house. Third, the effect of emitting area on odor concentration and emission might be partly confounded with that of headspace volume (HSV). Actually, the two factors are highly correlated (r = 0.96).

The effects of emitting area and manure dilution ratio could not be fully separated from the effect of headspace volume in this study. The changes in headspace volume were due to adding dilution water and changing emitting area. In practice, similar confounding happens. Manure pits have different emitting areas and are recharged with different amount of manure and water. This creates different headspace volumes. Headspace volume might affect the air velocity above the emitting area, and thereby influence odor emission.

However, when adding headspace volume to the model that already contained temperature and ventilation rate, the effect of headspace volume was not significant. In addition, it is worth mentioning that adding headspace volume to the model that already contained emitting area (fitted terms: T, V, E, and HSV) or manure dilution ratio (fitted terms: T, V, D, and HSV) did not change the percentage of variance accounted for in model 1 (fitted terms: T, V, D, and E). All had the same percentage of variance and accounted for 63.3% of odor emission variance. From the preceding discussion, we conclude that headspace volume in our study had very little effect on odor emission from the manure vessel.

EFFECTS OF ENVIRONMENTAL FACTORS ON N LOSSES AND MANURE CHARACTERISTICS

Table 4 presents manure characteristics before and after the experiment. These include pH, dry matter, ash, VFA, ammonium-N, total-N, and total-N loss. Means and standard deviations (in parentheses) are presented to give a range of expected values of manure characteristics. The concentrations of individual VFA, total VFA, ammonium-N, total-N, and dry matter were

calculated per kg of manure and per kg of ash. The latter excludes the effect of dilution of the manure with water. After 7 days of running the experiment, the total VFA concentration per kg of ash was reduced by 69%, individual VFA concentrations per kg of ash was reduced in the range from 50% (iso-butyric acid) to 85% (propanoic acid), ammonium-N and total-N concentrations per kg of ash were reduced by 19% and 13%, respectively, and pH increased by 0.76.

Table 4. Descriptive statistics of manure characteristics before dilution of the manure and after the experiment

Variables	Before Dilution (n = 4)		After Experiment (n = 54)		Percent change per kg ash
	Per kg manure, Mean (SD)[a]	Per kg ash, Mean (SD)	Per kg manure, Mean (SD)	Per kg ash, Mean (SD)	
Dry matter (g kg^{-1})	34.72 (0.73)	2408 (49)	24.5 (7.4)	2244 (93)	-7
Ash (g kg^{-1})	14.42 (0.07)	--[b]	11.0 (3.4)	--	--
Total VFA (g kg^{-1})[c]	6.06 (0.15)	420 (10)	1.37 (0.87)	128.4 (72.1)	-69
Acetic acid (g kg^{-1})	4.11 (0.14)	285 (9)	0.97 (0.53)	92.1 (47.4)	-68
Propanoic acid (g kg^{-1})	1.09 (0.02)	75.4 (1.6)	0.13 (0.15)	11.65 (11.97)	-85
Butyric acid (g kg^{-1})	0.32 (0.02)	22.2 (1.8)	0.07 (0.07)	6.74 (6.14)	-70
Pentanoic acid (g kg^{-1})	0.048 (0.004)	3.30 (0.27)	0.010 (0.009)	0.92 (0.75)	-72
Iso-butyric acid (g kg^{-1})	0.193 (0.004)	13.42 (0.32)	0.07 (0.05)	6.70 (3.91)	-50
Iso-pentanoic acid (g kg^{-1})	0.302 (0.006)	20.98 (0.46)	0.11 (0.09)	10.37 (6.27)	-51
Total-N (g kg^{-1})	2.78 (0.01)	193.1 (1.6)	1.81 (0.49)	167.8 (17.4)	-13
Ammonium-N (g kg^{-1})	1.89 (0.02)	131.6 (1.1)	1.14 (0.31)	106.3 (16.4)	-19
pH	7.49 (0.06)	--	8.25 (0.41)	--	--

[a] SD = standard deviation.

[b] -- = not applicable.

[c] Total VFA=acetic acid+propanoic acid+butyric acid+pentanoic acid+iso-butyric acid+iso-pentanoic acid.

The pH of manure after the experiment can be explained in relationship with the ammonium and total VFA concentrations (both in g kg $^{-1}$) in the manure. The regression model is given in equation 6 (values in parentheses are standard errors):

$$pH = 7.88\,(0.15) + 0.932\,(0.16)\,\text{ammonium} - 0.50\,(0.06)\,\text{totalVFA} \qquad R^2 = 61\% \quad (6)$$

The model explained 61% of the variance in pH. Our study confirmed the results of Sommer and Husted (1995), Aarnink *et al.* (1996), and Canh *et al*. (1998a), who stated that pH of the manure is mainly affected by concentrations of ammonium and total VFA.

The VFA pool was largely dominated by short straight-chain VFA (acetic, propanoic, and butyric acids), which comprised 91% and 86% of total VFA in the manure before and after the experiment, respectively. This confirms the results of Miller and Varel (2003) and Otto *et al.* (2003). Acetic acid was the main VFA contributing to total VFA in the manure (68% and 70.6%, respectively, before and after the experiment), confirming the results of Farnworth *et al.*

(1995) and Canh *et al.* (1998c). Short branched-chain VFA contributed minimally to the total VFA concentration in the manure.

Dry matter of the manure before the experiment was 34.7 g kg^{-1}. This is a rather low concentration when compared to other studies, for example, Bakker *et al*. (2004), in which it was about 80 g kg^{-1}. There are two possible reasons for this observation. First, pigs may have played with the water drinker, resulting in water spillage on the floor and then into the manure pit. Second, manure was collected from the manure pit, which had not been emptied for two months, and conversions within the manure during storage could reduce its dry matter.

Table 3 presents the effect of environmental factors on total-N loss and manure characteristics during the experiment. The concentrations of total-N, ammonium-N, total VFA, and individual VFA were calculated per kg ash. Temperature influenced the concentrations of total-N, ammonium-N, total-N loss, and pH ($P < 0.01$), but not the concentrations of total VFA and individual VFA ($P > 0.05$), except for pentanoic and iso-butyric acids. When other independent factors were kept constant, total-N concentration decreased by 0.5% and total-N loss increased by 6% for each increase of 1°C. Ventilation rate had no significant effect on total VFA concentration, but a positive effect on total-N loss and a negative effect on pH ($P < 0.05$). When the other factors are kept constant, estimated total-N loss increased by 97.4% as ventilation rate increased from 0.5 to 1.5 L min^{-1}. Emitting area did not influence odor concentration, odor emission, total VFA concentration, and pH ($P > 0.05$). However, total-N concentration was reduced by 0.01% and total-N loss was increased by 0.09% ($P < 0.05$) as emitting area increased by 1 cm^2. This was expected because ammonia is soluble in water, and therefore its loss depends on emitting area. Increased dilution rate was associated with reduced total-N loss. Total-N loss decreased by 0.7% with each 1% increase in manure dilution. Manure dilution reduces ammonia concentration in the manure. According to Aarnink and Elzing (1998), ammonia emission is linearly related to ammonia concentration. Furthermore, manure dilution caused a lowering of the pH of the manure. A lower pH reduces ammonia volatilization as well (Sommer & Husted, 1995; Aarnink, 1997). The effects of two-way interactions of the environmental factors on manure characteristics were not consistent and are difficult to explain.

CONCLUSIONS

From the study on the effect of temperature, ventilation rate, emitting area, and manure dilution ratio on odor emission from manure and manure characteristics in a laboratory setup, we conclude the following:

- Increasing the temperature and the dilution ratio of the manure increased the odor concentration. When temperature increased from 10°C to 30°C and the manure dilution ratio increased from 0% to 100%, the odor concentration increased by 216% and 50%, respectively.
- Increasing the temperature and the dilution ratio of the manure increased the odor emission. When the temperature increased from 10°C to 30°C and the manure dilution ratio increased from 0% to 100%, the odor emission increased by 216% and 50%, respectively.
- Increasing the ventilation rate of the manure vessel reduced the odor concentration, but increased the odor emission. When the ventilation rate increased from 0.5 to 1.5 L min^{-1}, odor concentration decreased by 34% and odor emission increased by 97%.
- The emitting area of the manure surface did not influence odor concentration and emission.
- After running the experiment for seven days, total volatile fatty acid concentration decreased by 69%, total-N and ammonium-N concentrations decreased by 13% and 19%, respectively, and pH of the manure increased by 0.76.
- Total-N loss increased with temperature (6%/°C), with ventilation rate (an increase of 97.4% as ventilation increased from 0.5 to 1.5 L min^{-1}), and with emitting area (0.09% for each cm^2 larger area), but decreased with manure dilution ratio (0.7% for each 1% manure dilution).
- Temperature, ventilation rate, manure dilution ratio, and emitting area did not influence volatile fatty acid concentration.
- Increased ventilation rate and manure dilution ratio lowered the pH of the manure, but higher temperature increased the pH.
- Effects of manure dilution ratio and emitting area on odor emission and manure characteristics were partly confounded with headspace volume.

The results from this study confirmed the hypothesis that odor emission from pig manure can be reduced by altering environmental factors. Lowering the temperature and ventilation rate can be considered as possible measures to reduce odor emission from pig manure. We suggest that further studies on the effect of manure dilution ratio and emitting area on odor emission are required.

4

EFFECTS OF DIETARY CRUDE PROTEIN LEVEL ON ODOR FROM PIG MANURE

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ABSTRACT: *The objective of this study was to determine the effects of dietary crude protein (CP) level on odor emission, odor intensity, hedonic tone, and ammonia emission from pig manure and on manure composition (pH, total nitrogen, ammonium, volatile fatty acids, indolic, phenolic and sulfur-containing compounds). An experiment was conducted with growing pigs (n = 18) in a randomized complete block design with 3 treatments in 6 blocks. Treatment groups were 12%, 15% and 18% CP diets. Barley was exchanged for soybean meal. Crystalline amino acids (AA) were included in the 12% CP diet up to the level of pigs' requirement; the same amount of AA was added to the 15% and 18% CP diets. Pigs with an initial body weight (BW) of 36.5 ± 3.4 kg (mean ± SD) were individually penned in partly slatted floor pens and offered a daily feed allowance of 2.8 x maintenance requirement for net energy (NE: 293 kJ/$BW^{0.75}$). Feed was mixed with water, 1/2.5 (w/w). Feces and urine of each pig were accumulated together in a separate manure pit under the slatted floor. After an adaptation period of 2 wk, the manure pits were cleaned and manure was collected. In the 5th wk of the collection period, air samples for odor and ammonia analyses, and manure samples were collected directly from each manure pit. Odor samples were analyzed for odor concentration and for hedonic value and intensity above odor detection threshold. Manure samples were analyzed for volatile fatty acids, and indolic, phenolic and sulfurous compounds, ammonium and total N concentrations. Reducing dietary CP from 18% to 12% lowered odor emission (P = 0.04) and ammonia emission (P = 0.01) from pig manure by 80% and 53%, respectively. Reduced dietary CP decreased total N, methyl sulfide, carbon disulfide, ethanethiol, phenol, 4-ethyl phenol, indole and 3-methyl indole concentrations in the manure (P < 0.05). Volatile fatty acids and cresols concentrations in the manure of pigs fed different dietary CP levels were similar. A reduction of dietary CP and at the same time providing essential AA is an option to reduce odor emission as well as ammonia emission from pig manure.*

Key words: *Protein, Diet, Growing Pig, Odor*

INTRODUCTION

Odor emission from pig production facilities causes serious nuisance in the surrounding areas, and should therefore be reduced. Odor is mainly generated by the microbial conversion of feed in the intestinal tract of pigs and by microbial conversion of pigs' excreta under anaerobic conditions in manure storages. A great number of odorous compounds have been identified from animal production facilities. O'Neill and Phillips (1992) summarized 168 compounds in livestock wastes or in the surrounding air. Recently, Schiffman *et al*. (2001) identified a total of 331 different compounds in the air and lagoon water from pig production facilities. They can be classified into 4 main groups: (1) sulfurous compounds, (2) indolic and phenolic compounds, (3) volatile fatty acids (VFA), (4) ammonia and amines. Many of these compounds are intermediate or end products of protein degradation (Le *et al*., 2005a). Therefore, protein is probably the main dietary compound that should be altered to reduce odor strength and offensiveness. Odor is evaluated through its strength (odor concentration and odor intensity) and offensiveness (odor hedonic value).

Diets generally contain more protein than the pigs' requirement. The main reason is that the amino acids (AA) composition of dietary protein from feeds does not match the animal's requirement and thus the dietary levels are formulated to supply the minimum level of the most essential and limitting AA. This gives a surplus of other AA in the diet. Usually a large part of dietary protein and its metabolites is excreted via urine and feces (Aarnink, 1997; Blair *et al*., 1999). Reducing protein or N concentration in excreta decreases the availability of substrates that microbes can metabolize to odorous compounds. It is clear from the literature that ammonia from animal production facilities can be decreased considerably by reducing the amount of protein in the diet (Canh *et al.*, 1998; Hobbs *et al.*, 1998; Zervas & Zijlstra, 2002) or by adding crystalline AA to the diet. However, in the case of odor strength and offensiveness the situation is not so straightforward.

Up to now, scientists mainly focused on certain specific odorous compounds in the manure or in the odorous air, e.g. Hobbs et al. (1996), and Sutton *et al*. (1998). Little attention has been given to the effect of dietary crude protein (CP) levels on odor strength and odor offensiveness measured by olfactometry. The main objective in this study was to determine the effects of dietary crude protein levels on odor strength, odor offensiveness, and ammonia emission from manure of growing pigs and on manure characteristics.

MATERIALS AND METHODS

ANIMALS, EXPERIMENTAL DESIGN, AND DIETS

A randomized complete block arrangement with 3 treatments in 6 blocks was used to study effects of dietary CP level on odor concentration, odor emission, odor intensity, odor hedonic tone, and ammonia emission from growing pig manure and on manure characteristics (pH, and concentrations of total N, ammonium, VFA, and indolic, phenolic and sulfurous compounds). There were 3 dietary CP levels: 12, 15, and 18%. Each treatment was replicated 6 times, 1 replicate in each of 6 blocks, of which a block consisted of samples collected on the same day and from animals with similar initial body weight (BW).

In total 18 growing barrows, Great Yorkshire x (Great Yorkshire x Dutch Landrace) with an initial BW of 36.5 ± 3.4 kg (mean ± SD) were randomly allocated to one of the 3 diets within each of the 6 blocks. Pigs were penned individually in galvanized steel pens (2.1 x 0.96 m) with a slatted floor at the rear (0.97 x 0.96 m). There was a separate manure pit under the slatted floor of each pen. The volume of the manure pit was 1.35 x 0.91 x 0.36 m (length x width x depth). Pigs were penned in a mechanically ventilated and temperature-controlled room. Temperature and relative humidity were recorded every 5 min. The average temperature and relative humidity of the room during the experimental period were 21.0 ^{0}C ± 0.84 and 50.0 % ± 5.32 (mean ± SD), respectively.

Three diets with dietary CP levels of 12, 15 and 18% were formulated. Barley was exchanged for soybean meal to increase the CP level. Diets had similar contents of net energy (NE), minerals, and vitamins but differed in CP levels by varying the amount of extracted soybean meal (Tables 1 and 2). Diets were supplemented with essential AA e.g. Lys, Trp, Thr and Met. The method of supplementing amino acids (AA) was as follows: first, we supplemented the 12% CP diet with these mentioned AA up to the level of animal requirement based on apparently ileal AA digestibility (CVB- Animal feed product board, 2004). Then, we supplemented 15 and 18% CP diets with the same amount of these AA as supplemented in the 12% CP diet. This was done to study solely the effect of the CP levels and prevent confounding effects with AA supplementation. Other constituents, for example, fibrous components, and dietary electrolyte balance (dEB), which could possibly affect odor production, were equalized.

Experimental diets were analyzed for AA composition, ash, dry matter (DM), CP, minerals, crude fiber, fat, starch, sugar and gross energy. The AA (except Met, Cys, and Trp) were assayed by ion-exchange column chromatography after hydrolysis for 23 hours in HCl (6

mol L^{-1}). Cystine and Met were determined as cysteic acid and methionine sulfone after oxidation with performic acid before hydrolysis (Schram *et al*., 1954). Tryptophane was determined according to Sato *et al*. (1984).

Table 1. Ingredient composition of experimental diets, as-fed basis

Composition (%)	Diet		
	12%CP	15%CP	18%CP
Barley	48.98	38.60	28.23
Tapioca meal (starch 62.5-65.7%)	30.00	30.00	30.00
Soybean meal extracted (crude fiber <5%)	11.30	19.28	27.25
Wheat middlings	1.48	3.84	6.20
Cane molasses (sugar< 47.5%)	3.00	3.00	3.00
K_2CO_3	0.57	0.29	0.00
Soybean oil	1.52	1.94	2.36
$CaCO_3$	0.99	0.98	0.97
Monocalcium phosphate.H_2O	0.71	0.63	0.55
Salt	0.49	0.49	0.49
Premix[a]	0.20	0.20	0.20
L-Lysine HCl	0.41	0.41	0.41
DL-Methionine	0.15	0.15	0.15
L-Threonine	0.15	0.15	0.15
L-Tryptophan	0.04	0.04	0.04

[a] The vitamin-mineral premix supplied per kg feed included 7,000 IU vitamin A, 1,700 IU vitamin D3, 20 IU vitamin E, 1.5 mg vitamin K, 1.5 mg vitamin B1, 4 mg vitamin B2, 11 mg d-pantothenic acid, 18 mg niacine, 18 µg vitamin B12, 0.1 mg folium acid, 1.0 mg vitamin B6, 100 mg choline chloride, 75 mg Fe, 10 mg Cu, 65 mg Zn, 30 mg Mn, 0.15 mg Co, 0.75 mg I, 0.30 mg Se.

Starch content was determined enzymatically according to the amyloglucosidase/ hexokinase method (NEN 3574). Sugar was assayed according to the non-starch polysaccharides (NSP) procedure (Gelder *et al*., 1992). Crude fiber was determined gravimetrically after treatment with sulfuric acid and potassium hydroxide (ISO/DIS 6895). For total fat, samples were hydrolyzed with chloric acid, followed by extraction of fat with petroleum ether. The Ca, P, Mg, Na, K, Cu and Zn contents were determined using the inductively coupled plasma atomic emission spectrometry (ICP-AES). The Cl content was determined by potentiometric titration of water-diluted solid samples with the chloride specific ion electrode (Jenway Chloride Meter, model PCLM3). For sulfate, samples were extracted with chloric acid. Sulfate was separated with ion chromatography using a water // sodium hydroxide gradient and an Ionpac AS 11 (Dionex) as column. Detection takes place by

suppressed electric conductivity. Identification and quantification occurs using an external standard solution. The DM was determined gravimetrically after 4 h at 103°C (ISO 6496). The content of ash was determined gravimetrically after ashing at 550 °C (ISO 5984). Nitrogen content was determined by the Kjeldahl method (ISO 5983). The analyzed dietary CP levels of 12%CP, 15%CP and 18%CP on an as-fed basis were 12.3, 14.2 and 18.0%, respectively (Table 2).

Table 2. Nutrient composition of experimental diets, as-fed basis

Composition	Diets			
	Unit	12%CP	15%CP	18%CP
Calculated composition				
Crude protein (CP)	%	12.00	15.00	18.00
Fecal digestible CP	%	9.48	12.41	15.33
Ileally digestible CP	%	8.92	11.58	14.23
NE	kcal/kg	2,175	2,175	2,175
NSP[a]	%	17.31	17.99	18.67
Digestible NSP	%	8.96	9.97	10.99
Illegally digestible Amino acids				
Lys	%	0.74	0.93	1.12
Met	%	0.29	0.32	0.36
Cys	%	0.15	0.18	0.22
Met+ Cys	%	0.44	0.51	0.58
Thr	%	0.43	0.53	0.64
Trp	%	0.14	0.18	0.21
Analyzed composition				
Dry matter	%	86.80	86.04	87.15
CP (Nx6.25)	%	12.28	14.24	18.03
Ash	%	6.38	6.03	6.37
Crude fiber	%	3.26	3.56	3.45
Crude fat	%	1.99	2.37	3.88
Gross energy	kcal/kg	3,668	3,693	3,873
Sugar	%	5.71	5.99	6.49
Starch	%	41.07	37.98	34.85
NSP	%	19.36	19.43	17.53
Na	%	0.20	0.19	0.20
K	%	1.06	1.04	1.07
Sulfate	%	0.11	0.11	0.11
Chlorine	%	0.52	0.46	0.46
dEB[b]	meq/kg	213	220	227
dEBS-a[c]	meq/kg	190	196	204

Amino acids				
Ala	%	0.59	0.66	0.85
Arg	%	0.70	0.90	1.22
Asp	%	1.04	1.35	1.95
Cys	%	0.18	0.19	0.25
Glu	%	2.20	2.51	3.36
Gly	%	0.53	0.62	0.76
His	%	0.49	0.54	0.72
Ile	%	0.46	0.57	0.77
Leu	%	0.82	0.97	1.30
Lys	%	0.83	0.99	1.25
Met	%	0.27	0.30	0.34
Phe	%	0.58	0.70	0.91
Pro	%	0.73	0.79	1.00
Ser	%	0.60	0.73	0.90
Thr	%	0.55	0.64	0.73
Trp	%	0.17	0.20	0.25
Tyr	%	0.40	0.50	0.67
Val	%	0.58	0.68	0.89

[a] Non-starch polysaccharides (NSP) were calculated as organic matter–(CP+crude fat+starch+ sugar).

[b] dEB was determined as mEq = Na + K – Cl.

[c] dEBS-a was determined as mEq = Na+K–Cl–2S. dEBS-a does not take into account S present in AA.

Pigs were fed 2.8 times the maintenance NE requirement (293 kJ/$BW^{0.75}$). Water was restrictedly provided by mixing feed with water in the ratio of 1/2.5 (w/w). Apart from water with feed no additional water was given to the pigs. So it was aimed to have the same amount of feed and water intake by the pigs to excrete almost a similar amount of manure. Pigs were fed 2 times per day at 0800 and 1500. The amount of feed provided was adjusted each day according to the expected BW gain of 750 g d^{-1}. Feed intake was recorded every day. Pigs were weighed at the beginning and at the end of the experimental period just before the morning feeding. Daily weight gain and feed efficiency were obtained from the feed intake and the increase in BW during the experimental period.

After an adaptation period of 2 wk to allow pigs to acclimatize to the experimental diets, pens and manure pits were cleaned. Subsequently, feces and urine were accumulated together in the manure pit. In the 5th wk of the collection period, air samples for odor and ammonia measurements and manure samples were collected for subsequent analyses.

ODOR SAMPLE COLLECTION AND MEASUREMENT OF ODOR CONCENTRATION, ODOR HEDONIC TONE AND ODOR INTENSITY

Collection of odor samples. Odor samples were used to measure odor concentration, odor hedonic tone and odor intensity. Odor samples were collected directly from air above the manure in the pit. A schematic view of the odor sampling set up is shown in Fig. 1. A vessel without a bottom was placed in the middle of the manure pit. The bottom of the vessel touched the bottom of the manure pit. The net surface of the vessel was 595 cm^2; and the diameter was 28 cm. The vessel was divided into 2 compartments by a lid. The net height of the lower compartment was 40 cm and the net height of the upper compartment was 20 cm.

Air entering the upper compartment of the vessel from a pressurized cylinder was odor-free air. Air entered the lower compartment of the vessel via 24 holes of one mm diameter each, located at the edge of the lid. Air was exhausted from the vessel by a hole of five mm diameter in the middle of the lid.

The outgoing odor air from the vessel was split into 2 streams. One stream was used to collect the odor sample. It was connected to an odor-sampling bag placed in a rigid container. This container was connected to a critical glass capillary, which had a flow rate of 0.5 L min^{-1}, and then to a vacuum pump. The other stream was used to collect ammonia and was connected to 2 connected impingers. The outgoing air from the impinger was connected to a critical glass capillary, which had a flow rate of 0.5 L m^{-1}, and then to the vacuum pump.

Odor samples were collected according to the European standard (CEN standard 13725, 2003). The sampling method for delayed olfactometry was applied using the 'lung principle'. A 40 liter Nalophaan odor sampling bag was placed in a rigid container. The sample bag had been flushed with compressed and odorless air 3 times before it was placed in a rigid container for collection of the odor sample. The sample bag was used once for each odor sample as recommended by European standard (CEN standard 13725, 2003). The air was removed from the container by the vacuum pump. The lower pressure in the container caused the bag to fill with a volume of sample air equal to the volume removed from the container (Fig. 1).

One odor sample was collected from each manure pit. During transport and storage, odor samples were kept at a temperature above the dew point of the sample to avoid condensation. This was achieved by warming the rigid container of the odor bag to about 4 ^0C above the ambient temperature. The interval between sampling and measuring odor concentration did not exceed 30 h, as recommended by European standard (CEN standard 13725, 2003).

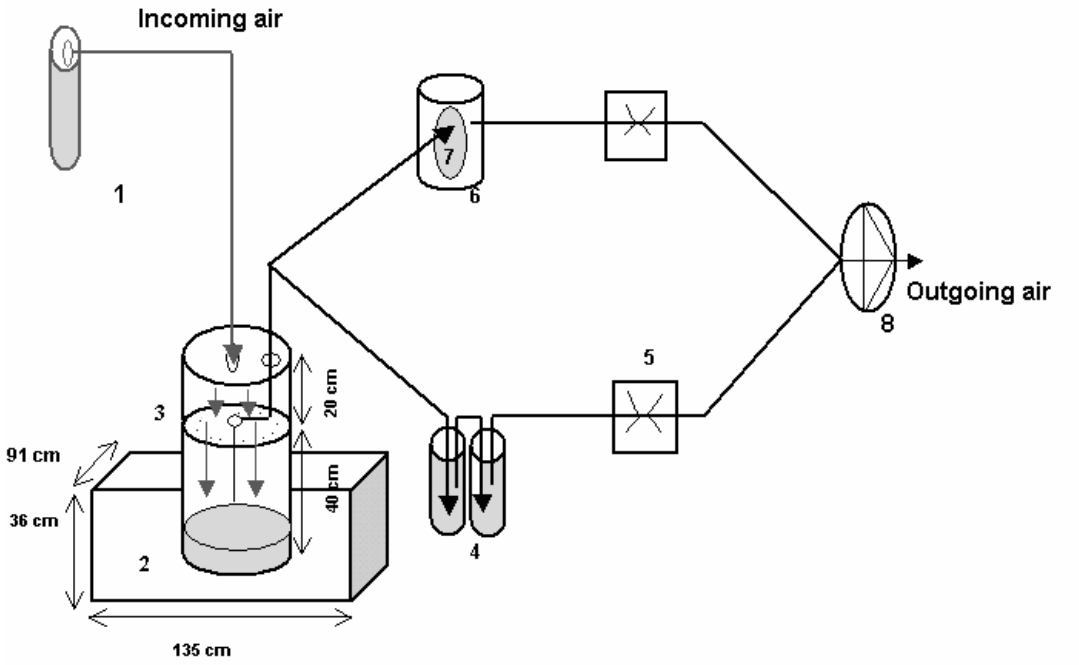

Fig. 1. Schematic view of the odor and ammonia sample collection (1 = odor free air pressurized cylinder, 2 = manure pit, 3 = vessel, 4 = impingers, 5 = critical glass capillary, 6 = rigid container, 7 = odor bag, 8 = vacuum pump)

Measurement of odor concentration. Odor concentration was measured by olfactometry according to the European standard (CEN standard 13725, 2003) as described in detail by Le *et al*. (2005b). Odor concentrations of the examined samples were expressed in European odor units per cubic meter air ($ou_E\ m^{-3}$). One odor unit is defined as the amount of odor-causing gases which, when diluted in 1 m^3 of air, can just be distinguished from clean air by 50% of the members of an odor panel.

Odor emission was defined as the number of odor units emitted from a manure surface per second and it was obtained by multiplying the ventilation rate with the corresponding odor concentration (Equation 1).

$$E_{odor} = (C_{odor} \times V \times 10{,}000) / (60 \times 1{,}000 \times 595) \quad [1]$$

where E_{odor} = odor emission $s^{-1}\ m^{-2}$ ($ou_E\ s^{-1}m^{-2}$), C_{odor} = odor concentration ($ou_E\ m^{-3}$), and V = ventilation rate (L min^{-1}), 10,000 = $cm^2\ m^{-2}$, 60 = sec min^{-1}, 1,000 = liters m^{-3}, and 595 = the cm^2 surface area of the manure pit.

Measuring odor hedonic tone and odor intensity. Hedonic tone (H) is used to evaluate the odor offensiveness, which is a measure of the unpleasantness or pleasantness of the perceived odor above the odor detection threshold. Odor intensity (I) refers to the magnitude of the odor sensation and is a measure of the intensiveness of the odor above the odor detection threshold. Odor intensity and hedonic tone were measured at the same time by olfactometry and

were determined by the same panel members as for odor concentration. The principle of the measurement is to vary the odor concentration and thus to vary hedonic value and intensity. The odor concentration varied randomly in 5 dilution factors above the detection threshold. At each presentation, each panelist was asked to indicate the perceived hedonic value, using a 9-point hedonic scale ranging from - 4, extremely unpleasant or offensive through 0, neither pleasant nor unpleasant or neutral odor to + 4, extremely pleasant. The panelist was also asked to indicate the perceived odor intensity using a 7-point intensity scale ranging from 1, no odor through 2, very faint odor to 7, overwhelming odor. For each odor sample, the hedonic tone and the odor intensity at each odor concentration level above the detection threshold were calculated as the average of the hedonic tone and the odor intensity perceived by all panelists, and plotted against the logarithm of the odor concentration. From the regression lines obtained, the odor concentration at H = -1 (mildly unpleasant), H = -2 (moderately unpleasant), I = 1 (no odor), I = 2 (very faint odor), I = 4 (distinct odor) were derived. Regression lines of the hedonic tone and the odor intensity were also plotted against the logarithm of the odor concentration for all samples in the same treatment.

MEASURING AND CALCULATING AMMONIA EMISSION

Samples for determining ammonia emission were collected at the same time as odor samples. Fig. 1 gives a schematic view of the ammonia emission measurement and collection procedure. Ammonia in the outgoing air was removed by passing through 2 impingers (ammonia trap), each containing about 20 ml 0.5 M HNO_3 solution. The system was run for about 90 min. The ammonia concentration and the volume of the liquid were determined in the first and the second impingers. Ammonia emission per time unit and surface unit was calculated with Equation 2.

$$MNH_3 = (CNH_3 \times V \times 10{,}000) / (T \times 60 \times 595) \quad [2]$$

where MNH_3 = ammonia emission (mg s^{-1} m^{-2}), CNH_3 = ammonia concentration (mg mL^{-1} HNO3), V = volume of HNO_3 solution (mL), 10,000 = cm^2 m^{-2}, T = sampling time (min), 60 = s min^{-1}, and 595 = the cm^2 surface area of the manure pit.

COLLECTING AND ANALYZING MANURE SAMPLES

Manure samples were analyzed to evaluate the effect of the diets on manure characteristics. Analyses included DM, ash, total N, ammonium, pH, VFA (acetic, propionic, butyric, pentanoic, iso-butyric, iso-pentanoic, hexanoic, and heptanoic acid), indolic (indole and 3-methyl indole) and phenolic compounds (phenol, 4-ethyl phenol, and cresols), and sulfurous

compounds (carbon disulfide, methyl sulfide, methyl disulfide, and ethanethiol). Immediately after collecting odor samples, manure in each manure pit was mixed thoroughly before a sample of about 1 kg was collected. Manure samples were stored at - 20 ^{0}C until analysis.

Ammonium-N was determined spectrophotometrically according to NEN 6472 (Derikx *et al*., 1994). Volatile fatty acids were measured using a Packard 427 gas chromatograph, equipped with a flame ionization detector. Manure pH was measured by a pH electrode. For determination of indolic and phenolic compounds and sulfurous compounds, 2.5 g fresh manure was extracted with 15 ml 50% methanol for two hours. The sample was centrifuged and the supernatant was analyzed by the HPLC. The HPLC conditions were a water-methanol gradient as elution solution and Alltima C18 (Alltech) as column. Detection was done by UV-absorption at 200 nm. For identification and quantification, an external standard solution was used.

STATISTICAL ANALYSIS

The effect of dietary CP levels on daily gain, daily feed intake, feed efficiency, odor emission, odor hedonic value, odor intensity, ammonia emission, and manure characteristics were analyzed using ANOVA of GenStat statistical package 7th version (GenStat VSN International Ltd., 2004) with the following model.

$y_{ij} = \mu + \rho_j + \alpha_i + e_{ij}$ where: y_{ij}: dependent variables, μ: overall mean, ρ_j: effect of block, j = 1-6, α_i: effect of diet i = 1, 2, 3, e_{ij}: experimental error.

Data are presented as either arithmetic or geometric mean. A natural log transformation was applied to odor emission, concentrations of VFA, total N and ammonium-N, indolic and phenolic, and sulfurous compounds since they were skewed and not normally distributed.

In each treatment, odor hedonic tone and odor intensity were plotted against the natural logarithm of odor concentration; and odor hedonic tone was plotted against odor intensity. The differences between slopes and between intercepts were tested to decide whether there should be separate regression lines for treatments or a common line for all treatments. The relationship between ammonia emission and odor emission was determined by linear regression.

RESULTS

EFFECTS OF DIETARY CRUDE PROTEIN LEVEL ON DAILY GAIN, DAILY FEED INTAKE AND FEED EFFICIENCY

To detect whether there are any effects of different dietary CP levels on production parameters, the daily gain, daily feed intake and feed efficiency are summarized in Table 3. No

effects of the protein levels on average daily feed intake, daily gain, and feed efficiency were observed ($P > 0.05$), although feed efficiency approached significance.

Table 3. Effects of dietary CP level on daily gain, feed intake and feed efficiency

Variables	Diets			SEM[a]	*P* value
	12%CP	15%CP	18%CP		
Initial BW, kg	36.7	36.2	36.5	0.4	0.66
Final BW, kg	65.7	66.9	68.6	1.2	0.26
ADFI, kg/ day	1.7	1.7	1.7	0.03	0.76
ADG g/ day	629	668	697	22.3	0.15
G:F, g/kg	371	387	409	9.7	0.052

[a] SEM = Standard errors of the means with 10 df for error.

EFFECTS OF THE DIETARY CRUDE PROTEIN LEVEL ON ODOR STRENGTH AND OFFENSIVENESS

Descriptive statistics and analysis of variance of effects of the dietary CP levels on odor strength and offensiveness from pig manure are given in Tables 4 and 5, respectively. Geometric means of odor concentration and odor emission from pig manure were highest at the 18%CP treatment, 31,888 $ou_E\ m^{-3}$ and 4.46 $ou_E\ s^{-1}\ m^{-2}$, respectively. The 12%CP treatment had the lowest odor concentration and lowest odor emission, 7,259 $ou_E\ m^{-3}$ and 1.03 $ou_E\ s^{-1}\ m^{-2}$, respectively.

Table 4. Odor strength (concentration and intensity), odor emission and offensiveness (hedonic tone) from manure of growing pigs fed different dietary CP levels (*n = 18*). Geometric and arithmetic means are given

Odor variables	Diets					
	12%CP		15%CP		18%CP	
	GM[a]	AM[b]	GM	AM	GM	AM
Concentration, $ou_E\ m^{-3}$	7,259	8,360	13,226	21,218	31,888	40,904
Emission, $ou_E\ s^{-1}m^{-2}$	1.03	1.18	1.85	2.94	4.46	5.76
Concentration at H[c] = -1, $ou_E\ m^{-3}$	2.04	2.33	3.52	3.71	1.30	1.47
Concentration at H = -2, $ou_E\ m^{-3}$	5.26	5.71	9.68	10.07	4.71	4.98
Concentration at I[d] = 1, $ou_E\ m^{-3}$	0.61	0.74	0.90	0.95	0.50	0.51
Concentration at I = 2, $ou_E\ m^{-3}$	1.32	1.53	2.01	2.08	1.20	1.23
Concentration at I = 4, $ou_E\ m^{-3}$	6.23	6.74	9.87	10.20	7.10	7.47

[a] GM = geometric mean; [b] AM = Arithmetic mean; [c] Hedonic tone; [d] Intensity.

Table 5. Effects of the dietary crude protein levels on odor strength (concentration and intensity (I)) and offensiveness (hedonic tone (H)) from growing pig manure

Variables	Diets			SEM[a]	*P* value
	12%CP	15%CP	18%CP		
ln[b] (Odor concentration)	8.89[c]	9.49[cd]	10.37[d]	0.35	0.04
ln (Odor emission)	0.03[c]	0.62[cd]	1.49[d]	0.35	0.04
ln (Odor concentration at H = -1)	0.71[c]	1.26[c]	0.26[d]	0.16	0.004
ln (Odor concentration at H = -2)	1.66[c]	2.27[d]	1.55[c]	0.16	0.02
ln (Odor concentration at I = 1)	-0.49	-0.10	-0.69	0.21	0.2
ln (Odor concentration at I = 2)	0.28	0.70	0.18	0.19	0.2
ln (Odor concentration at I = 4)	1.83	2.29	1.96	0.17	0.2

[a] SEM = Standard errors of the means with 10 df for error.

[b] Natural logarithm.

[c, d] Means within rows missing a common superscript letter are different at $P < 0.05$.

Analyses of variance show that the dietary CP level affected both odor concentration and odor emission from pig manure ($P = 0.04$) (Table 5). Further analyses show that the 18%CP treatment had a higher odor concentration and odor emission than the 12%CP treatment ($P < 0.05$). The 15%CP treatment had intermediate results, but did not differ from 12%CP treatment nor from 18%CP treatment ($P > 0.05$).

At H = -1, odor concentration of manure of pigs fed the 18%CP diet was lower than that of the other two diets ($P = 0.004$), while it was similar for the other 2 diets. At H = -2, there were no differences in odor concentration of manure of pigs fed either the 12%CP or 18%CP diet. Odor concentration (at H = - 2) of the air from manure of pigs fed either the 18%CP or 12%CP diet was lower than that of the 15%CP diet ($P < 0.05$). The odor concentration of the air from manure of pigs fed different dietary CP levels was similar at different levels of odor intensity measured above detection threshold (Table 5).

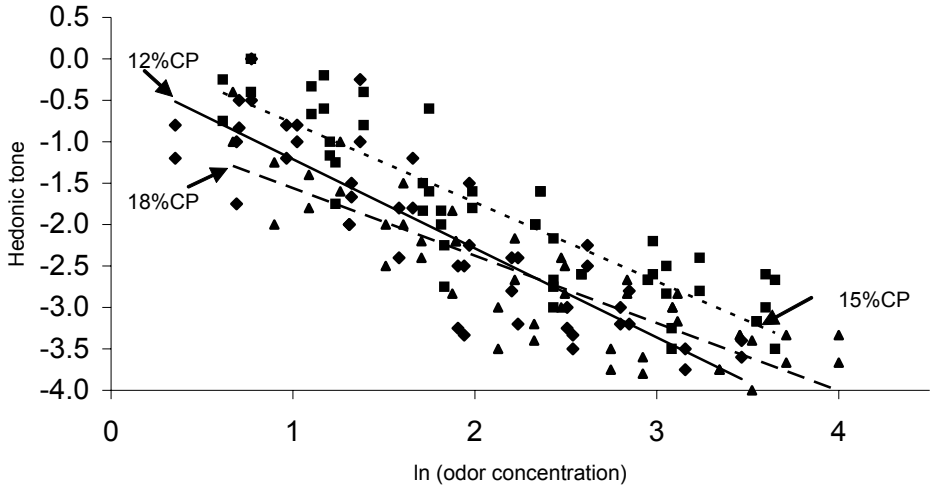

Fig. 2. Hedonic tone (H) as a function of odor concentration with regression lines, $H_{12\%CP}$ = -0.09 (0.16) – 1.11 (0.08) ln (odor concentration), indicated by ___ ♦; $H_{15\%CP}$ = 0.18 (0.18) – 0.95 (0.07) ln (odor concentration), indicated by ……■; $H_{18\%CP}$ = -0.87 (0.18) – 0.75 (0.07) ln (odor concentration), indicated by __ __▲, R^2 = 78.3%

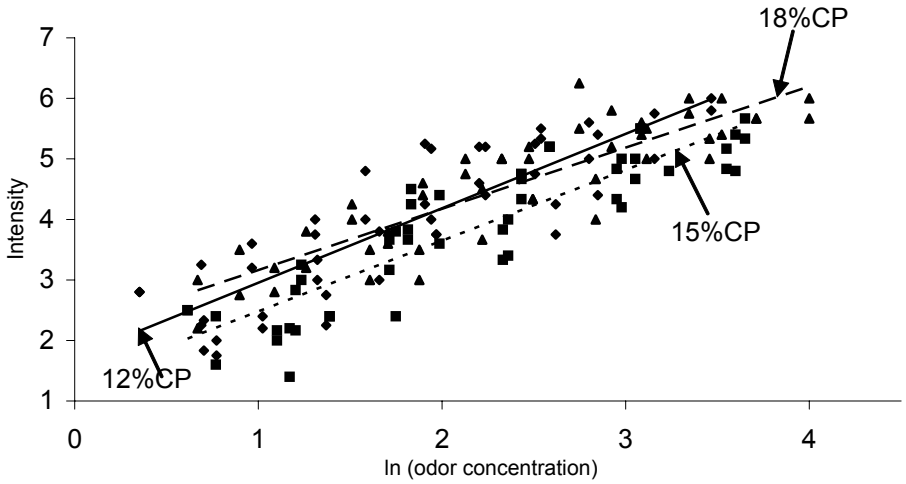

Fig. 3. Odor intensity (I) as a function of odor concentration with regression lines, $I_{12\%CP}$ = 1.95 (0.13) + 1.13 (0.05) ln (odor concentration), indicated by ___ ♦; $I_{15\%CP}$ = 1.40 (0.14) + 1.13 (0.05) ln (odor concentration), indicated by ……■; $I_{18\%CP}$ = 1.83 (0.15) + 1.13 (0.05) ln (odor concentration), indicated by __ __▲, R^2 = 77.5%

Relationships between odor concentration and hedonic tone, between odor concentration and intensity, and between intensity and hedonic tone are shown in Figs 2, 3 and 4,

respectively. Both intercept and slope of the relationship between hedonic tone and odor concentration were different among treatments ($P < 0.05$). Only the intercepts of the relationship between hedonic tone and intensity and between intensity and odor concentration were different among treatments ($P < 0.05$).

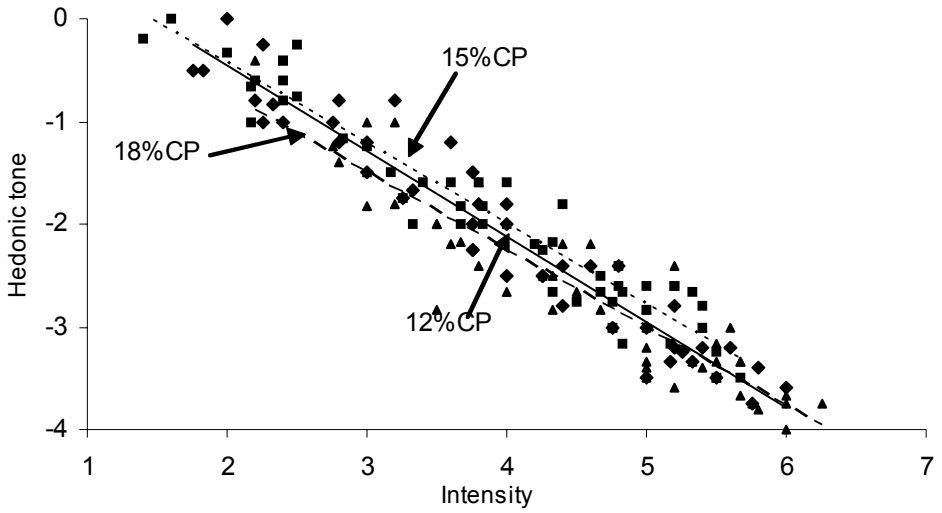

Fig. 4. Hedonic tone (H) as a function of odor intensity (I) with regression lines, $H_{12\%CP}$ = 1.03 (0.09) - 0.79 (0.02) I, indicated by ___ ♦; $H_{15\%CP}$ = 1.13 (0.09) – 0.79 (0.02) I, indicated by ……■; $H_{18\%CP}$ = 0.91 (0.10) – 0.79 (0.02) I , indicated by __ __▲, R^2 = 91.8%

EFFECTS OF DIETARY CP LEVEL ON MANURE CHARACTERISTICS AND AMMONIA EMISSION

Tables 6 and 7, respectively, show the descriptive statistics and the analyses of variance of the effects of different dietary CP level on manure characteristics including VFA, indolic and phenolic and sulfurous compounds, total N and ammonium-N concentrations, pH and ammonia emission. Reduced dietary CP levels decreased total N, methyl sulfide, carbon disulfide, ethanethiol, phenol, indole, 3-methyl indole, and 4-ethyl phenol concentrations ($P < 0.05$), pH ($P < 0.01$) and ammonia emission from pig manure ($P = 0.01$). Reduced dietary CP levels caused a tendency to decrease ammonium-N concentration in pig manure ($P = 0.07$).

Table 6. Descriptive statistics of manure characteristics and ammonia emission from manure of pigs fed different dietary CP levels (*n = 18*)

Variables	Diets		
	12%CP	15%CP	18%CP
Dry matter, g kg^{-1}	128.2 (24.09)[a]	119.6 (16.95)	123.4 (10.09)
Ash, g kg^{-1}	41.3 (4.41)	37.4 (3.82)	37.8 (2.47)
Total VFA[b], g kg^{-1}	7.41 (3.73)	6.35 (1.89)	7.58 (2.14)
Acetic acid, g kg^{-1}	4.23 (1.94)	3.98 (0.99)	4.65 (1.12)
Propionic acid, g kg^{-1}	1.72 (0.95)	1.25 (0.5)	1.47 (0.5)
Butyric acid, g kg^{-1}	1.0 (0.82)	0.5 (0.32)	0.7 (0.4)
Iso-butyric acid, g kg^{-1}	0.13 (0.05)	0.20 (0.06)	0.25 (0.11)
Iso-pentanoic acid, g kg^{-1}	0.33 (0.08)	0.45 (0.14)	0.53 (0.18)
Total N, g kg^{-1}	5.78 (0.97)	6.24 (1.09)	7.25 (0.73)
Ammonium-N, g kg^{-1}	1.91 (0.48)	2.51 (0.5)	2.86 (0.65)
pH	7.10 (0.34)	7.52 (0.17)	7.83 (0.27)
Ammonia emission, mg $s^{-1}m^{-2}$	0.008 (0.005)	0.009 (0.001)	0.017 (0.001)
Phenol, mg kg^{-1}	9.10 (2.5)	17.32 (6.97)	32.73 (12.49)
Cresols, mg kg^{-1}	38.18 (8.41)	39.58 (9.72)	41.42 (7.3)
4-ethyl phenol, mg kg^{-1}	1.60 (0.60)	5.84 (1.38)	11.75 (1.58)
Indole, mg kg^{-1}	5.96 (0.92)	9.77 (3.8)	10 (1.7)
3-methyl indole, mg kg^{-1}	5.30 (1.0)	4.80 (0.9)	8.74 (2.21)
Carbon disulfide, mg kg^{-1}	3.33 (0.56)	7.05 (0.71)	9.06 (1.67)
Methyl sulfide, mg kg^{-1}	1.35 (1.42)	8.13 (2.17)	6.48 (2.83)
Ethanethiol, mg kg^{-1}	78.82 (19.1)	81.9 (16.8)	104.6 (14.5)

[a] Standard deviation.

[b] Total VFA = acetic acid + propinoic acid + butyric acid + iso-butyric acid + iso-pentanoic acid.

Dietary CP levels did not influence cresols, and total and individual VFA concentrations in pig manure. Although branched-chain VFA concentrations in pig manure: iso-butyric, iso-pentanoic acids were reduced by decreasing dietary CP levels, they were not statistically different, $P = 0.08$ and $P = 0.1$, respectively. Methyl disulfide, hexanoic, heptanoic and pentanoic acids were not detected in the manure of the different treatments. The detection limits of methyl disulfide and the three VFAs are 0.5 mg/kg and 0.1 g/kg, respectively. The correlation between ammonia and odor emission was 0.1.

Table 7. Effects of dietary crude protein levels on manure characteristics and ammonia emission, only significant effects ($P < 0.05$) are presented

Variables	Diets			SEM[a]	*P* value
	12%CP	15%CP	18%CP		
ln[b] (total N)	1.73[c]	1.82[cd]	1.98[d]	0.06	0.03
ln (ammonium-N)	7.54	7.81	7.93	0.11	0.07
ln (methyl sulfide)	0.7[c]	2.19[d]	1.96[d]	0.2	< 0.01
ln (carbon disulfide)	1.19[c]	1.9[d]	2.2[e]	0.04	< 0.01
ln (ethan ethiol)	4.3[c]	4.4[c]	4.6[d]	0.08	0.04
ln (phenol)	2.18[c]	2.8[d]	3.43[e]	0.14	< 0.01
ln (3-methyl indole)	1.65[c]	1.55[c]	2.14[d]	0.08	< 0.01
ln (indole)	1.77[c]	2.22[d]	2.29[d]	0.35	0.02
ln (4-ethyl phenol)	0.20[c]	1.64[d]	2.46[e]	0.19	< 0.01
pH	7.1[c]	7.52[d]	7.83[d]	0.4	< 0.01
ln (ammonia emission)	-5.03[c]	-4.70[c]	-4.21[d]	0.15	0.01

[a] Standard errors of the means with 10 df for error.

[b] Natural logarithm.

[c,d,e] Means within rows missing a common superscript letter are different at $P < 0.05$.

DISCUSSION

Odor nuisance from pig production facilities is of growing concern in residential areas. It is preferable that odor abatement solutions are done at the source instead of end-of-pipe. Diet is the first step in the odor production chain from feed to manure. It is generally accepted that dietary alterations can significantly reduce odor from pig manure (Le *et al*., 2005a). It is well-documented that odor is generated by microbial conversion of nutrient residues in the gut of animals and during storage of manure. Dietary protein is one of the most important precursors for odor production thus it is expected that odor produced from pig manure will be reduced as dietary crude protein (CP) level decreases. In addition, it is expected that odor is decreased when most unabsorbed AA are used for microbial growth, provided that sufficient carbohydrates are available for microbes. If not, then AA are used as an energy source. In this study, odor concentration and emission decreased nearly 80% by reducing dietary CP from 18% to 12%. Literature supplies very little information on the effects of dietary CP level on odor concentration and odor emission from pig manure as measured by olfactometry. It is difficult to compare absolute values of odor concentration and emission among different studies, because they use different types of measuring standards, sampling methods, animal types, diet composition, feeding strategies, housing systems, environmental conditions, etc. All these

factors could affect odor concentration and emission from pig manure and the pig housing facilities. This study confirmed the finding of Hayers *et al*. (2003) who found that odor emission reduced by 31% and 33%, respectively, by decreasing dietary CP content from 19% to 16% and 13%. Obrock *et al*. (1997) however found no difference in odor concentration between finishing pigs fed 13% and 9% CP with AA supplemented diets. Possibly, the protein levels in the study of Obrock *et al*. (1997) were so low that they did not cause any difference in odor concentration from pig manure or had sufficiently high level of fermentable carbohydrates.

Reduced dietary CP levels decreased the concentrations of indolic and phenolic compounds (phenol, indole, 3-methyl indole and 4-ethyl phenol) and sulfurous compounds (methyl sulfide, carbon disulfide, ethanethiol) in pig manure. Protein is the precursor for the production of these compounds in the gut of animals and in the manure. Odorous compounds produced in the large intestine of animals are excreted in manure via two ways: (i) absorbed by the gut wall and transferred to the liver, where they are detoxified to glucuronides and sulfates and then excreted via urine. Odorous compounds are released when glucuronides and sulfates in urine come into contact with feces and (ii) direct excretion of odorous compounds or odor precursors from the large intestine of animals via feces after being formed. Therefore, a reduction in the dietary CP level would result in a decrease in the concentration of these compounds in the manure.

When sufficient non-starch polysaccharides (NSP) are available, microbes will use NSP as an energy source and protein or AA as a nitrogen source for their biomass synthesis. If the amount of NSP is relatively low compared to that of protein, the microbes will use protein or AA as an energy source. This situation with insufficient NSP creates more odor than when adequate NSP is available. It is important to notice that the 3 diets had a rather low and similar NSP levels, therefore increased CP level resulted in a higher ratio between fermentable protein and NSP in the gut of animals and in the manure. It means more protein or AA was used by microbes as an energy source resulting in a higher concentration of odorous compounds in the gut of animals and in the manure.

So far only limited research has been conducted on the impact of dietary CP reduction on odorous compounds in manure, for examples, indolic, phenolic, and sulfurous metabolites. There is hardly any research that correlates the impact of dietary CP reduction on odor strength and offensiveness of the odorous air and that of the excretion of odorous compounds in manure or in the air emitting from the manure. Previous studies have shown a reduction of phenol, 4-ethyl phenol, indole, 3-methyl indole concentrations in pig manure (Hobbs *et al*., 1996) as dietary CP levels were reduced approximately 20 to 13%, however, no measurements of odor

strength and offensiveness of the odorous air were conducted. Sutton et al. (1998) reported a reduction of sulfurous compounds (carbon disulfide, dimethyl sulfide, dimethyl disulfide) as dietary CP levels were reduced from 13% to 8%. The reduction of these odorous compounds is supposed to decrease odor strength and offensiveness from pig manure. This interpretation should, however, be handled with care, because the relationships between the concentration of a single odorous compound or a group of odorous compounds in manure and/or in the odorous air and the odor strength and offensiveness of the odorous air measured by olfactometry are not clear. In addition, odor is a complex mixture of many compounds, for example, 331 compounds as reported by Schiffman *et al*. (2001), the number of compounds analyzed in our experiment or in those of others are probably too few to give a general picture of odor strength and offensiveness measured by olfactometry.

According to Canh *et al*. (1998) fermentable NSP are the most important dietary components determining VFA concentration in the manure. The VFA pool was largely dominated by the short straight–chain VFA such as acetic, propionic and butyric acids which comprised 91% of total VFA in the manure confirming the results of Otto *et al*. (2003) and Le *et al*. (2005b). Branched-chain VFA are only produced from protein metabolism. That could be the reason for the increase of iso-butyrics and iso-pentanoic acid concentrations in the manure as dietary CP levels increased from 12% to 18%, although they were not statistically different ($P = 0.08$ and $P = 0.1$, respectively).

The main objective of this study was to determine the effect of dietary CP levels on odor strength and offensiveness, but ammonia emission was considered as well because it is a serious environmental problem. Odor abatements are only of interest if they do not increase other environmental problems as ammonia. Ammonia emission (mg $s^{-1}m^{-2}$) was decreased by 53 % as dietary CP levels were reduced from 18.0% to 12.2% (analyzed CP values), about 9.2 % ammonia emission reduction for each 1% unit reduction in dietary CP combined with AA supplementation. This figure is very similar to the 10% that was reported by Canh *et al*. (1998), Kay and Lee (1997), and Sutton *et al*. (1997). Ammonia emission is largely influenced by ammonium concentration, pH and temperature (Aarnink & Elzing, 1998). In this experiment, temperature was controlled so the effect of temperature was excluded. Ammonia emission reduction seems to have mainly resulted from manure ammonium concentration and pH reduction. Lowering dietary CP level from 18.0% to 12.2% resulted in a decrease of the ammonium-N concentration in the manure by 33.2% and a decrease of manure pH by 0.73 unit.

So far, almost all odor studies have focused on concentration and emission of odor and odorous compounds. The odor concentration limits the question of ‘how strong and

unpleasant an odor is' to a detection threshold and the original odor is characterized in odor units or multiples of the concentration at detection threshold. However, this approach has a limitation in considering this odor relative to others. Odor concentration does not take into account the different characteristics of odor (Power & Stafford, 2001). Obviously not all odors are similar in their ability to cause annoyance. In our study, not only odor concentration but also hedonic tone and intensity were measured. The latter two criteria can answer the question how strong and unpleasant an odor is. By using dynamic olfactometry to determine odor concentration and then odor intensity and odor hedonic tone, suitable relationships between them can be determined, allowing different odor types to be compared. The use of odor concentration, odor hedonic tone and odor intensity can give an overall comparison between odors.

A higher odor concentration at H = - 1 (mildly unpleasant) of odorous air from manure of pigs fed lower CP diets implies that pigs fed the 12%CP or 15%CP diets produce a less offensive odor from the manure than the 18%CP diet. A higher odor concentration at H = - 2 (moderately unpleasant) of odorous air from manure of pigs fed 15%CP diet implies that pigs fed the 15%CP diets produce a less offensive sensory response than the 18%CP and 12%CP diets. The effect of dietary CP levels on intensity of odorous air from pig manure was not significant, in other words odorous air from manure of pigs fed different dietary CP levels is similar in the magnitude of odor sensation. It is important to recall that odor hedonic tone and intensity are measured at odor concentrations above the detection threshold. Samples with a high odor concentration are diluted more before the odor detection threshold is reached and vice versa. From Fig. 2 the overall effect of a sample on the hedonic tone can be calculated. When we assume an odor concentration at a certain distance from the animal house for diet 15%CP of 2 at logarithmic scale (7.4 $ou_E\ m^{-3}$), then from Table 4 (based on the ratio of the odor concentration between treatments) it can be calculated that concentrations at the same distance from the animal house when feeding 12%CP or 18%CP diets at logarithmic scale would be 1.4 (4.1 $ou_E\ m^{-3}$) and 2.9 (18.2 $ou_E\ m^{-3}$), respectively. From Fig. 2 it can be calculated that the corresponding hedonic tones are - 1.6, - 1.7 and - 3.0 for diets 12%CP, 15%CP, and 18%CP, respectively. Briefly, the unpleasantness of the odor from the animal house would be similar when diets 12%CP or 15%CP would be fed, while the odor would be clearly more unpleasant when diet 18%CP would be fed.

This study shows that the correlation between ammonia and odor emission is very low (0.1). It can be explained by the fact that odor is a complex mixture of various compounds such as sulfur-containing compounds, indolic and phenolic compounds, VFA, and ammonia and

volatile amines while ammonia is a single compound. In addition, ammonia is not a very offensive odor (Oldenburg, 1989). This result implies that ammonia emission may contribute minimally to odor emission and strategies that have been demonstrated to be successful in reducing ammonia emission may not have a similar impact on odor.

The relationship between ammonia and odor emission from pig manure and pig production facilities has been questioned by scientists. Inconsistent findings were found in literature and between our finding and others. Schulte *et al*. (1985) and Miner (1995) found a high correlation between ammonia and odor emission from pig production facilities. On the other hand Williams (1984), Oldenburg (1989), Liu *et al*. (1993) and Verdoes and Ogink (1997) found only a low correlation between ammonia and odor emission from pig houses. The inconsistencies in the relationship between ammonia and odor emission likely comes from the fact that ammonia and odor samples were collected from different farms and at different times. Farms are different in animal types, housing design, and dietary composition, especially fermentable carbohydrates which may vary a lot among diets. Different times of sample collection and farms might have different environmental factors. These farms and environmental factors play key roles in influencing odor and ammonia emission (Le *et al*., 2005a; Le *et al*., 2005b) and consequently the relationship between them. In our study, these sources of variances were prevented, because we collected odor and ammonia samples from the manure of the different treatments in the same animal house, at the same time, and with the same air flow rate.

IMPLICATIONS

This study demonstrates that feeding a diet that more closely meets the protein/amino acids requirement of the pigs reduces odor concentration, odor emission, odor offensiveness and ammonia emission from pig manure. This can be achieved by reducing the crude protein content of the diet and supplementing the diet with essential amino acids. The results of this study were obtained under rather controlled conditions, therefore, they should be validated in a conventional pig facility, measuring not only odor from manure, but from other sources in the animal house, as well.

5

EFFECTS OF CRYSTALLINE AMINO ACID SUPPLEMENTATION TO THE DIET ON ODOR FROM PIG MANURE

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ABSTRACT: *The objective of this study was to determine the effects of specific crystalline amino acids (AA) supplementation to a diet on odor emission, odor intensity, odor hedonic tone, and ammonia emission from pig manure, and on manure characteristics (pH, ammonium, total nitrogen, sulfurous, indolic and phenolic compounds and volatile fatty acid concentrations). An experiment was conducted with growing pigs (n = 18) in a randomized complete block design with 3 treatments in 6 blocks. Treatment groups were 1) 15% crude protein (CP) basal diet with 3 times the requirement of sulfur-containing AA (14.2 g/kg diet, as fed basis); 2) basal diet with 2 times the requirement of Trytophan (Trp), and Phenylalanine (Phe)+Tyrosine (Tyr) (2.9 and 20.4 g/kg diet, as fed basis, respectively); and 3) basal diet with AA supplementation to levels sufficient for maximum protein gain. Pigs with an initial body weight (BW) of 41.2 ± 3.4 kg (mean ± SD) were individually penned in partly slatted floor pens and offered a daily feed allowance of 2.8 x maintenance requirement for net energy (293 kJ/BW$^{0.75}$). Feed was mixed with water, 1/2.5 (w/w). Feces and urine of each pig was allowed to accumulate in separate manure pits under the slatted floor. After an adaptation period of 2 wk, and after cleaning the manure pits, manure was subsequently collected. In the 5th wk of the collection period, separate samples were collected directly from each manure pit for odor, ammonia, and manure composition analysis. Odor samples were analyzed for odor concentration, and for hedonic tone and odor intensity above odor detection threshold. Results showed that supplementing crystalline S-containing AA in surplus of the requirements increased odor emission ($P < 0.001$) and odor intensity ($P < 0.05$), and reduced odor hedonic tone ($P < 0.05$) from air above the manure pits. Supplementing crystalline Trp, Tyr, and Phe in surplus of recommended requirements did not affect odor emission, odor intensity, or odor hedonic tone. Regardless of dietary treatment, all pigs had similar performance levels. No differences were observed in ammonia emission from manure of pigs fed different levels of AA supplementation ($P = 0.20$). It is concluded that in order to reduce odor from pig manure the S-containing AA should be minimized to just meet recommended requirements.*

Key words: *Crystalline Amino Acids, Diet, Growing Pig, Odor*

INTRODUCTION

Odor emission from pig production facilities can cause serious nuisance for residents in the surrounding areas of pig operations. Odor is generated by the microbial conversion of feed in the intestinal tract of pigs and by microbial conversion of pig excreta under anaerobic conditions in manure storages. There are a great number of odorous compounds identified in air and manure from animal production facilities. O'Neill and Phillips (1992) summarized 168 compounds in livestock wastes or in the surrounding air. Recently, Schiffman *et al*. (2001) identified a total of 331 different odorous compounds in the air and lagoon water from pig production facilities. Odorants can be classified into four main groups: (1) sulfurous compounds, (2) indolic and phenolic compounds, (3) volatile fatty acids (VFA), and (4) ammonia and amines. Many of these compounds are intermediate or end products of amino acid (AA) metabolism. Therefore, AA are important dietary nutrients that should be considered to reduce odor emission. Odor is evaluated through its strength (concentration and intensity) and offensiveness (hedonic tone).

In a literature review Le *et al*. (2005a) found that that the sulfurous compounds and the aromatic compounds of indoles and phenols are considered most important for odor nuisance in the air and in manure from pig production facilities. This hypothesis mainly relied upon the concentration of these compounds in manure and/or in the air and their detection threshold. Tryptophan (Trp), Phenylalanine (Phe) and Tyrosine (Tyr) are main substrates for the synthesis of indolic and phenolic compounds. The sulfur-containing AA, Methionine (Met) and Cystine (Cys), are the main substrates for the synthesis of sulfurous compounds such as methanethiol and hydrogen sulfide (Mackie *et al*., 1998). A change in the concentration of these AA in the diet may alter the level of odorous compounds produced in the gut of animals and in the manure.

Since there are few studies on effects of supplemented crystalline AA types in the diet on odor strength and offensiveness of air from pig manure, our objective was to determine whether AA type influences odor emission, odor strength, odor offensiveness, and ammonia emission from pig manure and manure characteristics (pH, ammonium, total nitrogen, sulfurous, indolic and phenolic compounds, and VFA concentrations). In addition in this study the odor emission from pig manure in practical situations was simulated by collecting odor samples directly from the manure pit.

MATERIALS AND METHODS

ANIMALS, EXPERIMENTAL DESIGN, AND DIETS

A randomized complete block design with 3 treatments in 6 blocks was used to study effects of amino acids (AA) types in the diet on odor concentration, odor emission, odor intensity, odor hedonic value, and ammonia emission from growing pig manure, and on manure characteristics. Three groups of pigs were fed different diets: 1) 15% crude protein (CP) basal diet with 3 times the requirement of sulfur-containing AA (SAA) (14.2 g/kg diet, as fed basis); 2) basal diet with 2 times the requirement of Trp, and Phe+Tyr (TAA) (2.9 and 20.4 g/kg diet, as fed basis, respectively); and 3) basal diet with AA supplementation to levels sufficient for maximum protein gain (no more added than needed = NOAA). In all diets, additional AA were supplemented to the diets in crystalline form. Each treatment was replicated 6 times, 1 replicate in each of 6 blocks, of which a block consisted of samples collected on the same day and from animals with similar initial body weight (BW).

In total 18 growing barrows (Great Yorkshire x Dutch Landrace) with an initial BW of 41.2 ± 3.4 kg (mean ± SD) were allocated to 6 blocks, with blocks based on initial BW. Pigs were penned individually in galvanized steel pens (2.1 x 0.96 m) with a slatted floor at the rear (0.97 x 0.96 m). There was a separate manure pit under the slatted floor of each pen. The size of the manure pit was 1.35 x 0.91 x 0.36 m (length x width x depth). Pigs were housed in a mechanically ventilated and temperature controlled room. Temperature and relative humidity were recorded every 5 min. The average temperature and relative humidity of the room during the experimental period were 21.0 ^{0}C ± 0.84 and 50.0 % ± 5.32 (mean ± SD), respectively.

Diets were formulated to have similar contents of net energy (NE), non-starch polysaccharides (NSP), electrolyte balance (dEB), minerals, and vitamins (Tables 1 and 2). The basal diet (NOAA) was formulated to contain 15% CP with AA supplementation to just meet the requirement for the pig based on ileal AA digestibility (CVB- Animal feed product board, 2004). To formulate the SAA diet, additional Met was added to that sulfur-containing SAA in the diet was 3 times the recommended level. To formulate the TAA diet, additional Trp, Phe, and Tyr was added to provide 2 times the requirement of these AA of Trp, and Tyr + Phe (TAA) based on ileal digestibility. Analyzed AA composition of the diets are presented in Table 2 with concentrations of sulfur-containing AA 0.49, 1.42 and 0.51%; Trp, 0.19, 0.20 and 0.29%; and Phe + Tyr, 1.13, 1.15 and 2.04%; in NOAA, SAA and TAA diets, respectively.

Table 1. Ingredient composition of experimental diets, as-fed basis

Composition (%)	Diet[a]		
	NOAA	SAA	TAA
Wheat	50.00	50.00	50.00
Tapioca meal (starch 62.5-65.7%)	13.13	12.12	11.99
Wheat gluten meal	4.00	4.00	4.00
Potato protein	2.53	2.53	2.53
Wheat middings	15.00	15.00	15.00
Palm kernel expeller (crude fibre<22%)	6.60	6.60	6.60
Cane molasses, < 47.5% sugar	3.00	3.00	3.00
Soybean oil	1.69	1.69	1.69
K_2CO_3	0.93	0.93	0.93
$CaCO_3$	1.15	1.15	1.15
Monocalcium phosphate·H_2O	0.68	0.68	0.68
Salt	0.48	0.48	0.48
Premix[b]	0.20	0.20	0.20
L-Lysine HCl	0.45	0.45	0.45
DL-Methionine	0.06	1.07	0.06
L-Threonine	0.09	0.09	0.09
L-Tryptophan	0.01	0.01	0.15
L-Phenylalanine	0.00	0.00	0.60
L-Tyrosine	0.00	0.00	0.40

[a] SAA = 3 times requirement of sulfur-containing AA; TAA = 2 times requirement of Trp, and Tyr + Phe; NOAA = supplementation of these AA up to requirement.

[b] The vitamin-mineral premix supplied per kg feed: 7,000 IU vitamin A, 1,700 IU vitamin D3, 20 IU vitamin E, 1.5 mg vitamin K, 1.5 mg vitamin B1, 4 mg vitamin B2, 11 mg d-pantothenic acid, 18 mg niacin, 18 µg vitamin B12, 0.1 mg folic acid, 1.0 mg vitamin B6, 100 mg choline chloride, 75 mg Fe, 10 mg Cu, 65 mg Zn, 30 mg Mn, 0.15 mg Co, 0.75 mg I, 0.30 mg Se.

Experimental diets were analyzed for nutrient composition: AA, ash, dry matter (DM), CP, minerals, fiber, fat, starch, sugar and energy. The analyses were conducted as described in P. D. Le *et al.* (Chapter 4).

Pigs were fed 2.8 times the maintenance NE requirement (293 kJ/$BW^{0.75}$). Water was restrictedly provided by mixing feed with water in the ratio of 1/2.5 (w/w). Apart from water with feed no additional water was given to the pigs. So it was aimed to have the same amount of feed and water intake by the pigs to excrete almost a similar amount of manure. Pigs were fed 2 times per day at 0800 and 1500. The amount of feed provided was adjusted each day according to an assumed BW gain of 780 g d^{-1}. Feed intake was recorded every day. Pigs were

weighed at the beginning and at the end of the experimental period just before the morning feed. Daily gain and feed efficiency were derived from the feed intake and the increase of BW during the experimental period. After an adaptation period of 2 wk to allow the pigs to acclimatize to the experimental diets and pens, the manure pits were cleaned. Subsequently, feces and urine accumulated in the manure pit. In the 5th wk of the collection period, odor, ammonia and manure samples were collected for subsequent analysis.

Table 2. Nutrient composition of experimental diets, as-fed basis

Composition	Unit	Diet[a]		
		NOAA	SAA	TAA
Calculated composition				
Crude protein (CP)	%	15.00	15.57	15.60
Digestible CP	%	12.18	12.77	12.80
Ileal digestible CP	%	12.17	12.74	12.78
NE	kcal/kg	2183	2178	2187
NSP[b]	%	17.99	17.85	17.83
Digestible NSP	%	9.00	8.94	8.93
Illeal digestible amino acids				
Lys	%	0.74	0.74	0.74
Met	%	0.27	1.27	0.27
Cys	%	0.23	0.23	0.23
Thr	%	0.43	0.43	0.43
Trp	%	0.14	0.14	0.28
Phe	%	0.59	0.59	1.18
Tyr	%	0.40	0.40	0.79
Analyzed composition				
Dry matter	%	86.11	86.68	86.60
Crude protein (N x 6.25)	%	14.81	15.36	15.24
Gross energy	kcal/kg	3,804	3,901	3,837
Ash	%	5.41	5.55	5.59
Total fat	%	2.76	3.81	3.88
Crude fiber	%	3.61	3.68	3.86
Starch	%	40.14	39.29	39.64
Sugar	%	3.89	4.04	4.12
NSP	%	19.09	18.63	18.11
Na	%	0.19	0.21	0.22
K	%	1.15	1.18	1.16
Sulfate	%	0.11	0.11	0.11
Cl	%	0.45	0.46	0.44
dEB[c]	meq/kg	251	263	270

dEBS-a[d]	meq/kg	227	241	246
Amino acids				
Ala	%	0.56	0.56	0.58
Arg	%	0.71	0.74	0.75
Asp	%	0.90	0.91	0.92
Cys	%	0.25	0.27	0.27
Glu	%	3.45	3.48	3.51
Gly	%	0.60	0.60	0.61
His	%	0.34	0.35	0.36
Ile	%	0.51	0.52	0.52
Leu	%	0.98	0.99	1.00
Lys	%	0.60	0.75	0.76
Met	%	0.24	1.15	0.24
Phe	%	0.65	0.66	1.20
Pro	%	1.19	1.37	1.30
Ser	%	0.64	0.64	0.67
Thr	%	0.54	0.54	0.54
Trp	%	0.19	0.20	0.29
Tyr	%	0.48	0.48	0.84
Val	%	0.65	0.66	0.67

[a] SAA = 3 times requirement of sulfur-containing AA; TAA = 2 times requirement of Trp, and Tyr + Phe; NOAA = supplementation of these AA up to requirement.

[b] Non-starch polysaccharides (NSP) were calculated as organic matter–(CP+crude fat+starch+ sugar).

[c] dEB was determined as mEq = Na + K – Cl.

[d] dEBS-a was determined as mEq=Na+K–Cl–2S. dEBS-a does not take into account S present in AA.

COLLECTING ODOR SAMPLES AND MEASURING ODOR CONCENTRATION, ODOR HEDONIC TONE, AND ODOR INTENSITY

Odor samples were collected as described by P. D. Le *et al.* (Chapter 4). The schematic view of the odor sample collection is shown in Fig. 1. One odor sample was collected directly from manure in each manure pit. This odor sample was measured for odor concentration according to CEN standard 13725 (2003) as described in detail by Le *et al.* (2005b). Odor concentrations of the examined samples were expressed in European odor units per cubic meter air ($ou_E\ m^{-3}$). One odor unit is defined as the amount of odor-causing gases which, when diluted in 1 m^3 of air, can just be distinguished from clean air by 50% of the members of an odor panel.

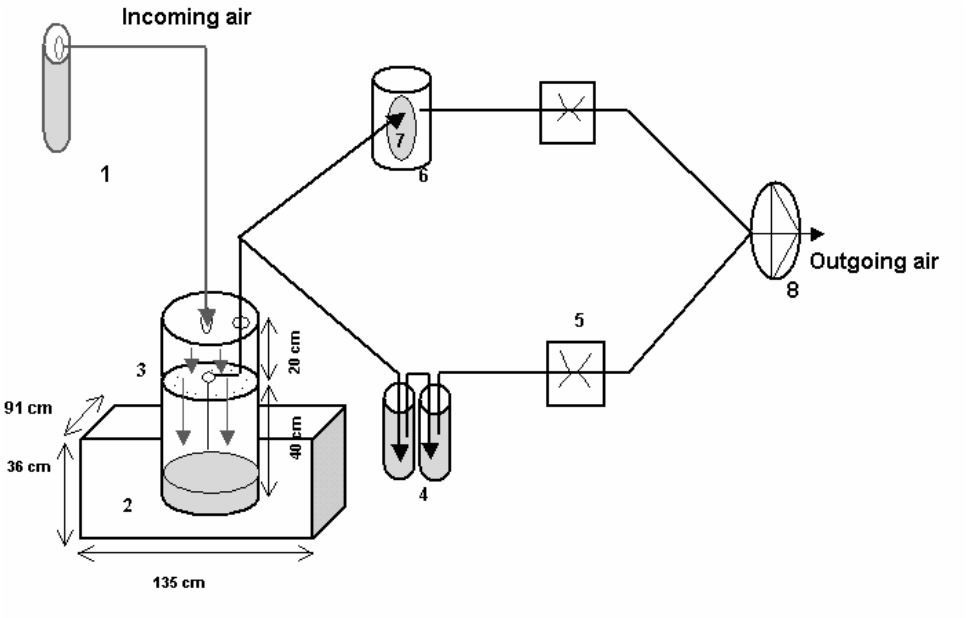

Fig. 1. Schematic view of the odor and ammonia sample collection (1 = odor free air pressurized cylinder, 2 = manure pit, 3 = vessel, 4 = impingers, 5 = critical glass capillary, 6 = rigid plastic container, 7 = odor bag, 8 = vacuum pump)

Odor emission was defined as the number of odor units emitted from a manure surface per s. It was calculated by multiplying the ventilation rate with the corresponding odor concentration (Equation 1):

$$E_{odor} = (C_{odor} \times V \times 10{,}000) / (60 \times 1{,}000 \times 595) \quad [1]$$

where E_{odor} = odor emission s^{-1} m^{-2} (ou_E $s^{-1}m^{-2}$), C_{odor} = odor concentration (ou_E m^{-3}), and V = ventilation rate (L min^{-1}), 10,000 = cm^2 m^{-2}, 60 = sec min^{-1}, 1,000 = liters m^{-3}, and 595 = the cm^2 surface area of the manure pit.

Measuring odor hedonic tone (H) and odor intensity (I) was carried out as described by P. D. Le *et al*. (Chapter 4). Hedonic tone is used to evaluate odor offensiveness which is a measure of the unpleasantness or pleasantness of the perceived odor. Odor intensity refers to the magnitude of odor sensation and is a measure of the intensiveness of the odor above the odor detection threshold. Hedonic tone was measured by the scores of the panel members. They used a 9-point hedonic scale ranging from -4, extremely unpleasant or offensive; 0, neither pleasant nor unpleasant or neutral odor; to +4, extremely pleasant. Odor intensity was measured by using a 7-point intensity scale ranging from 1, no odor; 2, very faint odor; 7, overwhelming odor. For each odor sample, the hedonic tone and odor intensity at each odor concentration level above the detection threshold were calculated as the average of the perceived hedonic tone and the odor intensity of all panelists, and plotted against the logarithm of the odor concentration. From the regression lines obtained, the odor concentration at H = -1 (mildly

unpleasant), H = -2 (moderately unpleasant), I = 1 (no odor), I = 2 (very faint odor), I = 4 (distinct odor) were derived. Regression lines of the odor hedonic tone and the odor intensity were also plotted against logarithm of the odor concentration for all samples in the same treatment.

COLLECTING AND MEASURING AMMONIA EMISSION

Samples for determining ammonia emission were collected at the same time with the same system as odor samples (Fig. 1). One ammonia sample was collected from each manure pit. Ammonia in outgoing air was removed by passing through two ammonia traps (impingers), each containing about 20 ml 0.5 M HNO_3 solution. The system was run for about 90 min. The ammonia concentration and the volume of the liquid were determined in the first and the second impingers. Ammonia emission per time unit and surface unit was calculated as (Equation 2):

$$MNH_3 = (CNH_3 \times V \times 10{,}000) / (T \times 60 \times 595) \quad [2]$$

where MNH_3 = ammonia emission (mg s^{-1} m^{-2}), CNH_3 = ammonia concentration (mg mL^{-1} HNO_3), V = volume of HNO_3 solution (mL), 10,000 = cm^2 m^{-2}, T = sampling time (min), 60 = s min^{-1}, and 595 = the cm^2 surface area of the manure pit.

COLLECTION AND MEASUREMENT OF MANURE CHARACTERISTICS

Manure samples were analyzed to evaluate the effect of the diets on manure characteristics. These include DM, ash, total N (total-N), ammonium-N, pH, VFA (acetic, propionic, butyric, pentanoic, iso-butyric, iso-pentanoic, hexanoic, and heptanoic acid), indolic (indole and 3-methyl indole) and phenolic compounds (phenol, 4-ethyl phenol, and cresols), and sulfurous compounds (carbon disulfide, methyl sulfide, methyl disulfide, and ethanethiol). One manure sample was collected from each manure pit. Manure samples were collected and analyzed as described by P. D. Le *et al.* (Chapter 4).

STATISTICAL ANALYSIS

The effect of AA types on daily gain, daily feed intake, feed efficiency, odor emission, odor hedonic value, odor intensity, ammonia emission, and manure characteristics were analyzed using ANOVA of GenStat statistical package 7th version (GenStat VSN International Ltd., 2004) with the following model:

$y_{ij} = \mu + \rho_j + \alpha_i + e_{ij}$ where: y_{ij} = dependent variables, μ = overall mean, ρ_j: effect of block (j = 1-6), α_i: effect of diet, (i = 1, 2, 3) and e_{ij}: experimental error.

Data were presented as either arithmetic or geometric mean. A natural log

transformation was applied to odor emission, concentrations of VFA, total-N, ammonium-N, indolic and phenolic compounds, and sulfur-containing compounds since they were skewed and not normally distributed. In each treatment, odor hedonic tone and odor intensity was plotted against the natural logarithm of odor concentration, and odor hedonic tone was plotted against odor intensity. The differences between slopes and between intercepts were tested to decide whether there should be separate regression lines for treatments or a common line for all treatments. The relationship between ammonia emission and odor emission was determined by linear regression.

RESULTS

EFFECTS OF AMINO ACID SUPPLEMENTATION ON DAILY GAIN, DAILY FEED INTAKE AND FEED EFFICIENCY

To detect whether there are any effects of treatments on production parameters, the effects of AA supplementation to the diet on daily gain, daily feed intake, and feed efficiency are summarized in Table 3. Average daily feed intake, daily gain and feed efficiency were similar among treatments ($P > 0.05$).

Table 3. Effects of amino acid supplementation to the diet on daily gain, daily feed intake and feed efficiency

Variables	Diet[a]			SEM[b]	*P* value
	NOAA	SAA	TAA		
Initial BW, kg	41.4	40.8	41.4	0.4	0.40
Final BW, kg	73.7	73.4	74.4	1.1	0.79
ADFI, kg/ day	1.87	1.87	1.87	0.00	0.30
ADG, g/ day	702	708	716	23	0.91
G:F, g/kg	375	379	383	12.5	0.90

[a] SAA = 3 times requirement of sulfur-containing AA; TAA = 2 times requirement of Trp, and Tyr + Phe; NOAA = supplementation of these AA up to requirement.

[b] SEM = Standard errors of means with 10 df for error.

EFFECTS OF AMINO ACID SUPPLEMENTATION ON ODOR STRENGTH AND OFFENSIVENESS

Descriptive statistics and analysis of variance of effects of the diets on odor strength and offensiveness from pig manure are shown in Tables 4 and 5, respectively. Geometric odor concentration and odor emission from pig manure were highest from pigs fed the SAA treatment, 111,302 $ou_E\ m^{-3}$ and 15.48 $ou_E\ s^{-1}\ m^{-2}$, respectively. NOAA treatment had the lowest

odor concentration and odor emission, 13,224 $ou_E\ m^{-3}$ and 1.88 $ou_E\ s^{-1}\ m^{-2}$, respectively.

Table 4. Geometric and arithmetic means of odor strength (concentration & intensity) and offensiveness (hedonic tone) from manure of pigs supplemented AA to the diets

Odor variable	Diet[a]					
	NOAA		SAA		TAA	
	GM[b]	AM[c]	GM	AM	GM	AM
Concentration, $ou_E\ m^{-3}$	13,224	16,423	111,302	118,369	16,318	21,234
Emission, $ou_E\ s^{-1}m^{-2}$	1.88	2.33	15.48	16.50	2.23	2.99
Concentration at H[d] = -1, $ou_E\ m^{-3}$	3.19	3.83	1.57	1.86	3.56	3.83
Concentration at H = -2, $ou_E\ m^{-3}$	9.39	11.58	5.70	6.25	11.36	13.06
Concentration at I[e] = 1, $ou_E\ m^{-3}$	0.75	0.90	0.29	0.36	0.83	0.91
Concentration at I = 2, $ou_E\ m^{-3}$	1.72	2.00	0.84	0.96	1.92	2.06
Concentration at I = 4, $ou_E\ m^{-3}$	9.03	10.08	6.89	7.24	10.27	11.24

[a] SAA = 3 times requirement of sulfur-containing AA; TAA = 2 times requirement of Trp, and Tyr + Phe; NOAA = supplementation of these AA up to requirement.

[b] GM = geometric mean; [c] AM = arithmetic mean; [d] Hedonic tone; [e] Intensity.

Table 5. Effects of amino acid supplementation to the diets on odor strength (concentration and intensity) and offensiveness (hedonic tone) from pig manure

Variables	Diet[a]			SEM[b]	*P* value
	NOAA	SAA	TAA		
ln[c](Odor concentration)	9.49[d]	11.62[e]	9.70[d]	0.26	< 0.001
ln (Odor emission)	0.63[d]	2.74[e]	0.83[d]	0.26	< 0.001
ln (Odor concentration at H = -1)	1.16[d]	0.45[e]	1.27[d]	0.12	0.002
ln (Odor concentration at H = -2)	2.24[d]	1.74[e]	2.43[d]	0.16	0.04
ln (Odor concentration at I = 1)	-0.29[d]	-1.24[e]	-0.19[d]	0.20	0.007
ln (Odor concentration at I = 2)	0.54[d]	-0.18[e]	0.65[d]	0.14	0.004
ln (Odor concentration at I = 4)	2.20[d]	1.93[e]	2.31[d]	0.10	0.05

[a] SAA = 3 times requirement of sulfur-containing AA; TAA = 2 times requirement of Trp, and Tyr + Phe; NOAA = supplementation of these AA up to requirement.

[b] SEM = Standard errors of the means with 10 df for error.

[c] Natural logarithm.

[d,e] Means within rows missing a common superscript letter are different at $P < 0.05$.

Analyses of variance show that diets affected odor concentration and odor emission from pig manure ($P < 0.001$). Further analyses show that pigs fed the SAA treatment had a higher odor concentration and odor emission than pigs fed the TAA and NOAA treatments ($P < 0.01$). No differences were observed in odor concentration and odor emission between pigs fed the

TAA and NOAA treatments. At the same level of odor hedonic tone or odor intensity, odor concentration from the pigs fed the SAA treatment was lowest ($P < 0.05$). No differences in odor concentration at different levels of hedonic tone and odor intensity were found between pigs fed the TAA and NOAA treatments.

Relationships between odor concentration and odor hedonic tone, between odor concentration and intensity, and between intensity and hedonic tone are shown in Figs 2, 3 and 4, respectively. In each figure, a difference in intercepts ($P < 0.05$) of different regression lines was observed. The intercept of the pigs fed the SAA treatment differed from that of pigs fed the TAA and NOAA treatments ($P < 0.05$), but it was similar for pigs fed the TAA and NOAA. The regression lines were similar in slopes. There was a strong linear relationship between hedonic tone and the natural logarithm of odor concentration (R^2 = 66%), between odor intensity and the natural logarithm of odor concentration (R^2 = 71%) and between hedonic tone and intensity (R^2 = 89%). Hedonic tone decreased while odor intensity increased as the odor concentration increased. Hedonic tone decreased as odor intensity increased.

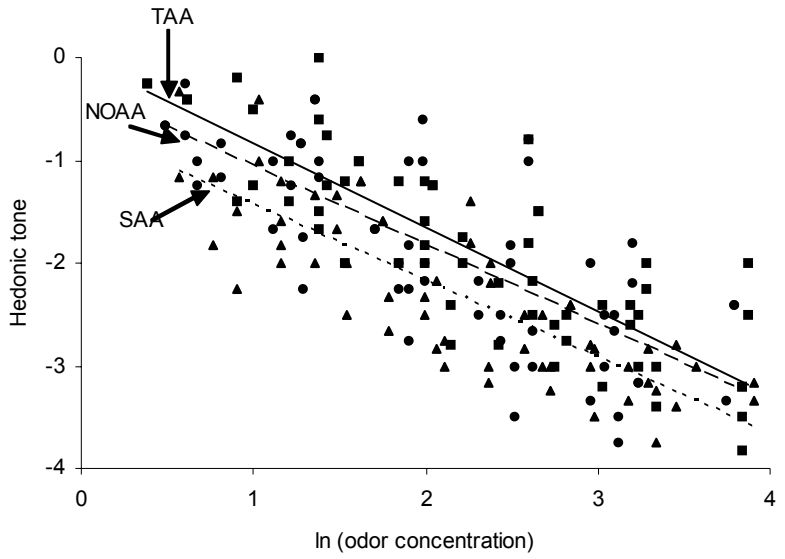

Fig. 2. Hedonic tone (H) as a function of odor concentration with regression lines, SAA = 3 times requirement of sulfur-containing AA; TAA = 2 times requirement of Trp and Tyr + Phe; NOAA = supplementation of these AA up to requirement. H_{SAA} = -0.62 (0.12) – 0.78 (0.04) ln (odor concentration), indicated by ……▲; H_{TAA} = -0.10 (0.12) – 0.78 (0.04) ln (odor concentration), indicated by _____■; H_{NOAA} = -0.27 (0.11) – 0.78 (0.004) ln (odor concentration), indicated by ___ ___ ●; R^2 = 66%

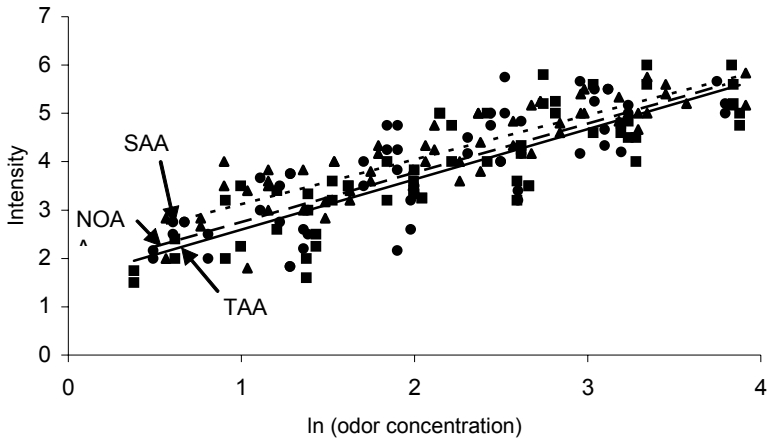

Fig. 3. Odor intensity (I) as a function of odor concentration with regression lines, SAA = 3 times requirement of sulfur-containing AA; TAA = 2 times requirement of Trp and Tyr + Phe; NOAA = supplementation of these AA up to requirement. I_{SAA} = 2.04 (0.13) + 0.99 (0.05) ln (odor concentration), indicated by ……▲; I_{TAA} = 1.66 (0.13) + 0.99 (0.05) ln (odor concentration), indicated by _____■; I_{NOAA} = 1.78 (0.13) + 0.99 (0.005) ln (odor concentration), indicated by ___ ___ ●; R^2 = 71%

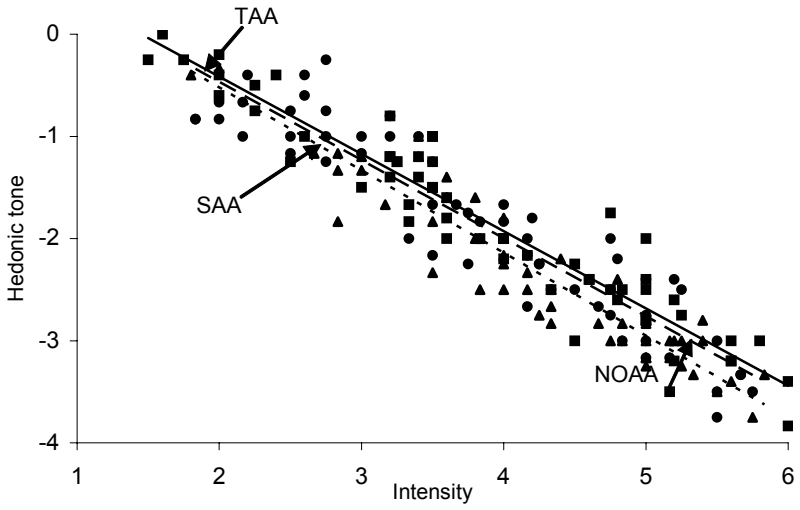

Fig. 4. Hedonic tone (H) as a function of odor intensity (I) with regression lines, SAA = 3 times requirement of sulfur-containing AA; TAA = 2 times requirement of Trp and Tyr + Phe; NOAA = supplementation of these AA up to requirement. H_{SAA} = 0.94 (0.09) – 0.77 (0.02) I, indicated by ……▲; H_{TAA} = 1.16 (0.09) – 0.77(0.02) I, indicated by _____■; H_{NOAA} = 1.09 (0.09) – 0.77(0.02) I, indicated by ___ ___ ●; R^2 = 89%

EFFECTS OF AA SUPPLEMENTATION ON MANURE CHARACTERISTICS AND AMMONIA EMISSION

Table 6. Descriptive statistics of manure characteristics and ammonia emission from manure of pigs supplemented different types of AA to the diets (*n* = 18)

Variables	Diet[a]		
	NOAA	SAA	TAA
Dry matter, g kg^{-1}	114.1 (4.05)[b]	116.9 (12.49)	110.6 (9.82)
Ash, g kg^{-1}	30.5 (1.04)	33.5 (3.27)	29.4 (2.19)
Total VFAs[c], g kg^{-1}	6.38 (1.39)	5.00 (1.04)	5.65 (1.27)
Acetic acid, g kg^{-1}	4.15 (0.82)	3.30 (0.72)	3.82 (0.84)
Propionic acid, g kg^{-1}	1.40 (0.32)	1.03 (0.22)	1.13 (0.29)
Butyric acid, g kg^{-1}	0.42 (0.25)	0.23 (0.10)	0.30 (0.14)
Iso-butyric acid, g kg^{-1}	0.12 (0.04)	0.12 (0.04)	0.12 (0.04)
Iso-pentanoic acid, g kg^{-1}	0.3 (0.06)	0.28 (0.1)	0.28 (0.04)
Total N, g kg^{-1}	6.0 (0.21)	6.62 (0.29)	6.17 (0.53)
Ammonium-N g kg^{-1}	2.61 (0.16)	3.14 (0.19)	2.87 (0.24)
pH	7.75 (0.17)	7.65 (0.22)	7.93 (0.07)
Ammonia emission, mg $s^{-1}m^{-2}$	0.014 (0.003)	0.012 (0.004)	0.015 (0.003)
Phenol, mg kg^{-1}	16.15 (1.68)	17.15 (3.04)	20.50 (5.61)
Cresols, mg kg^{-1}	40.93 (9.7)	44.82 (6.78)	67.32 (16.6)
4-ethyl phenol, mg kg^{-1}	0.89 (0.28)	1.17 (0.26)	0.40 (0.24)
Indole, mg kg^{-1}	9.85 (3.81)	3.52 (1.13)	9.90 (2.24)
3-methyl indole, mg kg^{-1}	5.22 (1.16)	4.45 (0.94)	5.50 (0.95)
Carbon disulfide, mg kg^{-1}	0.53 (0.19)	0.40 (0.11)	0.47 (0.21)
Methyl sulfide, mg kg^{-1}	1.22 (1.4)	2.36 (1.82)	3.78 (2.21)
Ethanethiol, mg kg^{-1}	80.0 (6.7)	99.6 (22.6)	82.3 (16.6)

[a] SAA = 3 times requirement of sulfur-containing AA; TAA= 2 times requirement of Trp, and Tyr + Phe; NOAA = supplementation of these AA up to requirement.

[b] Standard deviation.

[c] Total VFA = acetic acid + propinoic acid + butyric acid + iso-butyric acid + iso-pentanoic acid.

Descriptive statistics and analyses of variance of the impacts of AA supplementation on manure characteristics and ammonia emission are shown in Tables 6 and 7, respectively. Volatile fatty acids, carbon disulfide, ethanethiol, phenol, and 3-methyl indole concentrations in the manure of pigs fed diets supplemented with different kinds of AA were similar. Manure from pigs fed SAA diet had the highest total N and ammonium-N concentrations ($P < 0.01$). Ammonia emission and manure pH were similar between manure of pigs fed different diets. Diets affected cresols ($P < 0.01$), 4-ethyl phenol ($P = 0.03$), indole ($P < 0.01$), and methyl

sulfide (P = 0.04) concentrations in the manure. Methyl disulfide, hexanoic, heptanoic, and pentanoic acids were not detected in the manure of the different treatments. The detection limits of methyl disulfide and the three VFAs are 0.5 mg/kg and 0.1 g/kg, respectively. The correlation between ammonia emission and odor emission was – 0.3.

Table 7. Effects of amino acid supplementation on manure characteristics, only significant effects are presented

Variables	Diets[a]			SEM[b]	*P* value
	NOAA	SAA	TAA		
ln[c] (total N)	1.8[d]	1.9[e]	1.8[d]	0.02	0.04
ln (ammonium-N)	7.87[d]	8.05[e]	7.96[e]	0.03	< 0.01
ln (cresols)	3.69[d]	3.79[d]	4.18[e]	0.08	< 0.01
ln (indole)	2.22[d]	1.22[e]	2.27[d]	0.47	< 0.01
ln (4-ethyl phenol)	-0.16[de]	0.02[e]	-0.75[d]	0.42	0.03
ln (methyl sulfide)	0.61[d]	1.06[de]	1.48[e]	0.21	0.04

[a] SAA = 3 times requirement of sulfur-containing AA; TAA = 2 times requirement of Trp, and Tyr + Phe; NOAA = supplementation of these AA up to requirement.

[b] Standard errors of the means with 10 df for error.

[c] Natural logarithm.

[d,e] Means within rows missing a common superscript letter are different at $P < 0.05$.

DISCUSSION

Odor nuisance from pig production facilities is a growing concern for the society and consequently, it needs to be reduced. It is preferable that odor abatement solutions are done at the source of production. Diet is the first step in odor generation in animal husbandry (Le et al., 2005a) and it is generally accepted that by dietary manipulation, odor from pig manure can be influenced. Odor is a complex mixture of various compounds, in which sulfurous compounds, indolic, and phenolic compounds are considered the most important compounds in terms of odor strength and offensiveness (O'Neill & Phillips, 1992; Mackie *et al.*, 1998). Reducing precursors of these compounds in the diet should reduce odor strength and offensiveness. Therefore, the primary objective of this study was to determine manure odor strength and odor offensiveness from pigs fed diets with different types of AA supplementation. In addition, ammonia emission and manure characteristics were evaluated in this study.

We hypothesized that the surplus of sulfur-containing AA in the diet would provide precursors for the production of odor sulfurous compounds, such as hydrogen sulfide and

methanethiol, both of which can volatilize from the manure and create odor. As expected, manure of pigs fed the SAA diet had a higher odor concentration, and thus odor emission, than pigs fed the NOAA and TAA diets.

Manure from pigs fed the SAA diet had consistently lower odor concentration at different levels of odor hedonic tone and odor intensity than the TAA and NOAA diets. This implies that pigs fed the SAA diet produces a strong and offensive sensory response at lower levels of odor concentration than manure from pigs fed the TAA and NOAA treatments. Literature shows that the surplus of sulfur-containing AA in the diet provides precursors for many odorous compounds in manure and in the odorous air such as hydrogen sulfide (Ren, 1999; Sutton *et al*., 1999), methanethiol (Inoue *et al.*, 1995; Yoshimura *et al*., 2000), dimethyl sulfide (Kelly *et al.*, 1994), dimethyl disulfide (Bonnarme *et al*., 2001), and dimethyl trisulfide (Chin & Lindsay, 1994). In addition to sulfurous compounds which have a low odor detection threshold, concentrations of sulfurous compounds in the odorous air can be relatively high. Furthermore, the nature of the smell of sulfurous compounds is more offensive than that of other odorous compounds. This explains why manure from pigs fed the SAA diet had a higher odor concentration and higher odor emission, and a higher odor intensity and lower hedonic tone (more unpleasant) than manure from the pigs fed the TAA and NOAA diets.

It is generally accepted that the crystalline sulfur-containing AA are absorbed completely by the time digesta reaches the terminal ileum. The excess sulfur-containing AA are absorbed in the small intestine of animals and ended up as pyruvate (from Cys), Succinyl CoA (Met), and SO_4^{2-}. Sulfates are excreted via urine; in the manure sulfates are quickly converted to sulfur odorous compounds, mainly hydrogen sulfide and methanethiol. According to Spoelstra (1980), sulfate-reducing bacteria produce a trace amount of carbon disulfide, methyl sulfide, and ethanethiol.

Considering manure characteristics, analyzed sulfurous compounds: carbon disulfide, methyl sulfide, and ethanethiol concentrations in the manure of pigs fed the SAA treatment were not higher than in the other two treatments. A possibility is that these three compounds are not important in terms of mass concentration compared to other sulfurous compounds. In this experiment, precursors for sulfurous compounds in manure are mainly sulfates from the urine. Probably, carbon disulfide, methyl sulfide, and ethanethiol are mainly produced from metabolism of sulfur-containing AA in the intact protein in the large intestine of animals and in manure. This is supported by the finding of the experiments in Chapter 4 where increased dietary CP levels resulted in higher concentrations of these compounds in the manure.

According to Banwart and Bremmer (1975) hydrogen sulfide and methanethiol (methyl

mercaptan) represented 70 to 90% of the total S volatilized in the manure while Beard and Guenzi (1983) stated that most of the S emitted is in the form of hydrogen sulfide (39%) and methanethiol (34%). In addition, according to O'Neill and Phillips (1992), carbon disulfide and methyl sulfide are not among the compounds having the lowest odor detection threshold, as methanethiol and hydrogen sulfide were the compounds having the lowest odor detection threshold, 0.0003 and 0.1 $\mu g/m^3$, respectively. These two compounds were not analyzed in this experiment, because they have boiling points (6 and – 60.7 ^{0}C, respectively) too low to be captured in the manure samples for subsequent analysis. Furthermore, it is difficult to analyze these compounds by a normal gas chromatography.

From the results of odor strength and offensiveness and the concentrations of sulfurous compounds in the manure of pigs fed different diets, it is difficult to correlate the concentrations of single odorous compounds in the manure and the odor strength and offensiveness of the air emitting from the manure. Therefore, it is necessary to analyze odorous compounds in the odorous air. We feel the focus should be on volatile sulfurous compounds since this better reflects the relationship between odor sulfurous compounds and odor strength and offensiveness. Techniques to collect and to analyze odorous compounds in the air are, however, still under development.

Supplementation with a surplus of Trp and Phe + Tyr to a level twice the requirement estimate did not increase odor concentration, emission, and intensity, nor did it reduce hedonic tone (more unpleasant) from pig manure. These AA are precursors for phenol (Hammond *et al*., 1989; Sutton *et al*., 1999); 4-methylphenol (Hengemuhle & Yokoyama, 1990); 4-ethylphenol (Spoelstra, 1977; Hengemuhle & Yokoyama, 1990); indole, and 3-methylindole (Honeyfield & Carlson, 1990; Jensen & Jørgensen, 1994). There are two possible reasons. First, the excess of absorbed Trp, Tyr and Phe were degraded to carbon chain and nitrogen where excess would show up as increased urea excretion in urine. If this is the case then the excess Trp, Tyr and Phe absorbed in the small intestine of animals will not cause much odor nuisance from the manure. Second, although these compounds are thought to be mainly responsible for the smell in the headspace and ventilation air of pig houses (Schaefer, 1977; Williams, 1984; O'Neill & Phillips, 1992), these phenolic and indolic compounds may not be as important in causing odor nuisance as expected. The hypothesis for the importance of these compounds within previous studies was mainly based on their concentration in the air and/or in manure from pig production facilities and their olfactometry detection threshold. Odor is a complex mixture of various compounds, for example, Schiffman et al. (2001) reported 331 compounds, in which the relationship between each individual odor compound or a group of odor compounds and the odor strength

and offensiveness of the mixture of the odor air is not yet clear. In addition, by reviewing the literature, Le et al. (2005a) found that there was a large variation in the concentration of an odorous compound and its detection threshold. Nearly all olfactometry studies in literature have focused on odor concentration and emission. As discussed by P. D. Le *et al.* (Chapter 4), odor evaluation based on odor concentration has a limitation in comparing the odor relative to others. The use of odor concentration a lone, odor hedonic tone, and odor intensity jointly as in this experiment can give an overall comparison between odors.

Although the main objective of this study was to determine the effect of AA supplementation to the diet on odor strength and offensiveness, ammonia emission was also considered because it is a serious environmental problem. Odor abatements are only of interest if they do not increase other environmental problems such as ammonia emission. It is well-documented that ammonia emission from pig manure is mainly influenced by pH and ammonia concentration. These two factors are mainly driven by dietary protein content and electrolyte balance (Canh *et al.*, 1998a; Canh *et al.*, 1998). The similar ammonia emission from manure of pigs fed the diets supplemented with different types of AA can be explained by the fact that the pigs fed the NOAA, SAA, and TAA diets had similar CP and electrolyte balance concentration (Table 2). In addition, although total N and ammonium-N concentrations in the manure from pigs fed the SAA treatment were higher than in manure from pigs fed the NOAA and TAA treatments, differences were small and partly compensated by small differences in pH (Table 6).

This study shows that the correlation between ammonia emission and odor emission is low and negative (-0.3). According to P. D. Le *et al*. (Chapter 4) the correlation between ammonia emission and odor emission in a controlled environment is very low, because odor is a complex mixture of various compounds, while ammonia is a single compound. In addition, ammonia is not a very offensive odor (Oldenburg, 1989). As reported by P. D. Le *et al*. in Chapter 4 inconsistent findings were found in literature about the relationship between ammonia and odor emission from pig manure or from pig production facilities. The inconsistencies likely come from the differences in environmental factors, housing design, dietary composition, and animal types between experiments. In this study and other studies (Chapter 4, 5, 6 &7), these sources of variances were prevented, because ammonia samples were collected from the same animal house, at the same time, and with the same air flow rate.

IMPLICATIONS

This study demonstrates that supplementing crystalline sulfur-containing amino acids to the diet above the requirement for the pig increases the odor strength and offensiveness from pig manure. Therefore, to reduce odor from pig manure sulfur-containing amino acids should be formulated very near the requirement for the animal. Supplementing crystalline phenylalanine, tyrosine, and tryptophan to the diet, above requirement does not increase odor strength and offensiveness from pig manure. Ammonia emission has a low correlation with odor emission, so strategies that have demonstrated to be successful in reducing ammonia emission may not have a similar impact on odor emission. From this study it is clear that sulfurous compounds contribute significantly to odor nuisance.

6

EFFECTS OF FERMENTABLE PROTEIN LEVEL ON ODOR FROM PIG MANURE

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ABSTRACT: *An experiment was conducted in growing pigs to determine the effects of levels of potentially fermentable protein (apparent ileal non-digestible protein) on odor emission, odor intensity, odor hedonic tone, and ammonia emission from pig manure, and on manure characteristics (pH, ammonium, total N, indolic, phenolic and sufurous compounds, and volatile fatty acid concentrations). Pigs (n = 18) were allocated in a randomized complete block arrangement having 3 treatments in 6 blocks. Treatments had different levels of fermentable protein in the diet: 28, 38, 48 g/kg feed as-fed basis. Pigs with an initial body weight (BW) of 41.3 ± 3.2 kg (mean ± SD) were penned individually in partly slatted floor pens and offered a daily feed allowance of 2.8 x maintenance requirement for net energy (293 kJ/BW$^{0.75}$). Feed was mixed with water, 1/2.5 (w/w). Feces and urine of each pig were accumulated in separate manure pits under the slatted floor. After an adaptation period of 2 wk, and after cleaning the manure pits, manure was subsequently collected. In the 5th wk of the collection period, separate samples for odor, ammonia, and other measurements were collected directly from each manure pit. Odor concentration, and hedonic tone and odor intensity above odor detection threshold were measured by olfactometry. Manure samples were analyzed for volatile fatty acids (VFA), indolic, phenolic, sulfurous compounds, ammonium and total N concentrations. Data were evaluated using analysis of variance. Regardless of dietary treatment, all pigs had similar performance. Results showed no effects of fermentable protein levels on odor emission, odor intensity, and hedonic tone of the odorous air nor on ammonia emission from the pig manure. Fermentable protein levels did not influence ammonium, VFA, cresols, and indole concentrations in the manure, but increased fermentable protein levels enhanced the concentrations of total N, methyl sulfide, carbon disulfide, ethanethiol, phenol, 3-methyl indole, and 4-ethyl phenol in the manure (P <= 0.01). It can be concluded that reduction in fermentable protein levels does not decrease odor emission, odor intensity and odor hedonic tone. To reduce odor by means of protein, the levels of fermentable protein should not be considered alone, it should be considered together with total dietary crude protein level and ileal digestible crude protein level.*

Key words: *Fermentable Protein, Diet, Growing Pig, Odor*

INTRODUCTION

Intensive pig operations are recognized as potential sources for odor nuisance for residents in the surrounding areas. Odor is evaluated through its strength (concentration and intensity) and offensiveness (hedonic tone). Odor is mainly generated by the microbial conversion of feed in the gut of pigs and by microbial conversion of pig excreta under anaerobic conditions in manure storages. There are a great number of odorous compounds identified in air and manure from animal production facilities. O'Neill and Phillips (1992) summarized 168 compounds in livestock wastes or in the air around pig production facilities. Recently, Schiffman *et al*. (2001) identified a total of 331 different odorous compounds in the air and lagoon water from pig production facilities. The odorous components can be classified into four main groups, namely (1) sulfurous compounds, (2) indolic and phenolic compounds, (3) volatile fatty acids (VFA), and (4) ammonia and amines.

Many odorous compounds are intermediate or end products of protein metabolism in the large intestine of the animals and in the manure storages (Le *et al.*, 2005a). In addition, it is generally accepted that odorous compounds e.g. sulfurous, indolic and phenolic compounds, and branched- chain VFA produced from protein fermentation (protein used as an energy source by microorganisms) have a higher offensive sensory response (or a smaller value of hedonic tone) and a higher magnitude of odor sensation (or a higher value of odor intensity) than straight-chain VFA produced from fermentable carbohydrates. Therefore, protein is the first dietary component that should be altered in order to reduce odor. One strategy is to reduce the amount of protein which is subject to bacterial breakdown both in the large intestine of the animals and in the manure. This strategy can be achieved by (1) reducing the amount of protein, at the same time supplementing essential amino acids (AA) to the diet and (2) selecting feedstuffs with a high ileal digestible protein so a small amount of ileal non-digestible protein goes into the large intestine of the animals or/and in the manure. In this paper, the apparently ileal non-digestible protein is defined as potentially fermentable protein (FP). It can be assumed that protein entering the large intestine can be potentially used for odor formation.

The effects of reduced crude protein (CP) and AA supplemented diets on odor strength and offensiveness from the growing pig manure were reported by P. D. Le *et al*. (Chapter 4). They found that odor concentration and odor emission were reduced by 80% when dietary CP was reduced from 18% to 12% while maintaining the same AA composition. Available literature contains no information on the effects of fermentable protein levels in the diet on odor strength and odor offensiveness from pig manure. The main objective in the present study was to

determine whether fermentable protein levels influence odor strength and odor offensiveness and ammonia emission from pig manure and manure characteristics. We hypothesize that odor strength and offensiveness are reduced by decreasing the amount of fermentable protein. This study simulated the odor emission from pig manure by collecting odor samples directly from the manure pit.

MATERIALS AND METHODS

ANIMALS, EXPERIMENTAL DESIGN, AND DIETS

A randomized complete block arrangement with 3 treatments in 6 blocks was used to study effects of fermentable protein levels on odor strength (odor concentration and odor intensity) and odor offensiveness (odor hedonic tone), ammonia emission from growing pig manure and manure characteristics. There were three fermentable protein levels: 28 (LFP), 38 (MFP), 48 (HFP) g/kg diet, as-fed basis. Treatment had 6 replicates with 1 replicate in each of 6 blocks, of which a block consisted of samples collected on the same day and from animals with similar initial body weight (BW).

In total 18 growing barrows (Great Yorkshire x Dutch Landrace) with an initial BW of 41.3 ± 3.2 kg (mean ± SD) were allocated to 6 blocks with blocks based on initial BW. Pigs were penned individually in galvanized steel pens (2.1 x 0.96 m) with a slatted floor at the rear (0.97 x 0.96 m). There was a separate manure pit under the slatted floor of each pen. The size of the manure pit was 1.35 x 0.91 x 0.36 m (length x width x depth). Pigs were housed in a mechanically-ventilated and temperature-controlled room. Temperature and relative humidity were recorded every 5 min. The average temperature and relative humidity of the room during the experimental period were 21.0 ^{0}C ± 0.84 and 50.0 % ± 5.32 (mean ± SD), respectively.

Diets had a similar content of CP (150 g/kg diet as-fed) but different contents of fermentable protein. This was achieved by using protein sources with different ileal digestibility. In addition, diets had similar contents of net energy (NE) according to CVB (2004), non-starch polysaccharides (NSP), dietary electrolyte balance (dEB), mineral content, and vitamins (Tables 1 & 2). Diets were supplemented with essential AA: Lys, Trp, Thr, Phe, Tyr and Met up to the level of animal requirement based on apparent ileal digestibility (CVB-Animal Feed Product Board, 2004).

Experimental diets were analyzed for AA composition, ash, dry matter (DM), CP, minerals, crude fiber, fat, starch, sugar and gross energy. The chemical analyses were conducted as described in P. D. Le *et al*. (Chapter 4).

Table 1. Ingredient composition of the experimental diets, as-fed basis

Composition (%)	Diets[a]		
	LFP	MFP	HFP
Wheat	50.00	25.00	0.00
Maize	0.00	21.09	41.79
Tapioca meal (starch 62.5-65.7%)	13.13	15.12	17.11
Wheat gluten meal	4.00	2.00	0.00
Potato protein	2.53	1.27	0.00
Wheat middings	15.00	7.50	0.00
Soybean meal extract (crude fiber <5%)	0.00	6.12	12.24
Beans (phaseolus), heat treated	0.00	7.50	15.00
Alafa meal	0.00	3.75	7.50
Palm kernel expeller (crude fibre < 22%)	6.60	3.30	0.00
Cane molasses, < 47.5% sugar	3.00	3.00	3.00
K_2CO_3	0.93	0.52	0.11
Soybean oil	1.69	1.08	0.47
CaCO3	1.15	0.96	0.76
Monocalcium phosphate.H_2O	0.68	0.67	0.66
Salt	0.48	0.48	0.48
Premix[b]	0.20	0.20	0.20
L-Lysine HCl	0.45	0.35	0.25
DL-Methionine	0.06	0.13	0.19
L-Threonine	0.09	0.08	0.07
L-Tryptophan	0.01	0.03	0.04
L-Phenylalanine	0.00	0.04	0.08
L-Tyrosine	0.00	0.02	0.04

[a] Fermentable protein (FP) level LFP = 28g/kg diet ; MFP = 38 g/kg diet; HFP = 48 g/kg diet.

[b] The vitamin-mineral premix supplied per kg feed included 7,000 IU vitamin A, 1,700 IU vitamin D3, 20 IU vitamin E, 1.5 mg vitamin K, 1.5 mg vitamin B1, 4 mg vitamin B2, 11 mg d-pantothenic acid, 18 mg niacine, 18 µg vitamin B12, 0.1 mg folic acid, 1.0 mg vitamin B6, 100 mg choline chloride, 75 mg Fe, 10 mg Cu, 65 mg Zn, 30 mg Mn, 0.15 mg Co, 0.75 mg I, 0.30 mg Se.

Pigs were fed 2.8 times the maintenance NE requirement (279 kJ/$BW^{0.75}$). Water was restrictedly provided by mixing feed with water in the ratio of 1/2.5 (w/w). Apart from water with feed, no additional water was given to the pigs. So, it was aimed to have the same amount of feed and water intake by the pigs giving a similar amount of manure. Pigs were fed 2 times per day at 0800 and 1500. The amount of feed provided was adjusted each day according to an assumed BW gain of 780 g d^{-1}. Feed intake was recorded every day. Pigs were weighed at the beginning and at the end of the experimental period just before the morning feeding.

Daily gain and feed efficiency were derived from the feed intake and the increase in BW during the experimental period.

Table 2. Nutrient composition of experimental diets, as-fed basis

Composition	Unit	Diets[a]		
		LFP	MFP	HFP
Calculated composition				
Crude protein (CP)	%	15.00	15.00	15.00
Fecal digestible CP	%	12.18	12.03	11.88
Ileal digestible CP	%	12.16	11.18	10.20
Fermentable protein (FP)	%	2.80	3.80	4.80
NE	kcal/kg	2183	2183	2183
Digestible NSP[b]	%	9.00	9.44	9.87
Illealy digestible amino acids				
Lys	%	0.74	0.74	0.74
Met	%	0.27	0.32	0.36
Cys	%	0.23	0.19	0.14
Met + Cys	%	0.50	0.50	0.50
Thr	%	0.43	0.43	0.43
Trp	%	0.14	0.14	0.14
Phe	%	0.59	0.59	0.59
Tyr	%	0.40	0.40	0.40
Phe + Tyr	%	0.99	0.99	0.99
Analyzed composition				
Gross energy	kcal/kg	3,804	3,887	3,950
CP (Nx6.25)	%	14.81	15.41	16.10
Dry matter	%	86.11	86.70	86.88
Ash	%	5.41	5.66	5.52
Crude fiber	%	3.61	4.27	4.96
Total fat	%	2.76	4.25	5.62
Sugar	%	3.89	4.75	5.12
Starch	%	40.14	39.25	38.21
NSP	%	19.09	17.38	16.31
Ca	%	0.64	0.64	0.59
Mg	%	0.15	0.16	0.17
P	%	0.49	0.47	0.44
Na	%	0.19	0.22	0.21
K	%	1.15	1.19	1.15
Sulfate	%	0.11	0.12	0.16
Chloride	%	0.45	0.48	0.43
dEB[c]	meq/kg	251	267	265

dEBS-a[d]	meq/kg	227	241	232
Amino acids				
Ala	%	0.56	0.68	0.79
Arg	%	0.71	0.86	0.98
Asp	%	0.90	1.27	1.63
Cys	%	0.25	0.25	0.24
Glu	%	3.45	3.11	2.71
Gly	%	0.60	0.63	0.66
His	%	0.34	0.39	0.44
Ile	%	0.51	0.59	0.66
Leu	%	0.98	1.15	1.30
Lys	%	0.60	0.85	0.95
Met	%	0.24	0.32	0.35
Phe	%	0.65	0.76	0.82
Pro	%	1.19	1.08	1.04
Ser	%	0.64	0.71	0.75
Thr	%	0.54	0.59	0.64
Trp	%	0.19	0.21	0.23
Tyr	%	0.48	0.53	0.62
Val	%	0.65	0.71	0.76

[a] Fermentable protein levels: LFP = 28g/kg diet ; MFP = 38 g/kg diet; HFP = 48 g/kg diet.

[b] Non-starch polysaccharides (NSP) were determined as organic matter–(CP+crude fat+starch+sugar).

[c] dEB was determined as mEq = Na + K – Cl.

[d] dEBS-a was calculated as mEq=Na+K–Cl–2S. dEBS-a does not take into account S present in AA.

After an adaptation period of 2 wk to allow the pigs to acclimatize to the experimental diets and pens, the manure pits were cleaned. After that, feces and urine accumulated in the manure pit. In the 5th wk of the collection period, odor, ammonia and manure samples were collected for subsequent analysis.

COLLECTING ODOR SAMPLES AND MEASURING ODOR CONCENTRATION, ODOR HEDONIC TONE AND ODOR INTENSITY

Odor samples were collected as described by P. D. Le *et al*. (Chapter 4). The schematic view of the odor sample collection is shown in Fig. 1. One odor sample was collected directly from manure in each manure pit for approximately 90 min. Odor sample was measured for odor concentration according to CEN standard 13725 (2003) as described in detail by Le *et al*. (2005b). Odor concentrations of the examined samples were expressed in European odor units per cubic meter air ($ou_E\ m^{-3}$). One odor unit is defined as the amount of odor-causing

gases which, when diluted in 1 m^3 of air, can just be distinguished from clean air by 50% of the members of an odor panel.

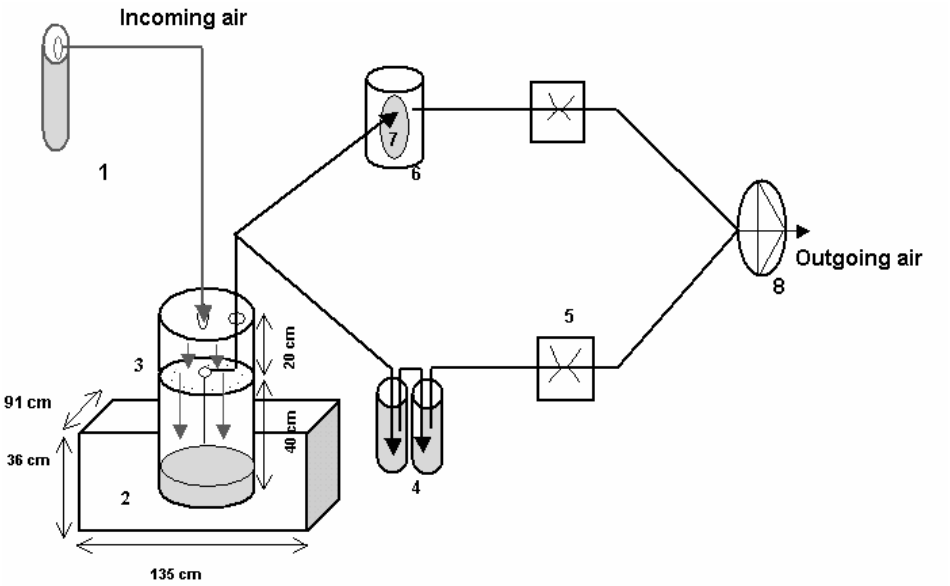

Fig. 1. Schematic view of the odor and ammonia sample collection (1 = odor free air pressurized cylinder, 2 = manure pit, 3 = vessel, 4 = impingers, 5 = critical glass capillary, 6 = rigid container, 7 = odor bag, 8 = vacuum pump)

Odor emission was defined as the number of odor units emitted from a manure surface per second. It was calculated by multiplying the ventilation rate with the corresponding odor concentration (Equation 1).

$$E_{odor} = (C_{odor} \times V \times 10{,}000) / (60 \times 1{,}000 \times 595) \quad [1]$$

where E_{odor} = odor emission s^{-1} m^{-2} (ou_E $s^{-1}m^{-2}$), C_{odor} = odor concentration (ou_E m^{-3}), and V = ventilation rate (L min^{-1}), 10,000 = cm^2 m^{-2}, 60 = sec min^{-1}, 1,000 = liters m^{-3}, and 595 = the cm^2 surface area of the manure pit.

Measuring odor hedonic tone (H) and odor intensity (I) was carried out as described by P. D. Le *et al*. (Chapter 4). Hedonic tone is used to evaluate odor offensiveness and is a measure of the unpleasantness or pleasantness of the perceived odor. Odor intensity refers to the magnitude of the odor sensation. Hedonic tone was measured by using a 9- point hedonic scale ranging from – 4, extremely unpleasant or offensive through 0, neither pleasant nor unpleasant or neutral odor to + 4, extremely pleasant. Odor intensity was measured by using a 7- point intensity scale ranging from 1, no odor through 2, very faint odor to 7, overwhelming odor. For each odor sample, the hedonic tone and the odor intensity at each odor concentration level above the detection threshold were calculated as the average of the hedonic tone and the

odor intensity of perceived by all panelists, and plotted against the logarithm of the odor concentration. From the regression lines obtained, the odor concentration at H = -1 (mildly unpleasant), H = -2 (moderately unpleasant), I = 1 (no odor), I = 2 (very faint odor), I = 4 (distinct odor) were derived. Regression lines of hedonic tone and intensity were also plotted against logarithm of the odor concentration for all samples in the same treatment.

COLLECTING AND MEASURING AMMONIA EMISSION

Samples for determining ammonia emission were collected at the same time and for the same duration of time as odor samples (Fig.1). Ammonia in outgoing air was removed by passing through 2 ammonia traps (impingers), each containing about 20 ml 0.5 M HNO_3 solution. The system was operated for about 90 min. The ammonia concentration and the volume of the liquid were determined in the first and the second impingers. Ammonia emission per time unit and surface unit was calculated with Equation 2.

$$MNH_3 = (CNH_3 \times V \times 10{,}000) / (T \times 60 \times 595) \quad [2]$$

where MNH_3 = ammonia emission (mg s^{-1} m^{-2}), CNH_3 = ammonia concentration (mg mL^{-1} HNO_3), V = volume of HNO_3 solution (mL), 10,000 = cm^2 m^{-2}, T = sampling time (min), 60 = s min^{-1}, and 595 = the cm^2 surface area of the manure pit.

COLLECTION AND MEASUREMENT OF MANURE CHARACTERISTICS

Manure samples were collected to evaluate the effect of the diets on manure characteristics. These include DM, ash, total-N, ammonium-N, pH, VFA (acetic, propionic, butyric, pentanoic, iso-butyric, iso-pentanoic, hexanoic, and heptanoic acid), indoles (indole, and 3-methyl indole) and phenol compounds (phenol, 4-ethyl phenol, and cresols), and sulfurous compounds (carbon disulfide, methyl sulfide, methyl disulfide, and ethanethiol). One manure sample was collected in each manure pit. Manure samples were collected and analyzed as described in P. D. Le *et al*. (Chapter 4).

STATISTICAL ANALYSIS

The effects of the fermentable protein levels on daily gain, daily feed intake, feed efficiency, odor concentration, odor emission, odor hedonic tone, odor intensity, ammonia emission, and manure characteristics were analyzed using ANOVA of GenStat statistical package 7th version (GenStat VSN International Ltd., 2004) with the following model.

$$y_{ij} = \mu + \rho_j + \alpha_i + e_{ij}$$

where: y_{ij}: dependent variables, μ = overall mean, ρ_j = effect of block, j = 1-6, α_i = effect

of fermentable protein levels, i = 1, 2, 3, and e_{ij} = experimental error.

Data was presented either as arithmetic or as geometric mean. A natural log transformation was applied to odor emission, concentrations of VFA, total N, ammonium-N, indolic and phenolic compounds, and sulfurous compounds since they were skewed and not normally distributed.

For each treatment, odor hedonic tone and odor intensity was plotted against the natural logarithm of odor concentration, and odor hedonic tone was plotted against odor intensity. The differences between slopes and between intercepts were tested to decide whether there should be separate regression lines for treatments or a common line for all treatments. The relationship between ammonia emission and odor emission was determined by linear regression.

RESULTS

EFFECTS OF THE FERMENTABLE PROTEIN LEVELS IN THE DIET ON DAILY GAIN, DAILY FEED INTAKE AND FEED EFFICIENCY

The effects of different fermentable protein (FP) levels on production parameters, daily gain, daily feed intake and feed efficiency of pigs fed diets with different fermentable protein levels are summarized in Table 3. Average daily feed intake, daily gain and feed efficiency were similar among treatments.

Table 3. Effects of the fermentable protein levels on daily gain, daily feed intake and feed efficiency

Variables	Diets[a]			SEM[b]	*P* value
	LFP	MFP	HFP		
Initial BW, kg	41.4	41.2	41.3	0.3	0.95
Final BW, kg	73.7	75.4	75.2	0.6	0.20
ADFI, kg/ day	1.87	1.87	1.86	0.007	0.27
ADG, g/ day	702	742	736	15	0.20
G : F, g/kg	376	398	397	7.5	0.11

[a] Fermentable protein levels: LFP = 28g/kg diet ; MFP = 38 g/kg diet; HFP = 48 g/kg diet.

[b] SEM = Standard errors of means with 10 df for error.

EFFECTS OF THE FERMENTABLE PROTEIN LEVELS IN THE DIET ON ODOR STRENGTH AND OFFENSIVENESS

Descriptive statistics and analysis of variance of the effects of fermentable protein levels on odor strength and offensiveness from growing pig manure are given in Tables 4 and 5,

respectively. Geometric means of odor concentration and odor emission ranged from 13,224 $ou_E\ m^{-3}$ and 1.88 $ou_E\ s^{-1}\ m^{-2}$, respectively for LFP treatment to 19,732 $ou_E\ m^{-3}$ and 2.78 $ou_E\ s^{-1}\ m^{-2}$, respectively for HFP treatment. Analysis of variance shows that fermentable protein levels did not affect odor concentration nor odor emission from the manure. The least significant differences of means (5% level) of odor concentration and odor emission are 1.24 and 1.23 (in logarithmic scale), respectively. Odor concentration at different levels of hedonic tone and odor intensity of LFP, MFP, and HFP treatments were similar.

Table 4. Descriptive statistics of odor strength (concentration and intensity) and offensiveness (hedonic tone) from manure of growing pigs fed diets with different fermentable protein levels (*n = 18*). Geometric and arithmetic means are given

Odor variables	Diets[a]					
	LFP		MFP		HFP	
	GM[b]	AM[c]	GM	AM	GM	AM
Concentration, $ou_E\ m^{-3}$	13,224	16,424	14,045	22,529	19,732	28,190
Emission, $ou_E\ s^{-1}m^{-2}$	1.88	2.33	1.98	3.16	2.78	3.91
Concentration at H^d = -1, $ou_E\ m^{-3}$	3.19	3.83	1.73	2.29	1.65	3.11
Concentration at H = -2, $ou_E\ m^{-3}$	9.39	11.58	6.69	9.03	7.39	10.01
Concentration at I^e = 1, $ou_E\ m^{-3}$	0.75	0.90	0.47	0.50	0.50	0.77
Concentration at I = 2, $ou_E\ m^{-3}$	1.72	2.00	1.25	1.31	1.35	1.81
Concentration at I = 4, $ou_E\ m^{-3}$	9.03	10.08	8.76	9.68	9.87	11.02

[a] Fermentable protein levels: LFP = 28g/kg diet ; MFP = 38 g/kg diet; HFP = 48 g/kg diet.

[b] Geometric mean; [c] Arithmetic mean; [d] Hedonic tone; [e] Intensity.

Table 5. Effects of fermentable protein levels in the diet on odor strength (concentration and intensity) and offensiveness (hedonic tone) from manure of growing pigs

Variables	Diets[a]			SEM[b]	*P* value
	LFP	MFP	HFP		
ln[c](Odor concentration)	9.49	9.55	9.89	0.39	0.75
ln (Odor emission)	0.63	0.68	1.02	0.39	0.75
ln (Odor concentration at H^d = -1)	1.16	0.55	0.50	0.37	0.47
ln (Odor concentration at H = -2)	2.24	1.90	2.00	0.25	0.62
ln (Odor concentration at I^e = 1)	-0.29	-0.76	-0.69	0.25	0.38
ln (Odor concentration at I = 2)	0.54	0.22	0.30	0.20	0.50
ln (Odor concentration at I = 4)	2.20	2.17	2.29	0.14	0.85

[a] Fermentable protein levels: LFP = 28g/kg diet ; MFP = 38 g/kg diet; HFP = 48 g/kg diet.

[b] SEM = Standard errors of the means with 10 df for error.

[c] Natural logarithm; [d] Hedonic tone; [e] Intensity.

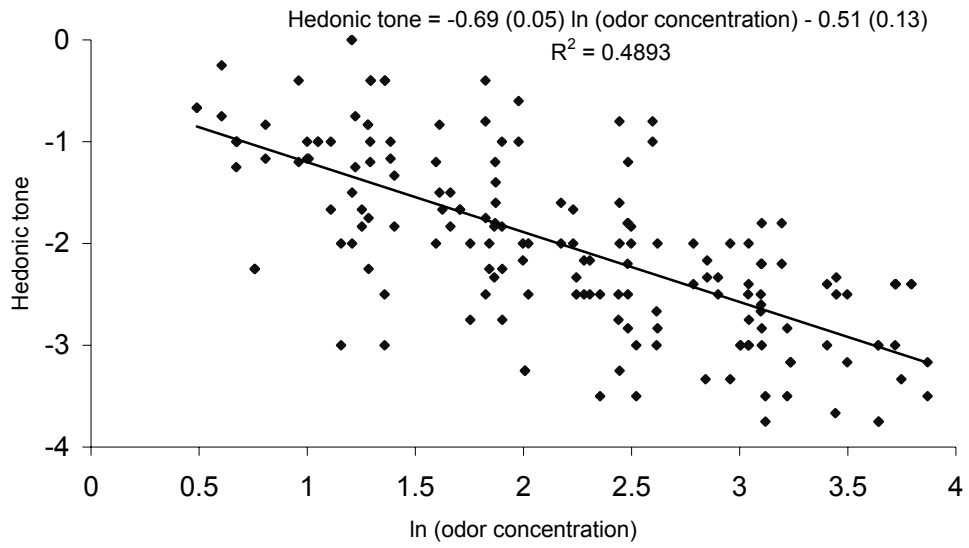

Fig. 2. Hedonic tone as a function of odor concentration with regression line

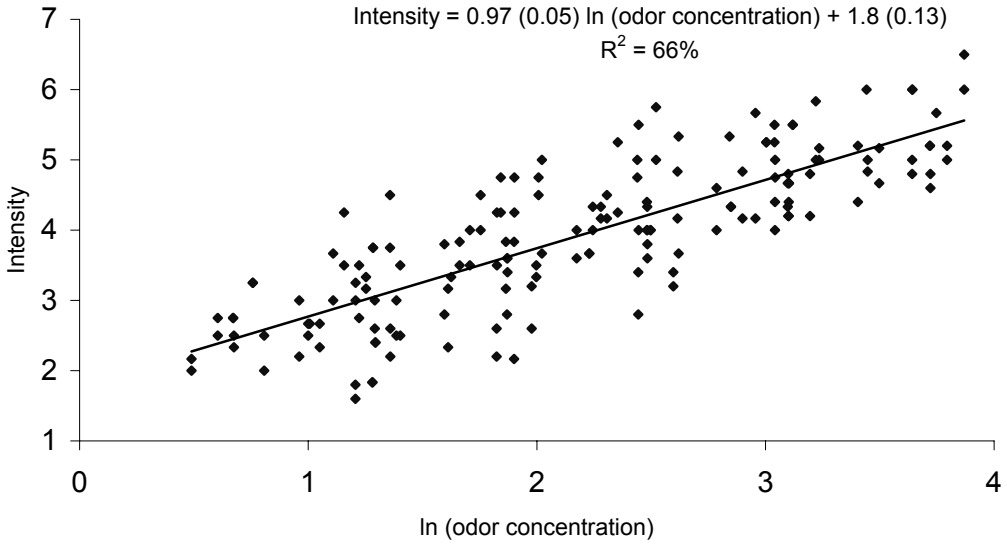

Fig. 3. Odor intensity as a function of odor concentration with regression line

Relationships between odor concentration and hedonic tone, between odor concentration and intensity, and between intensity and hedonic tone are shown in Figs 2, 3 and 4, respectively. Intercepts and slopes were not affected by fermentable protein levels, therefore one regression line is given in each respective relationship. Fig. 2 shows that increased odor concentrations resulted in lower hedonic tones which means that the odorous air becomes more unpleasant. It can be seen from Fig. 3 that increased odor concentrations resulted in higher odor intensities which mean higher magnitudes of odor sensation. Similar to odor concentration, increased odor intensity caused lower hedonic tones or more unpleasant odor (Fig. 4).

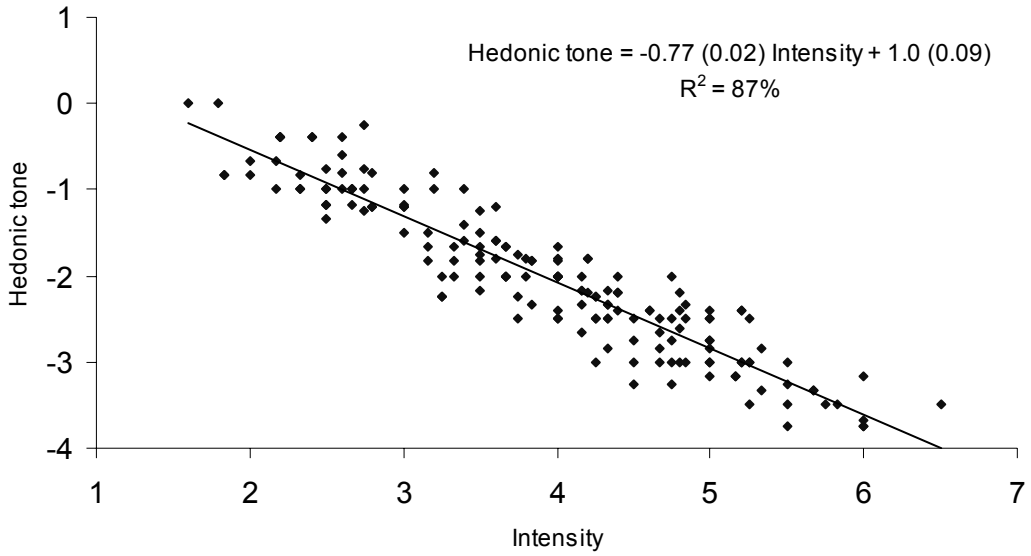

Fig. 4. Hedonic tone as a function of odor intensity with regression line

EFFECTS OF THE FERMENTABLE PROTEIN LEVELS IN THE DIET ON MANURE CHARACTERISTICS AND AMMONIA EMISSION

Descriptive statistics and analyses of variance of the effects of fermentable protein levels on manure characteristics and ammonia emission from pig manure are presented in Tables 6 and 7, respectively. Different fermentable protein levels did not influence individual and total VFA, ammonium-N, cresols and indole concentrations and pH of the manure. Increased fermentable protein levels resulted in higher total N, methyl sulfide, carbon disulfide, ethanthiol, phenol, 3-methyl indole, and 4-ethyl phenol concentrations in the manure ($P <= 0.01$). Methyl disulfide, hexanoic, heptanoic and pentanoic acids were not detected in the manure of the different treatments. The detection limits of methyl disulfide and the three VFA are 0.5 mg/kg and 0.1 g/kg, respectively. The correlation between ammonia emission and odor emission was 0.14.

Table 6. Descriptive statistics of manure characteristics and ammonia emission from manure of pigs fed diets with different fermentable protein levels (*n = 18*)

Variables	Diets[a]		
	LFP	MFP	HFP
Dry matter, g kg^{-1}	114.1 (4.05)[b]	115.2 (11.68)	121.8 (10.42)
Ash, g kg^{-1}	30.5 (1.04)	30.8 (3.02)	34.1 (2.66)
Total VFA[c], g kg^{-1}	6.4 (1.39)	5.8 (1.6)	5.9 (1.29)
Acetic acid, g kg^{-1}	4.2 (0.82)	3.8 (0.45)	3.9 (0.77)
Propionic acid, g kg^{-1}	1.4 (0.32)	1.2 (0.35)	1.1 (0.24)
Butyric acid, g kg^{-1}	0.42 (0.25)	0.33 (0.13)	0.38 (0.12)
Iso-butyric acid, g kg^{-1}	0.12 (0.04)	0.13 (0.05)	0.13 (0.08)
Iso-pentanoic acid, g kg^{-1}	0.30 (0.06)	0.38 (0.05)	0.38 (0.12)
Total N, g kg^{-1}	6.0 (0.21)	6.3 (0.62)	7.0 (0.46)
Ammonium-N, g kg^{-1}	2.6 (0.16)	2.6 (0.25)	2.7 (0.29)
pH	7.75 (0.17)	7.89 (0.11)	7.85 (0.14)
Ammonia emission, mg $s^{-1}m^{-2}$	0.014 (0.003)	0.011 (0.003)	0.013 (0.07)
Phenol, mg kg^{-1}	16.2 (1.68)	20.6 (4.75)	26.5 (5.92)
Cresols, mg kg^{-1}	40.9 (9.7)	52.4 (11.65)	49.3 (9.45)
4-ethyl phenol, mg kg^{-1}	0.89 (0.28)	5.32 (2.45)	16.15 (3.5)
Indole, mg kg^{-1}	9.8 (3.81)	10.7 (2.22)	11.8 (3.85)
3-methyl indole, mg kg^{-1}	5.2 (1.16)	6.4 (1.55)	8.7 (1.69)
Carbon disulfide, mg kg^{-1}	0.53 (0.19)	9.5 (0.62)	20.7 (2.72)
Methyl sulfide, mg kg^{-1}	1.2 (1.4)	2.1 (3.47)	73.9 (69.5)
Ethanethiol, mg kg^{-1}	80 (6.7)	96.7 (14)	105.4 (20.1)

[a] Fermentable protein levels: LFP = 28g/kg diet ; MFP = 38 g/kg diet; HFP = 48 g/kg diet.

[b] Standard deviation.

[c] Total VFA = acetic acid+propionic acid+butyric acid + *iso*-butyric acid +*iso*-pentanoic acids.

Table 7. Effects of the fermentable protein levels on manure characteristics and ammonia emission, only significant effects ($P < 0.05$) are presented

Variables	Diets[a]			SEM[b]	*P* value
	LFP	MFP	HFP		
ln[c] (total N)	1.79[d]	1.84[e]	1.94[f]	0.03	0.01
ln (methyl sulfide)	0.61[d]	0.64[d]	3.86[e]	0.5	< 0.01
ln (carbon disulfide)	- 0.69[d]	2.25[e]	3.02[f]	0.1	< 0.01
ln (phenol)	2.78[d]	3.01[e]	3.26[f]	0.07	< 0.01
ln (3-methyl indole)	1.63[d]	1.83[d]	2.15[e]	0.08	< 0.01
ln (4-ethyl phenol)	-0.16[d]	1.59[e]	2.76[f]	0.15	< 0.01
ln (ethanethiol)	4.35[d]	4.56[e]	4.64[e]	0.06	0.01

[a] Fermentable protein level: LFP = 28g/kg diet ; MFP = 38 g/kg diet; HFP = 48 g/kg diet.

[b] Standard error of the mean with 10 df for error.

[c] Natural logarithm.

[d,e,f] Means within rows missing a common superscript letter are different at $P < 0.05$.

DISCUSSION

Odor nuisance from pig production facilities is a growing concern for downwind neighbors and residential areas, consequently it should be reduced. Perhaps the greatest opportunity for reducing odor nuisance from pig production facilities is through prevention of the formation of odorous compounds or by altering the compounds formed. Diet is the first step in the odor production chain from feed to manure. Dietary alteration is a relatively unexplored field, but it has a significant potential to reduce odor from pig manure (Le *et al*., 2005a). Odor compounds in the manure come from three main sources: precursors excreted via urine, precursors excreted via feces, and odor compounds excreted directly via feces. Dietary manipulation may alter the amount of precursors and/or odor compounds in urine, feces or both and so alter odor production in the manure.

Protein metabolites are main contributors to odor nuisance (Schaefer, 1977; Spoelstra, 1980; Mackie *et al.*, 1998). Protein that arrives into the large intestine can be subject to bacterial fermentation and is called fermentable protein. The fermentation of protein in the hindgut creates odorous compounds and provides precursors for odor formation in the manure. Thus, it was expected that reducing the amount of fermentable protein could reduce odor production as a result of less protein for an energy source for bacterial utilization.

In the current experiment, although increased fermentable protein levels resulted in increased odor concentrations and odor emissions from manure of growing pigs, they

were not statistically different ($P > 0.05$). Literature supplies no information on effects of fermentable protein levels on odor concentration and emission from pig manure thus no comparisons or confirmations can be made. There are two possible reasons leading to the non-effects of fermentable protein levels on odor concentration and emission. First, the differences in the amount of fermentable protein between diets might be too small to create a clear contrast in odor concentration and emission. Table 8 shows the estimated amount of protein at different parts from feed to feces. It can be seen from the table that the contrast in fermentable protein levels between treatments is from 10 g to 20 g/kg diet, or from 6 to 13% of the crude protein in the diet that is relatively small. Second, increased fermentable protein levels resulted in a higher amount of protein fermented in the large intestine of animals, 0.2, 8.5 and 16.8 g/kg diet as-fed basis, respectively for LFP, MFP and HFP treatments. Consequently, more odorous compounds or odor precursors are expected from the HFP treatment than from LFP treatment. Odorous compounds produced from protein fermentation in the large intestine of animals are absorbed via the gut wall and transferred to the liver where they are detoxicated and excreted via urine in the form of glucoronides and sulfates. In the manure, odorous compounds are quickly released from glucuronides and sulfates. However, it seems that the fermentable protein may not be the only source of odor precursors for odor production in the manure. It can be seen from Table 8 that the three treatments differed in the apparent ileal digestible protein, 121.6, 111.8 and 102.0 g/kg diet, respectively for LFP, MFP, and HFP. Pigs fed these diets had similar daily weight gain so it is expected that (1) pigs fed diets with a higher ileal digestible protein excreted more odorous precursors via urine for odor production in the manure. At the same time, diets with a high ileal digestible protein level had a smaller amount of protein entering the large intestine and a smaller amount of protein, which could act as protein precursor for odor production in the large intestine of animals. (2) the situation is the other way around for pigs fed diets with a lower apparent ileal digestible protein content. As a result, the effects of different fermentable protein levels on excretion of odor precursors and consequently odorous compounds might have been equalized/compensated by odor precursors excreted via urine.

In the large intestine of animals and in the manure, microbes prefer to use NSP as an energy source and protein as a nitrogen source for their biomass syntheses. This process produces less odor than when protein is used as an energy source. The latter process prevails as the amount of NSP is relatively low when compared to protein. It is believed that there is an optimum ratio between protein and NSP. At this ratio odor production is minimized. For the matter of calculation, we assumed that 90% of protein in feces is in the form of bacterial protein. The calculated amount of protein in biomass is 25.4, 26.7 and 28.1 g/kg diet as-fed

basic, respectively for LFP, MFP and HFP treatments. So, the HFP treatment produced lightly more protein in biomass than the LFP treatment. This may partly reduce the effect of the HFP treatment on odor production from the manure.

Table 8. Estimated amount of protein and NSP at different parts from feed to feces (g/kg diet, as-fed basis)

Variables	Diets[a]		
	LFP	MFP	HFP
Crude protein in diet (1)	150	150	150
Ileal digestible protein (2)	121.6	111.8	102.0
Protein entering the large intestine (1 –2)	28.4	38.2	48.0
Fecal digestible protein (3)	121.8	120.3	118.8
Protein disappeared in the large intestine (3 – 2)	0.2	8.5	16.8
Protein in biomass [4 = (1 – 3)*0.9 [b]]	25.4	26.7	28.1
NSP in the diet (5)	191	174	163
Fecal digestible NSP (6)	90.0	94.4	98.7
Protein in biomass/fecal digestible NSP (4/6)	0.28	0.28	0.28

[a] Fermentable protein levels: LFP = 28g/kg diet ; MFP = 38 g/kg diet; HFP = 48 g/kg diet.

[b] It is assumed that 90% protein in feces are protein in biomass.

We found no effects of treatments on odor hedonic tone or odor intensity of the odorous air above manure, implying that diets having different fermentable protein levels did not create differences in the pleasantness of odor and in the magnitude of odor sensation. So far, olfactometry studies have focused on odor concentration and odor emission. Odor evaluation based on odor concentration has a limitation in considering the odor relative to others. The use of odor concentration, odor hedonic tone and odor intensity as in this experiment can give an overall comparison between odors.

Increased fermentable protein levels did increase the concentrations of methyl sulfide, carbon disulfide, ethanethiol, phenol, 3-methyl indole, and 4-ethyl phenol in the manure. Protein is the precursor of these compounds, and therefore, increased amount of protein in the large intestine of the animals resulted in higher concentrations of these odorous compounds in the manure. However, the odor strength and offensiveness of odorous air from pig manure were not significantly influenced by different fermentable protein levels. These odorous compounds are probably not representative enough for odorous compounds in the air to make a correlation between their concentrations in the manure and odor strength and offensiveness of the air sampled above the manure. It could mean that these odorous compounds are not contributing significantly to odor strength and offensiveness. It could also mean that odor strength and

offensiveness of these compounds were compensated by other odorous compounds not analyzed in the manure in this study. It may have a parallel with the study on AA supplementation above pigs' requirement by D.P. Le *et al*. (Chapter 5). In that study, the authors concluded that the precursors for very volatile sulfurous compounds such as methanethiol and hydrogen sulfide, were excreted via urine. The LFP diet may have had higher excretions of these volatile sulfurous compounds. Therefore, odorous compounds produced from protein fermentation in the large intestine of animals in the HFP diet might have been compensated by odorous compounds excreted via the urinary pathway in the low fermentable protein diet.

According to Canh *et al*. (1998d) NSP are the most important dietary components determining VFA concentration in the manure. In this experiment, diets had similar amounts of NSP (Table 2). Therefore, it is logical that the manure of pigs fed different levels of fermentable protein had similar total VFA concentrations. The VFA pool was largely dominated by the short straight–chain VFA such as acetic, propionic and butanoic acids which comprised 92% of total VFA in the manure. Acetic acid was the main VFA contributing to the total VFA in the manure (66%). These findings confirm the results of Otto *et al*. (2003) and Le *et al*. (2005b). Manure pH was not influenced by levels of fermentable protein. Manure pH is mainly affected by VFA and ammonium concentrations (Canh *et al.*, 1998b; Le *et al*., 2005b) and these were similar for the different levels of fermentable protein. Ammonia emission was not affected by levels of fermentable protein. The main factors influencing ammonia emission are pH and ammonium concentration (Canh *et al*., 1998a). These were similar for the different fermentable protein levels in this study.

This study shows that the correlation between ammonia emission and odor emission was very low (0.14). It confirms the findings of the other three studies of P. D. Le *et al*. (Chapters 4, 5, 7) and as explained by P. D. Le *et al.* (Chapter 4) that odor is a complex mixture of various compounds while ammonia is a single compound and in addition, ammonia is not a very offensive odor (Oldenburg, 1989).

IMPLICATIONS

This study demonstrates that, with the same crude protein level in the diets, fermentable protein level did not affect odor strength (concentration and intensity) and offensiveness (hedonic tone) from growing pig manure. Increased fermentable protein levels increased the concentrations of some odorous compounds in the manure: methyl sulfide, carbon disulfide, ethanethiol, phenol, 3-methyl indole, and 4-methyl phenol. However, these compounds may not

be dominant enough to affect odor strength and offensiveness or they may have been compensated by very volatile compounds in the urine not measured in this study. Although, it is very complicated, it is very meaningful that further studies focus on identifying the odor indicator compounds and their relation to diet. To reduce odor production by means of altering protein, the level of fermentable protein should not be considered alone, it should be considered together with dietary crude protein level and ileal digestible crude protein level, because odor precursors come from both feces and urine. In urine odor precursors include metabolic products of excess nutrients absorbed in the small intestine of animals and detoxicated products absorbed in the large intestine. Further studies should therefore also focus on odor from urine and feces separately. Ammonia seems to contribute minimally to odor emission, so strategies that have been demonstrated to be successful in reducing ammonia emission may not have a similar impact on odor.

7

INTERACTIVE EFFECTS OF DIETARY CRUDE PROTEIN AND FERMENTABLE CARBOHYDRATE LEVELS ON ODOR FROM PIG MANURE

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ABSTRACT: *The objective of this study was to determine the effects of dietary levels of crude protein (CP) and levels of fermentable carbohydrates (FC) and their interaction on odor emission, odor intensity, odor hedonic tone, and ammonia emission from pig manure, and manure characteristics. An experiment was conducted with finishing pigs (n = 36) in a 2x3 factorial randomized complete block arrangement with 6 treatment combinations in 6 blocks. There were 2 dietary CP levels (low 12%; high 18%, as-fed basis) and 3 digestible fermentable carbohydrates levels: (low 95.5; medium 145.5; and high 195.5 g kg^{-1} feed, as-fed basis). Pigs with an initial body weight (BW) of 57.7 ± 2.5 kg (mean ± SD) were penned individually in partly slatted floor pens. Daily feed allowance was adjusted to 2.8 x maintenance requirement for energy as net energy (293 $kJ/BW^{0.75}$). Feed was mixed with water, 1/2.5 (w/w). Feces and urine of each pig accumulated in separate manure pits under the slatted floor. After an adaptation period of 2 wk, and after cleaning the manure pits, manure was subsequently collected. In the 3rd wk of the collection period, fresh feces were collected for identifying fresh feces characteristics and organic matter, protein, fat and non-starch polysaccharides digestibility. In the 6th wk of the collection period air samples were collected directly above the manure in each pit: one for odor and one for ammonia concentration. Manure samples were taken for manure characteristics. Air samples were analyzed for odor concentration and for hedonic tone and odor intensity. Manure samples were analyzed for volatile fatty acids, indoles, phenoles, sulfurous compounds, ammonium, and total N concentrations. Dietary CP and FC levels affected fecal digestibility of protein, non-starch polysaccharides, and organic matter (P < 0.01). Dietary CP level and FC level did not affect odor emission, odor intensity and hedonic tone but their interaction affected odor emission at P=0.06. At a high dietary CP level, increased FC level decreased odor emission, while at a low CP level, increased FC level increased odor emission from pig manure. Total N and ammonium-N concentrations, and ammonia emission from pig manure were reduced at low dietary CP level (P < 0.001). High FC level led to low ammonia emission from pig manure (P = 0.01). Manure pH increased at high dietary CP level (P < 0.001) and decreased when FC level increased (P = 0.03). Total VFA concentration increased at high dietary CP level (P < 0.001) and when FC level increased (P = 0.001). Enhanced dietary CP increased the manure concentrations of phenol (P < 0.001), cresols (P = 0.01), indole (P < 0.001), 3-methyl indole (P = 0.08), 4-ethyl phenol (P < 0.001) and carbon disulfide (P < 0.001), but FC did not affect concentrations of these compounds (P > 0.05) in the manure. It is concluded that the interaction between dietary CP and FC plays a role in odor production and emission. Ammonia emission from pig manure can be reduced substantially by decreasing dietary CP and by increasing FC.*

Key words*: Crude Protein, Fermentable Carbohydrates, Diet, Odor, Finishing Pig*

INTRODUCTION

Odor nuisance from pig production facilities is a growing topic due to increasing complaints of neighbors of these facilities. Reduction in odor nuisance plays an important role for strategies concerning where to permit pig production facilities to be located and determines the maximum size of the facilities. So far, strategies to reduce odor mainly focused on technical approaches such as bio-filters (Hartung *et al.*, 2001; Sheridan *et al.*, 2002) and bio-scrubbers (Hahne *et al.*, 2003). In addition, studies have been made to limit microbial activities in manure or to mask odor with other odorous compounds (Stevens *et al.*, 1989). Minimizing odor at the source of production by dietary alterations is a relatively new strategy but may have a great potential (Sutton *et al.*, 1999). Odor is mainly produced by microbial conversion of feed residues and of endogenous products in the gut of animals and in the manure. Proteins are the main substrates for odor production. Proteins are the precursors for sulfurous, indolic and phenolic compounds and volatile fatty acids (Mackie *et al.*, 1998). Non-starch polysaccharides (NSP) and resistant starch (RS) can enter the large intestine and are available for fermentation by microbiota (Stephen *et al.*, 1983; Cummings & Macfarlane, 1991; Cummings & Englyst, 1993). Fermentable carbohydrates may modulate odor potential. Microbiota converts fermentable carbohydrates (RS and NSP) into short-chain volatile fatty acids (VFA), mainly acetic, propionic and butyric acids. Microbial activities and microbial ecology are changed when different types and various amounts of fermentable carbohydrates (FC) enter the large intestine.

Reduction of crude protein (CP) levels and formulation of diets with a minimum level of S-containing amino acids (AA) to the level needed by the animal decreases odor precursors in manure and thus limits odor production from pig manure (P. D. Le *et al*., Chapters 4 & 5). Dietary CP should be considered jointly with FC when considering odor reduction by dietary alterations. In the gut of animals and in manure storages, the microbiota generate energy from FC (Bergman, 1990). When the amount of FC is low or when protein is high compared to FC, the microbiota may use protein as an energy source (Reid & Hillman, 1999; Gibson & Roberfroid, 1995). We hypothesize that a low amount of FC and a large amount of CP in the large intestine may result in more odor precursors and more odorous compounds compared to a low amount of FC and a low amount of CP or a large amount of FC and a large amount of CP. So, dietary CP and FC interact with regard to odor precursor production.

Ammonia emission from pig production facilities contributes to environmental pollution (Tamminga, 1992; Jongbloed & Lenis, 1998). Consequently, nutrition or management measures

to reduce ammonia emission are necessary to ensure sustainable pig production. Dietary CP is the main source of ammonia emission from pig manure. It is well-documented that ammonia emission from pig manure can be reduced considerably by a decrease in dietary CP and an increase in FC (Kerr, 1995; Canh *et al.*, 1998; Zijlstra *et al.*, 2001). However, the relationship between ammonia and odor emission from pig manure is not clear.

The main objective in the present study was to determine which combination of dietary CP and FC influences odor emission, odor strength, odor offensiveness and ammonia emission from pig manure and which does not. In this study, we simulated odor emission from pig manure by sampling the air above the manure pit.

MATERIALS AND METHODS

ANIMALS, EXPERIMENTAL DESIGN, AND DIETS

A 2 x 3 factorial randomized complete block arrangement with 6 treatment combinations in 6 blocks was used to study effects of crude protein (CP) and fermentable carbohydrate (FC) level in the diet on odor concentration, odor emission, odor intensity, odor hedonic value, and ammonia emission from pig manure and fresh feces and manure characteristics. There were 2 dietary CP levels (low 120; high 180 g/kg as fed basis) and 3 digestible fermentable carbohydrates levels: (low 95.5; medium 145.5; and high 195.5 g/kg feed, as-fed basis). In this experiment, FC is defined as the sum of non-starch polysaccharides (NSP) and resistant starch (RS) entering the large intestine of pigs. Sugar beet pectin level represented NSP, and raw potato starch was the source of RS. The amount of RS was estimated based on ileal digestibility of raw potato starch of 58.7 % as determined by Smits & Jongbloed (1995). Among different types of resistant starch, raw potato starch is gelatinized poorly and hydrolyzed slowly by α-amylase. On this basis, we choose raw potato starch to supply RS to the large intestine of pigs. Both sugar beet pectin and raw potato starch have a high level of fermentation (Barry *et al.*, 1995; Lucile *et al.*, 1998) and a very low concentration of N. The starch content in potato starch was 751 g/kg.

Each treatment combination was replicated 6 times, 1 replicate in each of 6 blocks. A block consisted of samples collected on the same day and from animals with similar initial body weight (BW) and genotype.

In total 36 barrows with an initial BW of 57.7 ± 2.5 kg (mean ± SD) were allocated to 6 blocks. Blocks were based on initial BW and genotype. Pigs were penned individually in galvanized steel pens (2.1 x 0.96 m). The pen has a slatted floor at the rear (0.97 x 0.96 m).

There was a separate manure pit per pen below the slatted floor. The size of the manure pit was 1.35 x 0.91 x 0.36 m (length x width x depth). Pigs were housed in a mechanically ventilated and temperature-controlled room. Temperature and relative humidity were recorded every 5 min. The average temperature and relative humidity of the room during the experimental period were 19.0 ^{0}C ± 0.59 and 59.0 % ± 9.75 (mean ± SD), respectively.

Diets were formulated to have similar contents of net energy (NE), similar dietary electrolyte balances (dEB), and similar levels of minerals and vitamins (Tables 1&2). The low CP diets (12%) were supplemented with essential amino acids (AA) up to the level of animal requirement based on apparently ileal digestibility (CVB- Animal Feed Product Board, 2004). The high CP diets (18%) were supplemented with exactly the same amounts of AA as the low CP diets. This was done to prevent confounding effects between CP level and AA supplementation. Amino acids were supplemented in crystalline form.

Experimental diets were analyzed for dry matter (DM), ash, CP, AA composition, crude fiber, crude fat, starch and sugar, and minerals including Cr. The analyses were done as described in P.D. Le *et al*. (Chapter 4).

Pigs were fed 2.8 times the maintenance requirement for NE (293 kJ/BW$^{0.75}$). Water was restrictedly provided by mixing feed with water in the ratio of 1/2.5 (w/w). Apart from water with feed, no additional water was given to the pigs. So it was aimed to have the same amount of feed and water intake by the pigs. This will give almost a similar amount of manure. Pigs were fed 2 times per day at 0830 and 1500. The amount of feed provided was adjusted each day according to an estimated BW gain of 873 g d^{-1}. Feed intake was recorded every day. Pigs were weighed at the beginning and at the end of the experimental period just before the morning feeding. Daily gain and feed efficiency were derived from the feed intake and from the increase in BW during the experimental period.

After an adaptation period of 2 wk to allow the pigs to acclimatize to the experimental diets and pens, the manure pits were cleaned before collection started. Subsequently, feces and urine accumulated in the manure pit until the sampling period. In the 3rd wk of the experimental period, fresh feces were sampled. In the 6th wk of the collection period, odor, ammonia and manure samples were collected.

At the beginning of the period, some pigs got salmonellosis. All pigs were given Colistine (1200 W.O), 7 g day^{-1} pig^{-1} for 7 days, by mixing with drinking water. After 6 days, the salmonellosis was vanished.

Table 1. Ingredient composition of the experimental diets, as-fed basis

Composition (%)	Diets					
Crude protein level (CP)	Low CP (12%)			High CP (18%)		
Fermentable carbohydrate(FC)	Low	Medium	High	Low	Medium	High
Barley	40.00	40.00	40.00	20.00	20.00	20.00
Wheat	24.69	19.06	13.42	12.09	15.62	19.15
Soybean meal extr. (CF< 5%)	5.96	6.75	7.53	26.12	24.85	23.58
Wheat middlings	9.00	9.00	9.00	9.00	9.00	9.00
Cane molasses (Sugar < 47.5 %)	3.00	3.00	3.00	3.00	3.00	3.00
Potassium carbonate	0.70	0.60	0.50	0.15	0.08	0.00
Maize starch	12.31	6.16	0.00	25.82	12.91	0.00
Potato starch, native	0.00	5.65	11.29	0.00	5.28	10.55
Sugar beet pectin	0.00	4.30	8.60	0.00	4.02	8.03
Soybean oil	0.84	2.11	3.37	0.49	2.02	3.55
Limestone	1.25	1.21	1.17	1.20	1.18	1.15
Mono calcium phosphate.1H_2O	0.64	0.66	0.67	0.53	0.53	0.53
Salt	0.50	0.42	0.34	0.49	0.42	0.34
Chromic oxide-starch mixture (1:3)	0.03	0.03	0.03	0.03	0.03	0.03
Growth premix[a]	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine HCL	0.51	0.51	0.51	0.51	0.51	0.51
DL-Methionine	0.16	0.16	0.16	0.16	0.16	0.16
L-Threonine	0.20	0.20	0.20	0.20	0.20	0.20
L-Tryptophan	0.04	0.04	0.04	0.04	0.04	0.04

[a] The vitamin-mineral premix supplied per kg feed included 7,000 IU vitamin A, 1,700 IU vitamin D3, 20 IU vitamin E, 1.5 mg vitamin K, 1.5 mg vitamin B1, 4 mg vitamin B2, 11 mg d-pantothenic acid, 18 mg niacine, 18 µg vitamin B12, 0.1 mg folic acid, 1.0 mg vitamin B6, 100 mg choline chloride, 75 mg Fe, 10 mg Cu, 65 mg Zn, 30 mg Mn, 0.15 mg Co, 0.75 mg I, 0.30 mg Se.

FRESH FECES COLLECTION AND ANALYSES

Fresh feces were collected from the floor of the pen during 3 days, 2 times in the morning half an hour apart and 2 times in the afternoon half an hour apart. In the morning the floors were cleaned before collection. Fresh feces samples were put into small plastic bags, labled separately for each animal and for each time of collection. Fresh feces samples were stored in a freezer (-20 ^{0}C). On the last sampling day, the feces were weighed and pooled per 2 pigs and sent to the laboratory for analysis. The criterion for pooling the feces of 2 pigs was based on the amount of collected feces. The fresh feces were analyzed for dry matter (DM), ash, CP, fat and chromium, according to the protocols as described in P.D. Le *et al.* (Chapter 4).

Table 2. Nutrient composition of experimental diets, as-fed basis

Composition	Unit	Diets					
Crude protein level (CP)		Low CP (12%)			High CP (18%)		
Ferm. carbohydrate level (FC)		Low	Medium	High	Low	Medium	High
Calculated composition							
CP	%	12.00	12.00	12.00	18.00	18.00	18.00
Digestible CP	%	9.56	9.51	9.45	15.56	15.43	15.31
Ileally digestible CP	%	9.09	8.79	8.48	14.35	14.14	13.92
Net energy	kcal/kg	2,183	2,183	2,183	2,183	2,183	2,183
Starch, amyloglucosidase	%	46.21	42.19	38.17	40.47	35.63	30.79
NSP[a]	%	16.87	19.66	22.46	16.15	19.37	17.26
Digestible NSP+RS (fecal)	%	9.10	14.10	19.10	10.00	15.00	20.00
Ileally digestible AA							
Lys	%	0.74	0.74	0.75	1.17	1.15	1.13
Met	%	0.30	0.30	0.29	0.37	0.37	0.37
Cys	%	0.17	0.16	0.15	0.22	0.22	0.22
Met + Cys	%	0.47	0.46	0.44	0.59	0.59	0.59
Thr	%	0.45	0.45	0.44	0.68	0.67	0.66
Trp	%	0.14	0.14	0.14	0.21	0.21	0.21
Analyzed composition							
Dry matter	%	87.28	87.12	87.45	87.69	87.13	86.89
CP (Nx6.25)	%	12.85	12.56	12.44	18.18	18.41	18.41
Ash	%	4.80	4.68	4.68	4.85	4.89	4.84
Crude fiber	%	3.80	3.72	3.57	2.99	3.06	3.13
Crude fat	%	2.72	3.54	5.02	2.56	3.86	5.24
Sugar	%	4.66	4.50	4.60	6.14	5.99	6.01
Starch	%	43.79	39.64	36.17	40.51	33.59	29.01
NSP	%	18.45	22.21	24.52	15.45	20.39	23.39
Na	%	0.21	0.20	0.20	0.20	0.19	0.18
K	%	0.94	0.94	0.92	1.00	0.96	0.93
Sulfate	%	0.09	0.09	0.24	0.11	0.12	0.12
Chlorine	%	0.43	0.40	0.32	0.42	0.40	0.39
dEB[b]	meq/kg	212	214	232	225	216	208
dEBS-a[c]	meq/kg	193	195	182	202	192	184
Amino acids							
Ala	%	0.47	0.46	0.45	0.73	0.73	0.73
Arg	%	0.63	0.62	0.62	1.13	1.11	1.10
Asp	%	0.88	0.86	0.84	1.71	1.68	1.66
Cys	%	0.22	0.21	0.20	0.27	0.27	0.27
Glu	%	2.46	2.33	2.21	3.27	3.29	3.31
Gly	%	0.50	0.48	0.46	0.73	0.73	0.73

His	%	0.26	0.27	0.27	0.42	0.43	0.43
Ile	%	0.43	0.42	0.40	0.72	0.71	0.71
Leu	%	0.81	0.78	0.74	1.25	1.24	1.23
Lys	%	0.88	0.88	0.88	1.30	1.30	1.29
Met	%	0.31	0.30	0.28	0.35	0.35	0.35
Phe	%	0.55	0.53	0.51	0.85	0.85	0.84
Pro	%	0.86	0.82	0.78	0.98	0.98	0.98
Ser	%	0.53	0.51	0.50	0.83	0.83	0.83
Thr	%	0.58	0.57	0.56	0.80	0.80	0.79
Trp	%	0.20	0.19	0.18	0.27	0.27	0.26
Tyr	%	0.32	0.32	0.31	0.54	0.54	0.55
Val	%	0.55	0.53	0.52	0.80	0.81	0.82

[a] Non-starch polysaccharides(NSP) were determined as organic matter–(CP + crude fat + starch + sugar).

[b] dEB was determined as mEq = Na + K – Cl.

[c] dEBS-a was calculated as mEq=Na+K–Cl–S. dEBS-a does not take into account S present in AA.

ODOR SAMPLE COLLECTION AND MEASUREMENT OF ODOR CONCENTRATION, ODOR HEDONIC TONE AND ODOR INTENSITY

Odor samples were collected as described by P.D. Le *et al*. (Chapter 4). The collection system is shown in Fig. 1. The collection system was put directly in the manure pit. Air was sampled directly above the manure in each manure pit. Odor concentration of air samples was determined according to CEN standard 13725 (2003). This is described in detail by Le *et al.* (2005b). Odor concentrations of the examined samples were expressed in European odor units per cubic meter air ($ou_E\ m^{-3}$). One odor unit is defined as the amount of odor-causing gases which, when diluted in 1 m^3 of air, can just be distinguished from clean air by 50% of the members of an odor panel.

Odor emission was defined as the number of odor units emitted from a manure surface per second. It was calculated by multiplying the ventilation rate with the corresponding odor concentration (Equation 1).

$$E_{odor} = (C_{odor} \times V \times 10{,}000) / (60 \times 1{,}000 \times 595) \quad [1]$$

where E_{odor} = odor emission ($ou_E\ s^{-1}m^{-2}$), C_{odor} = odor concentration ($ou_E\ m^{-3}$), and V = ventilation rate (L min^{-1}), 10,000 = $cm^2\ m^{-2}$, 60 = sec min^{-1}, 1,000 = liters m^{-3}, and 595 = the cm^2 sampled surface area.

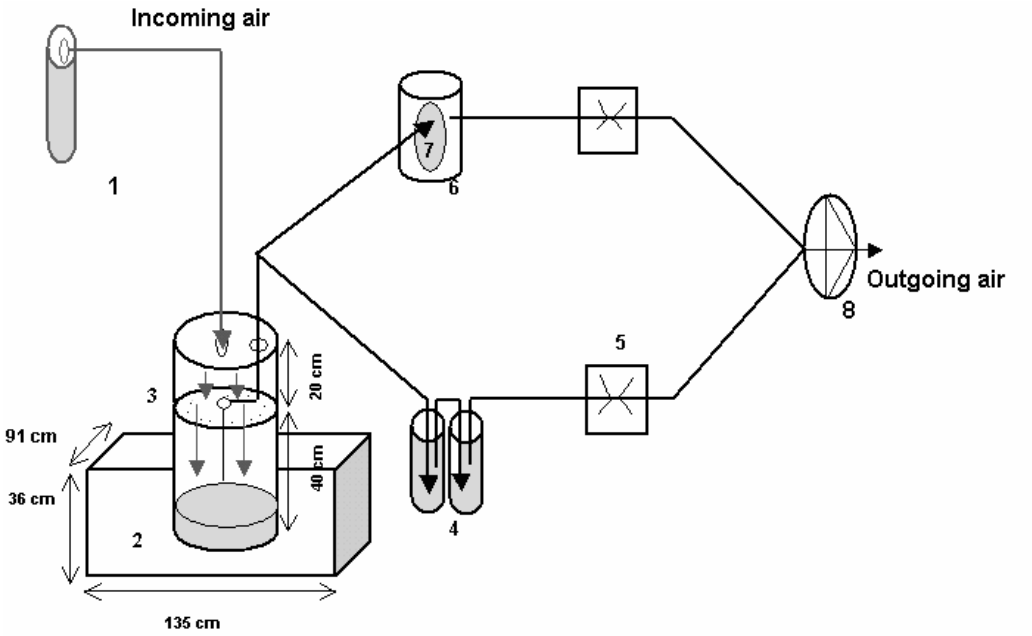

Fig. 1. Schematic view of the odor and ammonia sample collection (1 = odor free air pressurized cylinder, 2 = manure pit, 3 = vessel, 4 = impingers, 5 = critical glass capillary, 6 = rigid plastic container, 7 = odor bag, 8 = vacuum pump)

Odor hedonic tone (H) and odor intensity (I) was determined as described by P.D. Le *et al.* (Chapter 4). Hedonic tone is used to evaluate odor offensiveness which is a measure of the unpleasantness or pleasantness of the perceived odor. Odor intensity refers to the magnitude of odor sensation. Hedonic tone was measured by the scores of the panel members. They used a 9-point hedonic scale ranging from – 4, extremely unpleasant or offensive through 0, neither pleasant nor unpleasant or neutral odor to + 4, extremely pleasant. Odor intensity was measured by using a 7-point intensity scale ranging from 1, no odor through 2, very faint odor to 7, overwhelming odor. For each odor sample, the hedonic tone and odor intensity at each odor concentration level above the detection threshold were calculated as the average of the hedonic tone and the odor intensity perceived by all panelists, and plotted against the logarithm of odor concentration. From the regression lines obtained, the odor concentration at H = -1 (mildly unpleasant), H = -2 (moderately unpleasant), I = 1 (no odor), I = 2 (very faint odor), I = 4 (distinct odor) were derived. Regression lines of the odor hedonic tone and odor intensity were also plotted against the logarithm of the odor concentration for all samples in the same treatment.

COLLECTING AND MEASURING AMMONIA EMISSION

Air samples for determining ammonia emission were collected at the same time as odor samples (Fig. 1). One sample was collected from each manure pit. Ammonia in outgoing air

was removed by passing through 2 ammonia traps (impingers), each trap contained about 20 ml 0.5 M HNO_3 solution. The flow rate of the outgoing air was 0.5 $Lmin^{-1}$. The system was operated for about 90 min. Starting and ending time of collection were noted. The ammonia concentration and the volume of the liquid were determined in the first and the second impingers. Ammonia emission per time unit and surface unit was calculated with Equation 2.

$$M_{NH3} = (C_{NH3} \times V \times 10{,}000) / (T \times 60 \times 595) \quad [2]$$

where M_{NH3} = ammonia emission (mg s^{-1} m^{-2}), C_{NH3} = ammonia concentration (mg mL^{-1} HNO_3), V = volume of HNO_3 solution (mL), 10,000 = cm^2 m^{-2}, T = sampling time (min), 60 = s min^{-1}, and 595 = the cm^2 sampled surface area of the manure pit.

COLLECTION AND MEASUREMENT OF MANURE CHARACTERISTICS

Manure was sampled to evaluate the effect of the diet on manure characteristics. These characteristics included DM, ash, total N, ammonium, pH, VFA (acetic, propionic, butyric, pentanoic, iso-butyric, iso-pentanoic, hexanoic, and heptanoic), indolic (indole and 3-methyl indole) and phenolic compounds (phenol, 4-ethyl phenol, and cresols), and sulfurous compounds (carbon disulfide, methyl sulfide, methyl disulfide, ethanethiol). One manure sample was collected from each manure pit. Immediately after collecting odor samples, manure in each manure pit was mixed thoroughly before a sample of about 1 kg was taken. Manure samples were collected and analyzed as described by P.D. Le *et al.* (Chapter 4). Manure samples were stored at - 20 ^{0}C before being analyzed. The analysis of odorous compounds in manure was by gas chromatography.

STATISTICAL ANALYSIS

The effects of CP and FC levels and their interaction on daily gain, daily feed intake, feed efficiency, odor emission, odor hedonic value, odor intensity, ammonia emission, fecal digestibility of protein, fermentable carbohydrates, organic matter, fat, DM and ash and manure characteristics were analyzed using ANOVA of GenStat statistical package 7th version (GenStat VSN International Ltd., 2004) with the following model.

$$y_{ijk} = \mu + \rho_k + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where : y_{ijk} = dependent variable, μ = overall mean, ρ_k = effect of block, k = 1-6, α_i = effect of CP level, i = 1, 2, β_j = effect of FC level, j =1-3, $(\alpha\beta)_{ij}$ = the interaction between CP and FC level, e_{ijk} = experimental error.

Data were presented as either arithmetic or geometric mean. A natural log transformation was applied to odor emission, concentrations of VFA, total N and ammonium-N, indolic

and phenolic compounds, and S-containing compounds since they were skewed and not normally distributed. The relationship between ammonia emission and odor emission was determined by linear regression.

RESULTS

EFFECTS OF CP AND FC LEVELS ON DAILY GAIN, DAILY FEED INTAKE AND FEED EFFICIENCY

The effects of CP and FC levels on daily gain, daily feed intake and feed efficiency are summarized in Table 3. No effects of treatments on these traits were found. There was a tendency towards a higher ADG (P = 0.07) and feed efficiency (P = 0.10) at the medium FC level.

Table 3. Effects of CP and FC level on daily gain, daily feed intake and feed efficiency

CP level	Low CP			High CP			RSD[a]	P-value for		
Fer. carbohydrates	Low	Medium	High	Low	Medium	High		CP	FC	CPxFC
Initial BW, kg	57.7	58.2	57.6	58.4	57.3	56.1	1.5	0.27	0.11	0.16
Final BW, kg	103.8	106.8	104.3	104.3	105.6	103.3	3.2	0.59	0.15	0.77
ADFI, kg/ day	2.41	2.42	2.42	2.40	2.42	2.41	0.01	0.13	0.10	0.33
ADG, g/ day	824	869	835	819	862	844	44.2	0.97	0.07	0.90
G:F, g/kg	341	359	345	342	356	350	17.6	0.90	0.10	0.90

[a] Residual Standard Deviation.

EFFECTS OF DIETARY CP AND FC LEVELS ON ODOR STRENGTH AND OFFENSIVENESS

Descriptive statistics and analyses of variance of effects of dietary CP and FC on odor concentration, odor emission, odor intensity and odor hedonic tone are shown in Tables 4&5, respectively. Odor concentration and odor emission were in the range from 4,146 to 9,799 ou_E m^{-3}, and from 0.57 to 1.35 ou_E $s^{-1}m^{-2}$, respectively. Both dietary CP and FC did not affect odor concentration and odor emission (P > 0.05), but their interaction did at P = 0.06. At a low dietary CP level, when FC was increased from low to high level, odor concentration and odor emission increased from 4,817 to 9,799 ou_E m^{-3} and from 0.67 to 1,35 ou_E $s^{-1}m^{-2}$, respectively. At a high dietary CP level, odor concentration and odor emission decreased from 6,124 to 4,146 ou_E m^{-3} and from 0.85 to 0,57 ou_E $s^{-1}m^{-2}$, respectively when FC increased from low to high level. Odor concentrations at different levels of odor hedonic tone and odor intensity were similar (P > 0.05) for the different treatments.

Table 4. Geometric and arithmetic means of odor strength (concentration and intensity) and offensiveness (hedonic tone) from manure of pigs fed different levels of CP and FC (*n = 36*)

Crude protein	Fer. Carb.	Mean	Concentration, ou_E m^{-3}	Emission, ou_E $s^{-1}m^{-2}$	Odor concentration, ou_E m^{-3}, at				
					H[a] = -1	H = -2	I[b] = 1	I = 2	I = 4
Low CP (12%)	Low	GM[c]	4,817	0.67	2.94	8.0	0.79	1.79	9.03
		AM[d]	5,023	0.70	3.31	8.76	0.90	1.97	10.95
	Medium	GM	4,964	0.69	2.83	7.92	0.64	1.48	7.92
		AM	5,650	0.79	2.97	8.23	0.71	1.56	8.22
	High	GM	9,799	1.35	2.92	9.12	0.47	1.27	9.49
		AM	11,247	1.55	3.65	10.33	0.61	1.50	13.15
High CP (18%)	Low	GM	6,124	0.85	2.80	7.92	0.59	1.40	7.85
		AM	8,733	1.21	2.97	8.46	0.70	1.66	9.54
	Medium	GM	4,915	0.69	2.64	7.17	0.62	1.45	7.92
		AM	5,758	0.81	2.9	7.69	0.72	1.66	9.13
	High	GM	4,146	0.57	3.29	8.76	0.60	1.49	9.12
		AM	4,260	0.59	3.49	9.57	0.64	1.56	9.69

[a] Hedonic tone, [b] Intensity, [c] Geometric mean, [d] Arithmetic mean.

Table 5. Effects of CP and FC levels on odor strength (concentration and intensity) and offensiveness (hedonic tone) from pig manure

Crude protein	Fer. Carb.	ln[a](Odor concentration)	ln (Odor emission)	ln (odor concentration) at				
				H = -1	H = -2	I = 1	I = 2	I = 4
Low CP (12%)	Low	8.48	-0.40	1.08	2.08	-0.23	0.58	2.2
	Medium	8.51	-0.37	1.04	2.07	-0.45	0.39	2.07
	High	9.19	0.30	1.07	2.21	-0.76	0.24	2.25
High CP (18%)	Low	8.72	-0.16	1.03	2.07	-0.52	0.34	2.06
	Medium	8.5	-0.37	0.97	1.97	-0.48	0.37	2.07
	High	8.33	-0.56	1.19	2.17	-0.51	0.4	2.21
RSD[b]		0.57	0.57	0.49	0.42	0.50	0.44	0.42
P-value for	CP	0.27	0.29	0.91	0.72	0.90	0.82	0.68
	FC	0.55	0.58	0.82	0.58	0.46	0.74	0.66
	CPxFC	0.06	0.06	0.87	0.97	0.43	0.56	0.92

[a] Natural logarithm, [b] Residual Standard Deviation.

EFFECTS OF DIETARY CP AND FC LEVELS ON FECAL DIGESTIBILITY

Effects of diets with different CP and FC levels on fecal digestibility of nutrients are shown in Table 6. Dietary CP and FC levels additively affected DM, ash, organic matter (OM), protein and NSP digestibility ($P < 0.01$), but for crude fat only FC levels did ($P = 0.002$). With NSP given, the high FC diet had higher NSP ($P < 0.001$) and fat digestibility ($P = 0.002$) than the low FC diet. But, the high FC diet had lower apparent CP digestibility ($P = 0.008$), OM digestibility ($P < 0.001$) and DM digestibility ($P < 0.001$) than the low FC diet. Protein, NSP, OM and DM digestibility of the high CP diet were higher than the low CP diet ($P < 0.01$).

Table 6. Effects of dietary CP and FC levels on fecal digestibility (%)

Crude protein	Fermentable carbohydrates	Dry matter	Ash	Organic matter	Protein	Crude fat	NSP[a]
Low CP (12%)	Low	83.5	54.6	85.2	78.8	59.5	54.5
	Medium	83.0	55.0	84.6	75.1	71.9	61.5
	High	81.7	52.5	83.4	72.2	72.0	63.7
High CP (18%)	Low	85.7	53.5	87.6	81.3	62.9	61.9
	Medium	85.3	54.7	87.2	81.7	71.8	70.0
	High	83.3	54.6	85.0	77.0	73.9	71.2
RSD[b]		0.71	2.48	0.68	2.52	4.61	1.25
P-value for	CP	<0.001	0.80	<0.001	0.002	0.45	<0.001
	FC	<0.001	0.72	<0.001	0.008	0.002	<0.001

[a] Non-starch polysaccharides, [b] Residual Standard Deviation.

EFFECTS OF DIETARY CP AND FC LEVELS ON MANURE CHARACTERISTICS, AND AMMONIA EMISSION

Effects of dietary CP and FC level on manure characteristics and ammonia emission from manure are presented in Tables 7&8. Manure pH was lowered at low dietary CP ($P < 0.001$) and at high FC levels ($P = 0.02$). Total N and ammonium-N concentrations in manure were lowered when pigs had a low dietary CP diet or a low FC diet ($P <= 0.01$). Ammonia emission from pig manure was affected by dietary CP and FC levels. Lowering dietary CP from 18 to 12 % decreased ammonia emission from the manure by 62.4 % ($P < 0.001$). High FC diets resulted in low ammonia emission from pig manure ($P = 0.01$). On average, for each 100 g increase in digestible FC/kg diet, as fed basis, ammonia emission reduced by 29.4 %.

Table 7. Mean values of manure characteristics, and ammonia emission from manure

Crude protein level (CP)	Low CP (12%)			High CP (18%)		
Fer. carbohydrate level	Low	Medium	High	Low	Medium	High
Dry matter, g kg^{-1}	100.1	111.2	110.0	98.8	99.3	107.6
Ash, g kg^{-1}	24.4	25.2	23.7	27.5	26.5	27.2
Total VFA[a], g kg^{-1}	6.05	7.82	8.28	9.00	8.92	11.66
Acetic acid, g kg^{-1}	4.06	4.94	5.08	6.27	6.14	7.82
Propionic acid, g kg^{-1}	1.11	1.41	1.42	1.19	1.23	1.57
Butyric acid, g kg^{-1}	0.26	0.58	0.76	0.46	0.41	0.72
Iso-butyric acid, g kg^{-1}	0.23	0.30	0.33	0.38	0.40	0.53
Iso-pentanoic acid, g kg^{-1}	0.35	0.47	0.52	0.65	0.69	0.92
Pentanoic acid, g kg^{-1}	0.04	0.10	0.15	0.04	0.04	0.09
Total N, g kg^{-1}	5.21	5.57	5.82	8.06	8.00	9.15
Ammonium-N, g kg^{-1}	3.32	3.24	3.37	5.66	5.61	6.33
pH	7.26	7.05	7.02	8.38	8.44	8.15
Phenol, mg kg^{-1}	56.01	53.50	50.60	107.74	100.89	135.86
Cresols, mg kg^{-1}	23.60	45.00	36.60	37.20	37.20	49.70
4-ethyl phenol, mg kg^{-1}	1.92	3.75	3.65	17.20	19.36	17.35
Indole, mg kg^{-1}	7.26	8.50	8.48	13.34	10.16	15.18
3-methyl indole, mg kg^{-1}	5.77	7.02	6.30	8.06	9.01	8.74
Carbon disulfide, mg kg^{-1}	7.49	8.87	9.43	26.94	30.24	27.27
Ammonia emis., mg $s^{-1}m^{-2}$	0.013	0.008	0.011	0.036	0.031	0.024

[a] Total VFA = acetic acid + propinoic acid + butyric acid + iso-butyric acid + iso-pentanoic acid.

High FC levels resulted in high concentrations of individual and total VFA in the manure, e.g. acetic, propionic, butyric, pentanoic, iso-butyric, and iso-pentanoic acids ($P <= 0.01$). The high CP diet had higher acetic, iso-butyric, iso-pentanoic and total VFA concentrations in the manure ($P <= 0.01$) than the low CP diet. The high CP diet also had higher concentrations of phenol ($P < 0.001$), cresols ($P = 0.01$), indole ($P < 0.001$), 3-methyl indole ($P = 0.08$), 4-ethyl phenol ($P < 0.001$) and carbon disulfide ($P < 0.001$) in the manure than the low CP diet. Dietary FC level, however, had no effect on these concentrations in manure ($P > 0.05$). No interactions between dietary CP and FC on ammonia emission and manure characteristics were found.

Heptanoic and hexanoic acids were not detected in the manure of the different treatments. The detection limits of VFA were 0.1 g kg^{-1}. Methyl disulfide, methyl sulfide, and ethanethiol were not identified. The correlation between ammonia emission and odor emission was low (r = - 0.1).

Table 8. Effects of CP and FC level on manure characteristics, only significant effects are presented

Crude protein (CP)	Low CP (12%)			High CP (18%)			RSD[a]	*P*-value for	
Ferm. Carbohydrates	Low	Medium	High	Low	Medium	High		CP	FC
pH	7.26	7.05	7.02	8.38	8.44	8.15	0.20	< 0.001	0.02
ln[c] (total N)	1.65	1.71	1.76	2.09	2.08	2.21	0.09	< 0.001	0.01
ln (ammonium-N)	1.20	1.17	1.21	1.73	1.73	1.84	0.06	< 0.001	0.01
ln (total VFA)	1.79	2.04	2.11	2.19	2.18	2.45	0.16	< 0.001	0.01
ln (acetic acid)	1.39	1.58	1.62	1.83	1.81	2.05	0.15	< 0.001	0.01
ln (propionic acid)	0.10	0.33	0.35	0.17	0.20	0.45	0.16	0.88	0.01
ln (butyric acid)	-1.40	-0.65	-0.33	-0.90	-0.93	-0.35	0.39	0.64	< 0.001
ln (pentanoic acid)	-3.33	-2.54	-1.93	-3.20	-3.36	-2.63	0.70	0.06	0.01
ln (iso-butyric acid)	-1.50	-1.22	-1.11	-1.00	-0.92	-0.63	0.21	< 0.001	0.01
ln (iso-pentanoic acid)	-1.06	-0.75	-0.63	-0.45	-0.39	-0.09	0.16	< 0.001	< 0.001
ln (phenol)	3.97	3.92	3.86	4.64	4.60	4.85	0.29	< 0.001	0.59
ln (cresols)	3.40	3.62	3.44	3.66	3.78	3.93	0.35	0.01	0.43
ln (indole)	1.90	2.07	2.06	2.46	2.28	2.71	0.31	< 0.001	0.18
ln (3-methyl indole)	1.66	1.83	1.42	2.03	2.06	1.99	0.65	0.08	0.66
ln (4-ethyl phenol)	0.60	1.18	1.07	2.75	2.87	2.74	0.49	< 0.001	0.22
ln (carbon disulfide)	1.98	2.14	2.20	3.24	3.39	3.27	0.28	< 0.001	0.37
ln (ammonia emission)	- 4.30	- 4.77	- 4.55	- 3.34	-3.51	- 3.73	0.27	< 0.001	0.01

[a] Residual Standard Deviation, [b] Natural logarithm.

[d,e] Means within rows missing a common superscript letter are different at $P < 0.05$.

DISCUSSION

Protein and its metabolites are precursors for odor production in the gut of animals and in manure stores (Mackie *et al.*, 1998). In general, diets have more protein than the animals' requirement, leading to unutilized protein. Reduction in dietary crude protein (CP) and at the same time supplementating most essential amino acids (AA) to maintain AA balance is expected to reduce odor production from pig manure, but not have negative effects on pig performance. This experiment showed that pig performance was not affected at a low dietary CP level, 12% compared to 18%. Also, odor emission was not different at the two CP levels. The latter is inconsistent with the findings of Hayes *et al*. (2004) and P.D. Le *et al.* (Chapter 4), but confirming the finding of Clark *et al.* (2005a). The first two author groups found a reduction up to 80% in odor concentration and emission from manure of growing pigs when dietary CP was reduced by 6% level. The last author group could not find a reduction of odor emission from manure of finishing pigs when dietary CP was reduced from 17 to 14%. There could be 2

possible reasons for the non-effect of dietary CP on odor emission, hedonic tone and intensity of the odorous air above the manure pit in the present experiment. First, a sharp reduction in manure pH of 1.21 units occurred as dietary CP was reduced from 18 to 12%. This may affect the emission of odorous compounds. A high pH (8.30) of manure of pigs fed high dietary CP can inhibit the emission of some important or dominant odorous compounds in terms of odor concentration, odor intensity and odor hedonic tone such as, hydrogen sulfide. In this study we were unable to measure them. According to Shurson *et al.* (1998), when pH of manure is higher than 8, most reduced sulfur exists in solution as HS^- and S^{-2} ions, and the amount of free H_2S is so small that odor problems do not occur. At a pH below 8, equilibrium shifts rapidly toward formation of un-ionized H_2S and is about 80% un-ionized at pH 7. Under these conditions, a large fraction of hydrogen sulfide could emit from the manure. In addition, at a high pH some types of bacteria become less active. Further studies on effects of manure pH on odor concentration and odor emission from manure of pigs fed diets with similar dietary CP and FC are necessary to confirm this hypothesis. In addition, by measuring the concentration of odorous compounds, especially sulfurous compounds both in manure and in the air emitted from the manure, will also help to investigate the possible effects of pH on the emission of odorous compounds. The second reason for the non-effect of dietary CP level on odor emission could be that all diets supplied sufficient energy from FC for bacterial biomass synthesis. This led to few odorous compounds produced.

Increased FC did not lead to higher odor emission, although increased FC resulted in significantly higher VFA concentrations in manure (dominated by short-chain VFA). There are two possible reasons behind this finding. Volatile fatty acids may not cause a high odor concentration and may not be very offensive in the air above the manure pit. According to Spoelstra (1980) acetic, propionic and butyric acids were considered unimportant when investigating odor hedonic tone. Second, the correlation between VFA concentration in manure and in the air above the manure pit is very low. Willig *et al.* (2005) could not find a significant correlation between feces and headspace VFA concentrations. It means high concentrations of VFA in manure may not lead to high VFA concentrations in the air above the manure pit and may not lead to higher odor concentration and emission. This correlation is especially dominated by pH.

The interactive effect of dietary CP and FC on odor concentration and emission ($P = 0.06$) means both dietary CP and dietary FC should be considered simultaneously in dietary strategies to reduce odor production from pig manure. The diets with high CP and high FC or low CP and low FC had the lowest odor emission while diets with a low CP and a high FC or high CP and

low FC had high odor emissions. This means the ratio between protein and FC in the gut of animals and in the manure may play an important role in odor generation and emission. When energy is not a limiting factor for bacterial activities, gut bacteria obtain energy from FC and obtain nitrogen from dietary protein residues and from endogenous protein to synthesize their biomass. When energy from FC is a limiting factor, bacteria may switch to using protein as an energy source. In this case, more AA are degraded and odors may be released.

The purpose of measuring CP and FC fecal digestibility was to calculate how much CP and FC has been used to produce bacterial biomass and how much protein may have been converted to odorous compounds in the large intestine of pigs. From the concentration of CP and FC in the diet and their fecal digestibility, the ratio between biomass protein and digestible fermentable carbohydrates was estimated per kg diet as-fed (Table 9). For this estimation it was assumed that 90 % of protein in feces is present as bacterial biomass. The ratios between bacterial biomass protein and digestible FC of low and high CP diets were similar, 0.20 and 0.23, respectively. The amount of biomass produced per kg diet as-fed was 28 and 33 g, respectively for low and high CP diets. This means more protein in bacterial biomass was made at a high dietary CP level. This partly reduces odor precursors in manure of pigs fed a high CP diet.

Table 9. Protein and fermentable carbohydrates in the chain from feed to feces, calculated in g for each kg diet as-fed basic intake

Crude protein (CP)	Ferm. Carbo. (FC)	CP in the diet (1)	Fecal dig. protein (2)	FC in the diet (3)	Fecal dig. FC (4)[a]	Protein in biomass 5=(1-2)*0.9[b]	Ratio 5/4
Low CP (12%)	Low	128.5	101.3	184.5	100.6	24.5	0.24
	Medium	125.6	94.3	239.6	154.1	28.2	0.18
	High	124.4	89.8	280.2	191.2	31.1	0.16
High CP (18%)	Low	181.8	147.8	154.5	95.6	30.6	0.32
	Medium	184.1	150.4	220.3	159.1	30.3	0.19
	High	184.1	141.8	266.6	199.3	38.1	0.19

[a] Fecal digestible FC = digestible NSP + Resistant starch from potato native starch. It is assumed that 100% resistant starch is fermented in the large intestine of pigs.

[b] It is assumed that 90% protein in feces are bacterial protein.

Ammonia emission from pig manure is mainly influenced by manure pH and ammonium concentration. These factors are driven by dietary factors, e.g. CP and FC (Canh *et al.*, 1998; Canh *et al.*, 1998c). A low dietary CP level gave a low manure pH and ammonium concentration and consequently ammonia emission from pig manure. The results confirm the

findings of Canh *et al*. (1998) and Kay and Lee (1997) that for each 1% level dietary CP reduction ammonia emission from pig manure is reduced by approximately 10%. A high dietary FC level enhances microbial activities in the gut of the animal and in manure stores, resulting in higher VFA concentrations and a lower pH. This can explain how increased dietary FC reduces ammonia emission from pig manure and confirms the findings of Canh *et al*. (1998c), Kendall *et al*. (1999), and Shriver *et al*. (2003). According to Canh *et al*., (1999) increased dietary FC levels resulted in a clear shift of nitrogen excretion from urine to feces. However, in this study we did not find this. We found the ratios between ammonium-N concentration and total N in the manure of 0.68, 0.66, and 0.65, respectively for low, medium and high FC diets. According to Canh *et al.*, (1999) the shift of nitrogen excretion from urine to feces is mainly caused by cellulose and hemicellulose in the diet. Fermentable carbohydrates in the diets of our experiment were mainly composed of sugar beet pectin and resistant starch from raw potato. These carbohydrates have a higher level and rate of fermentablility compared to cellulose and hemicellulose. Different sources of FC used in their experiment and in ours may be therefore the reason for the differences in our finding and theirs.

Both CP and FC are precursors for VFA production in the gut of animals and in manure stores. Therefore, increased dietary CP and FC resulted in higher concentrations of VFA in manure. Acetic acid had the highest concentration and composed 61 to 67% to total VFA in manure. Short and straight-chain VFA dominated the VFA pool at levels from 87 to 90%. Branched-chain VFA contributed very little to VFA, from 10 to 12%. This confirms the findings of Otto *et al*. (2003), Le *et al.* (2005) and P.D. Le *et al.* (Chapter 4). Branched-chain VFA are produced from protein and its AA. Therefore, increased dietary CP resulted in increased concentrations of iso-butyric and iso-pentanoic acids in the manure and increased the proportion of branched-chain VFA in total VFA. Straight-chain VFA levels in manure increased as FC increased. This confirms the finding of Canh *et al*. (1998d).

Indolic, phenolic and sulfurous compounds in manure are produced from protein and come from three main pathways. First, protein is partly fermented in the large intestine of animals. The products of protein fermentation are indolic and phenolic and sulfurous compounds that are transferred to the liver where they are detoxified to glucuronides and then excreted via urine. Indolic, phenolic and sulfurous compounds are released when glucuronides come into contact with feces (Mackie *et al.*, 1998). Second, unfermented protein in the feces of animals can be fermented in the manure and indolic, phenolic and sulfurous compounds are produced. Third, a small proportion of indolic, phenolic and sulfurous compounds are excreted directly via feces. In addition, sulfurous compounds in the manure can be produced from sulfates excreted via

urine which mainly originate from excreta of excess S-containing AA absorbed in the small intestine of pigs. Increased dietary CP resulted in higher protein concentration in the large intestine of animals and in manure. Consequently higher concentrations of phenol, cresols, indole, 3-methyl phenol, 4-ethyl phenol and carbon disulfide in manure were found in manure of animals fed a high CP diet. This confirms the findings of Hobbs *et al.* (1996) and P.D. Le *et al*. (Chapter 4).

The high CP diet also had a higher concentration of carbon disulfide in the manure compared to the low CP diet. Carbon disulfide was the only sulfurous compound that could be measured in this experiment. Methyl sulfide, methyl disulfide, and ethanethiol were not identified. Other important odorous compounds in terms of strength and offensiveness, for example, hydrogen sulfide and methyl mercaptan could not be measured in this study, because they have a very low boiling point and easily escape from the manure.

No interaction between dietary CP and FC on ammonia emission was found. This means that dietary CP and FC affected ammonia emission in an additive way. This confirms the finding of Bakker and Smits (2002).

The correlation between ammonia and odor emission was very low, confirming the findings of P.D. Le *et al.* (Chapters 4, 5, 6). This was expected because ammonia is a single compound and not a very offensive compound (Oldenburg, 1989), while odor is a complex mixture of various compounds.

Briefly, increased dietary CP and FC led to a measured increase in concentrations of some odorous compounds in manure for which CP and FC are precursors. However, they did not directly increase odor concentration and emission from the manure. As discussed by P.D. Le *et al.* (Chapter 4), the relationship between the concentration of each odorous compound in manure or in air above the manure pit to strength and offensiveness of the odorous air is not clear yet. It is concluded that dietary CP and FC have an interactive effect on odor production and emission. Manure pH may play an important role in odor emission from the manure.

IMPLICATIONS

This study shows that dietary crude protein and fermentable carbohydrates have an interactive effect on odor concentration and emission from the manure of finishing pigs. Dietary strategies to alter odor production from pig manure should therefore focus simultaneously on both crude protein and fermentable carbohydrates. Manure pH seems to be a very important influencing factor determining odor emission. Therefore the effect of manure pH on odor

emission requires further study. Another important factor for odor production and emission from pig manure might be the amount of bacterial biomass produced in the large intestine and in manure. This might explain the role of protein and fermentable carbohydrates ratio in odor production process. Ammonia emission from pig manure can be reduced remarkably by reducing dietary crude protein and by increasing fermentable carbohydrates. More odorous compounds in manure and especially in the air above the manure pit should be analyzed to derive a satisfactory relationship between concentration of odorous compounds and odor concentration measured by olfactometry. The focus should be on some very volatile S compounds.

8

GENERAL DISCUSSION

Odor generated from pig production facilities is a major concern for the general public, producers, and policy makers. Odor problems have led policy makers to implement practices such as setback regulations in an attempt to control the maximum level of odor nuisance from pig farms. The primary goal of odor reduction is to maintain acceptance of intensive animal production by the general public and to allow a more sustainable development of pig production.

Odor is a mixture of various compounds, in which four main groups are associated with odor nuisance: (1) sulfurous compounds, (2) phenolic and indolic compounds, (3) volatile fatty acids (VFA), and (4) ammonia and volatile amines. In animal houses, odor may come from animal bodies, floors, and manure in the manure pit. According to Hansen (2005) the main source of odor is manure in the manure pit. In this study, we concentrated on reducing odor from manure in the manure pit.

Odor is mainly produced by the microbial conversion of feed components in the large intestine of pigs and by microbial conversion of urinary and fecal excreta in the manure. Odor precursors in manure originate from urine and feces. With regard to urine, odor precursors may include metabolic products of excess nutrients after being absorbed in the small intestine and also detoxicated products absorbed from the large intestine of animals and subsequently secreted in urine. Odor precursors in feces may include undigested feed components and endogenous products. These metabolic products, detoxicated products, undigested components and endogenous products are influenced by dietary composition. Therefore, odor production is presumably influenced by dietary factors, for example, level and type of protein/amino acids (AA) and fermentable carbohydrates (FC).

The fundamental starting point to reduce odor at the source of production by dietary approaches is to minimize potential odor precursors from protein and its metabolic products. This strategy can be achieved by the following approaches:

1. Reducing the amount of protein/amino acids in the diet by formulating diets that fit well to the animals' requirement so that fewer excess amino acids are absorbed in the small intestine of animals and less protein enters the large intestine of animals.

2. Balancing diets in such a way that non-utilized nutrients are optimally used for bacterial biomass synthesis so that less odor precursor is produced and converted to odorous compounds.

3. Using high ileally digestible ingredients so that less nutrient enters the large intestine of animals.

In this chapter, different dietary approaches, used in this study to reduce odor from pig

manure, will be discussed. Effects of dietary alterations on ammonia emission and its relation to odor emission will also be taken into account. Other odor-related issues such as key odorous compounds, translation of our findings to the animal house level, inputs from our findings of the environmental experiment for dietary experiments are discussed as well. Implications for odor reduction by dietary alterations, and further studies are proposed. The chapter ends with the primary general conclusions from this thesis.

POSSIBILITIES TO MANIPULATE ODOR FROM PIG MANURE BY DIETARY ALTERATIONS

CAN ODOR FROM PIG MANURE BE MINIMIZED BY DECREASING DIETARY CRUDE PROTEIN?

Generally, diets for pigs contain more nitrogen than their requirement for protein gain and maintenance. This results in unutilized protein. There are 3 main reasons for the presence of excess protein in animal diets. First, the AA pattern does not match the animals' requirement. Therefore, dietary crude protein (CP) is formulated to meet the minimum requirement of the most limiting essential AA. This leads to excess of the other AA. Second, diets are produced that meet the requirement of a large weight range of pigs. This means that if a diet meets the requirement for a growing pig, it will have an excess for a finishing pig. Third, there is variation in ileal digestibility of AA between feed ingredients. Excess of CP and AA causes production of precursors excreted via urine and feces and thus are present in the manure. The precursors are responsible for the production of sulfurous, indolic and phenolic compounds, volatile fatty acids (VFA), ammonia and volatile amines in the manure. Therefore, one of the dietary approaches to reducing odor from pig manure is to decrease dietary CP level. To balance AA pattern to maintain normal animal performance, essential AA are supplemented in the diet. By feeding reduced CP and simultaneously AA supplemented diets, less AA is deaminated, so less nitrogen is converted to urea and less odor precursor is excreted via urine and feces.

In the first dietary experiment (Chapter 4) reduction of dietary CP level from 18 to 12% and supplementation of crystalline AA decreased odor precursors. A low CP diet had less odor precursor from excess ileal absorbed AA and also less odor precursor from fermentable protein. Pigs fed a low CP diet had similar daily weight gain as the pigs on the high CP diet, so it is expected that a low CP diet resulted in less excess ileal absorbed AA and, as a consequence, less odor precursor excreted via urine. In addition, the low CP diet caused less degradation of protein in the large intestine than the high CP diet, 5.7 and 11.1 g/kg diet, respectively (Table 1). Degraded protein may be converted to odorous compounds. As a result, reducing dietary CP

level from 18 to 12% decreased odor emission of the manure of growing pigs by 80%. Odor hedonic tone was increased (less unpleasant odor). So it confirms that reduced CP diets can decrease odor nuisance from pig manure.

Table 1. Protein in diet, illeal digestible protein, protein disappeared in the large intestine (LI), fermentable carbohydrates in diet (FC) fermented carbohydrates (fCHO) and protein in bacterial biomass in feces of pigs fed different diets, calculated as g/kg diet as-fed and pH of manure

Chap	Treat.	CP in diet (1)	Ileal dig. protein (2)	Fecal dig. protein (3)	Protein disapp. in LI (3-2)	FC in the diet (4)	fCHO (5)	Protein in biomass (1-3)*0.9[a]	Manure pH
7	12% CP	126.1	92.4	95.1	2.7	234.8	148.6	28.0	7.1
	18% CP	183.3	144.0	146.7	2.7	213.8	151.3	33.0	8.3
4	12% CP	122.8	91.3	97.0	5.7	193.6	100.2	23.2	7.1
	18% CP	180.3	142.5	153.6	11.1	175.3	103.2	24.0	7.8

[a] It is assumed that 90% protein in feces are bacterial protein.

In the experiment described in Chapter 7, with different amounts of dietary fermentable carbohydrates, reducing dietary CP levels from 18 to 12% had no effect on odor emission from pig manure. It means that dietary CP is not the only factor influencing odor emission from manure. In addition, it gives an opportunity to formulate low odor-diets without having to minimize CP level. With respect to odor precursor production, odor precursors in manure are driven by 3 sources: (1) excess ileal absorbed AA, especially S-containing AA, (2) protein fermented/disappearing in the large intestine, (3) the amount of protein captured by bacteria in their biomass in the large intestine and in manure. From the 1st source, the 18% CP treatment was expected to produce more odor precursors than the 12 % CP treatment, because animals had similar daily weight gain. From the 2nd source, the two treatments produced a similar amount of odor precursors; about 2.7 g protein per kg of diet was apparently absorbed in the large intestine (Table 1). From the 3rd source, the 18% CP treatment produced more protein in biomass than the 12% CP treatment; 33 g compared to 28 g per each kg diet; so less odor precursor was produced than by the 12% CP treatment. So, it seems that the potential increase in amount of odor precursor in manure was compensated by the third source of odor precursors or an increase of bacterial biomass. Regarding odor emission from manure, pH seems to be an important factor driving odor emission from manure in this experiment (Chapter 7). Manure pH decreased from 8.30 to 7.10 as dietary CP reduced from 18% to 12%. A high pH inhibits microbial activities and reduces the emission of some key odorous compounds to the air. According to Shurson *et al*. (1998), when pH of manure is above 8, most of the reduced sulfur

exists in solution as HS^- and S^{2-} ions, and the amount of free H_2S is so small that odor problems do not occur. The effect of pH on odor emission from pig manure can be supported by our findings in the experiment reported in Chapter 3. In that experiment, we found that odor emission was increased when manure pH was lowered.

When comparing the odor emission from manure in the two experiments (Chapters 4 and 7), odor emission from the manure was lower in the latter experiment (Chapter 7) compared to the prior experiment (Chapter 4). From the dietary point of view, in the two experiments we did not use exactly similar levels and types of feed ingredients. The variation in these two experiments may make differences in the amount of protein in bacterial biomass and may create differences in formation of odor precursors in the gut of animals and in the manure. Different feed ingredients and the ratio between ingredients may influence the level and rate of fermentation, which finally affects odor production. It can be seen from Table 1 that in the latter experiment (Chapter 7) a similar amount of protein (2.7 g/kg diet as-fed basic) between 12% and 18% CP treatments disappeared in the large intestine of animals, which may be odorous compounds. This amount was lower than that in the former experiment (Chapter 4): 5.7 and 11.1 g/kg diet, respectively for 12 % CP and 18% CP treatments. This means diets in the former experiment (Chapter 4) produced more odor precursors in the large intestine than diets used in the Chapter 7. These precursors are excreted to manure via urinary and fecal pathways. This may partly contribute to the lower odor emission from the manure in the latter experiment. In addition, the amount of fecal digestible fermentable carbohydrates was higher in the latter experiment described in Chapter 7 than in the former experiment described in Chapter 4. Furthermore, the amount of protein in bacterial biomass produced, g per kg diet in the latter experiment (Chapter 7) was higher than in the former experiment (Chapter 4). Moreover, as mentioned above, manure pH seems to be an important driving factor for odor emission. In the former experiment (Chapter 4), manure pH dropped by 0.73 units, from 7.83 to 7.10 while it was 1.21 units in the latter experiment (Chapter 7), from 8.3 to 7.1.

Regarding experimental conditions, the former experiment was conducted on growing pigs while the latter experiment was conducted on finishing pigs. As discussed by Hobbs *et al.* (1996) and Pfeiffer (1993), it is suggested that finishing pigs have a larger hindgut and this prolongs bacterial fermentation, causing less precursors to be available for odor production in the manure. In addition, odor samples were collected after 4 wk of urine and feces collection in the former experiment, while a 5 wk period was used for the latter experiment. The difference in storage time might create some differences in odor emission from pig manure as discussed by Gralapp *et al*. (2002) and Clark *et al*. (2005b).

Based on theoretical knowledge of odor production and the practical finding in the first experiment, we may conclude that there is a great potential to reduce odor nuisance from pig manure by decreasing dietary CP levels. Manure pH among others seems to play an important role in odor emission from manure.

EXCESS S-CONTAINING AA IN THE DIET REMARKABLY INCREASE ODOR FROM PIG MANURE

Animals and bacteria use protein in the form of AA. A large part of protein or AA is absorbed in the small intestine of animals and utilized for muscle synthesis and other products. If not utilized, they are converted to carbon chains, sulfates (in case of S-containing AA) and urea. Also, excess protein may enter the large intestine of animals where they are either used as an energy source or as a nitrogen source for bacteria. In the large intestine of animals and in the manure, odorous compounds are produced from the excess protein or its metabolic products. Different types of AA provide different precursors for odorous compounds. From the literature review, we found that sulfurous, indolic and phenolic compounds can be considered as key odorous compounds. Sulfurous compounds are produced from S-containing AA (Met. and Cys.) by microbial fermentation (Kelly *et al.*, 1994; Yoshimura *et al.*, 2000). Microbial production of indolic and phenolic compounds results from AA metabolism, for example, Tryptophan (Trp), Phenylalanine (Phe) and Tyrosine (Tyr) (Spoelstra, 1977; Hengemuhle & Yokoyama, 1990; Jensen & Jørgensen, 1994).

Supplementing crystalline S-containing AA 3 times the animal requirement greatly increased odor emission and odor intensity and decreased odor hedonic tone (a less pleasant odor) of the air above the manure pit. This means that the excess crystalline AA or their metabolic products provided precursors for odor production in manure. It is generally accepted that crystalline AA are absorbed before they reach the end of the ileum and thus metabolic products of ileal absorbed AA excreted via urine are precursors for odor production in the manure. If sufficient Met or Cys are supplemented then no more extra Met compounds (epinephrine, choline, beatine, spermidine, spermine, carnitine, melantonin, creatinine and lanthionine) or Cys compounds (taurine, condriton, glutathionine and cyisine) will be made. In that case the excess of these AA (and their metabolites) will be converted to pyruvate (from Cys), succinyl CoA (Met), and SO_4^{2-}. In a normal situation, a rough estimate for the sulfur balance would be 65% retained (this includes all dietary S). Of the remaining sulfur approximately 55-60% is excreted in the feces and 40-45% in the urine. About 80% of urinary S is excreted as sulfate with smaller amounts of other S-containing compounds (mercaptolactate, mercaptoacetate, N-acetylcysteine, thiosulfate, thiocyanate, taurine, etc.)

(Kline *et al.*, 1971; Shurson *et al.*, 1998).

One may argue whether a part of crystalline AA is fermented at the end of the ileum or enters the large intestine of animals. If a part of crystalline AA is fermented at the end of ileum or enters the large intestine then less than 100% crystalline AA is absorbed before they reach the end of the ileum. In the large intestine, AA are fermented by bacteria and different S-compounds are formed. These compounds are partly absorbed through the gut wall and transferred to the liver, where they are detoxicated and excreted via urine in the forms of glucuronides or sulfates. A part of S- compounds can be excreted directly via feces. So, regardless of whether is absorbed in the small or in the large intestine of animals, excess S becomes S-containing compounds in the manure. The microbes in the manure metabolize it into various compounds, mainly hydrogen sulfide and methyl methanethiol.

In practice, diets are formulated with a wide range of raw materials. Some ingredients are rich in S, for example, blood meal, soybean meal, and molasses. To reduce odor from pig manure, these ingredients should be used at an appropriate level. In addition, S-containing AA should be formulated so they meet animals' requirement but no more. In some regions, drinking water is contaminated with S, which is also a precursor for odor production. Therefore, S in water should be controlled in the effort to minimize odor by reducing odor precursors.

Supplementing 2 times the requirement of Trp and Tyr + Phe did not increase odor strength and offensiveness of the odorous air above the manure pit. The excess of ileal absorbed Trp, Tyr and Phe were probably degraded to carbon chain and nitrogen. The excess nitrogen shows up as increased urea excretion via urine. This leads to a higher ammonium-N output compared to that of the control diet (Chapter 5). If this is the case then the excess Trp, Tyr and Phe absorbed in the small intestine will only give more ammonia and not cause odor nuisance. When these AA are part of protein and when these AA enter the large intestine of animals, indolic and phenolic compounds can be formed. The role of indolic and phenolic compounds in terms of odor strength and offensiveness will be further discussed in another section of this chapter.

CAN ODOR FROM PIG MANURE BE MINIMIZED BY REDUCING FERMENTABLE PROTEIN LEVELS?

Protein which escapes the small intestinal digestion may be used by bacteria in the large intestine of animals. It can be used for microbial growth or broken down to compounds which then may be absorbed through the gut wall and excreted via urine. The other part is voided into the manure via feces where it is also used by bacteria. Protein entering the large intestine is

named fermentable protein. To manipulate the amount of protein in the large intestine of animals one can use dietary ingredients which vary in ileal digestibility. In our experiment (Chapter 6), three levels of fermentable protein were used: 28, 38, and 48 g/kg feed. We did not find any effect of the fermentable protein levels in the diet on odor strength and offensiveness of the air above the manure surface. As explained in Chapter 6, odor may originate from odor precursors produced in the large intestine of animals and odor precursors excreted from the excess absorbed AA at ileum level. If so, dietary alterations to reduce odor from pig manure should consider different sources of odor precursors at the same time. Different diet ingredients have different patterns of digestion and absorption and thus differ in pathways of odor precursor excretion. In the large intestine, rapid fermentable ingredients are fermented at the beginning part of the tract and thus more odor precursors will be excreted via urine compared to continuous fermentation. Slow fermentable ingredients are fermented along the whole tract and may even continue to be fermented in the manure. So odor precursors may be voided from the large intestine in addition to the small intestine as a result of protein fermentation. It is necessary to study effects of dietary alterations on odor production and excretion from urinary and fecal pathways separately. This means that experiments should be designed in which odor from urine and feces is separately studied.

FERMENTABLE CARBOHYDRATES (FC) AND PROTEIN INTERACTIVELY AFFECT ODOR FROM PIG MANURE

The ratio between CP and FC may affect microbial activities in the large intestine of animals and in manure stores (Bergman, 1990; Reid & Hillman, 1999; Gibson & Roberfroid, 1995). Therefore, there will be a ratio between CP and FC at which odor production is minimized. At this ratio, not much protein is used as an energy source for bacteria. It is used to synthesize microbial biomass, so less protein is broken down to odor precursors and less odor is produced. Indeed we found an interactive effect between CP and FC on odor concentration and emission from pig manure ($P = 0.06$). Odor production from pig manure is low in cases when pigs are fed diets with a low CP and a low FC diet or a high CP combined with a high FC diet. The finding implies that CP and FC do affect odor production from pig manure in combination with each other. To increase the efficiency of odor reduction, a certain dietary factor must be considered in connection with other dietary factors. In practice, pig diets contain much more protein than the pigs' requirement; the effect of excess CP on odor production can be lowered by increasing the level of dietary FC.

Sugar beet pectin and potato native starch were used as sources of FC in the study

described in Chapter 7. Both have a high level of fermentability (Barry *et al.*, 1995; Lucile *et al.*, 1998); 97% pectin is fermentable, while for instance only 6-7% of cellulose and maize bran are fermentable. Carbohydrate sources that slowly ferment may prolong the fermentation in the large intestine of animals and in the manure pit. This can be useful in systems where manure remains in the manure pit for a longer period, e.g. 6 wk like in the Netherlands.

Bacterial biomass is a product of microbial growth. When growth occurs at a high level one can consider that this reflects the optimum ratio between CP and FC, in any case no shortage of sacharolytic energy for microbiota. If a large amount of bacterial biomass is produced less odor is expected from the manure. Bacterial biomass was not analyzed in the experiments presented in this thesis. In our study, we assumed that 90% of protein in feces is in the form of biomass. This indicator should be taken into account in future experiments where diets are altered to reduce odor from pig manure.

CONCLUDING REMARKS AND OTHER POTENTIAL APPROACHES TO REDUCE ODOR FROM PIG MANURE BY DIETARY MANIPULATION

Figure 1 shows a schematic view of odor precursor production and excretion. Proteins/AA are the main odor precursors. Odor precursors in the manure come from both urine and feces. Excess ileal absorbed AA produce odor precursors excreted via urine. Protein entering the large intestine can be fermented by bacteria resulting in odor precursors excreted via both urine and feces. Dietary manipulation aims at minimizing odor precursors excreted via urine and via feces. Consequently odor production from the manure will be reduced. To reduce odor precursors in the manure, excess AA, especially S-containing AA absorbed in the small intestine of animals should be minimized, the amount of protein entering the large intestine should be minimized, or protein in the large intestine should be captured in bacterial biomass. Excess crystalline sulfur-containing AA resulted in high odor strength and offensiveness from pig manure. So, a reduced CP and a small amount of supplemented sulfur-containing AA diets can prevent a high odor emission from pig manure. Odor emission from manure of pigs fed high CP diets can be reduced by increasing the level of fermentable carbohydrates in the diet. Manure pH seems to be an important factor driving odor emission from manure. Dietary alterations can be very effective to reduce odor emission from pig manure. It is important to note that the diets used in our studies are typical pig diets used in the Netherlands and in Europe.

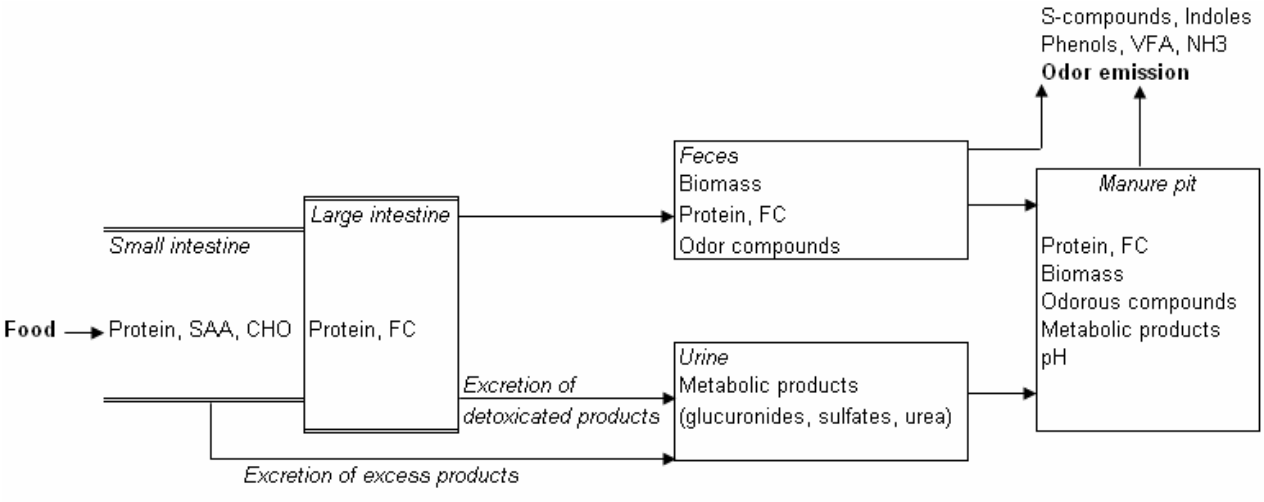

Fig. 1. Schematic view of odor precursors and pathways of odor production and excretion, AA: Amino acids, FC: Fermentable carbohydrates, CHO: Carbohydrates, SAA: Sulfur-containing AA, VFA: Volatile fatty acids

Another potential approach to managing odor from pig manure is by utilizing different feed formulation procedures. In many parts of the world, pig diets are formulated based on a total or on apparent ileal digestible AA basis. Standardized ileal digestibility formulation can be used to minimize excess AA in the diet. This formulation takes into account endogenous losses caused by specific feed ingredients and uses these values to determine AA values for particular feedstuffs (Rademacher, 2000). When developing a diet on standardized ileal AA, less AA is needed in the diet to meet the pigs' requirements. In addition, formulating diets that have optimal ratios of ileal absorbable AA will reduce excess AA in the diet. Other dietary approaches to reducing excess protein are phase feeding and individual feeding. These approaches also aim at maximizing nutrient utilization or formulating diets that meet well animals' dietary requirements and thus reducing excess nutrients for odor production.

CAN AMMONIA AND ODOR EMISSION BOTH BE REDUCED SIMULTANEOUSLY BY DIETARY ALTERATION?

The main objective of this study was to identify effects of dietary alterations on odor strength and offensiveness from pig manure by studying effects of ileal absorbed AA, fermentable protein levels, dietary CP and FC levels. Ammonia emission from manure was studied simultaneously as well, because it causes serious environmental problems. This study positively confirmed the findings of some previous studies in literature on the relationships between dietary alterations and ammonia emission. Reducing dietary CP levels and supplementing most essential AA to the diet decreased ammonia emission significantly and did not have any negative effect on animal performance. This study confirmed that for each 1 %

unit of dietary CP reduction, about 10% reduction of ammonia emission from pig manure can be achieved (Kay & Lee, 1997; Sutton *et al.*, 1997; Canh *et al.*, 1998). A high FC level stimulates bacterial activities in the hindgut of animals and in manure stores resulting in high short-chain VFA concentration and lower pH level and finally reduced ammonia emission (Canh *et al.*, 1998c; Kendall *et al.*, 1999; Shriver *et al.*, 2003). It is interesting that CP and FC additively influence ammonia emission so ammonia emission can be further reduced when more than one dietary component is altered, confirming the finding of Bakker and Smits (2002).

The correlation between ammonia and odor emission has been a concern in many studies in literature and in ours, as well. Inconsistent findings were found in the literature. We found a low and not significant correlation between ammonia and odor emission in the four dietary experiments, ranging from 0.1 to - 0.3. This means that dietary strategies which can reduce ammonia emission effectively may not work in the same manner for odor emission. Probably, manure pH is a driving factor behind the correlation between ammonia and odor emission. High manure pH increases ammonia emission. At the same time, at a high pH, hydrogen sulfide is in ionized form, which means that the emission of hydrogen sulfide is low. Bacterial activities are inhibited to a small extent at a high manure pH, and thus less odorous compound is produced. The role of manure pH should be further investigated. An experiment is required on the effects of acidifying or alkalizing the manure from pigs on ammonia and odor emission. In such an experiment, diets should be equal in CP and FC content which affect both ammonia and odor emission. Different levels of manure pH can be created by different dietary electronic balance (dEB) or supplementing diets with calcium salts, e.g. $CaCO_3$, $CaSO_4$, Ca-benzoate or $CaCl_2$.

It is worth mentioning that the correlation between ammonia and odor emission depends on environmental factors that may result in both positive and negative correlations. In our dietary experiments, environmental factors like temperature, air velocity, manure volume, and manure pit surface were similar for all treatments. Consequently, the correlation between ammonia and odor emission was driven by dietary factors alone. The correlation between ammonia and odor emission between animal houses or between farms in practice may be different from the correlations found in our studies, because, animal houses and farms differ in both climate and dietary factors from our standardized set-up.

EFFECTS OF DIETARY ALTERATIONS ON ODOR, FURTHER STUDIES AT THE ANIMAL HOUSE LEVEL

Odor is produced in the chain from feed to animal to urine and feces. This study focused

only on odor emission from pig manure in the manure pit, a mixture of urine and feces, because the most important source of odor is from manure (Hansen, 2005). However, findings on the effect of dietary factors on odor emission from pig manure should be tested at the animal house scale for a number of reasons.

First, odorous compounds produced in the large intestine of animals are partly excreted directly by flatus and via feces. Another part is absorbed via the gut wall and transferred to the liver where odorous compounds are detoxicated and excreted via urine in the form of glucuronides and sulfates. In addition, odor precursors in urine may include metabolic products from excess nutrients absorbed in the small intestine. When glucuronides and sulfates come in contact with feces, they are rapidly released (Spoelstra, 1979; Mackie *et al.*, 1998). In animal pens without slatted floor or with a fouled solid floor, odorous compounds that originate from the floor itself, in addition to odor from the manure pit, emit to the air. Measuring odor in the exhaust air from the animal house will include all sources of odor, e.g. feed, animal body, floor, and manure.

Second, odor emission from the animal house is influenced by environmental factors like temperature and ventilation rate or air velocity. Normally these environmental factors have interrelated effects on the odor emission process. In our experiments, these factors were controlled and therefore could not interact with dietary factors.

Third, in our experiments, feed and water were controlled and restricted and odor samples were collected from similar manure volumes in the manure pit. In practice pigs are fed *ad libitum* and have continuous free access to water. Reduction in dietary CP decreases water intake, water excretion, and manure volume (Pfeiffer & Henkel, 1991; Kay & Lee, 1997). This may change odor from pig manure compared to the values in our experiments.

In the Netherlands, efforts have been done to find out factors affecting odor emission at the animal house level. The effect of manure pH on odor emission at the animal house level has not yet received much attention. Studies described in this thesis show that manure pH may play an important role in odor emission from the manure. Therefore, future studies should focus on the effect of nutrition on manure pH and subsequently on odor emission at the animal house level.

DIETARY ALTERATION TO REDUCE ODOR FROM PIG MANURE: KEY ODOROUS COMPOUNDS

Odor measurements by olfactometry are time consuming and costly. Considerable effort has been made to elucidate the most important (key) odorous compounds in the odorous air or in manure in terms of odor strength and offensiveness or compounds whose concentration is correlated with odor nuisance. Identifying key compounds enable researchers to focus on

what compounds should be monitored and what strategies should be followed to control odor. A rather widely accepted list of key odorous compounds was summarized in Table 3 in Chapter 2. According to Hammond *et al.* (1989), Hobbs *et al.* (1995) and Sutton *et al.* (1999) the list of key odorous compounds can be summarized in 14 main compounds, namely, hydrogen sulfide, methanethiol, dimethyl sulfide, dimethyl disulphide, dimethyl trisulfide, phenol, 4-methyl phenol, indole, 3-methyl indole, acetic acid, propionic acid, butyric acid, 3-methyl butyric acid, and pentanoic acid.

Apart from measuring odor strength and offensiveness of the odorous air above the manure surface, we also measured some key odorous compounds in the manure. Increased dietary crude protein levels (Chapter 7) or fermentable protein levels (Chapter 6) resulted in higher concentrations of indolic and phenolic compounds in the manure. However, odor strength and offensiveness of the odorous air were not affected. Furthermore, according to Willig *et al*. (2005) there were high correlations between concentrations of some indolic and phenolic compounds in feces and in the headspace air: indole r = 0.59, skatole r = 0.67, 4methyl phenol r = 0.78. From all arguments discussed before in this chapter, we can conclude that indolic and phenolic compounds analyzed in this study may not be the key odorous compounds.

Manure from pigs fed a diet with 3 times the requirement of sulfur-containing AA had an extremely high odor strength and offensiveness (Chapter 5). Sulfur-containing AA are precursors for sulfurous compounds in manure and in the air above the manure. We can conclude that sulfurous compounds are key odorous compounds in terms of odor strength and offensiveness. However, we could not specifically show if some S compounds are more important than others. First, we were able to measure a few S compounds in the manure, and these compounds may not be representative enough for the most important odor compounds in the manure and in the air. Second, we did not measure S compounds in the odorous air above the manure surface, especially important odorous compounds, e.g. hydrogen sulfide, and methyl methanethiol. These compounds could not be measured in manure because they have a very low boiling point, have a low solubility in water, and are very reactive (Spoelstra, 1980). The techniques to sample and measure these compounds are still in development.

Ammonia can not be a key odorous compound, because we found a low correlation between ammonia emission and odor emission. This confirms the findings of Oldenburg (1989).

Volatile fatty acids concentrations in manure increased as dietary crude protein level or fermentable carbohydrate level increased (Chapter 7); however, odor strength and offensiveness

of the odorous air above the manure surface were not affected by dietary crude protein or fermentable carbohydrate levels. In addition, according to Willig *et al.* (2005), there was no significant correlation between VFA concentrations in feces and the headspace air. Furthermore, according to Schaefer (1977) VFA can not be key odorous compounds because correlation between the concentration of VFA and people's perception of odor is low. So, VFA analyzed in this study may not be one of the key odorous compounds in the odorous air.

In order to have a complete picture on key odorous compounds from pig production facilities, it is necessary to (i) simultaneously measure the concentration of odorous compounds in manure and in the odorous air above the manure, (ii) measure more odorous compounds than have been measured in our experiments, focusing special attention on very volatile sulfurous compounds, and (iii) measure odor strength and offensiveness of the odorous air above the manure pit by olfactometry as done in our studies.

INPUTS FROM THE ENVIRONMENTAL EXPERIMENT ON DIETARY EXPERIMENTS, ODOR CAN BE REDUCED BY ALTERING ENVIRONMENT

Dietary alterations mainly focus on reducing odor at the source of production. In other words, they prevent odor from being produced. Environmental alterations directly influence microbial activities and thus odor production. On the other hand, they also influence the emission of odorous compounds from manure to the air, or from the animal house to the environment. So, environmental alterations may influence both odor production and emission. An integrated approach to reducing odor from pig production facilities by altering both dietary and environmental factors may be more efficient than either dietary or environmental alteration is alone.

One of the objectives of the environmental experiment (Chapter 3) was to discover which environmental factors may influence odor concentration and emission. We found that temperature, ventilation rate and, to a less extent, manure dilution ratio and emitting surface area affected odor emission, ammonia emission and manure characteristics. Those factors were controlled in the dietary experiments (Chapters 4, 5, 6 and 7), so true effects of dietary factors were not confounded with environmental factors. In addition, the environmental experiment can be considered as a technical study, which provided knowledge and skills on standardization of sampling and measuring strategies for odor strength and on offensiveness of the odorous air from the manure.

In practice, environmental factors have a complex role in their influence on odor

production and on emission from the manure pit or from the animal house. For example, increased temperature in the animal house leads to increased ventilation rate. In addition, effects of environmental factors are confounded with animal body mass, animal activities and volume of manure in manure pits, etc. Furthermore, different environmental factors may not be homogenous in the entire animal house or in the manure pit. For example, the temperature of the manure pit is different at different layers in the manure. In our lab scale experiment (Chapter 3), temperature and ventilation rate were independently studied. Odor emission was reduced by 216% as temperature of the manure and air decreased from 30 to 10 ^{o}C. So lowering the temperature could be a possible solution to lower odor emission from pig manure. It is important to note that in our experiment, temperature was homogenous in the whole system, e.g. incoming air, headspace area, and manure. This principle can not be applied the same way in the animal house, because it requires too much energy and the temperature may be lower than the comfort zone of animals. Cooling the manure pit seems to be the best option in the light of altering temperature to reduce odor from pig manure. Cooling systems have been shown to be effective in reducing ammonia and odor emission from pig manure (Mol & Ogink, 2003). So, applying a cooling system can simultaneously lower odor and ammonia emission. Reducing ventilation rate is also an option for reducing odor emission.

Manure dilution ratio and emitting area were also studied in our experiment. However, their effects were not completely separated and were confounded by headspace volume. This situation is reflected in practice. Different farms or animal houses have different manure pits with different manure volume. However, it is important to recall that combined effects of emitting area and dilution ratio were neglectable compared to temperature and ventilation rate. We did not study the effects of humidity on odor from the manure, because, in the Netherlands, humidity in the animal house is fairly stable (60 to 80%). So, temperature and ventilation rate should be first choices in attempting to minimize odor by altering environmental factors. However, further studies should focus on independent effects of emitting area and dilution ratio on odor from pig manure. Determining the independent effects of temperature and ventilation rate on odor at animal house level should also be topics for further studies.

MAIN CONCLUSIONS

In general, we can conclude that there are great possibilities to reduce odor nuisance from pig manure by altering dietary factors. Altering multiple dietary factors and evaluating their correlations affecting odor production and emission is more efficient in odor nuisance reduction than altering a single dietary factor.

The specific conclusions are:

- There is a great potential to reduce odor nuisance by decreasing dietary crude protein level and supplementing with most essential amino acids to balance the amino acids pattern.
- Odor emission from pig manure is highly influenced by excess sulfur-containing amino acids in the diet. Increasing S-containing amino acids in the diet to 3 times the animal requirement significantly increases odor emission from pig manure. Increased sulfur-containing amino acids in the diet increases odor intensity and reduces odor hedonic tone of the odorous air above the manure.
- Dietary crude protein and fermentable carbohydrates have an interactive effect on odor emission from pig manure. Odor emission is reduced when pigs are fed low crude protein and low fermentable carbohydrate diets or high crude protein and high fermentable carbohydrate diets.
- When using similar diets as in our studies, sulfur-containing compounds are the most important key odorous compounds, while phenolic and indolic compounds and volatile fatty acids seem less important.
- Ammonia emission from pig manure can be reduced significantly by (i) feeding animals with a lower level of dietary crude protein and at the same time supplementing most essential amino acids to balance the amino acid pattern, (ii) increasing the fermentable carbohydrate level in the diet. Effects of dietary crude protein and fermentable carbohydrate levels on ammonia emission prove to be additive.
- The correlation between ammonia emission and odor emission in dietary experiments under controlled environmental condition is low. Dietary approaches that can reduce ammonia emission from pig manure may not be effective to reduce odor emission and even may have an opposite effect.

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SUMMARY

Odor emission from pig production facilities causes serious nuisance in the surrounding areas, and should therefore be reduced. Odor is mainly produced by the microbial conversion of feed components in the large intestine of pigs and by microbial conversion of urinary and fecal excreta in the manure. Proteins and fermentable carbohydrates are the main precursors for odor production. Odor is a complex mixture of various volatile compounds. Odorous compounds can be classified into four main groups: (1) sulfurous compounds, (2) indolic and phenolic compounds, (3) volatile fatty acids (VFA), and (4) ammonia and volatile ammines. Odor is evaluated through its strength (odor concentration and odor intensity) and offensiveness (odor hedonic value). A lot of efforts have already been made to minimize odor. Attempts were mainly focused on cleaning odorous compounds in the air emitted from pig production facilities. Efforts until now can be characterized as minimizing odor after it has been produced. In other words, they are end-of-pipe solutions.

Reducing odor at the source of production by altering dietary composition has a great potential but it is still a rather unexplored field. Effects of dietary crude protein level and fermentable carbohydrates on odor strength and offensiveness of the odorous air emitting from pig manure measured by olfactometry have not been studied well and the results were very inconsistent. In addition, the effects of crude protein and fermentable carbohydrates were studied independently whereas crude protein and fermentable carbohydrates may act in combination in order to affect odor production. Interactions are expected because in the large intestine of pigs and in the manure stores, microbiota uses protein as a nitrogen source and obtains energy from fermentable carbohydrates and also from crude protein for their biomass synthesis.

A division can be made between protein that is broken down in the small intestine to AA and absorbed in the small intestine and protein which escapes the small intestinal digestion, called ileal non-digestible protein or fermentable protein. Fermentable protein may be used by bacteria in the large intestine. It can be broken down to odorous compounds. A change in the level of fermentable protein may alter odor production in the large intestine of animals and in the manure. Effects of fermentable protein level on odor strength and offensiveness from pig manure have not yet been studied. Excess AA absorbed in the small intestine may also be converted to precursors for odor production. In this respect some AA seem to be more important than others. Sulfurous compounds and the aromatic compounds of indoles and phenols are considered most important for odor nuisance from pig production facilities. Tryptophan (Trp), Phenylalanine (Phe) and Tyrosine (Tyr) are main substrates for the synthesis of indolic and phenolic compounds. The S-containing AA, Methionine (Met) and Cystine (Cys)

are main substrates for the synthesis of S-compounds. A change in the concentration of these AA in the diet may alter the level of odorous compounds produced in the manure. The effects of these AA on odor strength and offensiveness have not yet been studied.

The overall goal of the present study is to assess the potential impacts of dietary factors on odor strength and offensiveness from pig manure, focusing on the effects in the ileum, in the large intestine and after excretion in the manure. The specific objectives are to test the hypotheses that:

1. Dietary CP level is an important factor in odor production from pig manure
2. Sulfur-containing AA and Trp, Phe and Tyr are important precursors for odor production from pig manure
3. Apparently ileal non-digestible protein (fermentable protein) fermented in the large intestine of pigs is an important source for odor production from pig manure
4. Dietary CP and FC levels do interact with regard to odor production from pig manure.

Ammonia emission causes serious environmental problems. Odor abatements by dietary alterations are only of interest if they do not increase ammonia emission from pig manure. Therefore, this study also evaluates the effects of these dietary factors on ammonia emission from pig manure and its relation to odor emission.

An extensive literature review was done to describe the state-of-the-art of knowledge on the relationship between odor and diet. Following this literature review, four dietary experiments were set up to test the hypotheses 1 to 4 (Chapters 4 to 7, respectively). Before conducting the dietary experiments, a study on the effects of environmental factors on odor from pig manure was done (Chapter 3). This was a technical study providing knowledge on sampling and measuring procedures determining odor emission from pig manure. In addition, it identified environmental factors having significant effects on odor production and emission from pig manure. These factors should be controlled in dietary experiments.

Chapter 4 presents a study on the effects of different dietary crude protein levels on odor from pig manure. Three dietary crude protein levels were used: 12, 15 and 18 % as-fed basic. In Chapter 5, a study on the effects of specific crystalline amino acid supplementation to the diet on odor from pig manure is described. This is to measure the effect of ileal absorbed amino acids on odor from pig manure. Treatment groups were 1) 15% crude protein basal diet with 3 times the requirement of sulfur-containing amino acids (14.2 g Met + Cys /kg diet, as fed basis); 2) basal diet with 2 times the requirement of Trp and Phe + Tyr (2.9 and 20.4 g/kg diet, as fed basis, respectively); and 3) basal diet with amino acid supplementation to levels

sufficient for maximum protein gain. Chapter 6 shows a study on the effects of different fermentable protein levels on odor from pig manure. This was to study the effect of the level of protein broken down in the large intestine on odor from pig manure. Three fermentable protein levels were used: 28, 38 and 48 g/kg feed as-fed basis. The three mentioned studies (Chapters 4, 5 and 6) were conducted on growing pigs and used a randomized complete block arrangement having three treatments in six blocks (n = 18/experiment). In Chapter 7, a study on the interactive effects of dietary crude protein and fermentable carbohydrate levels on odor from pig manure is presented. An experiment was conducted with finishing pigs (n = 36) in a 2x3 factorial randomized complete block arrangement with 6 treatment combinations in 6 blocks. There were 2 dietary crude protein levels (low 12%; high 18%, as fed basis) and 3 digestible fermentable carbohydrates levels: (low 95.5; medium 145.5; and high 195.5 g kg^{-1} feed, as-fed basis).

Results of the experiment described in Chapter 3 showed that temperature, ventilation rate and, to a less extent, manure dilution ratio and emitting surface affected odor emission, ammonia emission and manure characteristics. Those factors were controlled in the dietary experiments (Chapters 4, 5, 6 and 7), so true effects of dietary factors were not confounded with environmental factors. Feces and urine of each pig were accumulated together in a separate manure pit under the slatted floor. After an adaptation period of 2 wk, the manure pits were cleaned and manure was collected. In the 5^{th} wk (experiments described in Chapters 4, 5 and 6) or in the 6^{th} wk of the collection period (experiment described in Chapter 7), air samples for odor and ammonia analyses, and manure samples were collected directly from each manure pit. Dependent variables in the four dietary experiments were odor concentration and emission, odor intensity, odor hedonic tone, ammonia emission from pig manure, and manure characteristics: pH, ammonium, total N, indolic, phenolic, sulfurous compounds and volatile fatty acid (VFA) concentrations.

Results of the experiment described in **Chapter 4** show that reducing dietary crude protein from 18% to 12% lowered odor emission ($P = 0.04$) and ammonia emission ($P = 0.01$) from pig manure by 80% and 53%, respectively. Reduction in dietary crude protein levels decreased total N, methyl sulfide, carbon disulfide, ethanethiol, phenol, 4-ethyl phenol, indole and 3-methyl indole concentrations in the manure ($P < 0.05$). Reducing dietary crude protein and at the same time providing essential amino acids to balance the amino acid pattern is an option to reduce odor emission as well as ammonia emission from pig manure.

Results of the experiment described in **Chapter 5** show that supplementing crystalline S-containing amino acids (Met and Cys) three times animal requirement increased odor

emission by 723% ($P < 0.001$) from the manure. In addition, it increased odor intensity ($P < 0.05$), and reduced odor hedonic tone ($P < 0.05$) of the air sampled above the pig manure. Supplementing crystalline Trp, Tyr, and Phe in surplus of recommended requirement did not affect odor emission, odor intensity, or odor hedonic tone. No differences were observed in ammonia emission from manure of pigs fed different levels of amino acid supplementation ($P = 0.20$). The correlation between ammonia emission and odor emission was low (r = - 0.3) and non-significant. Regardless of dietary treatment, all pigs had similar performance levels. It is concluded that in order to reduce odor from pig manure the S-containing amino acids should be minimized to just meet recommended requirements. It is clear that sulfurous compounds contribute significantly to odor nuisance.

In **Chapter 6**, it is shown that fermentable protein levels had no effect on odor emission, odor intensity, and hedonic tone of the odorous air nor on ammonia emission from the pig manure. Increased fermentable protein levels enhanced the concentrations of total N, methyl sulfide, carbon disulfide, ethanethiol, phenol, 3-methyl indole, and 4-ethyl phenol in the manure ($P < 0.01$). The correlation between ammonia emission and odor emission was low (r = 0.14) and non-significant Regardless of dietary treatment, all pigs had similar performance levels. To reduce odor by means of protein, the level of fermentable protein should not be considered alone, it should be considered together with total dietary crude protein level and ileally digestible crude protein level.

As presented in **Chapter 7**, dietary crude protein level and fermentable carbohydrate level proved to have an interactive effect on odor concentration and emission at $P = 0.06$. At a high dietary crude protein level, increased fermentable carbohydrate level decreased odor emission from pig manure, while at a low crude protein level increased fermentable carbohydrate level increased odor emission. Total N and ammonium-N concentrations of the manure, and ammonia emission from the manure were reduced by lower dietary crude protein level ($P < 0.001$). A higher fermentable carbohydrate level led to lower ammonia emission from the manure ($P = 0.01$). Manure pH increased at the higher dietary crude protein level ($P < 0.001$) and decreased when fermentable carbohydrate level increased ($P = 0.03$). Total VFA concentration increased at the higher dietary crude protein level ($P < 0.001$) and when fermentable carbohydrate level increased ($P = 0.001$). Enhanced dietary crude protein increased the manure concentrations of phenol ($P < 0.001$), cresols ($P = 0.01$), indole ($P < 0.001$), 3-methyl indole ($P = 0.08$), 4-ethyl phenol ($P < 0.001$) and carbon disulfide ($P < 0.001$), but fermentable carbohydrate did not affect concentrations of these compounds ($P > 0.05$). The correlation between ammonia emission and odor emission was low (r = - 0.1) and non-

significant. Regardless of dietary treatment, all pigs had similar performance levels. It is concluded that the interaction between dietary crude protein and fermentable carbohydrate plays a role in odor production and emission. Ammonia emission from pig manure can be reduced substantially by decreasing dietary crude protein and by increasing fermentable carbohydrates.

Briefly, proteins/amino acids are the main odor precursors. Odor precursors in the manure come from both urine and feces. Excess ileal absorbed amino acids produce odor precursors excreted via urine. Protein entering the large intestine can be fermented by bacteria resulting in odor precursors excreted via both urine and feces. Dietary manipulation aims at minimizing odor precursors excreted via urine and via feces. Consequently odor production from the manure will be reduced. To reduce odor precursors in the manure, excess amino acids, especially S-containing amino acids (Met and Cys) absorbed in the small intestine of animals should be minimized, the amount of protein entering the large intestine should be minimized, or protein in the large intestine should be captured in bacterial biomass. Excess crystalline sulfur-containing amino acids resulted in high odor strength and offensiveness from pig manure. So, a reduced crude protein level and a minimized amount of supplemented sulfur-containing amino acids can prevent a high odor emission from pig manure. Some feed ingredients are rich in S, for example, blood meal, soybean meal, and molasses. To reduce odor from pig manure, these ingredients should be used at an appropriate level. Odor production and emission from manure of pigs fed a high crude protein diet can be reduced by increasing the level of fermentable carbohydrates in the diet.

In general, we can conclude that there are great possibilities to reduce odor nuisance from pig manure by altering dietary factors. Integrated approaches altering more than one dietary factor and considering the interaction between dietary factors may be more efficient in terms of minimizing odor nuisance than altering a single dietary factor.

- There is a great potential to reduce odor nuisance by decreasing dietary crude protein level and supplementing with most essential amino acids to balance the amino acids pattern.
- Odor emission from pig manure is highly influenced by excess sulfur-containing amino acids in the diet. Increasing sulfur-containing amino acids in the diet to 3 times the animal requirement significantly increases odor emission from pig manure. Increased sulfur-containing amino acids in the diet increases odor intensity and reduces odor hedonic tone of the odorous air above the manure.
- Dietary crude protein and fermentable carbohydrates have an interactive effect on odor

emission from pig manure. Odor emission is reduced when pigs are fed low crude protein and low fermentable carbohydrate diets or high crude protein and high fermentable carbohydrate diets.

- When using similar diets as in our studies, sulfur-containing compounds are the most important key odorous compounds, while phenolic and indolic compounds and volatile fatty acids seem less important.
- Ammonia emission from pig manure can be reduced significantly by (i) feeding animals with a lower level of dietary crude protein and at the same time supplementing most essential amino acids to balance the amino acid pattern, (ii) increasing the fermentable carbohydrate level in the diet. Effects of dietary crude protein and fermentable carbohydrate levels on ammonia emission prove to be additive.
- The correlation between ammonia emission and odor emission in dietary experiments under controlled environmental condition is low. Dietary approaches that can reduce ammonia emission from pig manure may not be effective to reduce odor emission and even may have an opposite effect.

SAMENVATTING

Geuremissie uit varkensstallen kan hinder veroorzaken voor de omgeving en moet daarom zoveel mogelijk worden beperkt. Geur wordt vooral veroorzaakt door microbiële omzetting van voercomponenten in de dikke darm van varkens en door microbiële omzetting van stoffen die via de urine en de feces worden uitgescheiden in de mengmest. Eiwitten en fermenteerbare koolhydraten zijn de belangrijkste precursors voor geurvorming. Geur is een complex mengsel van verschillende vluchtige componenten. Geurcomponenten kunnen in 4 groepen worden onderverdeeld: (1) zwavelhoudende componenten, (2) indolen en fenolen, (3) vluchtige vetzuren, en (4) ammoniak en vluchtige aminen. Geur wordt gekenmerkt door zijn sterkte (concentratie en intensiteit) en door de kwaliteit van de geur (aangenaam – onaangenaam, ofwel hedonische waarde). Er is al veel moeite gedaan om geurvorming en geuremissie tegen te gaan. In de varkenshouderij waren strategieën tot nu toe vooral gericht op het verwijderen van geurcomponenten uit de uitgestoten ventilatielucht. Deze maatregelen worden gekenmerkt door geurreductie nadat de geurcomponenten al zijn gevormd en zijn vrijgekomen uit de mengmest. Ze worden ook wel ‘end-of-pipe’ oplossingen genoemd.

Geuremissie kan ook worden gereduceerd door de voersamenstelling te wijzigen waardoor bepaalde geurcomponenten niet of minder worden gevormd. Dit lijkt een veelbelovende oplossingsrichting, echter over de mogelijkheden daarvan was tot nu toe weinig bekend. Effecten van eiwit en fermenteerbare koolhydraten op de geursterkte en geurkwaliteit, gemeten met de menselijke neus (olfactometrie), van lucht afkomstig uit de mestkelder zijn tot nu toe weinig onderzocht en de gevonden resultaten zijn vaak tegenstrijdig. Tevens zijn de effecten van ruw eiwit gehalte en gehalte aan fermenteerbare koolhydraten in het voer onafhankelijk van elkaar onderzocht, terwijl er waarschijnlijk een interactie bestaat tussen de effecten van deze twee factoren op de geurvorming. We verwachten een interactie, aangezien in de dikke darm van varkens en in de mestkelder de microben voor hun groei eiwit gebruiken als stikstofbron en fermenteerbare koolhydraten als energiebron.

Eiwit wordt voor een deel afgebroken in de dunne darm (tot aminozuren) en geabsorbeerd. Het deel dat niet in de dunne darm wordt afgebroken, ook wel fermenteerbaar eiwit genoemd, kan in de dikke darm door bacteriën worden omgezet en benut. Het kan ook worden omgezet tot geurcomponenten. Een verandering in het gehalte aan fermenteerbare koolhydraten zou invloed kunnen hebben op de productie van geurcomponenten in de dikke darm van de dieren en in de mengmest. Effecten van het gehalte aan fermenteerbare koolhydraten op geursterkte en –kwaliteit van varkensmest zijn nog niet onderzocht. Wanneer een overmaat aan aminozuren in de dunne darm wordt geabsorbeerd, kan dit ook tot vorming van precursors voor geurvorming leiden. Hierbij zijn sommige aminozuren waarschijnlijk belangrijker dan andere.

Zwavelhoudende componenten en de aromatische componenten van indolen en fenolen worden als de belangrijkste componenten gezien die geurhinder in de omgeving van varkensbedrijven veroorzaken. De aminozuren Tryptofaan (Trp), Phenylalanine (Phe) en Tyrosine (Tyr) zijn de belangrijkste substraten voor de vorming van indolen en fenolen. De zwavelhoudende aminozuren, Methionine (Met) en Cystine (Cys), zijn de belangrijkste substraten voor de vorming van zwavelhoudende geurcomponenten. Een verandering in concentratie van deze aminozuren in het voer zou de geurvorming in de mengmest belangrijk kunnen beïnvloeden. De effecten van deze aminozuren op de geursterkte en –kwaliteit zijn nog niet onderzocht.

De belangrijkste doelstelling van dit onderzoek was om het effect van verschillende voerfactoren op de sterkte en de kwaliteit van geur afkomstig van varkensmest te bepalen. Hierbij zijn vooral de processen in de dunne darm, in de dikke darm en in de mengmest van belang. De specifieke doelstelling was om de volgende hypothesen te testen:

1. Het ruw eiwitgehalte van het voer is een belangrijke factor voor de geurvorming in varkensmest.
2. Zwavelhoudende aminozuren en Trp, Phe en Tyr zijn belangrijke precursors voor vorming van geurcomponenten in varkensmest.
3. Schijnbaar ileaal niet verteerd eiwit (fermenteerbaar eiwit) dat gefermenteerd wordt in de dikke darm is een belangrijke bron voor geurvorming in varkensmest.
4. Het ruw eiwit- en fermenteerbaar koolhydraatgehalte van het voer hebben een interacterend effect op de geurvorming in varkensmest.

Ammoniakemissie veroorzaakt veel milieuproblemen. Geurbestrijding via voeraanpassingen is alleen interessant als de ammoniakemissie uit varkensmest niet toeneemt. Daarom is in deze studie ook het effect op de ammoniakemissie bepaald en is de relatie tussen de ammoniak- en de geuremissie vastgesteld.

Het onderzoek is gestart met een uitgebreide literatuurstudie (hoofdstuk 2) naar de huidige stand van kennis ten aanzien van de relatie tussen voeding en geur. Na deze studie zijn 4 experimenten uitgevoerd om de hypotheses 1 tot en met 4 te testen. Deze experimenten zijn beschreven in hoofdstukken 4 tot en met 7. Voordat de voedingsexperimenten werden gestart is een studie gedaan naar de effecten van omgevingsfactoren op de geuremissie uit varkensmest (hoofdstuk 3). Dit was een technische studie om de monstername- en meetstrategie voor het bepalen van de geuremissie uit varkensmest vast te stellen. Verder is in deze studie vastgesteld welke omgevingsfactoren een belangrijke invloed hebben op de geuremissie. Het is belangrijk om deze factoren te controleren tijdens de voerexperimenten en gelijk te houden voor de

verschillende behandelingen.

In hoofdstuk 4 is het effect van verschillende eiwitgehalten op de geuremissie uit varkensmest beschreven. Drie eiwitgehalten zijn onderzocht: 12, 15 en 18 %. In hoofdstuk 5 is het onderzoek gerapporteerd naar het effect van specifieke synthetische aminozuren in het voer op de geuremissie uit varkensmest. Deze studie is gedaan om het effect van geabsorbeerde aminozuren in de dunne darm op de geuremissie uit varkensmest te onderzoeken. De behandelingen waren: 1) voer met 15% ruw eiwit en met 3 maal de minimale behoefte aan zwavelhoudende aminozuren (14,2 g Met + Cys /kg voer); 2) voer met 15% ruw eiwit en met 2 maal de minimale behoefte aan Trp en Phe + Tyr (respectievelijk 2,9 en 20,4 g/kg voer); en 3) voer met 15% ruw eiwit en toevoeging van aminozuren tot de minimale behoefte. Hoofdstuk 6 rapporteert de effecten van verschillende niveaus van fermenteerbaar eiwit in het voer op de geuremissie uit varkensmest. Deze studie was opgezet om het effect van eiwitafbraak in de dikke darm op de geuremissie te onderzoeken. Drie gehalten aan fermenteerbaar eiwit in het voer werden onderzocht: 28, 38 en 48 g/kg. De drie hiervoor genoemde studies (hoofdstukken 4, 5 en 6) werden uitgevoerd bij vleesvarkens. In deze studies werd een opzet gekozen met een volledig gerandomiseerde blokkenproef met drie behandelingen in 6 blokken (n = 18). In hoofdstuk 7 wordt een studie beschreven naar de interactie tussen ruw eiwitgehalte en gehalte aan fermenteerbare koolhydraten in het voer op de geuremissie uit varkensmest. Het experiment werd uitgevoerd met vleesvarkens (n = 36) in een 2 x 3 factoriele, volledig gerandomiseerde blokkenproef met 6 behandelingscombinaties in 6 blokken. De factoren waren: eiwitgehalte op 2 niveaus (laag 12%; hoog 18%) en gehalte aan fermenteerbare koolhydraten op 3 niveaus: (laag 95,5; midden 145,5; hoog 195,5 g kg^{-1} voer).

De resultaten van het experiment beschreven in hoofdstuk 3 laten zien dat temperatuur, ventilatie debiet en, in mindere mate, de verdunning van de mest met water en het emitterend oppervlak de geuremissie, de ammoniakemissie en de mestsamenstelling beïnvloedden. Voornoemde factoren werden daarom gelijk gehouden voor de verschillende behandelingen beschreven in hoofdstukken 4, 5, 6 en 7, zodat voereffecten niet verstrengeld zouden worden met omgevingsfactoren. In de voerexperimenten werden feces en urine van ieder varken apart verzameld in kleine mestkelders onder de roostervloer. Na een adaptatieperiode van 2 weken werden de mestkelders schoongemaakt en werd de mest verzameld. In de 5^{e} week (voor de experimenten beschreven in hoofdstukken 4, 5 en 6) of in de 6^{e} week (voor het experiment beschreven in hoofdstuk 7) werden lucht- en mestmonsters genomen in de mestkelder. Van de luchtmonsters werden de volgende variabelen bepaald: geurconcentratie en –emissie, geurintensiteit, hedonische waarde van de geur, en ammoniakemissie. In de mestmonsters

werden de volgende variabelen bepaald: pH, totaalstikstof, ammoniumstikstof, indolen, fenolen, zwavelhoudende componenten en vluchtige vetzuren.

Resultaten van het experiment beschreven in hoofdstuk 4 laten zien dat een verlaging van het ruw eiwitgehalte in het voer van 18% naar 12% een verlaging gaf van de geuremissie (P = 0,04) en de ammoniakemissie (P = 0,01) uit de mengmest van respectievelijk 80% en 53%. Verlaging van het ruw eiwitgehalte in het voer verlaagde de concentraties in de mengmest van totaalstikstof, methyl sulfide, carbon disulfide, ethanethiol, fenol, 4-ethyl fenol, indol en 3-methyl indol (P < 0,05). De productieresultaten van de varkens (groei, voeropname) waren vergelijkbaar voor alle behandelingen. Verlaging van het eiwitgehalte, onder toevoeging van limiterende aminozuren, is daarom een goede strategie om zowel de geuremissie als de ammoniakemissie uit varkensmest te reduceren.

Resultaten van het experiment beschreven in hoofdstuk 5 laten zien dat het toevoegen van synthetische zwavelhoudende aminozuren (Met en Cys) op een niveau van driemaal de behoefte een sterke toename gaf van de geuremissie uit de mengmest (+723%; P < 0,001). Tevens gaf het een toename van de geurintensiteit (P < 0,05) en verlaagde het de hedonische waarde van de geur (onaangenamere geur) (P < 0,05). Toevoeging van de synthetische aminozuren Trp, Tyr en Phe boven de behoefte had geen effect op de geuremissie, geurintensiteit en hedonische waarde van de geur. De verschillende aminozuurtoevoegingen hadden geen effect op de ammoniakemissie uit de mengmest (P = 0,20). De correlatie tussen ammoniakemissie en geuremissie was laag (r = -0,3) en niet significant verschillend van nul. De aminozuurbehandelingen hadden geen effect op de productieresultaten van de varkens. Geconcludeerd kan worden dat de zwavelhoudende aminozuren in het voer moeten worden geminimaliseerd, juist genoeg voor de behoefte van het dier, om de geuremissie te reduceren. De resultaten laten zien dat de zwavelhoudende geurcomponenten sterk lijken bij te dragen aan de geurhinder veroorzaakt door varkensstallen.

Resultaten in hoofdstuk 6 laten zien dat fermenteerbare eiwitniveaus geen invloed hadden op de geuremissie, geurintensiteit, de hedonische waarde van de geur en op de ammoniakemissie uit varkensmest. Een hoger gehalte aan fermenteerbaar eiwit gaf hogere concentraties van totaalstikstof, methyl sulfide, carbon disulfide, ethanethiol, fenol, 3-methyl indol, en 4-ethyl fenol in de mengmest (P < 0,01). De correlatie tussen ammoniakemissie en geuremissie was laag (r = 0,14) en niet significant verschillend van nul. Fermenteerbaar eiwitniveau in het voer had geen effect op de productieresultaten van de varkens. De conclusie van dit hoofdstuk is dat in relatie tot geuremissie het gehalte aan fermenteerbaar eiwit moet

worden bekeken in combinatie met het gehalte aan totaal ruw eiwit en het gehalte aan verteerbaar eiwit in de dunne darm.

Resultaten in hoofdstuk 7 laten zien dat het eiwitgehalte van het voer en het gehalte aan fermenteerbare koolhydraten een interactief effect hadden op de geuremissie uit de mengmest ($P = 0,06$). Bij een hoog ruw eiwitgehalte in het voer gaf een hoger gehalte aan fermenteerbare koolhydraten een verlaging van de geuremissie, terwijl bij een laag ruw eiwitgehalte een verhoging van het gehalte aan fermenteerbare koolhydraten een verhoging gaf van de geuremissie. Totaalstikstof en ammoniumstikstof concentraties in de mengmest en de ammoniakemissie uit de mengmest waren lager bij 12% dan bij 18% ruw eiwit in het voer ($P < 0,001$). Een hoger gehalte aan fermenteerbare koolhydraten gaf een lagere ammoniakemissie uit de mengmest ($P = 0,01$). De pH van de mengmest was hoger bij 18% dan bij 12% ruw eiwit in het voer ($P < 0,001$) en werd lager bij een toename van het gehalte aan fermenteerbare koolhydraten ($P = 0,03$). De totale concentratie aan vluchtige vetzuren was hoger bij 18% dan bij 12% ruw eiwit in het voer ($P < 0,001$) en nam tevens toe bij een toename van het gehalte aan fermenteerbare koolhydraten ($P = 0,001$). Het hoge eiwitgehalte gaf tevens hogere gehalten in de mengmest van fenol ($P < 0,001$), cresolen ($P = 0,01$), indol ($P < 0,001$), 3-methyl indol ($P = 0,08$), 4-ethyl fenol ($P < 0,001$) en carbon disulfide ($P < 0,001$). Het gehalte aan fermenteerbare koolhydraten had geen effect op de concentratie van deze componenten in de mengmest ($P > 0,05$). De correlatie tussen ammoniakemissie en geuremissie was laag ($r = -0,1$) en niet significant verschillend van nul. De verschillende behandelingen hadden geen significante invloed op de productieresultaten. Uit dit onderzoek kan worden geconcludeerd dat de interactie tussen ruw eiwitgehalte van het voer en het gehalte aan fermenteerbare koolhydraten een rol spelen in de geurvorming en –emissie. Verder kan worden geconcludeerd dat de ammoniakemissie uit varkensmest sterk verlaagd kan worden door het ruw eiwitgehalte van het voer te verlagen en door het gehalte aan fermenteerbare koolhydraten te verhogen.

In het kort kan worden geconcludeerd dat eiwitten / aminozuren de belangrijkste precursors zijn voor vorming van geurcomponenten. Deze precursors in de mengmest zijn zowel afkomstig van de urine als van de feces. Een overmaat aan geabsorbeerde aminozuren in de dunne darm veroorzaakt een hoge productie van precursors voor geurcomponenten die via de urine worden uitgescheiden. Eiwitten die in de dikke darm terecht komen kunnen door bacteriën worden gefermenteerd. Dit resulteert in precursors voor geurcomponenten die zowel via de urine als via de feces uitgescheiden kunnen worden. Een geurarm voer, een voer dat een lage geuremissie geeft uit de mengmest, zal dus zowel de excretie van precursors voor geurcomponenten via de urine als via de feces moeten reduceren. Om de uitscheiding van deze

precursors in de mengmest te verminderen zal het volgende moeten worden gedaan: (i) minimaliseren van de overmaat aan geabsorbeerde aminozuren in de dunne darm, vooral van de zwavelhoudende aminozuren (Met en Cys); (ii) minimaliseren van de hoeveelheid eiwit dat naar de dikke darm gaat; (iii) vastleggen van het eiwit dat in de dikke darm komt in biomassa, zodat het niet wordt gefermenteerd. Een overmaat aan synthetische zwavelhoudende aminozuren resulteerde in een zeer sterke, onaangename geur uit de mengmest van varkens. Daarom kan een verlaging van het ruw eiwitgehalte van het voer en een minimale hoeveelheid toegevoegde zwavelhoudende aminozuren leiden tot een reductie van de geuremissie uit varkensmest. Sommige grondstoffen voor varkensvoeders zijn rijk aan zwavel, zoals bloedmeel, sojameel en melasse. Om de geuremissie uit varkensmest te verminderen zullen deze componenten in een juiste (minimale) hoeveelheid moeten worden toegevoegd aan het mengvoer. Geuremissie uit mengmest van varkens die een hoog gehalte aan ruw eiwit in het voer hebben kan worden gereduceerd door meer fermenteerbare koolhydraten toe te voegen aan het voer.

De algemene conclusie van dit onderzoek is dat er goede mogelijkheden zijn om via voermaatregelen de geuremissie uit varkensmest te reduceren. Een integrale benadering is daarbij gewenst, aangezien sommige belangrijke factoren die van invloed zijn op de geuremissie, zoals eiwitgehalte en gehalte aan fermenteerbare koolhydraten, elkaar beïnvloeden. De specifieke conclusies van dit onderzoek zijn:

- Verlaging van het eiwitgehalte in het voer, onder toevoeging van limiterende aminozuren, kan een belangrijke verlaging van de geuremissie uit varkensmest bewerkstelligen en de hedonische waarde van de geur doen toenemen (minder onaangename geur).
- De geuremissie van varkensmest wordt sterk beïnvloed door een overmaat aan zwavelhoudende aminozuren in het voer. Verhoging van zwavelhoudende aminozuren in het voer tot driemaal de behoefte verhoogt de geuremissie en de geurintensiteit significant. Daarnaast geeft deze verhoging een onaangenamere geur.
- Eiwitgehalte en gehalte aan fermenteerbare koolhydraten hebben een interactief effect op de geuremissie uit varkensmest. De geuremissie is laag wanneer de varkens een voer krijgen met een laag ruw eiwitgehalte en een laag gehalte aan fermenteerbare koolhydraten of een voer krijgen met een hoog ruw eiwitgehalte en een hoog gehalte aan fermenteerbare koolhydraten.
- Bij gebruik van vergelijkbare voeders als in dit onderzoek zijn zwavelhoudende

componenten de belangrijkste sleutelcomponenten die de geuremissie bepalen, terwijl fenolen, indolen en vluchtige vetzuren minder belangrijk lijken te zijn.

- Ammoniakemissie van varkensmest kan significant worden gereduceerd door: (i) een verlaging van het ruw eiwitgehalte van het voer, onder toevoeging van limiterende aminozuren; (ii) een verhoging van het gehalte aan fermenteerbare koolhydraten in het voer. De effecten van ruw eiwitgehalte en gehalte aan fermenteerbare koolhydraten op de ammoniakemissie blijken onafhankelijk van elkaar te zijn en dus optelbaar.
- De correlatie tussen ammoniakemissie en geuremissie blijkt in deze voerproeven, onder gecontroleerde omstandigheden, laag te zijn. Voermaatregelen die de ammoniakemissie verlagen, verlagen daarom niet per definitie ook de geuremissie uit varkensmest. Effecten kunnen zelfs tegengesteld zijn.

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PEER-REVIEWED PAPERS

Le DP, Aarnink AJA, Jongbloed AW, van de Peet-Schwering CMC, Verstegen MWA., & Ogink NWM (2006a) Interactive effects of dietary crude protein and fermentable carbohydrate levels on odor from pig manure. *Journal of Animal Science* Submitted.

Le PD, Aarnink AJA, Jongbloed AW, van de Peet-Schwering CMC, Verstegen MWA & Ogink NWM (2006c) Effects of amino acid supplementation to the diet on odor from pig manure. *Journal of Animal Science* Submitted.

Le PD, Aarnink AJA, Jongbloed AW, van de Peet-Schwering CMC, Verstegen MWA & Ogink NWM (2006d) Effects of dietary crude protein level on odor from pig manure. *Journal of Animal Science* Submitted.

Le PD, Aarnink AJA, Jongbloed AW, van de Peet-Schwering CMC, Verstegen MWA & Ogink NWM (2006e) Effects of fermentable protein levels in the diet on odor from pig manure. *Journal Animal Science* Submitted.

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Le PD, Aarnink AJA, Ogink NWM & Verstegen MWA (2005d) Effects of Environmental Factors on Odor Emission from Pig Manure. *Transactions of the ASAE* **48**, 757-765.

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Cuốn luận văn này xin tặng Em và Con. Em và Con là nguồn động viên to lớn giúp anh hoàn thành công việc học tập của mình. Em và Con là điểm tựa tinh thần cho anh trong những lúc khó khăn. Cám ơn tình yêu của Em và Con đã dành cho anh.

Wageningen, October 2006

Le Dinh Phung

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Le Dinh Phung was born on 28th July 1974 in Thach Ban village, Le Thuy district, Quang Binh province, Vietnam. He started his study of Animal Sciences at Hué University of Agriculture and Forestry (HUAF) in 1992. He received his Bachelor degree in Animal Sciences with distinction at HUAF in 1997. In the same year, he started working as a researcher at the Center for Rural Development in Central Vietnam, HUAF. Also in the same year, he got a free admission to do a M.Sc study on Animal Sciences at HUAF where he received his M.Sc diploma in Animal Sciences with distinction in 1999.

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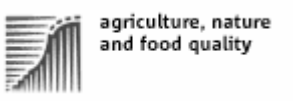
Training and Supervision Plan		**Graduate School WIAS**
Name	Le Dinh Phung	
Group	Animal Production Division & Animal Nutrition	
Daily supervisor(s)	Dr. Ir. André Aarnink & Dr. Ir. Nico Ogink	
Supervisor(s)	Prof. Dr. Ir. Martin Verstegen	
Project term	from March 2003 until April 2007	
Submitted	August 2006 first plan / midterm / **certificate**	

EDUCATION AND TRAINING (minimum 30, maximum 60 credits)	year	credits*
The Basic Package		
WIAS Introduction Course (mandatory)	2003	
Course on philosophy of science and/or ethics (mandatory)	2004	
Subtotal Basic Package		**3**
Scientific Exposure (conferences, seminars and presentations, minimum 8 credits)	year	
International conferences (minimum 3 credits)		
56th annual meeting of the EAAP, June 5-8, Uppsala, Sweden	2005	
European conference on environmental odor management, Cologne 17-19 November, Germany	2004	
ASAS-ADSA annual meeting, Minneapolis, USA, 8-14th July 2006	2006	
Seminars and workshops		
WIAS science day 2003, 2004, and 2005, Wageningen	2003-05	
WIAS seminar on Livestock genetics research at ILRI: Where we are and where we are going, 1 March 2004, Wageningen	2004	
Dietary protein:Physiological constraints to nutritive value + Seminar^{+}, 6 - 7 October 2004, Wageningen	2004	
PhD retreat 13-14 May 2004, Nijmegen	2004	
WIAS symposium on Choices for the Future, 25 February 2005, Wageningen	2005	
WIAS seminar Methane emissions in cattle: identification and evaluation of nutritional mitigation options, June 24th, Wageningen	2005	
WIAS seminar Approaches to sustainable development of animal production, 3rd October, Wageningen	2005	
WIAS farewell Prof.Dr. Verstegen, 30 March 2006	2006	
WIAS seminar on Animal Production Systems in Indonesia: Interaction of Livestock and Livelihoods	2006	
Presentations *(minimum 4 original presentations of which at least 1 oral, 1 credit each)*		
European conference on environmental odor management, Cologne Germany 17-19 November 2004 (Oral)	2004	
56th annual meeting of the EAAP, June 5-8, Uppsala, Sweden (Poster)	2005	
WIAS symposium "Choices for the Future", 25 February 2005, Wageningen (Oral)	2005	
ASAS-ADSA annual meeting, Minneapolis, USA, 8-14th July 2006 (Oral)	2006	
Subtotal International Exposure		**12**
In-Depth Studies (minimum 6 credits, of which minimum 4 at PhD level)	year	
Disciplinary and interdisciplinary courses		
Biology underpinning animal sciences: broaden your horizon, November 2003, Wageningen	2003	
Ecophysiology of the GI-tract, 28 February to 3rd March 2005, Wageningen	2005	
Advanced statistics courses		
Nonparametric statistics, Utrecht university, 27th May 2004, Utrecht	2004	
Factor analysis, Utrecht university, 5th November 2004, Utrecht	2004	
Basic & advanced statistical course, 9 days, June 2005, Production Ecology & Resource conservation, Wag	2005	
Design of animal experiment, 21-23 September 2005, WIAS course, Wageningen	2005	
Multivariate analysis, 19-27 April 2006, Production Ecology & Resource conservation, Wageningen	2006	
Longitudinal Data and Repeated Measurements, Utrecht University, 19-20 October 2006, Utrecht	2006	
Subtotal In-Depth Studies		**9**

Professional Skills Support Courses (minimum 3 credits)	year	
Course Techniques for Scientific Writing, 1-4 July 2003, WIAS course, Wageningen	2003	
Time planning and project management, March 2004, Wageningen	2004	
PhD workshop Scientific Publishing, 12 October 2004, Wageningen	2004	
Subtotal Professional Skills Support Courses		**3**
Research Skills Training (optional)	year	
Preparing own PhD research proposal	2003	
Subtotal Research Skills Training		**6**
Education and Training Total (minimum 30, maximum 60 credits)		**33**

* one ECTS credit equals a study load of approximately 28 hours



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