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Biochemical Identification and Biological Origin of Key Odor Components in Livestock Waste[1]

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ABSTRACT: Animal production results in conversion of feeds into valuable products such as meat, milk, eggs, and wool as well as into unavoidable and less desirable waste products. Intensification of animal numbers and increasing urbanization has resulted in considerable attention to odorous gases produced from animal wastes. It is clear that animal manure was, and still is, a valuable resource. However, it may be a major obstacle to future development of the animal industry if its impact on the environment is not properly controlled. Poor odor prevention and control from animal wastes is related to a lack of knowledge of the fundamental nature of odor and its production by farm animals. Odor, like noise, is a nuisance or disturbance and there is no universally accepted definition of an objectionable odor. Thus, regulation and control of odors in the environment is difficult because of the technical difficulties of defining odor limits and their measurement and evaluation. A variety of direct (sensory) and indirect (analytical instruments) methods for measuring odor intensity and determination of individual or key odor components are discussed. The biological origins of the four principal classes of odor compounds, namely branched- and straight-chain VFA, ammonia and volatile amines, indoles and phenols, and the volatile sulfur-containing compounds, are reviewed. Because more than 50% of N from animals is excreted as urea, one strategy to conserve N in waste is to inhibit the urease enzyme that converts urea to ammonia. Laboratory studies to evaluate di- and triamide compounds to control urea hydrolysis in slurries of cattle and swine wastes are presented. Finally, a brief overview of various intervention strategies is provided. Multiple combinations of nutritional management, housing systems, treatment options as well as storage and disposal of animal wastes will be required to reduce environmental pollution and provide for long-term sustainable growth.

Key Words: Animal Wastes, Anaerobic Conditions, Odors, Organic Compounds, Volatile Fatty Acids

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Introduction

Major changes have taken place in livestock production techniques over the past three decades. The use of chemical fertilizers on a large scale has resulted in a situation in which crop and animal production are no longer integrated and interdependent (Morse, 1995). Animal manure is no longer in demand for its fertilizer value. Also, transport of feed to animal production units is no limitation, resulting in developing of animal enterprises close to population centers and major markets on more expensive land. Development of intensive, confined housing and feeding practices followed, resulting in the generation of large amounts of liquid, rather than solid, manures, and associated odors. Thus, whereas the term manure was used in the past to describe excreta that was predominantly used as fertilizer and soil conditioner, manure is now considered a pollutant and a nuisance (Williams, 1995). Environmental pollution from animal manure is a global concern and is much more acute and serious in countries with high concentrations of animals on a limited land base for manure disposal.

The volume of manure generated today may be a major obstacle to future development of the animal industry if the impact on the environment is not properly managed and controlled. Within the United States, legislation is under consideration that most likely will restrict agricultural practices and penalize farmers for exceeding limits of waste disposal in an

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effort to control agricultural pollution. Poor odor prevention and control from animal wastes is related to a lack of knowledge of the fundamental nature of odor and its production by domestic animals. This review describes the current understanding of the biochemical identification and biological origin of key odor components in livestock wastes and briefly considers ways of reducing or controlling them.

Discussion

General Concepts

Waste Problems. Animal production results in conversion of feeds into valuable products such as meat, milk, eggs, and wool as well as into unavoidable and less desirable waste products. Animal wastes (feces, urine, and respiration and fermentation gases) are excreted in solid, liquid, and gaseous forms. Respiration and fermentation gases are lost to the environment soon after being produced by the animal. After excretion, solid and liquid animal waste is subjected to (mainly anaerobic) microbial conversions, which convert organic substrates into microbial biomass and soluble and gaseous end points. Intensification of animal numbers and increasing urbanization have resulted in considerable attention to odorous gases produced from animal wastes. In recent years, attention has also shifted to their impact on the environment and their effects on water quality, soil deterioration, air pollution, and the rural-urban interface (Morse, 1995; Williams, 1995).

Land application of excessive quantities of nutrients is subject to surface run-off and leaching that may contaminate ground or surface waters. Nitrate leaching is considered a major nitrogen (N) pollution concern on livestock farms. Ammonia toxicity to fish and altered effectiveness of chlorination are additional concerns related to N pollution. Phosphorus (P) entering surface waters from land application of animal manure can stimulate growth of algae and aquatic plants. Their subsequent decomposition results in an increased oxygen demand that interferes with the welfare of fish and wildlife. Excessive contributions of some minerals from animal manure can create high salt concentrations in the soil. Manure degradation can be a major source of methane (CH_4) and nitrogen oxides (NO_x), which contribute to accumulation of greenhouse gases. Volatilization of ammonia (NH_3) causes acid rain, which contributes to forest die-back in western Europe where animal sources are responsible for 50% of the acid precipitation (Lowe, 1995; Likens et al., 1996). Emissions of nitrous oxide (N_2O) during the nitrification-denitrification cycle can contribute to ozone depletion (Schulte, 1997). Manure can also be a source of odors that contribute to friction between urban and rural residents (Lowe, 1995).

Defining Odor. Odor is something that stimulates the olfactory system or sense of smell. Each compound has a characteristic smell, and humans are known to be able to detect over 10,000 odors despite being able to name only a few of them. Despite this acute sense of smell, it is often difficult to describe the smell of a compound, and each individual differs in his or her perception of odor. Odorants are of many types, but those of relevance to the current topic are foul-smelling, malodorous compounds. The human nose can detect and discriminate odors at concentrations even lower than those detectable by gas chromatography (e.g., hydrogen sulfide, H_2S). The maximum concentrations required for the detection of odors are termed odor threshold values (**OTV**). Generally, the lowest toxic values (**LTV**) of these compounds in air are at least a factor of 500 higher than OTV, and these odors are detected long before their concentration becomes a health risk (Table 1; Tamminga, 1992). It is worth noting that a mixture of odorants may smell different from the unmixed compounds and that, in general, pleasantness decreases as intensity increases. Many factors influence olfactory sensitivity (e.g., age, gender, and occupation), but none so much as individual variability. Thus, there is no universally accepted definition of an objectionable odor, nor are there any legally defined conditions under which livestock producers become legally required to reduce odor emissions emanating from the animal facility (NPPC, 1995). Also, there are no federal guidelines that regulate and control odors in the environment because of the technical difficulties of defining odor limits and their measurement and evaluation. Odor, like noise, is a nuisance or disturbance normally handled by state or local authorities, often in response to complaints. This is in contrast to pollution, for which legal limits can be set and enforced, often by federal and state agencies. Therefore, it is important to distinguish between odor and pollution because certain compounds in feces and urine make little contribution to odor but can contribute greatly to environmental pollution (e.g., N and P). Finally, high levels of odor compounds have been reported to reduce growth performance and increase susceptibility to disease (e.g., pigs in confinement housing; Tamminga, 1992). This also has an impact on the health of associated workers. Importantly, odor can elicit a wide range of physiological responses that range from irritation of the eyes, nose, and throat, to nausea, headache, and vomiting, to disturbance, annoyance, and depression (NRC, 1979). The psychological and sensory effects of livestock odor are described in detail by Schiffman (1998) in a related article from this symposium.

Odor Detection and Quantification. Odor nuisance is generally defined by the FIDO factors: Frequency, intensity, duration, and offensiveness, where frequency refers to the number of times an odor occurs, intensity refers to the strength of an odor, duration

Table 1. Odor threshold values (OTV) and lowest toxic values (LTV) of noxious compounds found in confinement houses[a]

Compound	OTV, ppb	LTV, ppb
Ammonia	4,700	25,000
Acetic acid	1,000	10,000
Phenol	5	5,000
Methyl mercaptan	2	500
Butyric acid	1	—
p-Cresol	1	5,000
Ethyl mercaptan	1	500
Dimethyl sulfide	1	1,000
Hydrogen sulfide	.5	10,000

[a]Source: Adapted from Tamminga (1992).

refers to the period of time an odor is encountered, and offensiveness refers to the unpleasantness or character of the odor (O'Neill and Phillips, 1992; NPPC, 1995; Schulte, 1997). Odor intensity has received the most attention in quantification of odor nuisance problems for regulatory purposes and also for research. Because odor intensity is generally considered the primary variable in determining odor, a variety of direct and indirect methods have been developed for measuring odor intensity. Direct (or sensory) methods involve the use of the human nose as a detector, generally in the form of a panel of trained observers, and indirect methods measure the concentration of volatile odorants or particles in air emissions and correlate these measurements to direct observations.

Direct methods, also referred to as sensory or olfactometric methods, can be divided into two major categories: scaling and dilution. Scaling involves rating the odor intensity on an arbitrary scale (Table 2) or referencing the odor intensity to the intensity of a known substance. Dilution methods are more objective and involve diluting the sample with a stream of odor-free air in order to determine odor threshold or detectability. Inclusion of a reference sample with an assigned magnitude is recommended to help compare values from different panelists. The advantage of scaling methods is that they are simple, easy to use, and do not need elaborate equipment. They do, however, have a number of disadvantages that have been outlined by Dravnieks (1979).

The referencing approach is useful for the scaling and dilution methods. With this approach, panelists compare the intensity of odor with the intensity of a series of different concentrations of a reference odorant and determine whether the given concentration of reference odorant is less than, greater than, or equal to the intensity of an odorous sample. The most commonly used reference odorant is *n*-butanol (C_4H_9OH), which is readily available in high purity, relatively nontoxic, stable, and has a reasonably agreeable odor that is unrelated to most other odors of interest.

All dilution methods are fairly similar in that they involve presenting the panelists with a range of dilutions of the odorous sample in liquid or vapor form, allowing a detection threshold to be determined. Detection threshold refers to the lowest concentration at which odor can be positively detected. The recognition threshold is often 1.5 to 10 times higher than the detection threshold. Results are expressed as a dimensionless ratio representing the volume of odorous and odor-free liquid or vapor to the volume of odorous liquid or vapor at the dilution representing the detection threshold. Liquid dilution has primarily been used in the assessment of odors in water and wastewater treatment effluents. Of more relevance to the evaluation of ambient odors emanating from livestock wastes are the use of the scentometer and dynamic olfactometers. The scentometer is a small, hand-held device designed specifically for field evaluation of ambient odors. This device consists of a rectangular, clear plastic box containing two chambers of activated charcoal (to produce odor-free air), two nasal ports (for detection by the nose), and a progressive series of holes from small to large (for dilution of the odorous air). The orifice size of the uncovered odorous air inlets determines the amount of odorous air in the chamber and expression of the dilution of odor-free air required to dilute the odorous sample to its threshold concentration.

Dilution olfactomers are probably the most common type of instrument in use for measuring odor intensity for research purposes. Dynamic olfactometers dilute odorous samples by mixing a stream of odorous air with odor-free air at known, measured flow rates. Some factors to consider in sensory odor measurements using a dynamic dilution olfactometer include the method of presentation (forced-choice method preferred), number and order of dilution steps, dilution rate and factor between dilutions, flow rates

Table 2. Non-objective (scaling) measurements of odor intensity and offensiveness[a]

Odor intensity	Strength of smell
0	No odor
1	Very faint
2	Faint
3	Distinct
4	Strong
5	Very strong
6	Extremely strong
Odor offensiveness	**Quality of smell**
0	Inoffensive
1	Slightly offensive
2	Fairly offensive
3	Definitely offensive
4	Strongly offensive
5	Very strongly offensive

[a]Adapted from Sneath (1994).

from sampling ports, source of dilution air, calibration gas, number of panelists, interstimulus times, minimum number of measurements per sample, sample bag material, and retention time of static samples (NPPC, 1995). However, there are a number of limitations to using sensory methods for measuring odor intensity (Kreis, 1978). These include rapid saturation of olfactory senses by some odorants, individual variation in sensitivity to different odor, fatigue as a result of adaptation, and changes in climatic variables (temperature, humidity, and wind speed) when measuring odors under field conditions, as well as effects of age, gender, health and personal habits on the sense of smell of individual panelists.

Biochemical methods that separate, identify, and quantify are therefore in demand. Techniques for determining the concentration of individual or key groups of odor components using instrumental or indirect methods have been developed and offer advantages of automated sampling and measurement. However, it is important to match chemical concentration with olfactometric measurement. Gas chromatography is the technique most commonly applied to separate and identify volatile and gaseous samples. Separation of biochemical compounds is achieved by injecting them onto specific columns that partition these compounds according to vapor pressure and solubility. Identification of the biochemical components is achieved using nonspecific detectors such as thermal conductivity flame ionization or electron capture with reference to a standard mixture of known components. In addition, peak areas and heights can be used to quantify the concentration of each component. The use of specific detectors, such as mass spectrometry, greatly improves the certainty with which compounds may be identified based on their ionized molecular fragment patterns (Zahn et al., 1997). This technique is sensitive and enables odorous compounds to be profiled or "fingerprinted." Gas chromatography-mass spectrometry is relatively expensive and is more commonly used for research and discovery rather than for routine monitoring, although some of the modern quadrupole instruments are more amenable to routine monitoring. Measuring changes in concentrations of specific odor components associated with odor emissions is critical in monitoring the effectiveness of different treatments for odor control. However, it is also important to correlate concentration of key odor components and their profiles with subject measurements of odor evaluation. A major difficulty in correlating concentration of key odor components with odor intensity is that mixtures of odorous compounds may be additive, subtractive, synergistic, or counteractive, which needs to be taken into account.

Suitable instruments that incorporate sensitivity and identification, together with a rapid, portable means of measuring odor concentration in air, are actively being developed for portable measurements in the field for abatement and control methods. The photo ionization detector (**PID**), a hand-held device, responds to compounds with a photo ionization potential equal to or less than that of the energy source (lamp). An energy of 10.2 eV from the lamp will exclude direct detection of major compounds in air such as water vapor, CH_4, and CO_2 but will detect volatile organic compounds as well as NH_3 and H_2S. Although sensitivity is low compared to olfactometry methods, PID has potential for measuring odor concentration from animal wastes and emissions. There has been a great deal of recent research into the development of electronic nose (**EN**) systems for odor detection and measurement. An EN is defined as an instrument that consists of an array of electronic chemical sensors with partial specificity and an appropriate pattern-recognition system capable of recognizing simple and complex odors. When presented with an odor, the EN would initially classify the odor type, based on pattern recognition of normalized response patterns. Then, using programmed knowledge about the relationship between sensor response and odor concentration for that odor type, the EN would give an integrated response or value for odor concentration. However, further work is required on sensors, response patterns and magnitudes and their relationship to odor concentration and type before this is a realistic possibility for fast, on-site measurement of odor type and concentration. Sensors have been developed for a number of applications, mostly in the food industry, such as grading coffee blends and beans, detection of off-flavors in lagers, and freshness in meat. Thus, a variety of indirect methods have been developed for odor measurement and evaluation, but so far none of these methods has received general acceptance over sensory methods using the human nose.

Sources of Odor Associated with Animal Production

Odor has long been associated with animal production. In general, feed and body odors are not regarded as offensive, but those generated from manure and its decomposition during collection, handling, storage, and spreading are considered offensive. Manure is a complex mixture of undigested dietary residues, endogenous secretions, and bacterial cells and their metabolic end points. Manure is subject to anaerobic degradation under a variety of moisture and temperature conditions, resulting in the generation of odorous volatile compounds. When manure undergoing degradation has a surface exposed to the atmosphere, volatile products and intermediates are emitted into the environment. This is the source of most odorous compounds that can absorb to particulate matter (dust), building surfaces, and clothing. Over 168 chemical compounds have been identified in the air of

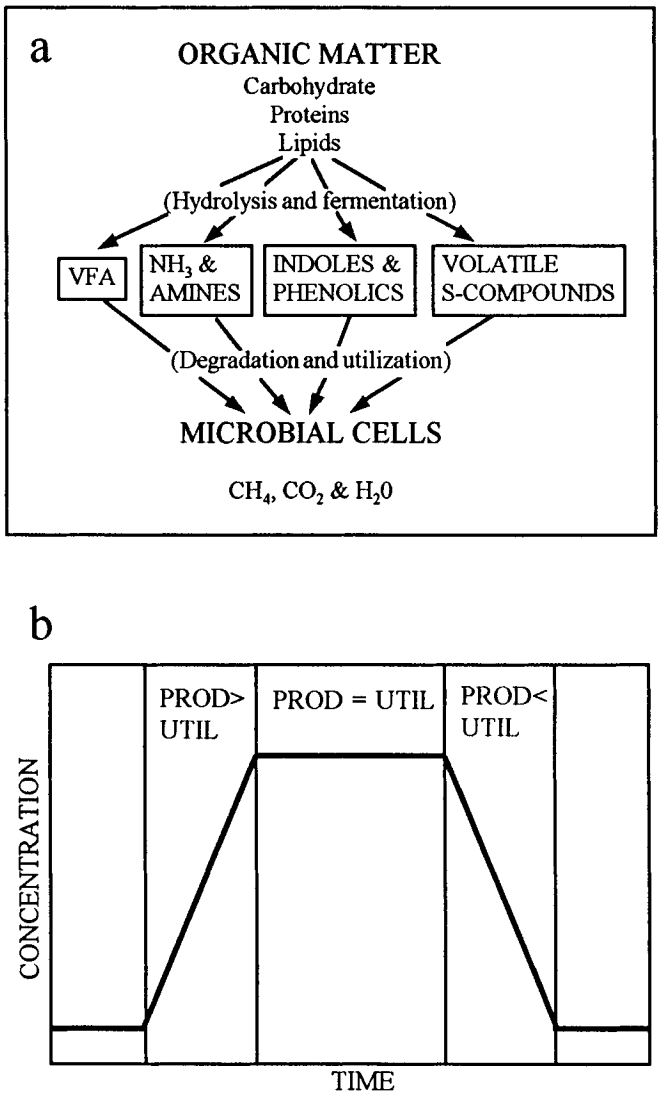

Figure 1. (a) Production and utilization of odor compounds under anaerobic conditions. (b) Odor concentration is dependent on the relative rates of production and utilization.

swine confinement buildings. These compounds are not only responsible for unpleasant odors but also affect the comfort, health, and production efficiency of animals as well as the comfort and health of human workers (Tamminga, 1992). Some principal odorous compounds are ammonia, amines, sulfur-containing compounds, volatile fatty acids, indoles, skatole, phenols, alcohols, and carbonyls (Curtis, 1993). The biological production and utilization of key odor components are summarized in the following section (Figure 1).

Volatile Fatty Acids. Anaerobic microbial fermentation contributes substantially to digestion and metabolism in farm animals, particularly the foregut of ruminants (cattle and sheep) and to a lesser extent the hindgut of nonruminants (pigs and poultry). Organic matter in feed, particularly structural carbohydrates, is fermented to VFA, mainly acetic (C_2), propionic (C_3), and butyric (C_4) acids and smaller amounts of valeric (C_5), hexanoic (C_6; caproic), and heptanoic (C_7; capric) acids (Figure 2). Microbial biomass, fermentation gases (CO_2 and CH_4), and some heat are also generated during anaerobic digestion. This process requires the presence of an electron sink, which is provided by CO_2 reduction, resulting in CH_4 formation:

$$CO_2 + 8H^+ \rightarrow CH_4 + 2H_2O.$$

Volatile fatty acids are absorbed into the blood, transported to organs and tissues, and subsequently metabolized as a source of energy, and CO_2 and CH_4 are removed from the rumen by respiration and eructation. Loss of CH_4 is largely determined by the fermentation pattern, and between 5 to 10% of the energy in feed is lost in this manner (Bryant, 1979). Although CH_4 is odorless, it contributes significantly to the greenhouse effect (Schulte, 1997). Worldwide CH_4 production is estimated at 400×10^6 t; animals are held responsible for 15 to 25% of this amount, 90% of this originating from ruminants (cattle, sheep, goats, and buffaloes). Within the United States, estimates indicate that CH_4 emissions from animal waste are responsible for 15% of the total, or almost 1% of worldwide, methane production from human activities. Livestock emissions in the United States have been primarily attributed to swine (50%) and dairy (30%) operations (USEPA, 1992).

During complete anaerobic degradation, organic matter is hydrolyzed and fermented, and approximately 90% of the energy available in the substrate is retained in an easily purified gaseous product, CH_4. The process involves many different kinds of interacting microbial species, most of which do not directly produce CH_4. Methane fermentation occurs in many anaerobic ecosystems such as sewage and organic waste digestors, aquatic sediments and marshes, flooded soils, and manure systems where the main electron acceptor, CO_2, is produced from the degraded organic substrates. It does not occur in environments where other electron acceptors such as oxygen, nitrate, or sulfate (sulfur) are readily available and outcompete CO_2 as an acceptor for reducing equivalents. Bryant was the first to propose a three-stage scheme for complete anaerobic degradation of organic matter and proceeded to isolate and characterize an intermediate group of fatty acid oxidizing bacteria (Bryant, 1979; McInerney and Bryant, 1981). The first stage, or acid-forming stage, involves a complex of species that hydrolyze the primary substrate polymers such as polysaccharides, proteins, and lipids and ferment them, producing fatty and other organic acids, alcohol, NH_3, S^-, CO_2, and H_2. Propionate and longer-chain fatty acids, some organic acids, and alcohols are subsequently degraded by a second intermediate group of bacteria called the obligate H_2-producing acetogenic bacteria. Finally, methanogens rapidly utilize the H_2 produced by other bacteria to reduce

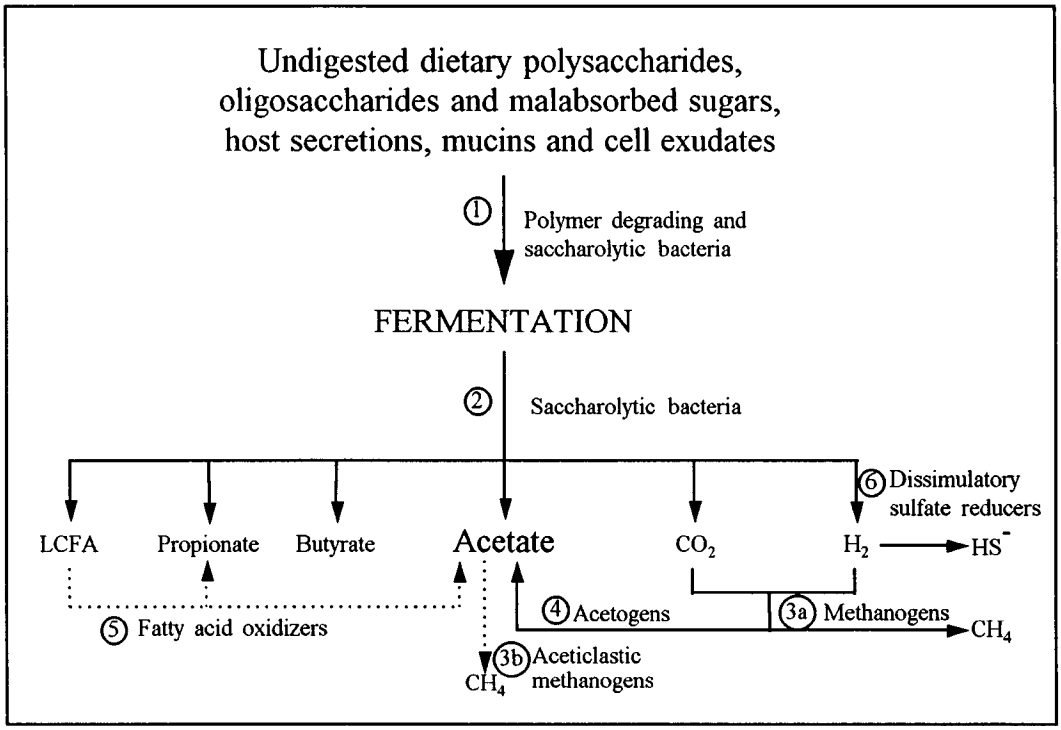

Figure 2. Simplified scheme showing carbohydrate fermentation and VFA formation in the intestinal tract. Processes involved in the complete anaerobic degradation of organic matter in stored wastes are marked with broken arrows (.....). The major functional groups of bacteria that participate in the sequential degradation, fermentation, and utilization of intermediates are numbered 1 to 6 (LCFA = long-chain fatty acids). Modified from Hudson and Marsh (1995).

CO_2 to CH_4, and the aceticlastic methanogens cleave acetate to CH_4 and CO_2. Acetate decarboxylation is a quantitatively important reaction because about 70% of CH_4 is derived from the methyl group of acetate in the above environments. Due to the short retention time of organic matter in certain gut compartments (rumen, cecum, and colon) only fermentative bacteria and H_2-utilizing methanogens grow fast enough to be maintained in the system. Thus, only a partial methane fermentation occurs, and acetate and longer-chained fatty acids accumulate and are absorbed from the intestinal tract where they contribute to the metabolizable energy requirements of the host. Efficiency of methane fermentation during complete anaerobic digestion is related to two important operational factors: the hydraulic retention time and the volumetric organic matter loading rate. The rate-limiting step in most fermentations is degradation of fatty acids.

Deamination and Decarboxylation of Amino Acids. Unlike carbohydrate availability in the hindgut of farm animals and in fresh waste, there is no shortage of organic N-containing compounds available for fermentation by the microbiota. In the gastrointestinal tract and manure a neutral pH (6 to 7) normally prevails. Under these conditions, deamination is the major pathway for metabolism of amino acids. Deamination results in the production of VFA, CO_2 plus H_2, as well as NH_3 (Figure 3 and Table 3). The general mechanism for oxidative deamination is as follows:

$$\begin{array}{l} \quad NH_2 \\ \quad | \\ R-CH-COOH + H_2O \rightarrow R-COOH + NH_3 + 4H + CO_2. \end{array}$$

However, in the gastrointestinal tract nonoxidative, ammonia lyase reactions are more common. Stickland-type reactions are also involved in amino acid catabolism to a lesser extent. Bacterial genera involved in this deamination activity include the following: *Bacteroides, Prevotella, Selenomonas, Butyrivibrio, Lachnospira, Eubacterium, Fusobacterium, Clostridium, Peptostreptococcus,* and *Acidaminococcus.*

Under certain conditions in the gastrointestinal tract and most likely during storage of fresh waste, slurries can undergo decarboxylation (Table 4). Amino acid decarboxylases are induced at pH 5 to 6 and are most likely involved in intracellular pH regulation by bacterial cells. The general reaction mechanism is shown below:

$$\begin{array}{l} \quad NH_2 \\ \quad | \\ R-CH-COOH \rightarrow R-CH_2-NH_2 + CO_2 + H. \end{array}$$

Bacterial genera with decarboxylase activity include *Bacteroides, Bifidobacterium, Selenomonas, Streptococcus,* and the enterobacteria.

Production of Indoles and Phenols. Microbial production of indoles and phenols results from amino acid

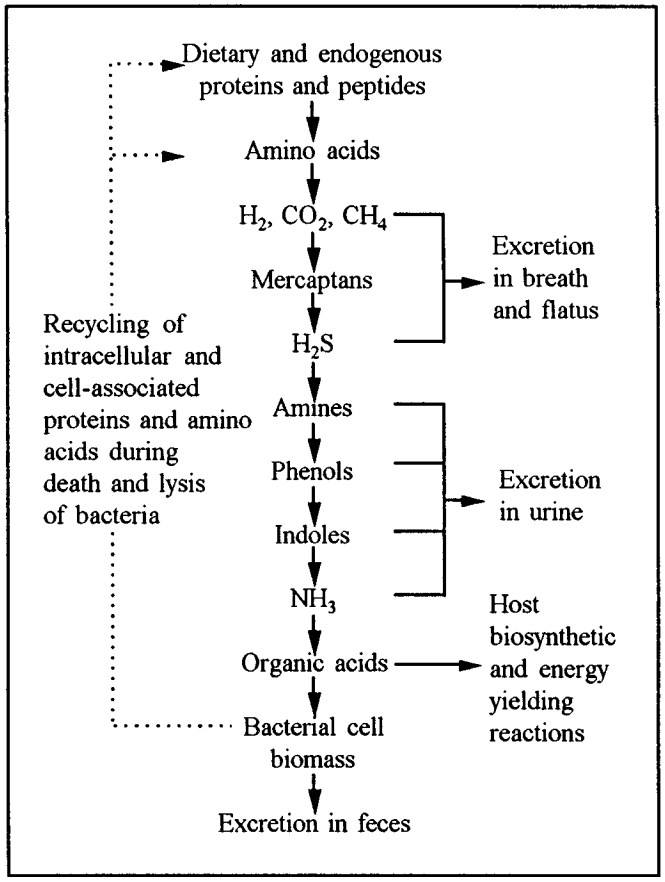

Figure 3. Overview of dietary and endogenous proteins, and peptides and amino acid fermentation in the intestinal tract (after Macfarlane and Macfarlane, 1995).

metabolism (Figure 4). Phenol, *p*-cresol, 4-ethyl phenol, and hydroxylated phenol-substituted fatty acids are the main products of tyrosine fermentation. Phenyl acetate and phenyl propionate are produced from phenylalanine, whereas indole and 3-methyl indole (skatole) are the principal end-products of tryptophan metabolism (Macfarlane and Macfarlane, 1995). Aromatic amino acids are metabolized by a variety of intestinal anaerobes including members of the genera *Bacteroides*, *Lactobacillus*, *Clostridium*, and *Bifidobacterium*. Further metabolism of the resulting ring compounds only occurs to a significant degree under aerobic conditions or in the presence of an inorganic electron acceptor.

Phenolic compounds formed in the colon are usually absorbed and detoxified by glucuronide and sulfate conjugation in the large intestinal mucosa and in the liver, where they are excreted in the urea. More than 90% of urinary phenols consist of *p*-cresol, the remainder being made up by phenol and, to a lesser extent, 4-ethyl phenol. Although little is known of the factors that control metabolism of aromatic amino acids in the large intestine, increasing dietary protein intake results in higher levels of amino acid fermentation in the colon as indicated by urinary phenol excretion and fecal ammonia concentrations. This effect was largely reduced by increasing the amount of fermentable carbohydrate (dietary fiber) in the diet. The tryptophan metabolites, indole and 3-methyl indole, are lipid-soluble and are rapidly absorbed from the colon. Within the body, absorbed skatole accumulates in adipose tissue, where it has been implicated as a major factor in boar taint. Dietary components such as antibiotics and dietary fiber indirectly affect skatole production through the gut microbiota. Sex hormones such as androsterone facilitate passage of lipophilic substances such as the indoles across biological membranes, leading to the sex/feeding interaction observed with boars, which have significantly higher concentrations of skatole in adipose tissue compared to castrates and gilts (Claus et al., 1994).

Table 3. Deamination reactions by anaerobic bacteria in the gastrointestinal tract and animal waste[a]

Amino acid	Corresponding VFA produced
Alanine, glycine, serine	Acetate
Threonine	Propionate
Glutamate, aspartate	Acetate, propionate, butyrate
Valine	Isobutyrate
Leucine	Isovalerate
Isoleucine	2-Methylbutyrate
Phenylalanine	Phenylacetate
Tyrosine	*p*-Hydroxyphenylacetate
Tryptophan	Indoleacetate → 3-methylindole
Tyrosine	Phenylacetate, phenylpropionate

[a]Source: Adapted from Mackie (1995).

Production of Sulfur-Containing Compounds. Production of sulfur compounds by anaerobic bacteria involves sulfate reduction and metabolism of sulfur-containing amino acids. Sulfate reduction proceeds via assimulatory or dissimulatory pathways. In the as-

Table 4. Decarboxylation reactions by anaerobic bacteria in the gastrointestinal tract and animal waste[a]

Amino acid	Corresponding amine produced
Glycine	Methylamine
Alanine	Ethylamine
α-Aminobutyrate	Propylamine
Ornithine	Putrescine → pyrrolidine[c]
Arginine[b]	Putrescine → pyrrolidine[c]
Norvaline	Butylamine
Lysine	Cadavenine → piperidine[c]
Arginine	Agmatine
Histidine	Histamine
Cysteic acid	Taurine
Tyrosine	Tyramine
Tryptophan	Tryptamine
Phenylalanine	Phenylethylamine

[a]Source: Adapted from Macfarlane and Macfarlane (1995).
[b]Decarboxylation and hydrolysis.
[c]Ring closure reaction.

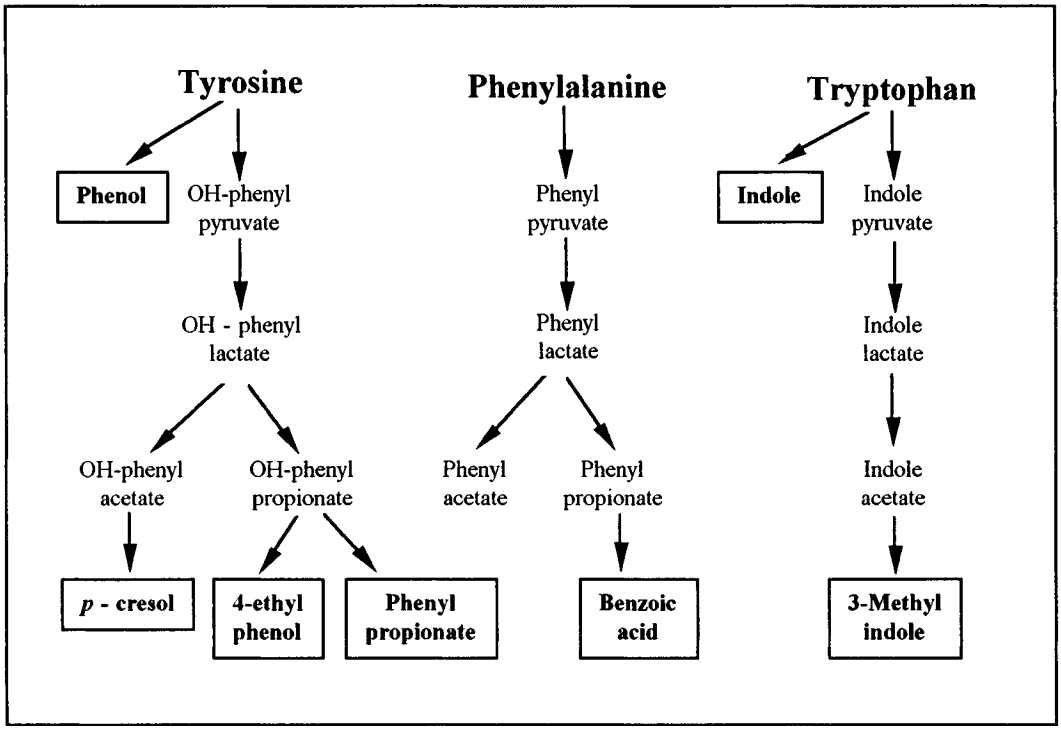

Figure 4. Metabolic pathways involved in the formation of phenolic and indolic compounds by intestinal anaerobic bacteria (after Macfarlane and Macfarlane, 1995).

simulatory process, bacteria produce enough reduced sulfur for cell biosynthesis of cysteine and methionine by transporting sulfate into the cell and activation to adenosine-5′-phosphosulfate. This is in contrast to the dissimulatory process in which sulfate is used as terminal electron acceptor and copious amounts of malodorus, toxic sulfide are produced as follows:

$$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4\ H_2O\ .$$

Sulfate can be supplied by dietary means or by depolymerization and desulfation of endogenously produced, sulfated glycoproteins such as mucins. Also, H_2 availability in the hindgut may influence competition between sulfate reducers and methanogens with non-limiting concentrations of H_2 allowing both processes to occur concomitantly. In the gut, the major hydrogenotrophic sulfate reducer is the genus *Desulfovibrio*, which is numerically dominant in feces. Assimulatory sulfate reducing bacteria are probably ubiquitous but include the genera *Veillonella*, *Megasphaera*, and the enterobacteria.

Metabolism of S-containing amino acids also gives rise to sulfide and mercaptans as follows:

$$\text{Cysteine} \rightarrow \text{Pyruvate} + NH_3 + H_2S;$$

$$\text{Methionine} \rightarrow \text{Pyruvate} + NH_3 + CH_3SH.$$

Other Sources of Ammonia. Ammonia, mainly present as NH_4^+ under pH conditions in the gut and manure, can be produced from urea and nitrate, in addition to amino acid deamination. Urea is hydrolyzed to ammonia by ureolytic bacteria. Urease activity is widely distributed among facultative anaerobes and also involves the activities of facultative anaerobes adherent on the gut epithelium. Normally, ammonia is absorbed from the hindgut and detoxified in the liver, where it is converted to urea. Nitrogen in urine is mainly excreted as urea in cattle, sheep, and swine and as uric acid in poultry. Thus, urinary urea is a major source of NH_3 in animal manure and wastes. Ammonia is an important nitrogen source for many species of anaerobic bacteria, and, in the presence of fermentable carbohydrate, ammonia is preferred to amino acids and peptides by many bacterial species.

In a situation analogous to sulfate reduction, nitrate reduction is mediated by assimilatory or dissimulatory nitrate reducers. Assimilatory nitrate reducing bacteria include the genera *Veillonella* and *Wolinella*. A wide range of bacteria are most likely involved in this process in manure slurries and waste digestors. Thermodynamically, this process is energetically favorable in terms of affinity for hydrogen but is most likely limited by nitrate availability.

Nature of Nitrogen Losses in Farm Animals

Farm animals consume considerable amounts of protein and other nitrogenous compounds with their feed. A portion of this protein is deposited in the body or secreted in milk, but usually a large part is excreted in feces and urine (Table 5). Nitrogen excreted in feces originates from feed, endogenous sources and in

Table 5. Fate of nitrogen consumed in feed by different categories of livestock[a]

Livestock category	Feed protein, g/kg DM	N partition, %				NH_3 loss, kg·animal^{-1}·yr^{-1}
		Body	Milk	Feces	Urine	
Lactating cows						8.8
Grazing	250	2	17	25	56	—
Stall fed	175	2	25	34	39	—
Beef cattle	150	22	—	30	48	5.7
Veal cattle	180	54	—	5	41	1.5
Lactating sow	160	5	20	20	55	8
Weanling pig	184	40	—	10	50	—
Growing pig	170	32	—	15	53	3
Laying hen	170	32	—	12	56	.2
Broiler	217	42	—	10	48	.1

[a]Source: Adapted from Tamminga (1992).

bacterial cells. Undigested feed N and endogenous N is mainly excreted as true protein (amino acids). Bacterial N is partly (15 to 20%) present in nucleic acids. Fecal N is partly excreted as NH_3. Nitrogen in urine is mainly excreted as urea. After excretion, feces and urine normally get mixed and degradation of N-containing compounds occurs due to the presence of large numbers of bacteria (Tamminga and Verstegen, 1992). An important end product of this degradation is NH_3, which, during storage or after soil application, may be converted to nitrate (nitrification) or nitrogen gas (denitrification). Threshold limit values for humans are 25 ppm for NH_3 at a daily subjection time of 8 h. Farm animals are subjected to these gases almost continuously and therefore lower threshold levels of between 2 and 10 ppm are considered safe.

Ammonia Emissions. Typically cattle feedlots and swine facilities lose 75% of the nitrogen excreted by the animals under current waste management systems (Vanderholm, 1985; Eghball and Power, 1994). The nitrogen cycle of a cattle feedlot is illustrated in Figure 5. Depending on the diet, feedlot cattle excrete approximately 60 to 80% of their nitrogen in urine and 20 to 40% in feces (Van Horn et al., 1996). Fecal nitrogen is 50% organic nitrogen and 50% ammonia; however, urine contains up to 97% urea nitrogen, which is readily converted by microbial urease to ammonia shortly after excretion (Mobley and Hausinger, 1989; Mobley et al., 1995). Depending on temperature, moisture, and pH, the majority of ammonia can volatilize into the atmosphere (Van Horn et al., 1996), which enhances the deposition of sulfate and nitrate, creating acid rain that acidifies soils and woodlands (Lowe, 1995; Likens et al., 1996). Some of the most extreme consequences of ammonia emissions have been experienced in The Netherlands, which has some of the most intensive agriculture in Europe (Lowe, 1995).

Besides the atmospheric and environmental consequences from ammonia emissions in livestock waste, large quantities of nitrogen, which could be used as fertilizer for crop production, are lost. Power et al. (1994) estimated that a feedlot containing 50,000 cattle could release 10,000 kg of ammonia nitrogen per day. Crops typically require a nitrogen to phosphorus (N:P) ratio of 5:1, which is the ratio in fresh cattle waste (Power et al., 1994). However, aged cattle feedlot waste contains a 1:1 N to P ratio, which creates a serious imbalance of N to P for crop production. Based on fertilizer cost, the nitrogen from beef feedlot waste alone in the United States is valued at approximately $111 million (Eghball and Power, 1994). Cattle and swine wastes lose 50 to 75% of their nitrogen from volatilization (Vanderholm, 1985; Eghball and Power, 1994; Van Horn et al., 1996).

Urease Inhibition. Because greater than 50% of the nitrogen from animals can be excreted as urea (Van Horn et al., 1996), one strategy to conserve N in waste is to inhibit the urease enzyme that converts urea to ammonia:

$$\begin{matrix} NH_2 \\ / \\ C \\ \backslash \\ NH_2 \end{matrix} = O + 2\ H_2O \xrightarrow{\text{urease}} 2\ NH_4^+ + HCO_3^- .$$

A number of urease inhibitors are available (Table 6). However, some are much more effective than others. Recent research in tropical rice production systems indicates that the phosphoryl di- and triamides, which are structural analogs of urea, can play an important role in inhibiting urease activity and increasing urea fertilizer efficiency (Byrnes and Freney, 1995). Three of these compounds, phenyl phosphorodiamide (**PPDA**), cyclohexylphosphoric triamide (**CHPT**), and N-(*n*-butyl) triphosphoric triamide (**NBPT**), have been evaluated in laboratory experiments for controlling urea hydrolysis in slurries of cattle and swine wastes. Results from these studies demonstrate that the di- and triamide analogs (PPDA, CHPT, and NBPT) can be successfully used to inhibit urease activity in laboratory studies (Varel, 1996). Further

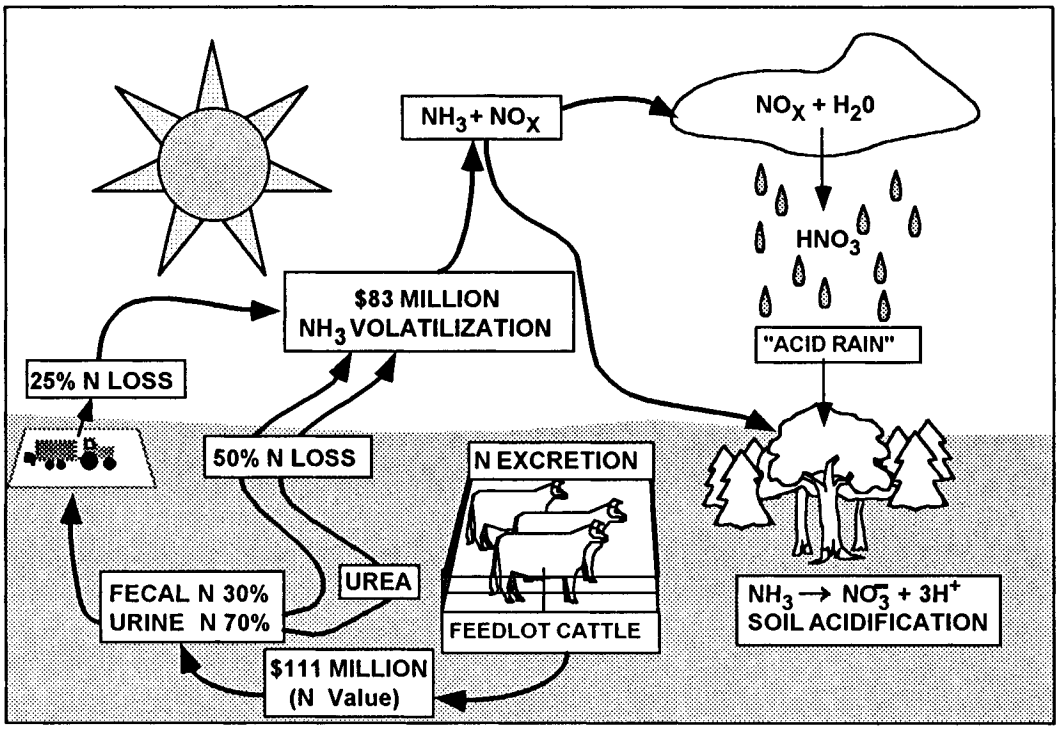

Figure 5. Loss of nitrogen from a feedlot.

studies have shown that these compounds can also be used to successfully inhibit urease activity in cattle feedlot manure, NBPT being the most effective compound tested (Varel et al., 1997). Further research to elucidate mechanisms that result in degradation or inactivation of these inhibitors is being undertaken in order to provide long-term control of ammonia emissions from animal wastes.

Intervention Strategies

Nutritional Management to Reduce Pollution and Odor from Manure. Many options are available to the animal industry to address environmental concerns related to water quality, soil degradation, air pollution, and rural-urban interface issues. Multiple combinations of nutritional management, housing systems, and treatment options as well as storage and disposal of animal wastes will be required to reduce odor and environmental pollution and provide for long-term sustainable growth. A brief review of intervention strategies is provided below. Feeding strategy or nutritional management is the basis for reducing environmental pollution from animal wastes. In the past, feeding programs and diet formulations were aimed at maximizing production performance without special concern for nutrient oversupply. Thus, feed composition and dietary regimens can be more closely adapted to an animal's nutritional requirements to avoid overfeeding and to reduce excretion of undigested components. This will decrease the available substrates that the microbes metabolize to odor compounds. Potential reductions in excretion of N and P can be achieved using the various measures listed in Table 7.

Fertilizer Option. As animal agriculture becomes more intensive, large amounts of animal manure that are distributed on small land holdings are global concerns. One of the most common applications of animal manure is to use it as a fertilizer or soil conditioner. Three concerns need to be evaluated when spreading manure on cropland. First, are pollution or odor problems likely to occur due to weather, topography, soil conditions, or rate of application? Second, does application schedule coincide with fertilization timetable? Third, is the manure application rate compatible with a fertilization rate? Farmers need adequate storage and access to enough cropland to comply with these concerns. The size of storage facilities depends on the amount of waste being generated daily, extra water that results from runoff, precipitation that falls directly into storage, and the amount of time required for waste storage.

Treatment Options. Options for treating animal manure depend on animal species (cattle, swine, poultry), housing systems (bedding vs flushing), manure handing systems (slurry vs conveyor belt), and purpose of treatment (disposal vs fertilizer). The purpose of manure treatment is to reduce the amount of nutrients and volume for disposal or spreading. An important reason for treating wastes is to reduce odor, so that it can be used as a fertilizer in populated areas. Some of the technologies applied to treatment of animal waste include composting, activated sludge, sequencing batch reactor and reverse osmosis systems, as well as biofiltration, bioscrubber, and soil filters.

Table 6. Inhibitors of purified *Klebsiella aerogenes* urease[a]

Compound class	Inhibitor
Substrate analog	Methylurea
	Thiourea
Hydroxamic acid	Acetohydroxamic acid
Phosphoroamide	Phenylphosphorodiamidate
Phosphate	H_3PO_4
Thiols	2-Mercaptoethanol
Boron-containing	Boric acid
Halogen	Fluoride
Inactivators	
Alkylating agent	Iodoacetamide
	Iodoacetic acid
	N-ethylmaleimide
Disulfide	DTNB

[a]Source: Adapted from Mobley and Hausinger (1989).

Use of Animal Waste as Feed. The nutrient content of animal waste depends on the animal species, type of feed, and bedding material used. An extensive list of publications covers this application and has been commercially practiced for many years (e.g., use of broiler litter in cattle feed). The conversion of manure to an acceptable biomass (after anaerobic fermentation) or single-cell protein (after aerobic processes) has been proposed but is not widely applied. It is worth noting that the animal industry contributes substantially to reducing potential pollutants that originate from the food processing industry, breweries, slaughter houses, and the grain milling industry (Van Horn et al., 1996).

Manure as Fuel. Waste-to-energy schemes that generate revenue from the energy produced and may provide fertilizer as a valuable by-product offer an alternative and environmentally acceptable means of disposal. Caloric value of manure depends on the composition of the waste and moisture content. Air-dried broiler litter has a caloric value of 13.5 GJ/ton, about half that of coal. Combustion produces an ash that retains most of the P and K present but little of the N from the original litter.

With the concern over future energy shortages and increasing costs of conventional fuels and electricity derived from them, much interest has developed in using anaerobic digestion as a source of renewable energy while providing acceptable waste management. Biogas (CH_4 plus CO_2) formed during anaerobic digestion is approximately 65% CH_4 and has a heat of combustion of 21.7 MJ/m^3 (20°C, 1 atm). The resulting sludge is much less odorous (stabilized) than raw manure and contains the nutrients (N, P, and K) that were in the original manure (Smith et al., 1979). This option is not widely applied because of the initial expense and operational requirements (Morse et al., 1996). It is important to note that very little odor is produced from properly managed anaerobic digestors and this may provide the impetus and incentive for their application to animal wastes in the future.

Implications

Livestock wastes, mostly manure, can be a valuable resource as well as a potential hazard to the environment. The change from asset to pollutant and nuisance has occurred as animal production systems have intensified over the last 20 yr. Poor odor control and prevention of environmental problems is related to a lack of knowledge of the fundamental nature of odor and its production by farm animals. In order to develop environmentally sound, sustainable animal production systems, scientists need to integrate research that focuses on modern analytical techniques

Table 7. Potential effects of nutritional management to reduce nitrogen (N) and phosphorus (P) content of animal manure[a]

	Estimated reduction (%) in animal manure	
Intervention strategy	N	P
Dietary options		
Protein restriction and supplementation with essential amino acids	20–25	—
Enzyme supplementation		
β-Glucanase plus xylanse	5	—
Phytase	—	25–30
Growth promotants	5	5
System options		
Use of fast-growing, efficient genotypes	5–15	5–15
Formulation closer to requirements	10–15	10–15
Phase feeding	10	10
Use highly digestible feed constituents	5	5

[a]Adapted from Anonymous (1992).

and latest sensory technology for measurement and evaluation of odor and pollution, together with a fundamental knowledge of animal and microbiological factors that are the basic units contributing to production of odor and pollutants. Without a thorough understanding of what odor is, how to measure it, and where it originates, odor will be difficult to control.

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