Livestock manure odor abatement with plant-derived oils and nitrogen conservation with urease inhibitors: A review[1]

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ABSTRACT: Confined animal feeding operations are under environmental scrutiny for production of large quantities of waste in a small area. The waste can result in odor, global warming gases, and the transfer of nutrients and pathogens to water and food sources. An incomplete anaerobic degradation of the carbohydrate, protein, and lipid components in waste is the primary cause of odor emissions. This incomplete degradation results in the formation of short-chain volatile fatty acids (VFA), amines and other nitrogenous compounds, and sulfur-containing compounds. Our objectives are to provide simple, cost-effective, and environmentally sound solutions to control odor and pathogens in livestock waste, with nutrient management a top priority. Previous studies have indicated that a urease inhibitor, N-(n-butyl) thiophosphoric triamide, can be used to reduce urea hydrolysis in beef cattle feedlot pens, conserve nitrogen, and inhibit ammonia emissions that contribute to odor. Our laboratory studies with antimicrobial plant-derived oils, thymol and carvacrol, at 2 g/kg of feedlot waste, demonstrated that production of VFA in flasks over 23 d can be completely inhibited. Fecal coliforms were reduced from 4.6×10^6 to 2.0×10^3 cells/mL 2 days after treatment and were undetectable within 4 d. Total anaerobic bacteria were reduced from 8.4×10^{10} to 1.5×10^7 cells/mL after 2 d and continued to be suppressed to that level after 28 d. These plant oils are not degraded under anaerobic conditions. However, our feedlot studies and the literature indicate these oils are degraded under aerobic conditions. This suggests that these generally recognized as safe (GRAS) chemicals, which are routinely used as preservatives in food and personal care products, should not accumulate in soils to which this waste is applied. It is concluded that chemical additives can be added to animal waste to prevent degradation, which in turn controls odor emissions, reduces pathogens, and conserves nutrients until the waste can be recycled as fertilizer. The economics and environmental effects of using thymol and carvacrol in livestock production facilities need to be determined.

Key Words: Animal Wastes, Essential Oils, Odors, Pathogens, Urease Inhibitors

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Introduction

Typically, cattle feedlots lose 75% of the nitrogen excreted by the animals under current management systems (Vanderholm, 1985; Eghball and Power, 1994; Bierman et al., 1999). This volatilization of ammonia nitrogen and related compounds contributes to offensive odor emissions. The nitrogen from feedlot manure in the United States is valued at approximately $111 million if it is available as a fertilizer (Eghball and Power, 1994). Besides this nitrogen loss, livestock waste can create other problems, which include nutrient enrichment of soil and water, emissions of greenhouse gas and odorants, and transmission of pathogenic microorganisms (Mackie et al., 1998; McCrory and Hobb, 2001). Most of these problems are related to microbial metabolism of the livestock waste. Thus, understanding the microbial activities occurring in the waste is critical to finding solutions to solve some of these problems.

This review will briefly describe some of the problems created by confined animal feeding operations (**CAFO**) in relation to the waste products generated and propose some solutions primarily associated with the use of chemical additives to control microbial fermentation of the waste. Specifically, the focus will be on 1) microbial metabolism occurring in feedlot waste and how urease inhibitors may affect nitrogen emissions and 2) microbial aspects of odor production and how natural antimicrobial additives, plant-derived essential oils, might be used to control odor emissions and pathogens in the waste.

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General Concepts

Problems Created with CAFO. Livestock production has evolved over the last few decades away from integrated farms to intensive systems using confined facilities. Thus, a large number of animals are reared in a small area, with the production of large quantities of excreta in a specific area. Many producers do not have adequate land immediately available to routinely and economically dispose of this waste. This may result in various methods to store the waste, including open stockpiles, compost piles, basins, or lagoons. These storage methods create problems associated with odor; production of the greenhouse gases carbon dioxide, methane and nitrous oxide; surface and ground water contamination; and transmission of pathogens. Dust can also be a major problem when the waste accumulates on the feedlot surface in a dry climate. Normally all of these storage methods result in considerable loss of nutrients from the waste. Nutrient management should be a high priority in determining solutions for problems associated with CAFO. This obviously begins with optimizing nutrient utilization by the animal to avoid excess nutrient excretion. A smaller mass of nutrients excreted means fewer substrates for microbial production of odor and other potential air, water, and soil contamination.

Feedlot Nitrogen Considerations

Schematics on the nitrogen cycle of a cattle feedlot (Varel, 1997) and nitrogen loss from a feedlot (Mackie et al., 1998) have been previously published. Depending on their diet, feedlot cattle excrete approximately 60 to 80% of their nitrogen in urine and 20 to 40% in feces (Van Horn et al., 1996; Bierman et al., 1999). 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 (Muck and Richards, 1980; Mobley and Hausinger, 1989; Mobley et al., 1995). Temperature, moisture, and pH greatly influence the volatilization of ammonia; however, the majority of ammonia will be emitted into the atmosphere (Van Horn et al., 1996), which enhances the deposition of nitrate, creating acid rain that acidifies soils and woodlands (Likens et al., 1996; McCory and Hobbs, 2001). Ammonia emissions in Europe have increased by more than 50% during the past 30 yr. Livestock production has been identified as the primary contributor to this increase (Pain et al., 1998; McCory and Hobbs, 2001). Many European countries have legislated the amount of livestock waste nitrogen that can be spread per unit of land area. This in turn requires increased storage capacity, which contributes to ammonia emissions.

Urease Inhibitors. Greater than 50% of ammonia in livestock slurries originates from urea hydrolysis (Van Horn et al., 1996). Thus, successfully inhibiting this process will potentially conserve nitrogen in waste.

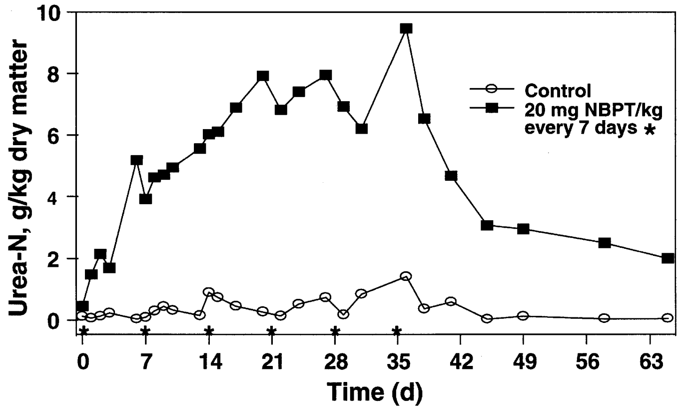

Figure 1. Effect of weekly topical applications of the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) on the accumulation of urea in feedlot manure. Adapted from Varel et al. (1999).

Studies have been conducted in the rumen to determine the effectiveness of various urease inhibitors (Jones and Mulligan, 1975; Whitelaw et al., 1991; Ludden et al., 2000). These results suggest that short-term inhibition in the rumen is possible, but the ruminal microflora appear to adapt to the inhibition and some of the inhibitors are absorbed by the rumen, which limit their practical use in improving utilization of dietary urea (Whitelaw et al., 1991; Ludden et al., 2000).

Urease inhibitors have been used successfully on a short-term basis as a soil additive to control urease activity when urea-based fertilizers are applied (Byrnes and Freney, 1995). Feedlot waste and the urea associated with it is more similar to the soil environment than the rumen environment, especially when the feedlot surface is dry. Initial laboratory studies with cattle waste slurries suggest that inhibitors prevent hydrolysis of urea for 4 to 11 d, and then a gradual hydrolysis occurs that is complete at 28 d (Varel, 1997). If the urease inhibitor is added weekly, 70% of the urea remains after 28 d.

Field studies (Varel et al., 1999) with the urease inhibitor N-(n-butyl) thiophosphoric triamide (**NBPT**) indicated that it could be used to increase the concentration of urea in feedlot pens with weekly treatments (Figure 1). These data indicate that little to no urea accumulates in feedlot manure when the urease inhibitor is not applied. This observation is important because 60 to 80% of the total nitrogen excreted by cattle is in urine, and urine contains up to 97% urea nitrogen (Van Horn et al., 1996; Bierman et al., 1999). Once this urea is converted to ammonia, it serves as a buffer, keeps the pH elevated, and enhances volatilization of ammonia (Varel et al., 1999). We estimate from our data in Figure 1 that at 30.7 d, urea nitrogen accumulation is in steady-state with degradation, and 17 g of urea/kg of DM is retained in the feedlot waste.

Controlling the environment on an open feedlot is not possible. Therefore, rainfall can easily reduce the concentration of an inhibitor applied to a feedlot surface and reduce its effectiveness. Microbial adaptation and degradation of urease inhibitors are also possible. McCrory and Hobbs (2001) concluded that urease inhibitors are currently too expensive and easily broken down or inactivated to bring any economic or practical benefit to livestock producers. Although the currently available compounds for inhibiting urease are very effective on a short-term basis (4 to 11 d), further studies are needed to prolong this inhibition. Future studies in which encapsulation of the inhibitors, which would limit the accessibility of the compounds to biological and chemical reactions and provide a prolonged and slow-release inhibition, may be fruitful. Also, a minor chemical modification of the compounds may reduce their susceptibility to microbial degradation (Mobley et al., 1995). In summary, urease inhibitors are initially very effective to conserve nitrogen in manure, but further processing such as injection of this manure into soil or transfer to an enclosed container must occur periodically to effectively recycle this nitrogen.

Microbial Aspects of Odor Production

Livestock excreta consists of undigested organic residues, including proteins, carbohydrates, and fats. These components can be degraded anaerobically, normally a slow process, or aerobically, which is a faster process. Under aerobic conditions the carbon, nitrogen, and sulfur compounds generally end up as carbon dioxide, nitrate, sulfate, and water. Under anaerobic conditions, if the organic matter is completely degraded, methane, carbon dioxide, hydrogen sulfide, ammonia, and water are the products. However, in most livestock environments the waste is in a dynamic state fluctuating between aerobic and anaerobic conditions. This results in the emissions of ammonia and associated N compounds, volatile fatty acids (VFA), sulfur compounds, various alcohols, and aromatic (indole, skatole, and cresol) compounds (Watts et al., 1994; Mackie et al., 1998). There are hundreds of volatile organic compounds (**VOC**) formed; most are offensive to the general public and are considered nuisance odor compounds.

There are many problems with defining odor because of the numerous compounds formed, accurately collecting and quantifying these compounds, and trying to determine whether the compounds analyzed by instrumentation correlate with sensory perception and evaluation (Zhu et al., 1999; Zahn et al., 2001). Ammonia, hydrogen sulfide, and VOC are considered the only volatile components from livestock production facilities with any appreciable odor. Ammonia and hydrogen sulfide are not well correlated with odor intensity. However, Zahn (1997) found a good correlation between air concentrations of VOC and odor offensiveness when 34 swine production facilities were examined. Therefore, controlling the formation and emission of the VOC should have a direct influence on odor release from animal production facilities.

The most critical point in controlling odor emissions is regulating the volatilization rate. There are six factors that influence the volatilization rate: source concentration, surface area, net radiation, air temperature, wind velocity, and relative humidity. By using a cover over a lagoon or waste storage basin we can reduce the volatilization rate by decreasing the solar radiation and direct wind velocity stripping off the VOC. Various other engineered processes can be used to affect the six factors controlling volatilization rate (Zahn et al., 1997).

If we can control the microbial formation of the VOC we will be even more successful in controlling the volatilization rate. In most livestock production facilities it is not possible to control the environment in which complete degradation of the waste to methane and carbon dioxide occurs. Conventional anaerobic digesters for production of methane were popular during the 1970s and 1980s; however, economics and the technical expertise required to operate these digesters have diminished their popularity (Morse et al., 1996). Similarly, aerobic treatment is not economically feasible, and it does little to conserve nutrients. Therefore, if we can slow down or stop the microbial fermentation of the organic matter in waste (Figure 2) before the hydrolytic and acetogenic bacteria become active, we can prevent the formation of VOC, which represent odor. This is supported by the studies of Zahn et al. (1997), in which they concluded that C2 through C9 organic acids from swine waste demonstrated the greatest potential for decreased air quality, because these compounds exhibited the highest transport coefficients and highest airborne concentrations. Thus, the use of antimicrobial chemicals should inhibit the fermentation, and in turn reduce odor produced, nutrients should be retained, pathogens may be destroyed, and production of greenhouse gases should be inhibited.

Natural Antimicrobial Additives

The requirements of chemicals that might be used to control the microbial fermentation of manure should include additives that are safe in the environment, inexpensive, and easy to apply. One group of chemical additives that fits these requirements is the naturally occurring antimicrobial plant-derived oils (Beuchat, 1994; Helander et al., 1998; Ultee et al., 1999). Essential oils are secondary metabolites of plants. They are volatile, consisting mostly of terpenes and oxygenated derivatives, and are used for flavor, fragrances, spices, and antiseptic and preservative action. Oils with the most effective antimicrobial activity, in descending order, are the phenolic compounds, alcohols, aldehydes, ketones, ethers, and hydrocarbons (Charai et al., 1996; Dorman and Deans, 2000). Two phenolic compounds we have studied are thymol (5-methyl-2-isopropylphenol) and carvacrol (5-isopropyl-2-methylphenol) (Figure 3). In practice, carvacrol is added to different products such as baked goods (16 ppm), nonalcoholic beverages (28 ppm/0.18 mM), and chewing gum (8 ppm) (Ultee et

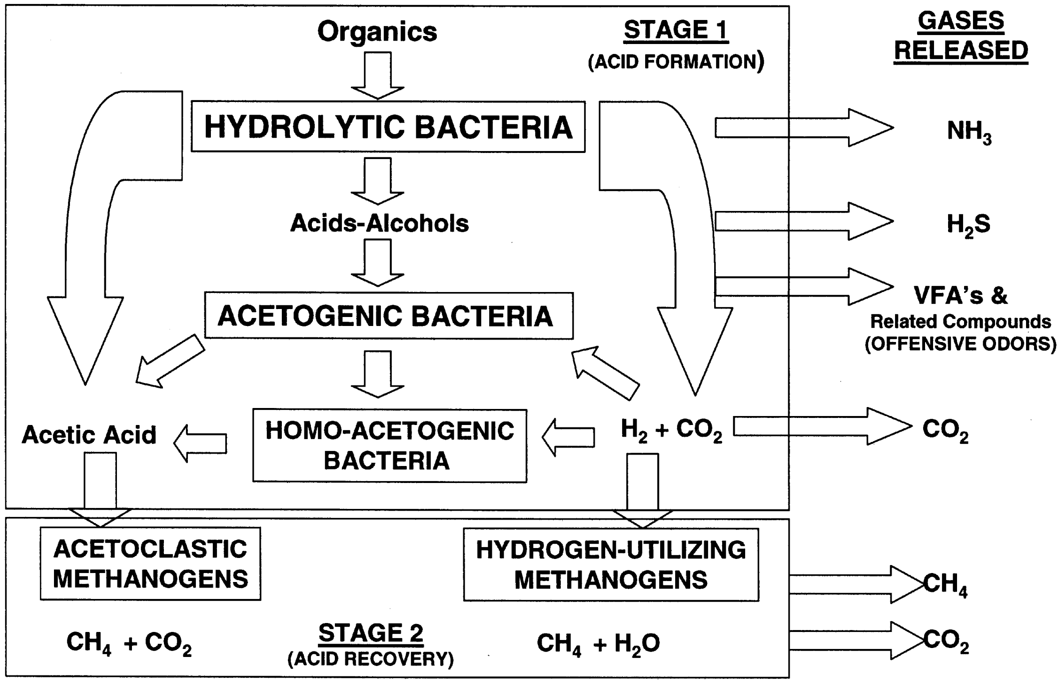

Figure 2. Anaerobic fermentation of organic matter. Adapted from Watts et al. (1994).

al., 1999). Thymol is a component in many different products, including soaps, toothpastes, shampoos, deodorants, and mouthwashes (Shapiro et al., 1994; Manou et al., 1998). These chemicals, like most plant essential oils, are generally recognized as safe (**GRAS**). Their antimicrobial mode of action consists of interactions with cell membranes that change the permeability for cations such as H^+ and K^+ (Ultee et al., 1999). The dissipation of ion gradients leads to loss of turgor pressure, inhibition of DNA synthesis, enzyme activity, and overall metabolic activities, and finally to cell death.

Numerous studies indicate that carvacrol and thymol are bactericidal to pathogenic microorganisms (Kim et al., 1995; Ultee et al., 1998; Hammer et al., 1999), and in particular to *Escherichia coli* 0157:H7 (Helander et al., 1998; Skandamis and Nychas, 2000) in pure culture. Helander et al. (1998) have shown that the minimum inhibitory concentration with carvacrol or thymol in a pure culture system is 3 m*M* and 1 m*M* for *E. coli* 0157:H7 and *Salmonella enterica* serovar *Typhimurium*, respectively. Kim et al. (1995) also found that 500 µg/mL (3.3 m*M*) of carvacrol will kill *E. coli* 0157:H7. Livestock waste contains many different types of microbial species; therefore, these in vitro studies may not be directly comparable to in vivo studies. Kim et al. (1995) found that a 1.5% solution of carvacrol was necessary to kill *S. enterica* serovar *Typhimurium* on fish cubes, a level that is significantly higher than the concentration (0.1%) needed to kill the organism in liquid medium.

Inhibition of Odor and Pathogens. We have evaluated various chemicals (Varel and Miller, 2000) and a number of plant essential oils (Varel and Miller, 2001) for their ability to control the production of short-chain fatty acids, L-lactate, gas production, and reduction of total anaerobic bacteria and fecal coliforms in stored waste. Some of these chemical additives, α-pinene, limonene, camphor, borneol, fenchol, eugenol, geraniol, 2-bromoethanesulfonic acid, anthraquinone, monensin, N,N^1-dicylohexylcarbodiimide, and methylglyoxal, had little inhibitory effect on the fermentation of cattle waste. Others, such as chlorhexidine diacetate, iodoacetic acid, and diphenyliodonium, were inhibitory to cattle but not swine waste (Varel, 2002).

Our studies suggest that thymol and carvacrol are the most effective in controlling the parameters listed

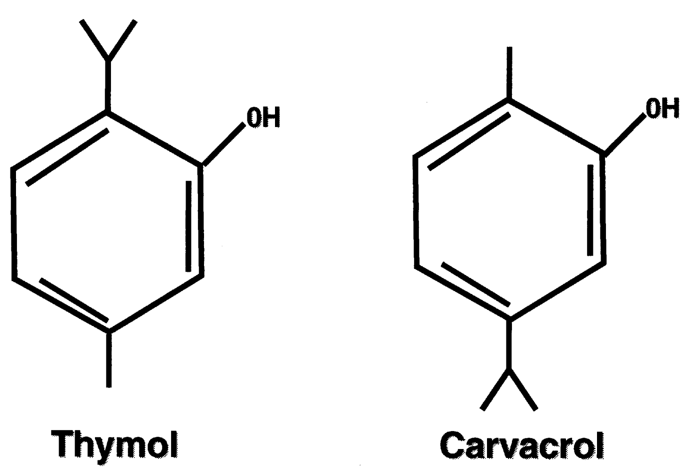

Figure 3. Structure of two plant essential oils, thymol (5-methyl-2-isopropylphenol) and carvacrol (5-isopropyl-2-methylphenol).

Table 1. Effect of pinene (P), carvacrol (C), and thymol (T) on total volatile fatty acid production from cattle waste in anaerobic flasks

	Total VFA produced (mM) in the treatment (mg/L) flasks[a]						
Incubation, h	Control (no additions)	P 500	C 1,000	T 1,000	C+T 500 + 500	C+T+P 1,000 + 1,000 + 500	SD
0	40	40	40	40	40	40	—
1	79	78	55	59	60	40	0.78
2	87	84	63	66	68	42	1.50
4	92	84	68	72	71	42	0.75
7	90	86	71	73	73	42	0.90
10	92	84	72	77	75	43	1.63
16	93	84	75	78	78	41	1.23
23	91[b]	88[b]	85[b]	94[b]	94[b]	41[c]	2.83

[a]Total VFA include acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and caproate; n = 3.
[b,c]Means with different superscripts differ ($P < 0.05$).

above. The data in Table 1 indicate that a combination of carvacrol and thymol, each at 1,000 mg/L, and 500 mg/L pinene will stop any new production of VFA in cattle waste for 23 d. Pinene is simply a masking agent and has no inhibitory effect on the fermentation when it is added by itself (Table 1). Data in Table 2 indicate that a combination of carvacrol, thymol, and pinene, at both concentrations evaluated, 750 or 1,000 mg/L each of carvacrol and thymol, reduced the number of viable anaerobic bacteria within 2 d in the waste compared with the control flasks. We did not see a complete bactericidal effect after 14 d, even at the higher concentration of carvacrol and thymol (1,000 mg/L); however, the number of organisms remained low, similar to the 2-d population. The population of fecal coliforms was reduced to nondetectable levels after 4 d when carvacrol and thymol were combined at 1,000 mg/L each (Table 3). The absence of fecal coliforms in the control flasks after 14 d was likely due to pH, which had dropped to 4.2 (Varel and Miller, 2001).

Previous studies have suggested that a combination of carvacrol and thymol would provide better antimicrobial action, rather than a higher concentration of one alone (Paster et al., 1995; Manou et al., 1998). However, further studies indicate that thymol or carvacrol can be used individually, and each is as effective as the combination as long as the total concentration added is the same (Varel and Miller, 2001; Varel, 2002). Based on cost, thymol would be the oil of choice. We have purchased thymol for $6.36/kg, which may even be less if a bulk purchase is made. Carvacrol is several times this amount.

We have initiated field studies with thymol in cattle feedlot pens with the objective of controlling pathogenic bacteria, in particular *E. coli* O157:H7, and odor. Our preliminary data indicate that the concentration of topically applied thymol decreases within days after application under aerobic conditions. This is consistent with other studies (Vokow and Liotiri, 1999) that suggest that essential oils are used as a carbon and energy source by ubiquitously occurring soil microorganisms, and these chemicals would not accumulate in soil if environmental conditions favor growth of these microorganisms. However, under anaerobic conditions, similar to those used in our laboratory studies, we have found thymol and carvacrol are not degraded for over 60 d (Varel, 2002). In livestock production facilities this appears to be ideal, because odor (VFA) is produced under anaerobic conditions, during which time we do not want these antimicrobial oils degraded. However, at the same time, we do not want these oils to build up in the soil environment. Therefore, it is speculated that

Table 2. Reduction of total anaerobic bacteria in cattle waste slurries incubated anaerobically after carvacol (C), thymol (T), and pinene (P) were added at two concentrations[a]

	Anaerobic bacteria (10^8 cells/mL)[b]			
Time, d	Control	C, T (750 mg/L each); P (500 mg/L)	C, T (1,000 mg/L each); P (500 mg/L)	SE
0	844	844	844	56
2	79.4[c]	4.0[d]	0.15[e]	0.8
7	78.3[c]	1.4[d]	0.06[e]	0.5
14	33.6[c]	7.6[d]	0.16[e]	0.9

[a]Adapted from Varel and Miller (2001), with permission.
[b]Means represent the average from three replicate flasks.
[c,d,e]Means in a row with different superscripts differ ($P < 0.05$).

Table 3. Reduction of total fecal coliforms in cattle waste slurries incubated anaerobically after carvacrol (C), thymol (T), and pinene (P) were added at two concentrations[a]

	Coliform bacteria (10^5 cells/mL)[b]			
Time, d	Control	C, T (750 mg/L each); P (500 mg/L)	C, T (1,000 mg/L each); P (500 mg/L)	SE
0	46	46	46	5.1
2	81[c]	1.5[d]	0.02[e]	0.4
4	2.9[c]	0.44[d]	None detected[f]	0.3
7	1	0.57		0.4
14	None detected[f]	0.03		0.005

[a]Adapted from Varel and Miller (2001), with permission.
[b]Means represent the average of three replicate flasks.
[c,d,e]Means in a row with different superscripts differ ($P < 0.05$).
[f]Detection limit is 1.0×10^2.

once the livestock wastes are spread on the soil surface (aerobic conditions), any essential oil would be broken down and metabolized by the soil microbes.

Elder et al. (2000) have shown that 28% of cattle may be carriers of *E. coli* O157 in their feces, and 11% of cattle presented for slaughter carry *E. coli* O157 on their hides. They suggest that there is a correlation between fecal prevalence and carcass contamination. Their data indicate that proper waste management may help control pathogens on the farm. Waste treated with carvacrol or thymol may reduce the spreading of this organism among cattle in the same pen and potentially reduce hide contamination at slaughter. Application of these chemicals is not something that would be required throughout the year. The peak season for cattle shedding *E. coli* O157 is late summer and early fall (Elder et al., 2000). This may be the most effective time to apply these chemicals to control pathogens. This seasonal period can also be an active time for odor production, because of high temperatures; thus, a reduction in odor emissions and pathogens could be accomplished simultaneously when both are most prevalent.

Feasibility of an Antimicrobial Approach. Potential limitations to the use of antimicrobial plant oils in livestock waste are that little to no degradation of the waste will occur, the cost-effectiveness of their use is unclear, and potential unknown adverse environmental effects must be studied further. Potential advantages of using these natural antimicrobial chemicals are that 1) microbial fermentation of waste is inhibited, which should reduce the rate of odor emissions, reduce the production of global warming gases, and conserve nutrients in the waste for increased fertilizer value; 2) pathogenic fecal coliforms should be destroyed; 3) the phenolic plant oils are stable under anaerobic conditions, although they are degraded under aerobic environments; 4) these products are classified as GRAS; and 5) these oils are used as pesticides, which could play a significant role in controlling flies in livestock waste.

Implications

Livestock production has changed over the last few decades and is now challenged with environmental and food safety issues. Nutrient management, odor emissions, and pathogenic microorganisms associated with livestock must be resolved with safe and cost-effective treatments. This requires a multidisciplinary approach and one solution is not universal to all production facilities. Microorganisms play a central role in many of the production issues, and therefore microbial activities should be addressed. Chemical additives that affect key metabolic pathways (urea hydrolysis), or additives that serve as antimicrobial agents (plant-derived oils), may offer multiple solutions for sustainable livestock production. Additives must be thoroughly evaluated and tested before one can expect producers to use them on a broad basis.

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