University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Roman L. Hruska U.S. Meat Animal Research Center | U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska

2004

Eugenol Stimulates Lactate Accumulation Yet Inhibits Volatile Fatty Acid Production and Eliminates Coliform Bacteria in Cattle and Swine Waste

V. H. Barel
USDA-ARS, varel@email.marc.usda.gov

D. L. Miller
USDA-ARS

Follow this and additional works at: http://digitalcommons.unl.edu/hruskareports

Barel, V. H. and Miller, D. L., "Eugenol Stimulates Lactate Accumulation Yet Inhibits Volatile Fatty Acid Production and Eliminates Coliform Bacteria in Cattle and Swine Waste" (2004). *Roman L. Hruska U.S. Meat Animal Research Center*. 309.
http://digitalcommons.unl.edu/hruskareports/309

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Roman L. Hruska U.S. Meat Animal Research Center by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Journal of Applied Microbiology 2004, **97**, 1001–1005 doi:10.1111/j.1365-2672.2004.02386.x

Eugenol stimulates lactate accumulation yet inhibits volatile fatty acid production and eliminates coliform bacteria in cattle and swine waste

V.H. Varel and D.L. Miller
Roman L. Hruska US Meat Animal Research Center, United States Department of Agriculture, Agricultural Research Service, Clay Center, NE, USA

2004/0357: received 30 March 2004, revised 19 May 2004 and accepted 26 May 2004

ABSTRACT

V.H. VAREL AND D.L. MILLER. 2004.

Aim: To determine how eugenol affects fermentation parameters and faecal coliforms in cattle and swine waste slurries stored anaerobically.

Methods and Results: Waste slurries (faeces : urine : water, 50 : 35 : 15) were blended with and without additives and aliquoted to triplicate 1-l flasks. Faecal coliforms were eliminated in cattle and swine waste slurries within 1 or 2 days with additions of eugenol at 10·05 mM (0·15%) and 16·75 mM (0·25%). At these concentrations volatile fatty acids (VFA) were reduced *ca* 70 and 50% in cattle and swine waste, respectively, over 6–8 weeks. Additionally, in cattle waste, eugenol stimulated the accumulation of lactate (>180 mM) when compared with thymol treatment (20 mM lactate). In swine waste, lactate accumulation did not occur without additives; eugenol and thymol stimulated lactate accumulation to concentrations of 22 and 32 mM, respectively.

Conclusions: Eugenol added to cattle waste may be more beneficial than thymol because not only does it effectively control faecal coliforms and odour (VFA production), it also stimulates lactate accumulation. This in turn, causes the pH to drop more rapidly, further inhibiting microbial activity and nutrient emissions.

Significance and Impact of the Study: Plant essential oils have the potential to solve some of the environmental problems associated with consolidated animal feeding operations. Thymol and eugenol reduce fermentative activity, thus, have the potential to reduce emissions of greenhouse gases and odour, and curtail transmission of pathogens in cattle and swine wastes.

Keywords: antimicrobial agents, cattle waste, eugenol, lactate, odour, pathogens, swine waste, thymol.

INTRODUCTION

Livestock raised in large confinement facilities present some challenges to the environment. Waste generated and stored in these facilities has the potential to emit nutrients, particulates, pathogens and odour to the environment. Contamination of surface and ground water is also possible. Incomplete degradation of the waste results in the production of offensive short-chain volatile fatty acids (VFA), aromatic chemicals, amines and other nitrogenous compounds, and sulphur-containing compounds (Mackie *et al.* 1998). Various amendments or additives such as organic acids, lactic acid and aluminum chloride have been explored with the objectives to control emissions from the waste (Berg and Hornig 1997; Hendriks and Vrielink 1997; Smith *et al.* 2004). Antimicrobial agents in plant oils, namely thymol and carvacrol, reduce pathogens and the production of volatile odour compounds in cattle and swine wastes (Varel and Miller 2001; Varel 2002). There are several advantages to using plant oils; they are natural products, are used in the food industry, and are generally recognized as

Correspondence to: V.H. Varel, USDA/ARS, PO Box 166, Clay Center, NE 68933, USA (e-mail: varel@email.marc.usda.gov).

© 2004 The Society for Applied Microbiology
No claim to original US government works

This article is a U.S. government work, and is not subject to copyright in the United States.

safe (Beuchat 1994; Ultee *et al.* 1999; Dorman and Deans 2000).

The objectives in the current study were to explore the use of eugenol (clove oil) as an additive to cattle and swine wastes. It was determined that it affects the metabolism of the micro-organisms in cattle waste differently than thymol or carvacrol. The latter oils inhibit lactate accumulation, whereas eugenol promotes lactate accumulation, which lowers pH and overall microbial activity more rapidly. In swine waste, all three oils were found to stimulate lactate accumulation.

MATERIALS AND METHODS

Chemicals

Chemicals were purchased from Sigma Chemical Company (St Louis, MO, USA), with the exception of eugenol which came from ICN Biomedicals, Inc. (Aurora, OH, USA).

Slurries of cattle and swine wastes

Swine and cattle wastes were processed similarly to earlier studies (Varel and Miller 2000, 2001). Briefly, cattle faecal waste, within 15 min of being excreted, was randomly collected from a pen of 40 animals fed a finishing diet of 85% ground corn and 15% forage. Swine faecal waste was randomly collected within 24 h of being excreted from animals fed a finishing diet of 85% corn and 11% soyabean meal. Cattle and swine urine were collected from catheterized animals. For both wastes, faeces, urine and distilled water in the ratio 50 : 35 : 15 (weight basis) were blended (Waring Inc., New Hartford, CT, USA) for 1 min. Three replicate samples were obtained from this slurry and analysed for various parameters, and were considered as time 0. The waste slurries were divided into 500 ml aliquots and antimicrobial plant oils were added directly at the desired concentration (thymol: 3·35, 6·7, 10·05, 13·4 and 16·75 mM; eugenol: 10·05, 13·4 and 16·75 mM). The slurry was blended for 1 min to provide a homogenous mixing of the antimicrobial oils and poured into a 1-l Erlemeyer flask, which was sealed with a rubber stopper and left stationary at ambient temperature (21°C). Treatments were in triplicate, and the contents of the flasks were gently swirled before being sampled at the days indicated in the figures and table. Faecal coliforms in cattle waste were determined from an independent group of flasks from which the VFA, lactate and pH data were determined; whereas with the swine waste only one set of flasks was set up for all data determinations.

Analytical methods

A 15-ml waste slurry sample was collected from each flask after briefly swirling the contents. The sample was mixed with 15 ml of 0·5 M H_2SO_4, centrifuged at 2000 *g* for 20 min at 4°C, and stored at −20°C until analysed (Varel and Miller 2000). L-lactate concentrations were determined with a membrane-immobilized system involving lactate oxidase (YSI autoanalyzer, Model 2700; Yellow Springs Instrument Co., Yellow Springs, OH, USA). Short-chain VFA (acetate, propionate, butyrate, valerate, isobutyrate, isovalerate) were determined in an aliquot from the original acidified sample as previously described (Varel 2002). Faecal coliforms were enumerated with 3M Petrifilm *Escherichia coli*: count plates (3M Microbiology Products, St Paul, MN, USA) according to previous methods (Varel and Miller 2001). Manure slurry pH was obtained from the contents in the flasks by using a combination pH electrode and pH meter.

Statistical analysis

Data were analysed as a split-plot in time with the GLM procedure of SAS (1989). Least squares mean are presented, and each mean represents samples from triplicate treatments ($n = 3$).

RESULTS

Thymol was used as a positive control in efforts to evaluate eugenol. Data in Fig. 1a confirm that thymol (13·4 mM) inhibits VFA production in cattle waste (75 mM compared with 199 mM at the end of 42 days). Similarly, the concentrations of eugenol evaluated indicated VFA inhibition was dose dependent, with 13·4 and 16·75 mM providing comparable inhibition to thymol. Eugenol at 10·05 mM was ineffective in controlling VFA production for the initial 2 days (>120 mM), however for the next 40 days VFA production increased <10 mM. Lactate accumulation in these treatments (Fig. 1b) was minimal for thymol (20 mM, day 42). This was in sharp contrast with the eugenol treatments in which lactate continued to increase for 42 days, reaching between 180 and 215 mM ($P < 0{\cdot}01$). These high lactate concentrations are reflected in the rapid decline (within 4 days) of the pH values 7·9–4·5 and 4·6 for 10·05 and 13·4 mM eugenol treatments, respectively (Fig. 1c). The pH of the thymol treatment was higher (5·7 after 42 days; $P < 0{\cdot}05$) than the eugenol treatment, because only 20 mM lactate accumulated in the thymol treatment.

In swine waste only one concentration of thymol and eugenol, 16·75 mM (0·25%), was evaluated over a 56-day period. Thymol was more inhibitory to VFA production (100 mM) compared with eugenol (180 mM; $P < 0{\cdot}05$) or no addition controls (340 mM; $P < 0{\cdot}01$) (Fig. 2a). Contrary to cattle waste (142 mM lactate, no addition treatment), only 5 mM lactate accumulated in the no addition treatment for swine waste (Fig. 2b). Lactate accumulation in swine waste

© 2004 The Society for Applied Microbiology,
No claim to original US government works

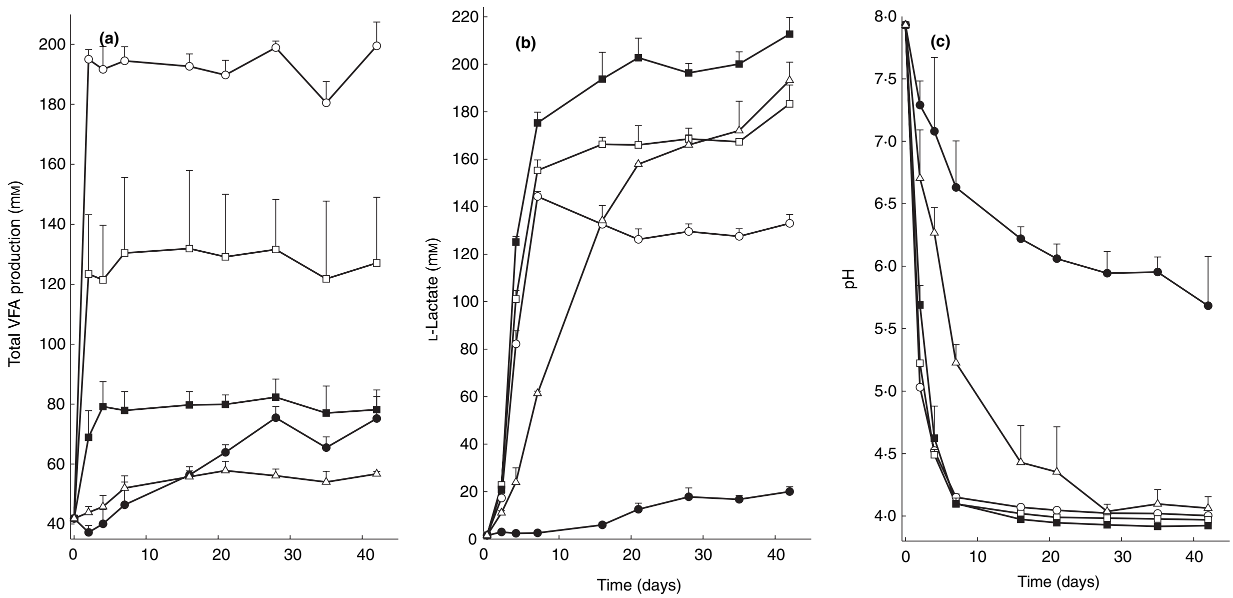

Fig. 1 Effect of thymol and eugenol treatments on VFA, lactate and pH in stored cattle waste. Treatments included: ○ control (no additions); ● thymol 13·4 mM; □ eugenol 10·05 mM; ■ eugenol 13·4 mM and △ eugenol 16·75 mM

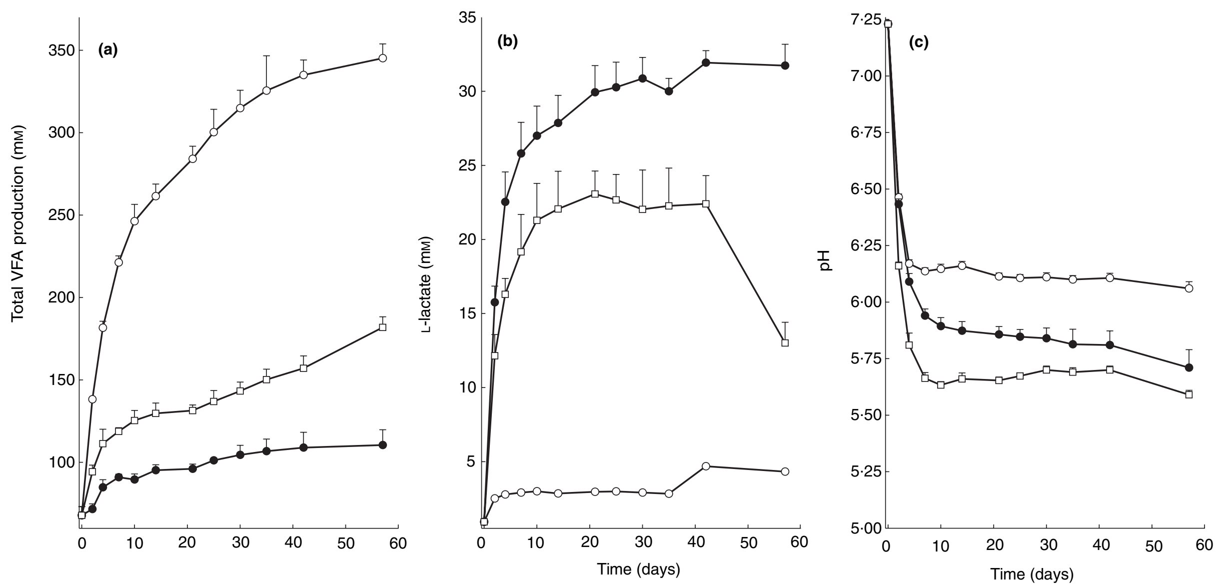

Fig. 2 Effect of thymol and eugenol treatments on VFA, lactate and pH in stored swine waste. Treatments included: ○ control (no additions); ● thymol 16·75 mM and □ eugenol 16·75 mM

was stimulated ($P < 0·05$) by thymol and eugenol (32 and 22 mM, respectively, at 42 days). Treatment pH values are indicated in Fig. 2c. In the no addition treatment, pH values remained above 6·0, because little lactate accumulated (5 mM). The pH values for the thymol and eugenol treatments were both lower ($P < 0·05$) than the control.

Faecal coliforms in cattle waste were eliminated with 10·05 and 6·7 mM thymol in 1 and 2 days, respectively (Table 1). Eugenol (13·4 mM) eliminated the coliforms within 1 day. In the no addition treatment, coliforms were still prevalent ($0·1 \times 10^5$ CFU ml^{-1}) after 42 days. In swine waste, the only concentration evaluated for thymol and

© 2004 The Society for Applied Microbiology,
No claim to original US government works

Table 1 Reduction of total faecal coliforms in cattle and swine waste slurries incubated anaerobically after various concentrations of thymol or eugenol were added

	Coliform bacteria (10^5 CFU ml^{-1})*							
	Cattle waste/additive concn (mM)					Swine waste/additive concn (mM)		
		Thymol			Eugenol		Thymol	Eugenol
Time (days)	No addition	3·35	6·7	10·05	13·4	No addition	16·75	16·75
0	10	10	10	10	10	7·8	7·8	7·8
1	TNTC	3·1	1·0	0†	0	ND	ND	ND
2	1500	2·5	0			10·3	0	0
4	1400	0·95				11·0		
8	1900	0·13				8·0		
11	1500	0·06				6·0		
14	1400	0·02				1·7		
21	130	0				0·07		
42	0·1					0		

TNTC, too numerous to count; ND, not determined.
*Mean represent the average from three replicate jars.
†Detection limit is $1{\cdot}0 \times 10^2$.

eugenol, 16·75 mM, eliminated the coliform bacteria in 1 day. The no addition treatment contained viable coliforms for 21 days but none were detected at 42 days.

DISCUSSION

Our previous studies (Varel and Miller 2001; Varel 2002) indicate that thymol was effective at controlling microbial metabolism in cattle and swine waste. Swine waste requires *ca* 3·5 mM thymol more than cattle waste to see a comparable inhibitory activity. Results from this study with cattle waste indicate that eugenol affects the microbial accumulation of L-lactate differently than thymol. Cattle waste stored anaerobically with no additions will produce high concentrations of VFA and lactate (Fig. 1a,b). When thymol is added to cattle waste it inhibits VFA production and lactate accumulation. When eugenol is added it inhibits VFA production, but it stimulates lactate accumulation. Lactate is produced by some micro-organisms and is metabolized by others. It is unclear in this study whether eugenol stimulated the production of lactate or inhibited its degradation to give the net accumulation.

This study also indicates that microbial populations or metabolism in cattle and swine wastes are different. Cattle waste with no additives accumulates high lactate concentrations, but little lactate accumulates in swine waste. This supports the hypothesis that differences in waste organic matter composition between cattle and swine will select the bacterial communities that reside in these waste slurries (Miller and Varel 2003). Results from swine waste also contrasted with cattle waste when thymol was added. Thymol stimulates lactate accumulation in swine waste; however, in cattle waste thymol inhibits lactate accumulation. Friedman *et al.* (2002) have demonstrated how various side chains, the position of double bonds, and isomeric compounds of plant oils can influence antimicrobial activities, which is consistent with our studies.

Various dietary additives and probiotics have been used to stimulate intestinal lactic acid producing bacteria, which promote intestinal well-being and prevent colonization of pathogenic bacteria (Valeur *et al.* 2004). Whether eugenol could serve this purpose in the pig intestinal tract is unclear. Eugenol fed to ruminants would likely have negative effects because it would reduce VFA production in the rumen, similar to what has been reported with thymol added to mixed ruminal cultures (Evans and Martin 2000).

Accumulation of lactate in livestock wastes has a desirable effect because it is ionically a strong acid that rapidly reduces pH. This is advantageous for conserving ammonia in the waste slurry (Burgess *et al.* 1998; Smith *et al.* 2004). The disadvantage of lowering slurry pH is that the VFA become more volatile. This happens in cattle waste slurries with no treatment, where high lactate and high VFA concentrations are generated with an end result of high odour being emitted. Zahn *et al.* (1997) have concluded that C2 to C9 volatile organic acids from swine waste demonstrate the greatest potential for decreased air quality (odour), as these compounds exhibit the highest transport coefficients and highest airborne concentrations. Lactate is a nonvolatile acid and not considered an odorous compound. Thus, an additive such as thymol or eugenol is desirable because they inhibit VFA production, which is considered by some to be responsible for the highest component in livestock odour (Zahn *et al.* 1997).

Several studies have been conducted with the objective to lower the pH in waste slurries in order to reduce ammonia emissions. Some have involved direct additions of organic acids and others have added glucose with the objective that micro-organisms such as *Lactobacillus plantarum* would convert the glucose to lactate (Hendriks and Vrielink 1997). Still others have directly added lactate acid (50% concentrate) at 5% by volume (Berg and Hornig 1997). It was concluded that lactate reduced ammonia and methane emissions from swine waste by 90%, and nitrous oxide emissions were eliminated. However, the quantity of lactate required to lower the pH to 4·5 is likely cost-prohibited, and there are hazards associated with handling acids. Therefore, addition of a plant oil, that naturally selects a microbial population which allows lactate accumulation in the waste slurry may be useful.

In conclusion, results from this study indicate that plant-derived oils may be effective in solving some of the environmental problems associated with consolidated animal feeding operations. Ammonia and odour emissions are two major issues of concern in animal production facilities. Eugenol suppresses microbial activity in cattle waste, inhibiting the production of short-chain VFA that are considered the predominant odour compounds emitted from livestock wastes. Additionally, eugenol stimulates accumulation of lactate in cattle and swine wastes, which lowers pH, and should conserve ammonia nitrogen in the waste. Thymol prevents short-chain VFA production in cattle and swine wastes; however, it also suppresses lactate formation in cattle waste. Therefore, eugenol may be the more effective oil to use with cattle waste. Field studies are needed to generate data for cost analyses on the use of these oils in livestock production facilities.

ACKNOWLEDGEMENTS

Technical assistance of Sue Wise and manuscript preparation by Debbie Brown are appreciated.

REFERENCES

Berg, W. and Hornig, G. (1997) Emission reduction by acidification of slurry-investigations and assessment. In *Proceedings of the International Symposium of Ammonia and Odour Control from Animal Facilities* ed. Voermans, J.A.M. and Monteny, G.J. pp. 459–466. The Netherlands: Vinkeloord.

Beuchat, L.R. (1994) Antimicrobial properties of spices and their essential oils. In *Natural Antimicrobial Systems and Food Preservation* ed. Dillon, V.M. and Board, R.G. pp. 167–179. Wallingford, UK: CAB International.

Burgess, R.P., Carey, J.B. and Shafer, D.J. (1998) The impact of pH on nitrogen retention in laboratory analysis of broiler litter. *Poultry Science* **77**, 1620–1622.

Dorman, H.J.D. and Deans, S.G. (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Bacteriology* **88**, 308–316.

Evans, J.D. and Martin, S.A. (2000) Effects of thymol on ruminal microorganisms. *Current Microbiology* **41**, 336–340.

Friedman, M., Henika, P.R. and Mandrell, R.E. (2002) Bacterial activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *Journal of Animal Science* **65**, 1545–1560.

Hendriks, J.G.L. and Vrielink, M.G.M. (1997) Reducing ammonia emissions from pig houses by adding or producing organic acids in pig slurry. In *Proceedings of the International Symposium of Ammonia and Odour Control from Animal Production Facilities* ed. Voermans, J.A.M. and Monteny, G.J. pp. 493–501. The Netherlands: Vinkeloord.

Mackie, R.I., Stroot, P.G. and Varel, V.H. (1998) Biochemical identification and biological origin of key odor components in livestock waste. *Journal of Animal Science* **76**, 1331–1342.

Miller, D.N. and Varel, V.H. (2003) Swine manure composition affects the biochemical origins, composition, and accumulation of odorous compounds. *Journal of Animal Science* **81**, 2131–2138.

SAS (1989) *SAS User's Guide: Statistics, Version 6*. Gary, NC: SAS Institute, Inc.

Smith, D.R., Moore, P.A., Jr, Haggard, B.E., Maxwell, C.V., Daniel, T.C., VanDevander, K. and Davis, M.E. (2004) Effect of aluminum chloride and dietary phytase on relative ammonia losses from swine manure. *Journal of Animal Science* **82**, 605–611.

Ultee, A., Kets, P.W. and Smid, E.J. (1999) Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. *Applied and Environmental Microbiology* **65**, 4606–4610.

Valeur, N., Engel, P., Carbajal, N., Connolly, E. and Ladefoged, K. (2004) Colonization and immunomodulation by *Lactobacillus reuteri* ATCC 55730 in the human gastrointestinal tract. *Applied and Environmental Microbiology* **70**, 1176–1181.

Varel, V.H. (2002) Carvacrol and thymol reduce swine waste odor and pathogens: stability of oils. *Current Microibology* **44**, 38–43.

Varel, V.H. and Miller, D.N. (2000) Effect of antimicrobial agents on livestock waste emissions. *Current Microbiology* **40**, 392–397.

Varel, V.H. and Miller, D.N. (2001) Plant-derived oils reduce pathogens and gaseous emissions from stored cattle waste. *Applied and Environmental Microbiology* **67**, 1366–1370.

Zahn, J.A., Hatfield, J.L., Do, Y.S., Dispirito, A.A., Laird, D.A. and Pfeiffer, R.L. (1997) Characterization of volatile emissions and wastes from a swine production facility. *Journal of Environmental Quality* **26**, 1687–1696.