The Effect of Disinfectants on the Density of Coliforms in Sawdust Bedding

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Summary

Three disinfectants were applied to sawdust bedding and their effect on coliform concentration determined. Orthophenylphenol, slaked lime and paraformaldehyde were applied to sawdust bedding twice at 7 day intervals.

Bedding in pens treated with slaked lime and paraformaldehyde showed a significant reduction in TBC and coliform count, while that in pens treated with orthophenylphenol was similar to the untreated control bedding. The moisture content of bedding treated with either slaked lime or paraformaldehyde was significantly reduced following each treatment. The three treatments showed a significant effect on the hydrogen ion concentration of the sawdust bedding. The coliform contamination of teat ends of cows bedded on sawdust treated with slaked lime and paraformaldehyde was significantly reduced. It was concluded that although slaked lime and paraformaldehyde had a similar effect on coliform concentration, slaked lime is preferred as it is effective, practical, and less likely to cause injury to the animals or workers.

Introduction

The teat ends of cows are in prolonged contact with bedding materials. The bacterial population in bedding can vary in amount and type and may influence the type and incidence of intramammary infection. (Rendos *et al.,* 1975)

Sawdust bedding has been implicated in the epizootiology of coliform mastitis (Dodd *et al.* 1970, Carrol and Jasper, 1980, and Eberhart, 1977) and Klebsiella mastitis in particular (Newman and Kowalski, 1973 and Bramley & Neave, 1975).

Treatment of bedding materials to control coliforms has been attempted. Paraformaldehyde spray (5%) reduced coliform count for 2-3 days but the count returned to predisinfection levels in a week (Bramley and Neave, 1975). Paraformaldehyde prill spread on a feedlot at the rate of 50 lb. per 3200 sq. ft. reduced the coliforms for about a week (Greenfield *et al.,* 1972). Bramley *et al.,* 1976 demonstrated a reduction in coliform count for only 24-48 hours after the application of 100 g. of paraformaldehyde prill into the bedding of each cubicle at the time of addition of new sawdust twice weekly.

Many factors contribute to coliform content of bedding. Temperature, moisture and pH are important variables. Coliforms have different growth optimums with regard to temperature than other organisms. Temperature in bedding tends to increase provided moisture is present. Sawdust bedding has more surface area than other wood products and would, therefore, have a greater capacity for water retention (Carrol, 1977). Disinfectants that reduce moisture absorption could reduce the growth of coliforms.

The object of this study was to apply a practical low cost disinfectant to sawdust bedding in an attempt to control coliform concentration by creating an unfavorable medium for coliform growth. A corresponding objective was to measure the effect of disinfection of sawdust on coliform contamination of teat ends.

Materials and Methods

A. *Disinfectants*
   1. Orthophenylphenol (1 gallon per 600 sq. ft. in 1:128 dilution)[1] (3 gallon per 600 sq. ft. in 1:3 dilution)[1]
   2. Slaked Lime - 8 lb./100 ft^2
   3. Five percent W/V paraformaldehyde suspension [to give a final concentration of 14.5 g. paraformaldehyde per sq. yd. (Bramley and Neave, 1975)]

B. *Housing and Animals*
   Six cows were confined in each of four 20'x30' pens, under roof, and bedded with new sawdust bedding material. An open concrete lot 30'x40' adjoined each pen.

C. *Application of the Disinfectants*
   Three pens were treated with a disinfectant twice at 7 day intervals. Slaked lime was mixed with the sawdust bedding, while orthophenylphenol and paraformaldehyde were sprayed on the surface. The fourth pen was left without treatment as a control. Orthophenylphenol was applied as a 1:128 dilution the first time and in a 1:3 dilution the second time.

D. *Bedding Samples*
   Bedding samples were collected at days 1 - 2 - 5 - 8 - 9 - 12 and 15. On days 1 and 8, the days disinfectants were applied, samples were collected twice, before and after application of disinfectants.

Samples of bedding in approximately 100 gram amounts were collected randomly from each pen and were examined

TABLE (1) Microbial Count of Disinfected Sawdust Bedding

Time of Sampling (Day)		Treatment											
		Control (No Treatment)			Orthophenylphenol			Slaked Lime			Paraformaldehyde		
		TBC/g	Coliform		TBC/g	Coliform		TBC/g	Coliform		TBC/g	Coliform	
		a	count/g[a]	type	a	count/g[a]	type	b	count/g[b]	type	b	count/g[b]	type
1*	First Application	$7.0x10^6$	$3.3x10^6$	Klebsiella	$8.6x10^6$	$4.0x10^6$	Klebsiella	$6.5x10^6$	$5.3x10^6$	Klebsiella	$8.7x10^6$	$2.9x10^6$	Klebsiella
1**	First Application	$7.0x10^6$	$3.3x10^6$	Klebsiella	$5.8x10^6$	$8.9x10^5$	Klebsiella	$7.0x10^2$	nil	- - -	$5.4x10^4$	$2.6x10^2$	Klebsiella
2	First Application	$8.5x10^6$	$5.2x10^6$	Klebsiella E. coli Enterobacter	$7.9x10^6$	$9.5x10^5$	Klebsiella E. coli Enterobacter	$9.5x10^2$	$3.2x10^2$	E. coli	$6.6x10^4$	$5.3x10^2$	E. coli
5	First Application	$7.9x10^7$	$4.4x10^7$	"	$4.2x10^7$	$3.1x10^6$	"	$5.7x10^5$	$2.5x10^4$	"	$6.2x10^5$	$2.1x10^4$	"
8*	First Application	$9.5x10^7$	$9.0x10^7$	"	$7.2x10^7$	$9.5x10^6$	"	$2.8x10^6$	$3.2x10^5$	"	$9.0x10^6$	$2.5x10^6$	E. coli Klebsiella Enterobacter
8**	Second Application	$9.5x10^7$	$9.0x10^7$	"	$9.0x10^6$	$3.0x10^6$	"	$2.5x10^2$	nil	- - -	$8.6x10^5$	$3.6x10^4$	E. coli
9	Second Application	$3.0x10^8$	$9.5x10^7$	"	$3.2x10^7$	$9.3x10^6$	"	$9.7x10^3$	$7.8x10^3$	E. coli	$8.9x10^5$	$7.4x10^4$	E. coli
12	Second Application	$9.6x10^8$	$3.8x10^8$	"	$3.5x10^8$	$6.5x10^7$	"	$4.9x10^5$	$5.3x10^4$	"	$7.5x10^6$	$6.3x10^5$	Enterobacter E. coli Klebsiella
15	Second Application	$4.0x10^9$	$3.5x10^9$	"	$3.8x10^9$	$3.1x10^9$	"	$3.4x10^7$	$6.0x10^5$	"	$2.3x10^7$	$7.5x10^5$	"

* = Before application of disinfectant.

** = After application of disinfectant.

a,b =Columns with different designation were significantly different at $P < .05$.

according to Rendos *et al.,* 1975 and Carrol and Jasper, 1980.

10 gram portions of sawdust were suspended in 90 ml saline. After thorough shaking, 1:10 serial dilutions were prepared in sterile saline. Eight serial, 1:10 dilutions were then plated in duplicate surface platings. In addition, 0.1 ml of each dilution was transferred to 0.5 ml of MacConkey broth and was incubated at 37°. If growth was indicated by pH change a loopful of content from the MacConkey broth was streaked on to Tergitol-7 agar (Difco). Colonies on this medium were then streaked on TSI agar slants and incubated. Organisms from the TSI were then subjected to the IMVIC test, ornithine decarboxylase and urea medium.

Total coliform counts were calculated from the last dilution which showed growth in MacConkey broth. The counts of individual coliform species were calculated from the last dilution from which that particular organism had been isolated. Dilutions of a colony count of 30-300 on one or both plates were used to calculate a count per gram of wet bedding material.

Teat Swabs:

Teat swabs were obtained at days 1 - 2 - 5 - 8 - 9 - 12 and 15. Teat swabs were obtained and examined according to Rendos *et al.,* 1975.

Prior to collecting teat swab samples, gross contamination was cleaned from the teats with a dry paper towel. To collect a sample, a swab was removed from the broth tube, rotated 3 times around the end of the teat and returned to the tube. Tubes were delivered directly to the laboratory. Broth solutions used for plating were prepared by shaking the tube, expressing excess broth from the swab and discarding the swab. Microbial counts were determined by the same methods as with the bedding samples.

The moisture content of the bedding samples was determined gravimetrically using 5 grams of each of bedding sample. The pH of each bedding sample was determined by making a slurry of 5 grams of bedding in 50 ml of distilled water and allowing the slurry to stand at room temperature for 30 minutes prior to measurement of hydrogen ion concentration. Bedding temperatures were obtained by mercury thermometer introduced 5 cm below the surface of the bedding.

E. Data were analyzed statistically using the Friedman test according to Holander and Wolfe, 1973.

Results

The effects of treating sawdust bedding with various disinfectants on the total bacterial count (TBC), coliform count, moisture content and pH of the bedding are presented in Tables 1 and 2 and Figures 1 and 2.

The TBC of the sawdust bedding treated with slaked lime was lowest except at the 15th day, when it was slightly higher than that treated with paraformaldehyde which had the lowest count at the 15th day.

The coliform count of the sawdust bedding treated with slaked lime was zero after each application and was lowest throughout the 15 days of the experiment. Statistical analysis of the TBC and coliform count showed a significant difference ($P < .05$) between the control bedding and beddings treated with slaked lime and paraformaldehyde. While there was no difference between the control bedding and bedding treated with orthophenylphenol except after the higher concentration (1.3) of orthophenylphenol was used in

TABLE (2) Moisture and pH of Disinfected Bedding

Time of Sampling (Day)		Treatment (Disinfection)							
		Control (untreated)		Orthophenylphenol		Slaked Lime		Paraformaldehyde	
		Moisture % a	pH c	Moisture % a	pH d	Moisture % b	pH d	Moisture % b	pH d
1*	First Application	40.6	6.70	40.2	6.70	41.2	6.70	40.6	6.70
1**		40.6	6.70	50.0	8.09	38.0	8.03	46.0	6.75
2		40.0	6.71	42.0	8.05	20.0	7.55	32.0	6.93
5		46.0	6.73	46.0	7.85	31.6	7.50	38.0	6.96
8*		54.0	6.75	50.0	7.2	40.0	7.52	40.0	7.01
8**	Second Application	54.0	6.75	53.0	8.09	36.0	8.35	39.6	7.00
9		50.0	6.79	46.0	8.01	20.0	7.9	32.0	7.01
12		50.0	6.84	42.0	7.4	42.2	7.63	42.6	7.04
15		53.0	6.90	52.0	7.05	47.0	7.58	47.4	7.2

* = Before application of disinfectant.

** = After application of disinfectant.

a,b,c,d = Columns with different designations were significantly different at P < .001.

the second application when a slight non significant reduction in the coliform count was achieved.

The coliform organisms isolated from the pre-treated sawdust were mainly Klebsiella species. Enterobacter, *Escherichia coli* (E. coli) and Klebsiella were isolated from all samples taken after day one in the control pen and in the orthophenylphenol treated pen. *E. coli* was isolated from the paraformaldehyde treated pen in all samples taken after day one whereas Klebsiella and Enterobacter were isolated intermittently. Klebsiella and Enterobacter were not isolated from bedding treated with slaked lime.

There was a significant difference (P< .001) in moisture content between the control bedding and beddings treated with slaked lime and paraformaldehyde. There was no difference in moisture content between the control bedding and bedding treated with orthophenylphenol. There was a significant difference (P< .001) between the pH of control bedding and treated beddings. The pH of control bedding increased slightly from 6.7 to 6.9 over the time of the trial. The pH of bedding treated with orthophenylphenol increased from 6.7 to 8.05 at the 2nd day, then fell to 7.2 at the 8th day. Following the 2nd application at day 8 the pH again rose from 7.2 to 8.09 at day 9 then fell to 7.05 at day 15. The pH of slaked lime treated bedding rose from 6.7 to 8.03 following treatment, then fell to 7.50 on day 5. After the 2nd application of lime on day 8, pH rose to 8.35 then declined to 7.58 at day 15.

Bedding pH in the paraformaldehyde treated pen rose from 6.7 to 7.01 on day 8 and to 7.2 at day 15.

The effects of treatments on the temperature of the bedding were not significant. The temperature of the weather during the experiment was variable between 0°C and 14.5°C. Bedding in the four treatment pens showed a wide variation in temperature between 2°C and 18°C.

The effect of treatment of the sawdust bedding on the microbial contamination of the teat ends is shown in Table 3. Statistical analysis of microbial contamination of the teat ends showed a significant reduction (P<.001) on TBC from teat ends of cows bedded on sawdust treated with paraformaldehyde, and on coliform count of teat ends of cows bedded on sawdust treated with paraformaldehyde and slaked lime, both of which showed no coliform contamination except at the 12th and 15th days.

Klebsiella organisms were not recovered from the teat ends of cows bedded on sawdust treated with slaked lime at any time during the trial. Klebsiella were recovered on the 5th day and later from those bedded on untreated sawdust (control) and sawdust treated with orthophenylphenol. Klebsiella were also recovered on the 15th day from those bedded on sawdust treated with paraformaldehyde. E. coli were recovered from teat ends of cows bedded on untreated (control) and treated sawdust. Enterobacter were also recovered, intermittently.

Fig. 1

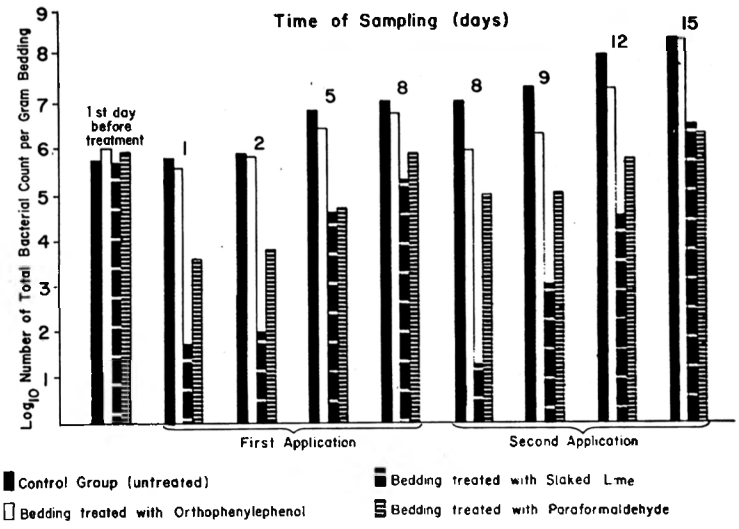

Fig. 2

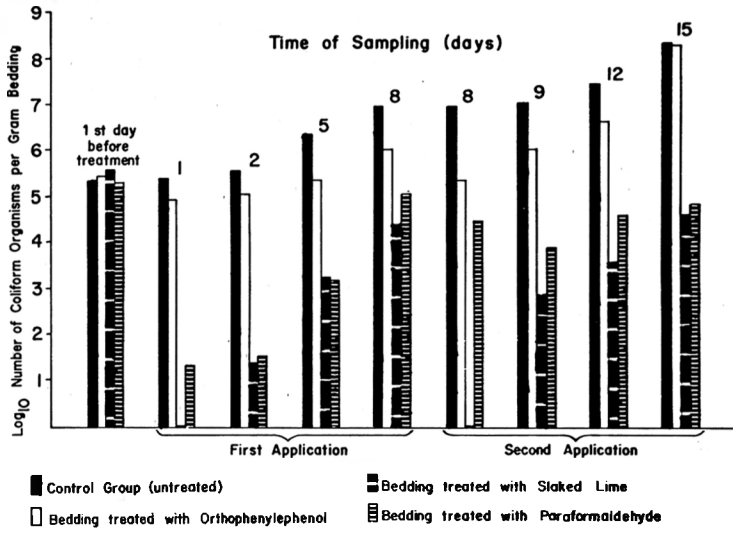

TABLE (3) Microbial Contamination of Teat Ends by Treatment

Time of Sampling	Treatment															
	Control (no treatment)				Orthophenylphenol				Slaked Lime				Paraformaldehyde			
	TBC a	Coliform c			TBC a	Coliform c			TBC a	Coliform d			TBC b	Coliform d		
	$\bar{x}$	count x	%	type	$\bar{x}$	count $\bar{x}$	%	type	$\bar{x}$	count $\bar{x}$	%	type	$\bar{x}$	count $\bar{x}$	%	type
1st day	6.67×10^5	7.70×10^3	50	E. coli	2.66×10^4	4.67×10^1	16.66	E. coli Entero-bacter	1.66×10^5	7.0×10^3	16.66	E. coli Entero-bacter	4.78×10^4	1.25×10^2	16.66	E. coli
2nd day	8.97×10^5	2.03×10^4	50	E. coli Entero-bacter	4.76×10^4	6.0×10^1	16.66	"	9.05×10^4	0	0	- - -	8.49×10^3	0	0	- - -
5th day	1.43×10^6	1.29×10^5	83.33	E. coli Kleb-siella	1.52×10^6	1.49×10^4	83.33	E. coli Kleb-siella	1.21×10^5	0	0	- - -	1.55×10^4	0	0	- - -
8th day	1.99×10^6	2.86×10^5	100	"	3.42×10^6	1.39×10^5	83.33	"	5.80×10^5	0	0	- - -	2.32×10^4	0	0	- - -
9th day	1.03×10^6	5.32×10^5	99.99	"	2.36×10^6	3.7×10^4	66.66	"	4.00×10^5	0	0	- - -	5.62×10^4	0	0	- - -
12th day	5.30×10^6	1.04×10^6	99.98	"	4.10×10^6	2.83×10^5	83.33	"	1.67×10^6	7.67×10^1	33.33	E. coli	7.07×10^4	4.67×10^1	16.66	E. coli
15th day	9.22×10^6	2.83×10^6	100	"	5.8×10^6	2.03×10^6	99.99	"	1.99×10^6	2.40×10^2	33.33	E. coli	1.03×10^5	9.0×10^1	33.33	E. coli Klebsiella

TBC = Total bacterial count.

% = Percentage of contaminated teat apices with coliforms.

a,b,c,d = Columns with different designations were significantly different at $P < .001$.

Discussion

The use of a disinfectant as a sawdust bedding treatment to reduce the coliform population would appear to have possible application on dairy farms for helping to control coliform mastitis.

Slaked lime and paraformaldehyde had a similar effect in reducing TBC and coliform count and in reducing Klebsiella and *E. coli* organisms in both sawdust bedding and on teat ends of cows bedded thereon. Reduction in TBC in sawdust treated with slaked lime lasted for about a week after each treatment and for 5-7 days after treatment with paraformaldehyde. The latter is in agreement with Bramley and Neave, 1975, and Greenfield *et al.*, 1972.

Coliform counts recovered from the teat ends of cows bedded on sawdust treated with slaked lime and paraformaldehyde were reduced for 12 days. Initially, the use of orthophenylphenol had no effect in reducing TBC or coliform. Following the use of increased quantity (3x) at the 2nd application, a slight reduction in coliform count occurred.

Moisture content of sawdust treated with slaked lime was reduced and pH was elevated. Both changes could account for reduced growth of mastitogenic coliform organisms. The reduction in moisture content may be controversial. It is not clear whether the reduction in moisture content was due to the presence of hydrated lime or whether it is the result of some other difference in experiment design or sample collection. Pens were adjacent and of the same design. Drainage and ventilation was the same for each pen. Water source and availability were the same for all pens. Number of animals was the same and size of the animals was similar. It does not appear to us that the design or sampling method accounts for the difference in moisture.

It may be that the application of lime reduces the absorbtion of water by sawdust. If this is true, and we can find no documentation to this effect, then water would leach through the bedding pack and enter the drainage system. At this time we believe this is what occurred. It may be that lime would have no effect if the drainage of the bedded area was poor so that water could not leach through.

Sawdust can be a major reservoir for coliform organisms which cause mastitis. Reducing the concentration of these organisms should have a beneficial effect on the incidence of coliform mastitis. Slaked lime can be used as a disinfectant for the sawdust bedding. It is effective, relatively low cost, and easily available. In addition, it is not harmful to the animal or the operator when used in the manner described. Paraformaldehyde use would be effective but would be associated with greater risk and greater cost. (Greenfield *et al.*, 1972, Bramley and Neave, 1975 and Bramley *et al.*, 1976).

References

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