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# Enterobacteria in Feedlot Waste and Runoff

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Samples of beef cattle feedlot waste (FLW), runoff from the pens, and water from a large drainage ditch at the feedlot were examined for *Enterobacteriaceae*. The drainage ditch receives the runoff but contains primarily subsurface drainage from fields on which FLW is spread for disposal. Plating and enrichment techniques with seven different media were used to isolate 553 cultures of enterobacteria. FLW contains about 50 million enterobacteria/g dry weight. More than 90% of these were *Escherichia coli*, none of which were enteropathogenic types as determined with multivalent sera. *Citrobacter* and *Enterobacter cloacae* were other organisms present in moderate numbers. Application of enrichment techniques broadened the spectrum of enterobacteria isolates to include the four *Proteus* spp., both *Providencia* spp., *Klebsiella*, *Enterobacter aerogenes*, *Arizona*, and a single isolate of *Salmonella* (serological group $C_2$). *Shigella* was not isolated. The wide spectrum of enterobacteria in FLW may be a hazard if unsterilized waste is refed. Fewer enterobacteria occurred in the runoff and in the drainage ditch; the most numerous species in FLW also were most numerous at these sites. However, neither *Salmonella* nor *Arizona* was isolated from runoff or drainage-ditch waters.

Cattle feedlots represent a serious, but largely undocumented, pollution hazard. Most studies on feedlot waste (FLW) concern disposal methods and movement of nutrients into surface and subsurface waters through runoff and percolation. Currently, refeeding is being investigated as a means for combating the increasing accumulation of waste from intensive animal production.

Surprisingly little attention has been paid to the microbiological aspects of either FLW or runoff. We have enumerated and categorized the microflora of FLW and associated sites (10). Gram-negative bacteria were the third most numerous group of organisms encountered; coliform counts were approximately $1 \times 10^7$/g dry weight of FLW and slightly less than $1 \times 10^5$/ml of runoff. These counts varied only slightly during 1 year. A brief report by Witzel et al. (12) gave coliform counts of $5 \times 10^5$/g wet weight in cattle manure, of which more than 95% were "typical" *Escherichia coli*. In an overall examination of coliforms in warm-blooded animals, Geldreich et al. (6) found a similar percentage of *E. coli* present when they examined strains from cattle manure by the IMViC tests. Miner et al. (9) isolated *Salmonella infantis* from litter and runoff at two feedlots, and the investigation by Bromel et al. (1) demonstrated transfer of antibiotic resistance from enteric bacteria of farm animals to those from human sources. The problem of identifying pollution from FLW in surface waters has led to the suggestion that *Streptococcus bovis* might be a better indicator than coliform organisms (8).

We evaluated the *Enterobacteriaceae* in FLW and runoff because methods for dealing with animal wastes must include consideration of potential health hazards.

## MATERIALS AND METHODS

**Samples.** Samples were collected in July from a cattle feedlot in central Illinois capable of sustaining 5,000 to 10,000 animals at a time. A detailed description of this commercial feedlot is given by Rhodes and Hrubant (10). Four types of samples were collected. (i) Composite FLW: 12 specimens of 3 to 5 g each were taken from scattered sites in two adjacent animal pens and combined to yield a 50- to 60-g composite sample. (ii) Individual FLW: 10 fresh manure deposits in seven different pens were individually sampled with paired sterile swabs for enrichment procedures. (iii) Runoff: multiple dips from a small drainage ditch adjacent to the pens were combined to yield a single 100-ml sample. (iv) Field ditch: a combined sample of 500 ml taken by multiple dip from the intersection of two drainage

ditches located about .5 mile from the pens. FLW had been spread on adjacent cornfields for several years. Runoff from the pens also emptied into this field ditch at the sample site via the drainage ditch of sample iii. All samples except sample ii were stored in cracked ice until analyzed in the laboratory within 4 hr of collection.

**Plate counts and FLW isolates.** The composite FLW sample i was diluted 1:3 (w/v) with sterile water and blended for 30 sec in a Waring Blendor. A 40-ml portion was then diluted with 60 ml of sterile 0.1% tryptone (Difco) to give a 1:10 dilution. Subsequent 10-fold dilutions were made with 0.1% tryptone; the 90-ml dilution blanks contained glass beads to aid dispersion. Colony counts were made from triplicate spread plates inoculated with 0.3 ml/plate; four dilutions were spread on each medium. Eosin methylene blue agar (EMB), deoxycholate lactose agar (DCL), sorbitol agar (Sorb), bismuth sulfite agar (BS), and *Salmonella-Shigella* agar (SS) were the plating media. All colonies on plates of the proper dilution were counted after incubation for 18 to 24 hr at 37 C. All colonies from one, two, or three plates of the countable dilution of each plating medium except EMB, selected to total 75 to 100 colonies, were transferred to Kliger's iron agar (KIA) slants. Of 250 colonies on EMB, 105 were transferred to KIA; the remaining 145 were pinpoint colonies and were not transferred to KIA. A total of 452 colonies were transferred to KIA from the count plates; from these, 352 isolates were obtained.

**Isolates from enrichment cultures.** Portions (10 ml) of composite FLW (1:10 dilution of sample i), runoff liquid (sample iii), and ditch water (sample iv) were added to 10 ml of double-strength brilliant green bile broth (BGB) and to selenite cystine broth (SC). Swabs of the individual FLW samples ii were added to BGB and to SC at the sampling sites. After incubation for 18 to 24 hr at 37 C, loopfuls of the BGB enrichment cultures were streaked on Sorb while the SC enrichments were streaked on BS and SS. The streak plates were incubated for 24 hr at 37 C, and colonies from each plate then were transferred to KIA. Ten colonies from each streak plate medium were transferred to represent the enrichment cultures of composite FLW, runoff, and ditch water (90 isolates). Each of the 10 individual FLW swab samples was represented by six subcultures on KIA from each streak plate medium (180 isolates).

**KIA and primary screen.** After the KIA slants were incubated for 18 to 24 hr, the isolates were grouped by their acid, gas, and $H_2S$ reactions. The media and tests used for subsequent differentiation of the *Enterobacteriaceae* were selected from those of Ewing and Davis (5). Cultures on KIA that were alkaline or exhibited no growth in the stab were transferred to pigment-enhancing media, glucose broth, and a repeat KIA test. These organisms were characterized no further. Presumed enterobacteria were all subjected to the following primary screen: indole production and motility on SIM medium; methyl red; Voges-Proskauer (VP) using the Barritt method for acetyl methyl carbinol; citrate utilization (Simmon's); urease production by the liquid method of Stuart et al. as cited by Ewing and Davis (5); mannitol fermentation; and growth in potassium cyanide broth (Difco).

**Secondary tests.** The isolates were regrouped after results of the primary screen were collated. Additional tests were selected for these groups from among the following: lysine and ornithine decarboxylase; arginine dehydrolase; phenylalanine deaminase; fermentation of sucrose, dulcitol, salicin, inositol, sorbitol, arabinose, rhamnose, arabinose plus dulcitol, and adonitol plus inositol plus sorbitol. All cultures were also checked for nitrate reduction in fluid medium with inverted insert vials. In a few instances, tests from the primary screen were repeated. Production of gas from glucose and the lactose fermentation were confirmed in carbohydrate fermentation media; urease was checked on Christensen's urea agar; and the VP, citrate, and potassium cyanide tests were repeated by the original techniques. Cultures were identified by their bio-

TABLE 1. *Number of Enterobacteriaceae in feedlot waste (composite FLW sample)*

| Cultures | Plating medium | | | | |
|---|---|---|---|---|---|
| | EMB[a] | DCL | Sorb | BS | SS |
| Total count/g (dry weight)[b] | $1.1 \times 10^8$ | $3.6 \times 10^8$ | $1.5 \times 10^8$ | $8.0 \times 10^5$ | $1.2 \times 10^6$ |
| Colonies transferred to KIA[c] | 250 | 82 | 89 | 101 | 75 |
| *Enterobacteriaceae* | 97 | 13 | 41 | 83 | 56 |
| Other organisms | 8 | 15 | 34 | 1 | 4 |
| No growth on KIA | 145[d] | 54 | 14 | 17 | 15 |
| *Enterobacteriaceae* count/g (dry weight)[e] | $4.4 \times 10^7$ | $5.6 \times 10^7$ | $6.8 \times 10^7$ | $6.4 \times 10^5$ | $8.8 \times 10^5$ |

[a] EMB, eosin methylene blue agar; DCL, deoxycholate lactose agar; Sorb, sorbitol agar; BS, bismuth sulfite agar; SS, *Salmonella-Shigella* agar; KIA, Kliger's iron agar.

[b] Triplicate spread plates per dilution; 0.3 ml/plate.

[c] One, two, or three plates of countable dilution used; all colonies from these plates transferred to KIA. For EMB only, number includes 145 pinpoint colonies counted but not transferred.

[d] Pinpoint colonies on EMB not transferred to KIA.

[e] *Enterobacteriaceae* count = (Number of *Enterobacteriaceae*/Number of colonies transferred) × total count/g (dry weight).

TABLE 2. *Isolates from composite feedlot waste sample by plating*[a]

| Isolate | Plating medium | | | | |
|---|---|---|---|---|---|
| | EMB | DCL | Sorb | BS | SS |
| *Escherichia coli* | 62 | 12 | 34 | | 20 |
| *E. coli*: indole − | 10 | | 2 | | |
| *E. coli*: citrate + | 4 | | 2 | | |
| *E. coli*: KCN + | 4 | | | 1 | 1 |
| *E. coli*: lactose − or (+) | 3 | | 2 | | |
| *Citrobacter* | 1 | | | 3 | 1 |
| *Citrobacter*: indole + | 2 | | | 18 | 4 |
| *Citrobacter*: citrate − | 5 | | | | 1 |
| *Citrobacter*: KCN − | 1 | | | | 1 |
| *Klebsiella* | | | | 9 | 1 |
| *Enterobacter cloacae* | 3 | | | 28 | |
| *E. cloacae*: KCN − | 2 | | | | |
| *E. cloacae*: ornithine − | | | 1 | 2 | 1 |
| *E. cloacae*: sorbitol − | | | | | 1 |
| *E. cloacae*: arabinose − | | | | 1 | |
| *E. cloacae*: rhamnose − | | | | 1 | |
| *Enterobacter aerogenes* | | | | | 1 |
| *Proteus vulgaris* | | | | | 7 |
| *Proteus mirabilis* | | 1 | | 1 | 3 |
| *Proteus morganii* | | | | | 2 |
| *Proteus morganii*: ornithine − | | | | | 2 |
| *Proteus rettgeri* | | | | | 2 |
| *Providencia stuartii* | | | | | 1 |
| Unidentified enterobacteria | | | | 18 | 4 |
| *Citrobacter*: phenylalanine +, indole + | | | | 1 | |
| *Proteus vulgaris*: ornithine + | | | | | 3 |
| Total enterobacteria | 97 | 13 | 41 | 83 | 56 |
| *Pseudomonas* sp. | 1 | | | | |
| *Bacillus* spp. | 2 | | | | |
| KIA: alkaline/no growth; no pigment; glucose − | 5 | 15 | 34 | 1 | 4 |
| Total isolates | 105 | 28 | 75 | 84 | 60 |

[a] In Tables 2-6, a blank space means that the specific organism was not isolated. For abbreviations, see footnote to Table 1.

chemical reactions and serotyping in accordance with the schema of Ewing (3, 4).

The carbohydrates used were from Sigma (St. Louis) or Difco (Detroit); all other media and media components were from BBL (Division of BioQuest, Cockeysville, Md.), except where noted.

## RESULTS AND DISCUSSION

FLW contains between 4.4 and 6.8 $\times$ $10^7$ enterobacteria/g dry weight (Table 1). These values represent only those cultures shown to be enterobacteria by biochemical tests. Total plate counts on BS and SS were about 100-fold lower than counts obtained on EMB, DCL, or Sorb. On these three media, total counts varied from 1.1 $\times$ $10^8$ organisms/g on EMB to 3.6 $\times$ $10^8$ on DCL. Pinpoint colonies and those that did not grow on transfer to KIA accounted for about 60% of colonies on EMB and DCL but only about 16% of those on Sorb. About 50% of the isolates from DCL and Sorb were demonstrably not enterobacteria by their reaction on KIA; in contrast, enterobacteria represented more than 90% of the isolates from EMB. The numbers of enterobacteria per gram of FLW, as calculated from plate counts on EMB, DCL, and Sorb, correspond to undifferentiated counts on EMB of cecal contents in cattle fed high-roughage diets (7).

More than 90% of the enterobacteria in FLW were *E. coli* (Table 2). *Citrobacter* and *Enterobacter cloacae* were also isolated from EMB. Single isolates of *Proteus mirabilis* and *E. cloacae* were obtained from DCL and Sorb, respectively. The inability of *E. coli* to grow on BS and SS is largely responsible for the difference between the total plate count on these media and that on EMB, DCL, and Sorb. Inhibition of most of the *E. coli* permitted isolation of *Enterobacter aerogenes*, *Klebsiella*, *Providencia stuartii*, and the four species of *Proteus* from BS and SS count plates. Between $10^5$ and $10^6$ of these organisms occur per gram of FLW. *Enterobacter* species are the

TABLE 3. *Isolates from composite feedlot waste sample by enrichment culture*

| Isolate | BGB →[a] Sorb | SC → BS | SC → SS |
|---|---|---|---|
| *Escherichia coli* | 5[b] | | |
| *E. coli*: citrate + | 3 | | |
| *Salmonella* Group $C_2$ | | 1 | |
| *Arizona*: KCN + | | 1 | |
| *Citrobacter* | | 4 | 1 |
| *Klebsiella* | 2 | | |
| *Enterobacter cloacae* | | 3 | |
| *Proteus mirabilis* | | | 1 |
| *P. mirabilis*: indole + | | | 1 |
| *Proteus morganii* | | | 1 |
| *Proteus rettgeri* | | | 1 |
| *Providencia stuartii* | | | 5 |
| Unidentified enterobacteria | | 1 | |

[a] Swabs incubated for 18 to 24 hr at 37 C in first designated medium (e.g., BGB); a loopful of this growth streaked on second medium (e.g., Sorb) and incubated 24 hr at 37 C. Ten colonies from these streak plates transferred to KIA as primary isolates subsequently characterized. BGB, brilliant green bile broth, SC, selenite cystine broth. For other abbreviations, see footnote to Table 1.

[b] Of 10 isolates picked from streak plate, number characterized as indicated.

TABLE 4. *Isolates from individual samples of feedlot waste by enrichment culture*

| Isolate | BGB →[a] Sorb | SC → BS | SC → SS |
|---|---|---|---|
| *Escherichia coli* | 48[b] | | |
| *E. coli*: indole − | 2 | 1 | |
| *E. coli*: citrate + | 6 | | |
| *E. coli*: KCN + | 1 | | |
| *E. coli*: lactose (+) | 1 | | |
| *Arizona* | 1 | 2 | 5 |
| *Citrobacter*: indole + | | 8 | 3 |
| *Citrobacter*: citrate − | 1 | | |
| *Enterobacter cloacae* | | 13 | |
| *E. cloacae*: ornithine − | | 1 | |
| *Proteus vulgaris* | | | 5 |
| *Proteus mirabilis* | | 12 | 35 |
| *P. mirabilis*: indole + | | 2 | |
| *P. mirabilis*: indole +, lactose + | | 3 | |
| *Proteus morganii* | | 3 | |
| *Providencia alcalifaciens* | | | 1 |
| *Providencia stuartii* | | 3 | 7 |
| Unidentified enterobacteria | | 2 | 1 |
| *Escherichia*: $H_2S$ + | | 2 | |
| *Citrobacter*: phenylalanine + | | | 1 |
| *Citrobacter*: phenylalanine +, indol + | | 5 | |
| *Proteus vulgaris*: ornithine + | | 1 | 1 |
| KIA: alkaline/no growth | | 2 | 1 |

[a] Swabs from 10 individual feedlot waste samples incubated for 18 to 24 hr at 37 C in first designated medium; a loopful of this growth streaked on second medium and incubated for 24 hr at 37 C. Six colonies from each streak plate transferred to KIA as primary isolates subsequently characterized (180 total isolates). For abbreviations, see footnote to Table 3.

[b] Of 60 isolates picked from streak plates, number characterized as indicated.

most numerous of the enterobacteria outside of the *E. coli* and *Citrobacter* groups; *Proteus* species are somewhat less abundant than are *Enterobacter*. EMB appears to be the best single medium for enumeration of coliform organisms in FLW and related sources. Both BS and SS agar are also required for determination of those enterobacteria present in small numbers compared to *E. coli*.

The results of enrichment culture techniques applied to the composite FLW (sample i) and to 10 individual FLW samples (sample ii) from seven pens are given in Tables 3 and 4, respectively. BGB enrichments, plated on Sorb, were used in attempting to isolate pathogenic *E. coli*. None were found although all sorbitol-negative isolates were tested with polyvalent OB sera which detect the 10 serotypes of *E. coli* most often implicated in infantile diarrhea. *Salmonella* and *Shigella* were sought in SC enrichments plated on BS and SS. One *Salmonella* group $C_2$ was isolated from FLW. Polyvalent O and group $C_2$ antisera confirmed the generic biochemical identification. *S. typhimurium* (group B) and *S. newport* (group $C_2$) appear to be the *Salmonella* most frequently isolated from cattle (2, 11). *S. infantis* (group $C_1$) was isolated from the litter and runoff at two experimental feedlots by Miner et al. (9). Several isolates from FLW were biochemically identified as *Arizona* strains. Neither *Shigella* spp. nor members of the alcalescens-dispar group were isolated. Although no numerical evaluation can be made from the enrichment procedure, frequency of isolation confirms the overall abundance of the types of enterobacteria found by plating.

Enrichment cultures of runoff and of field ditch water were similarly checked for enteric pathogens (Table 5). None were isolated. The infrequency of isolation indicates that *E. coli* does not survive well in these waters. Counts done on the runoff and ditch water show few coliforms compared to the numbers encountered in FLW (10).

Table 6 summarizes the classification of all isolates studied. The percentage of coliforms that are *E. coli* is similar to that reported previously in bovine feces (6, 12). Although *E. coli* constitutes more than 90% of the total enterobacteria in FLW, its poor survival in related waters indicates that it may have limited value as an indicator of pollution from feedlots. Middaugh (8) has suggested *S. bovis* as an indicator of pollution from bovine sources.

The presence of a broad spectrum of other enterobacteria in lesser numbers was demonstrated by enrichment culture techniques. Since these organisms, particularly the *Proteus* species, have poor assimilative capacity, they probably have a subordinate role in the degradation of FLW. However, coliforms and other enterics including *Proteus* and *Klebsiella* isolated from animal waste and a waste treatment lagoon were shown to be a potentially hazardous source of transferable R-factors carrying multiple antibiotic resistance (1). The isolation of a single *Salmonella* supports the position that agricultural wastes do have public health implications (2, 9, 11). The occurrence of a wide spectrum of enterobacteria also should give pause to proposals for indiscriminate refeeding of unsterilized FLW as a method of utilizing this waste.

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TABLE 5. *Isolates from runoff and field ditch water by enrichment culture*

| Isolate | Runoff | | | Field ditch water | | |
|---|---|---|---|---|---|---|
| | BGB →[a] Sorb | SC → BS | SC → SS | BGB → Sorb | SC → BS | SC → SS |
| *Escherichia coli* | 4[b] | | | 5 | | |
| *E. coli*: citrate + | 1 | | | | | |
| *Citrobacter* | | 4 | | 2 | 2 | 1 |
| *Klebsiella* | | 1 | | 1 | | |
| *Enterobacter cloacae* | | 1 | | | 4 | |
| *E. cloacae*: rhamnose − | | 2 | | | 2 | |
| *Enterobacter aerogenes* | 1 | | | | | |
| *Enterobacter liquefaciens* | 3 | | | | | |
| *Proteus mirabilis* | | | | | | 6 |
| *P. mirabilis*: indole +, lactose + | | | | | 1 | 1 |
| *Proteus morganii* | | | 1 | | | 1 |
| *P. morganii*: ornithine − | | | 1 | | | |
| *Proteus rettgeri* | | | 6 | | | |
| *Providencia alcalifaciens*: motility − | | | | | | 1 |
| Unidentified enterobacteria | 1 | | | | | |
| *Escherichia*: $H_2S$ + | | | 1 | 2 | | |
| *Pseudomonas* | | 1 | | | | |
| KIA: alkaline/alkaline | | 1 | 1 | | 1 | |

[a] Swabs incubated for 18 to 24 hr at 37 C in first designated medium; a loopful of this growth streaked on second medium and incubated for 24 hr at 37 C. Ten colonies from these streak plates transferred to KIA as primary isolates subsequently characterized. For abbreviations, see footnote to Table 3.

[b] Of 10 isolates picked from streak plate, number characterized as indicated.

TABLE 6. *Summary of enterobacteria isolated from feedlot waste (FLW), runoff, and field ditch water*[a]

| Characterization | FLW | | | | | | | Related sites | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | Composite FLW by plating | | | | | Composite FLW by enrichment | Individual FLW by enrichment | Runoff by enrichment | Field ditch by enrichment | |
| | EMB | DCL | Sorb | BS | SS | | | | | |
| *Escherichia* | 83[b] | 12 | 40 | 1 | 21 | 8 | 59 | 5 | 5 | 234 |
| *Salmonella* | | | | | | 1 | | | | 1 |
| *Arizona* | | | | | | 1 | 8 | | | 9 |
| *Citrobacter* | 9 | | | 21 | 7 | 5 | 12 | 4 | 5 | 63 |
| *Klebsiella* | | | | 9 | 1 | 2 | | 1 | 1 | 14 |
| *Enterobacter* | 5 | | 1 | 32 | 3 | 3 | 14 | 7 | 6 | 71 |
| *Proteus* | | 1 | | 1 | 16 | 4 | 60 | 8 | 9 | 99 |
| *Providencia* | | | | | 1 | 5 | 11 | | 1 | 18 |
| Unidentified | | | | 19 | 7 | 1 | 13 | 2 | 2 | 44 |
| Total *Enterobacteriaceae* | 97 | 13 | 41 | 83 | 56 | 30 | 177 | 27 | 29 | 553 |

[a] For abbreviations, see footnote to Table 1.

[b] No. of isolates characterized as indicated.

## LITERATURE CITED


1. Bromel, M., Y. N. Lee, and B. Baldwin. 1971. Antibiotic resistance and resistance transfer between bacterial isolates in a waste lagoon, p. 122-125. Int. Symp. Livestock Wastes, Columbus, Ohio.
2. Ellis, E. M. 1969. *Salmonella* reservoirs in animals and feeds. J. Amer. Oil Chem. Soc. **46**:227-229.
3. Ewing, W. H. 1969. Biochemical reactions given by *Enterobacteriaceae* in commonly used tests. Center for Disease Control, U.S. Dept. of Health, Education, and Welfare, Atlanta, Georgia.
4. Ewing, W. H. 1970. Differentiation of *Enterobacteriaceae* by biochemical reactions (revised and amended). Center for Disease Control, U.S. Dept. of Health, Education, and Welfare, Atlanta, Georgia.
5. Ewing, W. H., and B. R. Davis. 1970. Media and tests for differentiation of *Enterobacteriaceae*. Center for Disease Control, U.S. Dept. of Health, Education, and Welfare, Atlanta, Georgia.
6. Geldreich, E. E., R. H. Bordner, C. B. Huff, H. F. Clark, and P. W. Kabler. 1962. Type distribution of coliform bacteria in the feces of warm-blooded animals. Water Pollut. Contr. **34**:295-301.
7. Maki, L. R., and K. Picard. 1965. Normal intestinal flora of cattle fed high-roughage rations. J. Bacteriol. **89**:1244-1249.
8. Middaugh, P. R. 1971. Differentiation of ruminant from nonruminant fecal sources of water pollution by use of enteric bacteria, p. 126-128. Int. Symp. Livestock Wastes, Columbus, Ohio.
9. Miner, J. R., L. R. Fina, and C. Piatt. 1967. *Salmonella infantis* in cattle feedlot runoff. Appl. Microbiol. **15**: 627-628.
10. Rhodes, R. A., and G. R. Hrubant. 1972. Microbial population of feedlot waste and associated sites. Appl. Microbiol. **24**:369-377.
11. U.S. Dept. of Health, Education, and Welfare, Center for Disease Control, Report no. 108. *Salmonella* surveillance. 1971. Atlanta, Ga.
12. Witzel, S. A., E. McCoy, L. B. Polkowski, O. J. Attoe, and M. S. Nichols. 1966. Physical, chemical and bacteriological properties of farm wastes (bovine animals), p. 10-14. *In* Management of farm animal wastes. Amer. Soc. Agr. Eng. Publ. SP-0366.