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# Microbial Population of Feedlot Waste and Associated Sites

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A quantitative determination was made every 2 months for a year of the microflora of beef cattle waste and runoff at a medium-sized midwestern feedlot. Counts were obtained for selected groups of organisms in waste taken from paved areas of pens cleaned daily and, therefore, reflect the flora of raw waste. Overall, in terms of viable count per gram dry weight, the feedlot waste contained $10^{10}$ total organisms, $10^{9}$ anaerobes, $10^{8}$ gram-negative bacteria, $10^{7}$ coliforms, $10^{6}$ sporeformers, and $10^{5}$ yeasts, fungi, and streptomycetes. The specific numbers and pattern of these groups of organisms varied only slightly during the study in spite of a wide variation in weather. Data indicate that little microbial growth occurs in the waste as it exists in the feedlot. Runoff from the pens contained the same general population pattern but with greater variation attributable to volume of liquid. Comparable determinations of an associated field disposal area (before and after cropping), stockpiled waste, and elevated dirt areas in the pens indicate that fungi, and especially streptomycetes, are the aerobic organisms most associated with final stabilization of the waste. Yeasts, which are the dominant type of organism in the ensiled corn fed the cattle, do not occur in large numbers in the animal waste. Large ditches receiving runoff and subsurface water from the fields have a population similar to the runoff but with fewer coliforms.

Until recently, there has been little concern about the pollution hazard inherent in waste generated by livestock production. However, livestock wastes constitute a major and increasing problem. (i) The quantity of material is immense. In the United States, approximately 2 billion tons of manure is produced each year and more than 70% of this comes from cattle. In terms of solids, biochemical oxygen demand (BOD), or nitrogen, this waste represents 10 times that attributed to the human population. (ii) Animal waste has a high organic content and is only slowly degraded. Approximately 20% of it is solids; it has a BOD roughly 7 to 10 times that of domestic wastes and, generally, exhibits a chemical oxygen demand (COD)-BOD ratio between 4 and 10. (iii) Changes in farm technology have concentrated animal waste in limited areas often metropolitan. More than half the cattle waste is generated in feeding operations characterized by confinement of 1,000 to 100,000 animals in restricted areas (18 $m^2$/animal). Intensive farming has no use for manure as a fertilizer.

The extent of the animal waste problem has been abundantly documented, and cattle feedlot waste (FLW) is the greatest concern (1, 8). Accumulation of this waste has overtaxed the assimilative capacity of the environment, and runoff of high BOD liquid by rain wash and leaching endangers natural waters.

Almost all suggestions for resolving the animal waste problem involve some form of biological conversion: oxidative degradation or anaerobic stabilization by microorganisms, application to land, fermentative conversion to produce a useful product, enzymatic modification, and refeeding to the same or different animals. In spite of the crucial role of microorganisms in these propositions, there are only a few reports on the flora of cattle waste. Some are fragmentary; others do not reflect current husbandry practices or are directed toward detection of selected organisms of health significance (2, 4, 11, 12).

As part of research at the Northern Laboratory on the utilization of cattle FLW, we are studying its microbial flora as a basis for evaluating potential solutions. This information

can indicate the inherent capacity of indigenous microorganisms to effectively degrade the waste under controlled conditions (i.e., oxidation ditches or lagoons) or the extent to which they would compete with desired organisms in a directed fermentation under controlled, but septic, conditions (i.e., single-cell protein production, cellulolytic conversions, or methane generation). There is an evident need to assess the consequences of not only releasing these organisms into the environment through land application, but also confining them through refeeding.

In this report, we outline the aerobic microbial population of FLW and runoff in terms of groups of microorganisms and indicate its variability through a year; parallel determinations at sites associated with the feedlot are also given.

## MATERIALS AND METHODS

**Feedlot location.** Samples were taken from a medium-sized (ca. 5,000 to 10,000 head capacity) beef cattle feedlot located about 40 miles south of Peoria, Illinois. The arrangement of this feedlot and associated crop land is diagrammed in Fig. 1. The operation is managed by professional agriculturists in a highly mechanized manner. Feedlot pens and associated structures occupy about 50 acres whereas the 1,400-odd acres remaining are devoted to corn. The entire unit occupies a former floodplain lake of the adjoining Illinois River; this lake was drained about 60 years ago and the soil contains a high concentration of organic matter combined with fine silt and sand. The level fields are drained by two intersecting ditches, 15 to 30 m in width and 1.8 to 2.4 m deep, which roughly divide the area into quarters. Water surface in these ditches varies from .6 to 1.8 m below the surrounding land.

**Animal pens.** The pens are either entirely or par-

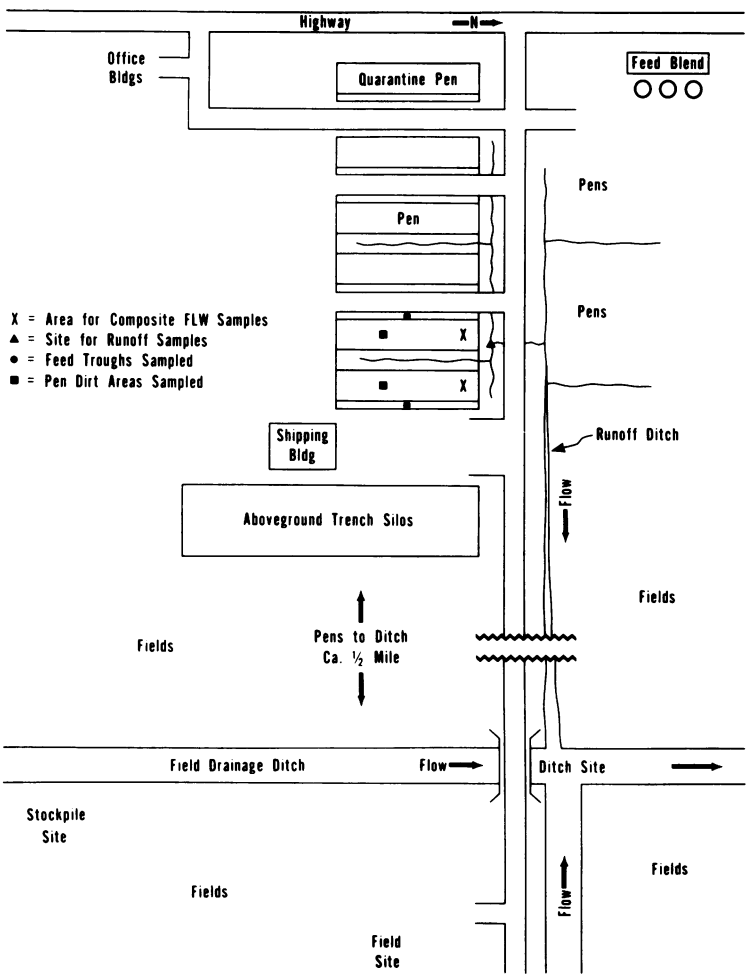


FIG. 1. *Diagram of feedlot and associated crop land.*

tially surfaced with concrete. A continuous concrete feed trough is located along one of the long sides of each pen with a concrete drive separating pairs of pens to facilitate mechanical dispersal of feed. An elevated soil area about 1 m high is maintained in the central portion of each pen for animal comfort; this soil is removed and replaced with fresh soil each year. The peripheral 9 to 15 m of the pens is cleaned daily or nearly so by a tractor-drawn self-loading scraper (small earth mover). The removed waste is hauled in the scraper directly to field areas where it is spread in a thin layer by the same machine.

**Animals and feeding practice.** Beef cattle of apparent Angus or Hereford breed occupied the sampled pens continuously during the year-long study. Approximately 200 animals occupied each 30 by 120 m pen (nominal size). Normally, new animals weigh ca. 225 kg when received. New arrivals are held briefly in quarantine pens and then are placed in the regular feeding pens. Usually, animals are sold at ca. 450 kg at the end of a 4- to 5-month feeding period. Presumably, three different groups of animals were the source of the feedlot waste and runoff from each pen during the year's study.

The animals were fed ad libitum a ration consisting of ensiled, cracked corn supplemented with protein and minerals; the ration contained antibiotic and steroid. Water and salt were continuously available. In this operation, shelled corn (ca. 30% moisture) is coarsely ground and ensiled in vast horizontal silos (ca. 30 by 180 m) constructed above ground of concrete and wood (walls ca. 8 m high). The wet corn is tightly packed by a bulldozer with a roller and covered with black plastic. The ensiled corn appears to undergo a mild yeast-lactic fermentation and has a palatable odor and appearance when fed.

**Samples.** Samples were collected every other month for a year. Each time, samples of FLW and associated runoff were taken, as well as a third sample from a related site. The month when the third sample was collected is given in the description below.

Weather conditions were fairly normal for the location. Temperatures ranged from −13 C (January) to 28 C (July) at the midmorning collection time. Precipitation was significantly lower than the normal 35 inches because of reduced rainfall during the late spring and summer period.

**FLW samples.** The same two representative pens were sampled throughout the study, and the area sampled was within the paved portion (Fig. 1). Six sites in each pen were randomly selected each time. A 3- to 5-g specimen was taken at each of the 12 sites; these specimens were placed together in a sterile container. This composite of 12 specimens was designated the FLW sample. Solids content average: 28% (range: 24% in January and March to 35% in May).

**Runoff samples.** Excess liquid from the pen areas sampled for FLW drains into a common ditch adjacent (Fig. 1). A culvert carries the runoff under an access road when sufficient runoff occurs during wet weather; otherwise, one or more stagnant accumulations occur in the ditch. Samples of runoff were taken either at the culvert entrance or from stagnant liquid, depending on the amount present. The sample consisted of about 100 ml obtained by repeated collection of small volumes by subsurface dipping. Solids content average: 0.78% (range: 0.41% in May to 1.75% in January).

**Related site samples.** Feed: A composite of 12 samples (3 to 5 g each) from along the length of the feed troughs of pens sampled for FLW. Solids content: 64%; November.

Stockpile: A composite of three cores (15 to 20 cm deep) from a site previously used for long-term storage of FLW before the advent of the mechanical scraper-spreader and continuous field application. Reportedly, this "composted" FLW is 0.5 to 1 m deep over several acres of low land between field and ditch; surface level with fields. Cores were taken from a 1-acre area which had no vegetation. Solids content: 29%; January.

Field—March: Composite of four cores (15 to 20 cm deep) from a field area used for disposal of FLW in a thin layer (ca. 5 to 8 cm) during the previous months and scheduled for planting (see Field—September). Solids content: 38%; March.

Field—September: Corresponding samples from the March site with mature corn at the harvest stage. Solids content: 38.5%; September.

Pen dirt: Composite of four cores (15 to 20 cm deep) from the elevated soil area within the two pens sampled for FLW; two cores from each pen. Solids content: 63%; May.

Field ditch: Composite of multiple subsurface dip samples from intersection of the two ditches which drain the fields. Solids content: 0.06%; July.

**Sample preparation.** All samples were immediately cooled by covering the container with cracked ice and were transported in an ice chest to the laboratory. All samples were prepared for plating by serial dilution in 90-ml dilution blanks of sterile 0.1% tryptone; a small quantity of glass beads was used in all dilution blanks to aid dispersion.

A 10-ml portion of well-mixed liquid samples was taken directly into a dilution blank to give the initial 1:10 dilution. FLW and other solid samples were weighed in entirety and washed into a sterilized blendor container with sufficient sterile water to make a 1:4 dilution (w/v). FLW samples were then blended for 30 sec; other solid samples were blended for 60 sec. A 40-ml portion of the 1:4 dilution was then diluted with 60 ml of tryptone diluent to give the initial 1:10 dilution.

A 50-ml portion of liquid samples or the 1:4 dilution of solid samples was weighed and then dried 24 hr at 100 to 103 C for dry weight determinations.

**Plating media and counts.** Enumeration of selected groups of organisms was done by spread plating 0.3 ml of appropriate dilutions onto previously prepared plates. Triplicate plates covering four to six dilutions were prepared for each counting medium. Plating was completed within 5 hr of collection. All plates were incubated at 28 to 30 C. The media and procedures used are outlined in Table 1. A number of other counting media were evaluated

TABLE 1. *Outline of counting procedure*

| Groups of microorganisms | Medium[a] | Incubation (days) | Comment |
|---|---|---|---|
| Total count | Eugon | 2 | Supports growth of most organisms |
| Anaerobes[b] | Eugon + 5 mg of sodium thioglycolate per ml | 4 | After absorption of diluent, plates overlayed with 15 ml of 1.5% water agar. Incubation in sealed jar containing ca. 25% by volume of germinating oats for anaerobiosis |
| Gram negative | EMB | 2 | Total colonies on medium |
| Coliform | EMB | 2 | Differential count on EMB based on colony appearance |
| Sporeformer | Eugon | 3 | 5-ml quantities of four dilutions heat-shocked in sterile screw-capped test tubes at 60 C for 30 min; chilled before plating |
| Yeast-fungi | Mycophil (*p*H 7) + 0.2 mg of dihydrostreptomycin sulfate and 330 $\mu$m of penicillin G per ml. | 3 | Differentiation of yeasts and fungi based on colony appearance; bacteria absent |
| Streptomycetes | Salts-starch agar + 0.5 mg/ml cycloheximide | 7 | Count based on characteristic colony appearance in restricted background of bacterial growth |

[a] Eugon, eosin methylene blue (EMB), and Mycophil media are BBL products (BBL, Division of BioQuest, Cockeysville, Md.). The salts-starch agar is that of Pridham et al. (13).

[b] No attempt was made to enumerate the strict anaerobes by more refined methods.

in a preliminary trial with waste from this feedlot. These included Tergitol-7 for coliforms, Eugon agar plus azide for gram-positive bacteria, Eugon agar plus benzyl penicillin for gram-negative bacteria, Mycophil agar plus cycloheximide for streptomycetes, and Mycophil agar at *p*H 4.7 for yeasts and fungi. None were superior to those used for evaluation of FLW flora.

Triplicate plates of dilutions containing 30 to 300 colonies were counted. Sometimes, streptomycetes, yeasts, and fungi were counted on plates containing 20 to 50 colonies because only these afforded discrete growth. Yeast and fungal counts are presented together because some of the yeasts encountered formed pseudomycelia difficult to distinguish from immature fungus colonies. All counts are expressed as the $\log_{10}$ per gram of sample dry weight (solid samples) or per milliliter of original liquid samples.

Representative organisms were isolated from the counted plates of all samples. Their characterization will be reported in future publications, but results indicate that the groups counted are the type indicated.

## RESULTS

The numbers of organisms in feedlot waste are shown in Fig. 2. Total counts averaged 10 billion/g on a dry weight basis. Overall, the populations of all groups of organisms found in our study is 10- to 1,000-fold higher than previous reports would indicate (2, 9, 10, 14).

In terms of numbers, the indigenous flora is remarkably constant through the year. Although absolute numbers vary somewhat with seasonal conditions, the relative population of the groups of organisms is almost constant. The most abundant single aerobic organism in FLW samples was a yellow pigmented organism, which appears to be in the corynebacterium-mycobacterium group; it always constituted 30 to 50% of the total plate count. In general, counts were lowest in the January sample when −18 to −7 C temperatures were common. Assuming that microbial growth was impossible under these conditions, the similarity of counts in January compared to those in warmer weather indicates that feedlot organisms do not proliferate appreciably in the waste after deposition in the pens. The most consistent seasonal variation was the increasing number of streptomycetes through the summer months.

Gram-negative organisms were the second

most numerous group encountered. The coliform group of organisms, everywhere associated with the feedlot operation, varied between 10 and 40% of the gram-negative population. A complete study of the *Enterobacteriaceae* in this feedlot has been done (5).

No attempt was made to enumerate the strict anaerobes present in the flora of the rumen (3, 6) or intestine. Because the anaerobic procedure used was not adequate for strict anaerobes, representative isolates from anaerobic plates were most often facultative orga-

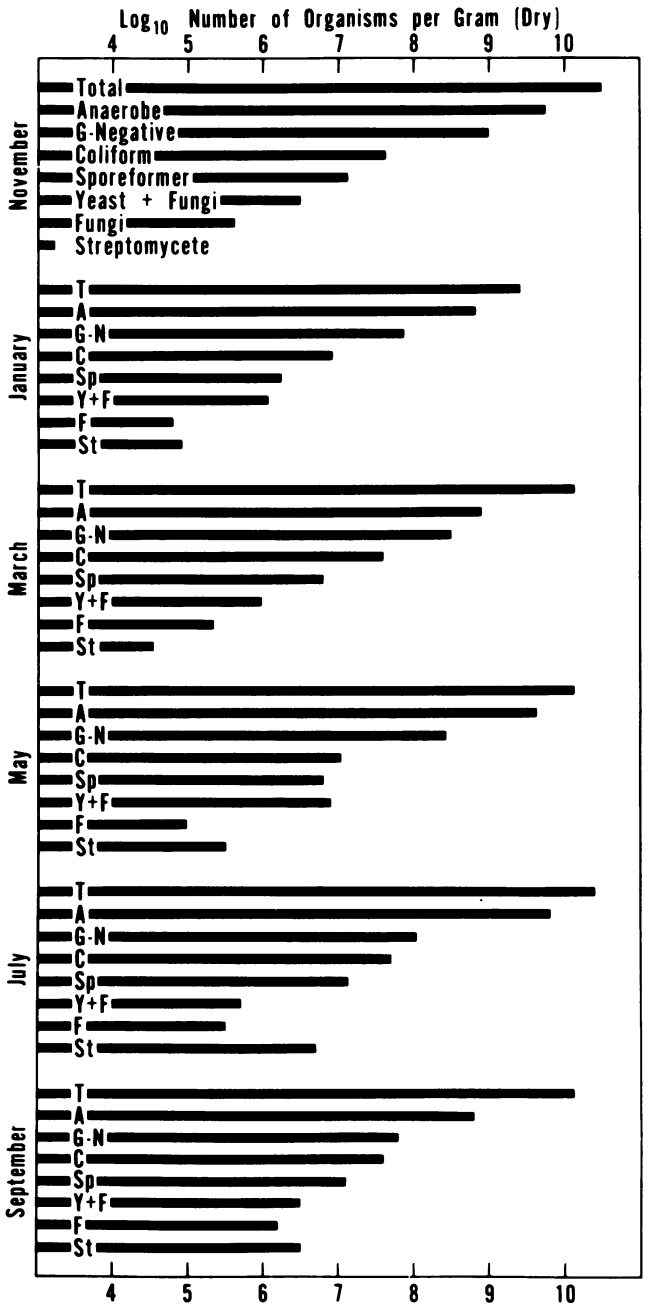


FIG. 2. *Microorganisms in feedlot waste. Numbers are expressed as the $\log_{10}$ of counts per gram of sample on a dry weight basis.*

nisms; microaerophilic or obligate anaerobes were present but less common. About 10% of the colonies did not grow upon transfer from the anaerobic count plates.

As measured by the occurrence of spores in the waste, the number of aerobic sporeforming bacteria does not vary appreciably more than does that of other bacteria. Essentially, only the genus *Bacillus* is enumerated by the method used.

Overall, runoff from the feedlot pens has the same pattern of organisms found in FLW (Fig. 3). Quantitatively, the population varies more than that of FLW because the volume of runoff

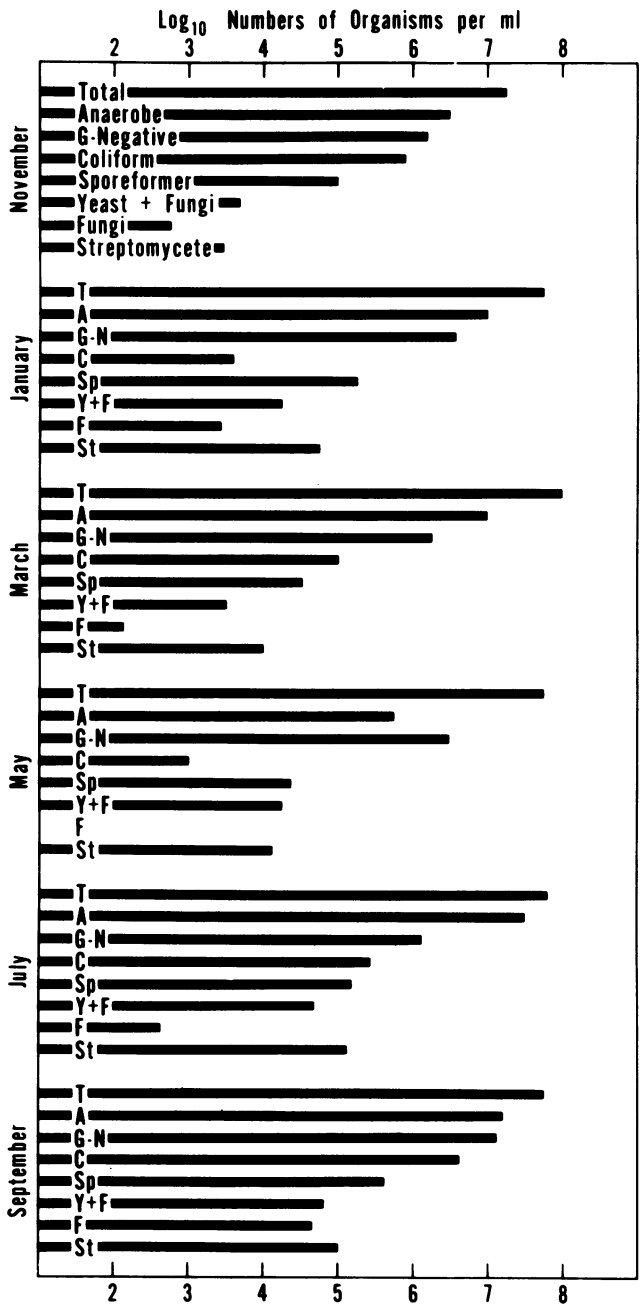


FIG. 3. *Microorganisms in runoff from feedlot pens. Numbers are expressed as the $\log_{10}$ of counts per milliliter of original liquid sample.*

varies. The fewest number of organisms occurred in the November sample when no flowing water was present and temperatures were relatively low. The sample taken as ice in January is not unusual, except for a reduced coliform count. Samples taken in dry months from stagnant liquid closely approximated those of the feedlot, except for fewer coliforms and fungi. Slightly higher counts in stagnant liquid generally reflected higher solids content under these conditions.

The pattern of organisms varied most among the samples taken from the sites related to the feedlot pens (Fig. 4). Of these sites, the low

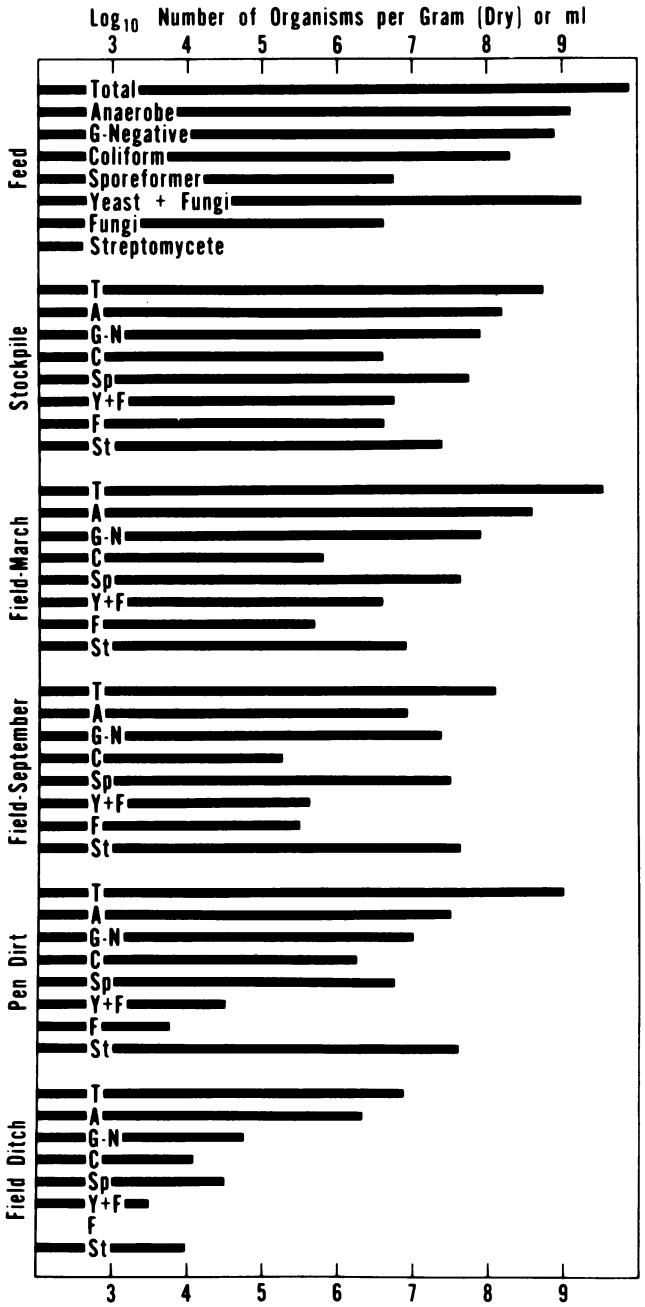


FIG. 4. *Microorganisms at sites associated with the feedlot operation. Numbers are expressed as the $\log_{10}$ of counts per gram of sample on a dry weight basis (solid materials) or per milliliter of original liquid (fluid samples).*

numbers of coliforms and the absence of fungi in the field drainage ditch were reminiscent of runoff samples and quite distinct from the pattern found in samples from the surrounding fields. Direct runoff from the fields is minimal because of the flat terrain.

The field sites had greater numbers of fungi, streptomycetes, and sporeforming bacteria than occurred in the feedlot. However, neither the season of year nor presence of a crop markedly influenced the overall pattern of microorganisms. The 6-month interval since the last application of FLW reduced the numbers of all groups except streptomycetes, but did not otherwise change the pattern typical of field samples. The stockpiled waste most closely resembled field samples. This site of thoroughly stabilized waste had the highest streptomycete count encountered. The pattern of microorganisms in the mounded dirt of the pens resembled that of the FLW itself, except for a greater number of sporeformers and much more abundant streptomycetes.

## DISCUSSION

In many ways the feedlot studied represents efficiently run commercial cattle-feeding operations. However, its specific circumstances provided an opportunity to study several related aspects of the waste problem not often found at a single location. These include: (i) daily removal of FLW from paved surface so that samples represented fresh waste in the state which would be involved in conversion, refeeding, or disposal treatments; (ii) a discrete runoff channel of limited slope so that runoff liquid was always present; (iii) cropped fields used over a period of years for land disposal of the waste at a low application rate with subsurface drainage from these fields into ditches; (iv) an elevated packed earth area in the pens similar to the situation in unpaved feedlots; (v) feed produced and processed at the feedlot location with opportunity for "recycling" microorganisms.

Because of the turnover of animals and their varied ages through the study period, the flora encountered is probably characteristic of raw waste generated by this type of feeding operation. There appears to be only minimal proliferation of FLW organisms in the liquid which drains from the pens. This condition also is indicated by the low number of organisms (ca. $10^7$/ml) reported in lagoons used for treatment of cattle waste (2, 14). It is of interest that coliforms persisted at the field site through the 6-month interval between samples and were often reasonably abundant in water samples. *Escherichia coli* ordinarily does not survive well in either soil or water, apparently because suitable energy sources are limited (7). Nutrient availability, as well as continuous inoculation, probably is responsible for the relative abundance of coliforms at sites receiving FLW. Bromel et al. (2) found a large coliform population (ca. $10^6$/ml) in an animal waste lagoon and in a river receiving drainage from feedlots among other sources. Contrary to the finding of McCalla and Viets (9) that *E. coli* dies rapidly when manure is incubated, coliforms were present in large numbers in the FLW stockpile site.

Dominance of yeasts is the most striking microbial characteristic of the feed. It is evident that they do not proliferate in the animal gut and that comparatively few persist into the waste. The feed also contains a large number of facultative anaerobes, gram-negative, and coliform organisms. Some appear to be lactics from the ensiling process. Streptomycetes are few. Possibly, the gram-negative population in the feed results from inoculation during grain ensiling and feed preparation. The yeasts found at other sites in this study appear to be of only two or three types; they probably arise in the feed.

The pattern of microorganisms in the field, pen, and stockpile sites probably represents the role of certain organisms in the stabilization of the waste after soluble and readily metabolized components are removed by leaching or bacterial action.

Overall, streptomycetes and fungi were the most numerically varied organisms encountered. The numbers of these organisms are related to the time in which the FLW occurred on the site and indicate their role in the ultimate stabilization of the material. The characterization of representative isolates of all groups in terms of their possible role in the disposal or utilization of FLW will be the subject of future reports.

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