Role of Excreted Chlortetracycline in Modifying the Decomposition Process in Feedlot Waste

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Water and air pollution resulting from improper management and disposal of animal wastes is an ever increasing problem in the feedlot industry. The biological stabilization and ultimate recycling of animal wastes is dependent on the activities of varied types of microorganisms as well as environmental conditions. The rate at which the microbial decomposers stabilize the waste is affected by many physical and chemical factors such as moisture, temperature, pH, amount and type of available nutrients, and the presence of metabolic inhibitors.

Dietary antibiotic supplementation has been reported to increase weight gains (1), increase feed conversion efficiency (2), alter rumen microflora (3), and help to maintain animal health in cattle feedlot operations (4,5). Current evidence suggests that the increased occurrence of drug-resistant pathogens in animals receiving constant low levels of antibiotic supplement is a potential public health hazard (6). The results of our studies on feedlot waste decomposition suggest that excreted antibiotic residues may also alter the stabilization process in animal wastes and increase their environmental pollution potential.

Materials and Methods

1. Animal maintenance and sample collection. Two groups of yearling steers were maintained on a high concentrate ration with the experimental group (group B) receiving 70 mg/head/day chlortetracycline. The control animals (group A) received unsupplemented rations.
2. Chlortetracycline extraction and bioassays. Chlortetracycline was extracted from feedlot manure with acid-acetone and bioassayed according to the method of the Association of Official Agricultural Chemists (7) for the measurement of chlortetracycline in animal feeds. Aliquots of the extracted material were autoclaved at pH 4.5, 5, 6 or 7 and assayed with one-quarter inch paper discs substituted for assay cups.
3. Chromatography and bioautography. Acid-acetone extracts of fresh feedlot manure, pharmaceutical grade

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chlortetracycline (American Cyanamid), and the chlortetracycline feed supplement (Aureo Super 500 Premix, American Cyanamid) were examined by descending paper chromatography in a solvent system of n-butanol, acetic acid, and water in the ratio 4:1:5. Paper chromatograms were subjected to bioautography according to the general procedure described by Doskočilová (8).

4. Biochemical oxygen demand studies. All biochemical oxygen demand (BOD_5) determinations were performed with the azide modification of the iodometric method described in Standard Methods for the Examination of Water and Wastewater (9).

Results

1. Characterization of the inhibitory substance. Pooled manure samples from the pens of feedlot cattle receiving 70 mg/head/day chlortetracycline feed supplement were extracted with acid-acetone and bioassayed. The dose response curves of the manure extracts were parallel to those produced by the pharmaceutical grade chlortetracycline. Manure extracts and standards autoclaved at pH 4.5 and 5 produced inhibition zones whereas no inhibition zones were produced by samples autoclaved at pH 6 or 7. Chlortetracycline characteristically loses its biological activity during heating at a neutral pH.

Acid-acetone extracts of feedlot manure and standards were also subjected to paper chromatography and bioautography. Under exposure to short wavelength ultraviolet light, paper chromatograms of manure extracts and standards showed intensely fluorescing zones with equivalent R_f values. Upon subsequent bioautography, growth of the standard test organism was inhibited at the chromatographic positions of the fluorescent materials present in the extracts and in the chlortetracycline standards.

The amount of excreted antibiotic present in the feedlot wastes was determined by quantitative bioassays of fresh feedlot manures. The bioassays demonstrated a chlortetracycline concentration of 14 µg/gm of fresh manure. Antibiotic bioassays of aged manures demonstrated a chlortetracycline concentration of 0.34 µg/gm of manure. Information concerning the persistence of antibiotic residue in manure was obtained in half-life studies on samples incubated at 4, 28 or 37 C. The half-life of the antibiotic residue in feedlot manure was one week at 37 C and greater than 20 days at 4 or 28 C.

2. Effect of dietary antibiotic supplementation on the BOD_5 values of manure. Standard BOD_5 values for fresh manures from the control (group A) and treated (group B) steers were determined. The results demonstrated that

38 percent more of the organic matter was oxidized in group A manure than in manures from animals receiving dietary chlortetracycline. The activities of the microflora present in fresh manure from the control and antibiotic treated animals in the stabilization of the two types of fresh manure substrates were evaluated through cross-seeding BOD_5 experiments. The group A and group B manures were evaluated as substrates for their endogenous microflora by the standard BOD procedure. In addition, each manure was heated to inactivate its microflora and then inoculated with the endogenous microflora or the microflora from the other type of manure. The results of the cross-seedings demonstrated that the group B microflora oxidized 40 percent more of the organic matter in group A manure than in group B manure. When group B manure was compared as the common substrate, the group A microflora removed 31 percent more of the BOD_5 than the group B microflora.

In order to simulate feedlot conditions where the antibiotic concentration was 14 µg/gm in fresh manure, cross-seeding BOD_5 determinations were also conducted in the presence of added pharmaceutical grade chlortetracycline. The final antibiotic concentration was 15 µg/ml of distilled water. The results demonstrated that the group A microflora removed 42 percent more of the BOD_5 of control manure than the microflora from cattle receiving dietary chlortetracycline. The data also revealed that the group A microflora oxidized 143 percent more of the organic matter in group A manure than in group B manure.

Discussion

Quantitative bioassays of fresh feedlot manure revealed that approximately 75 percent of the dietary chlortetracycline was excreted. The antibiotic concentration was 14 µg/gm of fresh feedlot manure, and 0.34 µg/gm of aged feedlot manure. Hungate *et al.* (3) demonstrated that ingested chlortetracycline alters the rumen microflora, hence the possibility that excreted chlortetracycline might also alter the nature and activities of the microflora participating in the decomposition process was further evaluated through comparative BOD_5 determinations on manures from steers in a controlled feeding experiment.

Standard BOD_5 values on manure from control steers and manures from steers receiving dietary chlortetracycline demonstrated that antibiotic supplementation of animal feeds may alter the microflora participating in the stabilization of feedlot manure. Cross-seeding BOD_5 experiments indicated that the effect of ingested chlortetracycline in modifying the subsequent biodegradation of excreted wastes from feeder cattle is

two fold: 1) ingested antibiotic selects for a microbial population relatively inefficient in the stabilization process, and 2) antibiotic supplementation apparently alters the digestive processes in the animal, resulting in manures which are less biodegradable as measured by the standard BOD_5 procedure.

Contamination and eutrophication of domestic water supplies resulting from liquid run-off of feedlot wastes is of public health significance. The reduced stabilization rate as well as reduced biodegradability of manures from animals receiving dietary chlortetracycline, increases the environmental pollution potential of these wastes.

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