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Determination of water quality variables, endotoxin concentration, and *Enterobacteriaceae* concentration and identification in southern High Plains dairy lagoons[1]

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ABSTRACT

The objectives of this study were to determine the concentration of endotoxin, determine 20 water quality variables, and identify and enumerate fungal and bacterial pathogens from United States southern High Plains dairy lagoons and control lakes during summer and winter. Water samples were collected in triplicate from the north, south, east, and west quadrants of each body of water. The mean (± SEM) winter dairy lagoon endotoxin concentration was significantly higher (9,678 ± 1,834 ng/mL) than the summer concentration (3,220 ± 810 ng/mL). The mean endotoxin concentration of the 2 control lakes (summer: 58.1 ± 8.8 ng/mL; winter: 38.6 ± 4.2 ng/mL) was significantly less than that of the dairy lagoons. Two hundred-one *Salmonella enterica* spp. isolates were identified, 7 serovars were recovered from the dairy lagoons, and 259 *Salmonella* ssp. were identified from 5 other dairy locations (milk barn, ditch effluent, settling basin, feed alley pad flush, and center pivots). Twenty-eight *Salmonella* spp. were identified from center pivot water. *Escherichia coli* O157:H7 pathogens were isolated from other dairy locations but not from lagoons. Neither *Salmonella* spp. nor *E. coli* O157:H7 were identified from control lakes. *Enterobacteriaceae* opportunistic pathogens were isolated from both dairies and control lakes. Important mesophilic and thermophilic catabolic (to manure biosolids) fungal isolates were identified from dairy effluent locations, but no thermophilic fungal isolates were cultured from the control lakes. Adequate curing of green forage following center pivot irrigation is important to kill lagoon water enteric pathogens, even though the lagoon water is mixed with fresh water. Recirculating lagoon water to flush the feed alley pad, where cows stand while eating, to remove manure and using lagoon water to abate dairy dust in loafing pens and unimproved dairy roads is inconsistent with good environmental practice management.

Key words: wastewater bacteria and fungi, dairy lagoon, endotoxin, *Salmonella* spp

INTRODUCTION

Increased regulation (both federal and state in the past 10 yr) has occurred because of the potential of concentrated animal feeding operations (**CAFO**) to contaminate the environment with feces and urine. Therefore, air, soil, and water pollution by CAFO have become important public issues. However, few published reports concern various aspects of fecal contamination from CAFO such as hormones, endotoxins, bacterial and fungal toxins, and infectious pathogens, to mention a few (Cole et al., 2000). This is especially true of fungal organisms in dairy lagoons on the United States southern High Plains (**SHP**), which includes Texas, New Mexico, and Oklahoma. It is our intention that facts concerning the concentrations of these pollutants be established in order to better understand the problems and how to solve them. Some questions should be asked. How long do these pollutants stay in the lagoon environment? How quickly are they degraded, and what microbes efficiently degrade these pollutants into harmless substances? What management practices best help speed up the process of biodegradation? What management practices help distribute these pollutants to a larger environmental area? What practices increase the chance of human and animal exposure to these pollutants? And finally, what problems do these contaminants present and how can dairy managers avoid exacerbating them? All of these questions need to be answered; however, we first need to know what types of pollutants exist in CAFO lagoons and their concentrations.

Large dairies with 2,500 to 7,000 head of milking cows are becoming commonplace on the SHP. This is similar to the feeder calf feedyard industry that moved from the Midwest to the SHP 50 yr ago (Uvacek, 1984).

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The present influx of dairies came for the same reasons as the feeder calf industry: greater undeveloped land area for buildings, animals and manure management, and a drier climate. Air and water pollution appeared to be less of a problem because fewer towns or large cities were present and there was less danger of polluting streams and rivers. Also, the presence of shallow lakes (playas) that were ephemeral and from which there was no drainage appeared to have advantages for runoff retention (Purdy et al., 2001b). The Ogallala Aquifer, which lies beneath the High Plains of the United States and runs from western Texas to South Dakota, is very deep (more than 500 feet) and ground water contamination appeared to be less of a problem. The Dockum, a minor aquifer underlying the Ogallala aquifer, extends into parts of western Texas and New Mexico. For reasons such as dust production and possible aquifer contamination, the CAFO are not as welcome as they once were. It is becoming increasingly difficult for dairies to find municipalities that offer favorable economic incentives and welcome them openly (Burchfield and Linderoth, 1999).

The tendency for CAFO is to become larger in order to become more profitable and to garner greater economic advantages. The larger CAFO become, the greater potential there is for environmental problems to develop (Centner, 2001). Pollution of air and water are of primary importance (Thorne, 2007). An important question for which answers are needed is: what types of pollutants exist in CAFO lagoons and in what concentrations are they found compared with control lakes?

Our hypothesis is that dairy lagoon water has sufficient pollutants and pathogens to warrant careful use to prevent the zoonotic spread of pathogens and to prevent pollution of humans and animals. The objectives of this study were to determine the concentration of endotoxin and pathogens and to identify and enumerate viable fungi from 4 SHP dairy lagoons and 2 control lakes during the summer and winter, as well as to determine 20 chemical and physical variables during the summer. A survey of potentially pathogenic fungi and bacteria as well as endotoxin in wastewater from dairies is required to better understand the potential for enhanced exposure to toxins and zoonotic pathogens where dairies use wastewater from lagoons for dust reduction and crop enhancement.

MATERIALS AND METHODS

Dairy Lagoons

Four dairy lagoons were designated as DL 3, DL 4, DL 5, and DL 6. Milk cow numbers per dairy ranged from 2,500 to 7,000 head. Dairy farms themselves were referred to as dairy 3, dairy 4, dairy 5, and dairy 6. The 4 dairies were located in the northeastern part of New Mexico. The mean (± SEM) surface area of the 4 dairy lagoons was 18,864 ± 3,708 m^2.

Control Lakes

Two control lakes were designated as CL 1 and CL 2. Water samples (8 samples/location, 4 in summer and 4 in winter) from the 2 control lakes compared against samples from the 4 dairy lagoons. The control lakes were 1.1 km apart and were 6.2 km from the nearest dairy studied (dairy 5). The surface area of CL 1 was 26,000 m^2 and the surface area of CL 2 was 15,300 m^2. Both control lakes contained fish, were inhabited by ducks and geese, and supported migratory water fowl.

Sample Collection for Endotoxin Analysis

Water samples for endotoxin analysis were collected (8 samples/location, 4 in summer and 4 in winter) in sterile 30-mL glass vials (Wheaton Scientific Co., Millville, NJ) previously heat-treated at 180°C for 3 h to ensure destruction of residual endotoxin. The vials were fitted with new vinyl fluted stoppers and aluminum seals (Wheaton Scientific Co.). Water samples were collected at sites 1 m from the shoreline at the air-water interface in each of 4 quadrants (north, south, east, and west) in each of the 4 lagoons and 2 control lakes, with no disruption of the bottom sediment (Purdy et al., 2001b).

Kinetic Limulus Lysate Assay

Endotoxin was measured by use of the kinetic chromogenic quantitative Limulus amebocyte lysate assay (Williams and Halsey, 1997), which is nonreactive to glucans (Bio Whittaker Inc., Walkersville, MD). Aliquots of the extracts were serially diluted in 10-fold increments with pyrogen-free water. A 100-μL aliquot of each dilution was mixed with 100 μL of freshly prepared Limulus amebocyte lysate containing chromogenic substrate in a pyrogen-free microtiter plate (Dynatech Corp., Chantilly, VA) that was kept at 37°C. Color development was monitored every 15 s with a microtiter plate-reading spectrophotometer (MR5000, Dynatech Corp.). The time interval required to reach 0.03 absorbency was determined and this interval was compared with a standard curve covering the range of 5 ng/mL to 0.5 pg/mL. Standards were linear over a 5-log range on a log-log plot and were used for data analysis. Unknown samples were calculated by linear interpolation. All dilutions were assayed in duplicate and a parallel dilution was spiked with 50 pg of en-