Analysis of Endotoxin in Feedyard Air and Playas: Endotoxin Effect on Market Stressed Feeder Calves

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Endotoxins (ET) are lipopolysaccharides which are derived from the cell walls of gram-negative bacteria and have toxic and pyrogenic effects when injected in vivo. Under mild acid hydrolysis ET is split into Lipid A and degraded polysaccharides, and it is the lipid A that is mainly responsible for the toxicity.

Under natural conditions the target organ for ET is the respiratory tract including the lungs. Endotoxin is a potent toxin which is in close association with organic dust, both of which are associated with acute respiratory illness in humans. A quantitative kinetic version of *Limulus* assay was used to determine the ET concentration of the samples. Samples were shaken for 1 hr and vortexed vigorously for 30 seconds. All samples were centrifuged for 10 min at 3000 x g. Aliquots of the supernatants were tested for the presence of enhancing or inhibiting factors by spiking recovery studies. When detected, these artifacts were eliminated by retesting at higher dilutions. One endotoxin unit (EU) is defined as the potency of the 0.10 ng of the EC5 reference material (U.S. Pharmacopeia).

Feedyard fugitive dust is the number one nuisance problem of feedyards, according to surrounding neighbors and small towns located downwind. Accompanying this problem is the increased pressure exerted by State and Federal regulatory agencies on feedyard owners regarding air, soil, and water quality. Our knowledge of fugitive organic dust is limited and we have essentially no knowledge of feedyard ET concentrations. Also, the impact of ET on the environment and wildlife is unknown. Therefore, establishing the concentration of ET, and determining the concentration and size of feedyard fugitive dust is important. What are the combined effects of organic dust and endotoxin on human and animal health? The search for some of the answers to these two unknowns in the feedyard makes this a worthy research study. In addition, finding a solution to the nuisance problem of feedyard fugitive dust has a high priority.

Endotoxin concentrations in feedyard air, upwind, on-site, and downwind.

The ET concentration of air at 1 meter height was determined for six feedyards, during the winter and summer, with one exception. One feedyard has yet to be analyzed during the summer. The air was analyzed for endotoxin at three positions (upwind, on-site, and downwind) simultaneously with Andersen two-stage air impactors. Two glass Petri dishes were filled with 20 ml of reverse osmosis water (ET background, 0.008 ng/ml) and placed in the Andersen two-stage air impactors (identified as plate 0 which separates out non-respirable particles, and plate 00 which separates out respirable size particles) at each of three positions in the feedyard. To evaluate the efficiency of ET collection with the Petri dishes, three traps were sometimes fitted on the downstream side of the dishes. The two stage impactor separates viable particles into two size ranges with the 50% cut-off diameter of plate 0 (stage-1) at 8.0 µm for spherical particles of unit density. The Andersen air impactors were run for 30 min (one ft^3 per min or 28.3 L per min) in the collection of ET samples. The upwind position served as a control to on-site and downwind positions.

The mean ET concentration of air (standard deviation) of six feedyard (FY) measured in ng/ml, sampled at three positions (upwind, on-site, and downwind) relative to the wind direction when sampled in the winter and summer were as follows: Winter, feedyard (n=6), Upwind-plate 0, 4.2 (7), plate 00, 3 (5); On-Site-plate 0, 6 (7), plate 00, 56 (83); Downwind-plate 0, 2 (2), plate 00, 1 (1); Summer, feedyards (n=5), Upwind-plate 0, 0.83 (1.42), plate 00, 0.17 (0.21); On-site-plate 0, 0.36 (0.43), plate 00, 0.41 (0.29); Downwind-plate 0, 0.10 (0.06), plate 00, 0.64 (0.68).

Endotoxin concentration in feedyard playas (shallow lakes).

The endotoxin concentration of seven feedyard playas and three control (non-feedyard) playas was determined during the winter and summer months over a two year period. Two exceptions occurred: one control playa and one feedyard playa remained dry during the summer months. The playas were sampled by taking 30 ml glass vial samples from just below the top of the water surface, from the north, south, east and west part of each playa.

The mean endotoxin concentration measured in ng/ml (standard deviation) of seven feedyard playas (FY) and three control (non-feedyard) playas (CP), in the winter and summer were as follows: Winter, control playas (n=3), North-mean 112 (27); South-mean 177 (38); East-mean 207 (49); West-mean 128 (16). Winter, feedyard playas (n=7), North-mean 7,245 (7,713); South-mean 7,898 (6,379); East-mean 8,909 (7,578); West-mean 7,953 (6,461); Summer, control playas (n=2), North-mean 224 (174); South-mean 288 (119); East-mean 285 (228); West-mean 270 (52); Summer, feedyard playas (n=6), North-mean 7,949 (6,168); South-mean 8914 (6,041); East-mean 8,923 (6,520); West-mean 8773 (7,294).

Endotoxin-organic dust study conducted on market stressed feeder calves.

A group of 105 market stressed feeder calves were purchased from 3 Eastern TN auction markets and held 5 days in an Eastern TN order buyer barn (OBB). While in the OBB the calves commingled, and were given ad libitum access to alfalfa hay and water. Calves were processed (castrated, dehorned, treated with ivermectin for parasites, vaccinated against infection with *Clostridium chauvoet, C. novyt* type B, *C. perfringens* types C and D, *C. septicum*, and *C. sordellii*, vaccinated against infectious bovine rhinotracheitis and parainfluenza 3 virus infection, weighed, eartagged, bled for sera, and a nasal turbinate mucus specimen was collected from each calf by use of a sterile cotton swab and finally, the calves' rectal temperatures were taken. Every other calf was administered a subcutaneous *Pasteurella haemolytica* vaccine and then shipped 1932 km to the USDA, Agricultural Research Service/Texas Agricultural Experimental Station research feedyard located at Bushland, TX.

After arrival at the feedyard the calves were unloaded, randomly sorted to three pens, fed, watered and allowed to rest overnight. On day one in the feedyard (FY), the calves temperatures were taken, they were weighed, bled for sera, and nasal mucus samples collected. The calves were similarly processed (as on day one) each week they were in the feedyard. The calves on day one were sorted to six pens, two pens for each of the three treatment groups (dust and equipment sound stress, no dust and equipment sound stress, and feedyard control calves).

A large tent (24 ft long x 16 ft wide x 11 ft high) with zipper doors at each end was used to administer the dust and equipment sound stress. The tent was equipped on the ouside with a blower and vacuum, portable industrial type motor (Cadillac products model HP33P) which sucked the dust from a venturi device and pushed the dust through a series of baffles inside the ceiling of the tent which created a uniform dusty environment. Oscillating fans were mounted on opposing corners at baffle height to enhance mixing the dust. Organic dust was made by passing feedyard manure naturally mixed with feedyard clay through a series of graduated screen wire openings and then ground with a motorized mortar and pestle until the dust was fine enough to aerosolize. The size of the dust particles were measured with a modified Coulter counter. Approximately 3 percent of the dust was under 3 μm in equivalent spherical diameter and 25 percent was under 10 μm in equivalent spherical diameter. Each gram of the dust contained 85,990 ng of ET.

After arrival in the feedyard the calves were monitored daily for the first 10 days for signs of acute respiratory tract disease. If a calf maintained a fever of ≥104 F for 48 hrs it was treated with antibiotics for three days. On FY day two 1/3 of the calves were treated with 300 g of the organic dust in the tent plus the equipment sound stress, 1/3 of the calves were submitted to the equipment sound stress in the tent with no dust, and 1/3 of the calves were left under feedyard environmental conditions. This process was repeated with the same calves on FY day three, and twice on FY day four.

Calves were grouped according to dust treatment and were analyzed for the following variables - *Pasteurella haemolytica* vaccination status, morbidity, mortality, treatment episodes, and rectal temperature. No significant differences were found. The total antioxidant capacity (mean=4,410 IU/ml of serum) of non-stressed calves (n=105) first entering the OBB was compared to the same calves after seven days of market stress (mean=3,999 IU/ml serum) on FY day two, and this difference was significant ($P \leq 0.002$). It may be possible to increase the total antioxidant capacity of calves prior to their entering market stress. Will this make the calves more resistant to acute respiratory tract disease when they arrive at the feedyard following marketing stress?