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Effects of intranasal administration of a lysozyme/zinc/carbopol preparation on health and performance of newly received beef cattle

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Introduction

Morbidity of lightweight cattle newly received to the feedlot environment results in major economic losses to the feedlot industry through the added costs of medication and decreased performance during the feeding period (Smith, 1998). The period of time that receiving cattle are most likely to develop bovine respiratory disease (BRD) is during the first 45 d at the feedlot (Edwards, 1996). Typically, metaphylactic medication protocols have been the methods of choice for producers to improve health during the days following receiving. These protocols are highly effective (Galyean et al., 1995; Duff et al., 2000); however, the potential exists to evaluate alternate methods to address respiratory infections.

During an inflammatory response, lysozyme, one component of the compliment system, is secreted (Tizard, 1996). Lysozyme has a low molecular weight (15 kD; Yoon, et al., 1999), and hydrolyzes the β-glycosidic bonds of Gram+ and Gram– bacterial cell walls (Tizard, 1996), thereby causing cell death. Data are lacking regarding the effects of lysozyme on the health of beef cattle. Therefore, we conducted an experiment to determine whether intranasal administration of lysozyme in a complex with zinc acetate and carbopol, would improve health and performance of lightweight, newly received cattle.

Experimental Procedures

Ninety-one crossbred (British x Continental) steer and bull calves (average BW 508.5 ± 38.6 lb) were shipped 1,370 km from an order buyer facility in Meridian, MS to the Texas Tech Burnett Center in New Deal, TX. The cattle were in transit approximately 15 h and were received at approximately 1000 on November 8, 2002. After unloading, cattle were kept in two soil-surfaced pens where they had access to water for approximately 2 h. Cattle were processed at approximately 1200, which included: individual BW measurement; ear-tagging with a uniquely numbered ear tag; horn tipping (as needed); identification of bulls; deworming with moxidectin (Cydectin, Ft. Dodge Anim. Health, Overland Park, KS); an IBR-PI3 vaccination (Prism 9, Fort Dodge Anim. Health); and vaccination with a clostridial vaccine (Vision 7 with Spur, Intervet, Millsboro, DE). At processing, the cattle were assigned randomly to treatment with an intranasal dose of a lysozyme/zinc/carbopol preparation, (**LYS**) or an intranasal dose of water/glycerol (**CON**). Treatments were administered via a pneumatic drenching gun (90 psi pressure) at the rate of 1 mL/nostril. One hundred milliliters of LYS contained 2.5 g of lysozyme, 2 g of zinc acetate, 1.25 g of Carbopol 940, and 75 mL of glycerin, brought to volume with deionized water, CON contained 75 mL of glycerin brought to 100 mL volume with deionized water. Cattle

were blocked based on processing order, the first 18 were in Block 1, the next 18 were Block 2, and so on until cattle were allotted to a total of five blocks. Each block consisted of two pens, and treatment (LYS or CON) were randomly assigned to the two pens within a block. Thus, there were five pens per treatment, with nine animals per pen (one CON pen consisted of 10 animals). After completion of processing, cattle were taken to their pens, (20 ft x 103 ft; with 16 ft of linear bunk space), where the pen was fed approximately 42 lb of long-stem alfalfa hay along with 36 lb of the 65% concentrate receiving diet (Table 1). The percentage of bulls per treatment did not differ ($P > 0.05$) using Chi-square analysis (24% bulls for LYS and 11% for CON), and bulls were left intact for the 28-d receiving period.

Each day at approximately 0730, cattle were examined by trained personnel for signs of BRD. To eliminate the potential for bias, these personnel were blind to treatment. Cattle displaying signs of BRD were removed from their pen and taken to the working area for a more thorough examination. When rectal temperature was $\geq$ 103.5 ^{o}F, the animal was administered tilmicosin phosphate (Micotil, Elanco Anim. Health, Indianapolis, IN) at the label dose, and flunixin meglumine (1 mL/100 lb of BW i.v.). Following treatment, the animal was returned to its pen. If a calf continued to display signs 2 d following the initial antimicrobial therapy, it was taken to the working area, and if its rectal temperature was $\geq$ 103.5 ^{o}F, it was administered a second antibiotic treatment (florfenicol, 6mL/100 lb BW; Nuflor, Schering Plough Anim. Health, Union, NJ). Cattle that were treated once for BRD were classified as a "single-treat," and those that were medicated two or more times were classified as "multiple treats."

Cattle were fed the receiving diet once daily at approximately 0745. Alfalfa hay was delivered at approximately 0900 for the first 6 d after arrival. The quantity of alfalfa hay fed was gradually decreased over the first 6 d, whereas the quantity of milled feed was increased. By d 7, all cattle were offered only milled feed. Weekly bunk samples were obtained to determine the DM content of the feed. On d 14, bunks were swept clean of orts, cattle were individually weighed, and each steer received a booster dose of IBR-PI3 (Prism 9, Fort Dodge Anim. Health). On d 28, cattle were individually weighed to end the receiving trial.

Performance data (ADG, DMI, and feed:gain ratio) were analyzed as a randomized complete block design using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC), with pen as the experimental unit. Block was the random effect. Health measurements were analyzed as binomially distributed data using PROC GENMOD of SAS.

Results and Discussion

Performance data are presented in Table 2. No effects ($P > 0.10$) of LYS were noted for ADG from d 0 to 14, d 14 to 28, or from d 0 to 28. Similarly, no treatment effects ($P > 0.10$) were noted for feed:gain during those periods. However, LYS tended to decrease DMI from d 0 to 14 ($P < 0.08$) and from d 0 to 28 ($P < 0.11$). Additionally, a trend ($P < 0.12$) for increased morbidity (Table 3) was observed when cattle were administered LYS at receiving. Moreover, administration of LYS increased morbidity ($P < 0.05$) 5 d following receiving (Table 3).

Based on the results of the present study, the administration of lysozyme intranasally increased morbidity, most notably later in the receiving period compared with CON cattle.

This likely resulted in the decreased DMI observed for LYS-treated cattle. These data disagree with those of Akinbi et al. (2000), who reported an improvement in health and survivability of mice with elevated concentrations of lysozyme protein in bronchoalveolar lavage fluid following exposure to respiratory bacteria. It should be noted, however, that the cattle used in the present study only received lysozyme in the naso-pharynx region, whereas the mice used by Akinbi et al. (2000) had the increased lysozyme deeper within the respiratory tract. Because lysozyme has been shown to be bactericidal against Gram+ and Gram– bacteria (Tizard, 1996), perhaps by introducing lysozyme into the naso-pharynx region, microorganisms present were killed, which may have facilitated subsequent colonization by *Mannheimia haemolytica* and/or *Pasturella multicoda.* This might explain why cattle that received LYS at processing had an increase in BRD morbidity later in the receiving period (i.e., d 5) than CON cattle.

Implications

Intranasally administering a complex of lysozyme, zinc, and carbopol decreased DMI and increased the percentage of morbidity from bovine respiratory disease. It is unclear, however, the extent to which lysozyme killed bacteria in the naso-pharynx region, which might have allowed other, potentially pathogenic, bacteria to colonize the region. Perhaps administration of lysozyme deeper within the respiratory tract may be of greater benefit. Further research is warranted to examine the exact effects of intranasal lysozyme on bacterial populations within the naso-pharynx region and to examine alternate methods to deliver lysozyme to ensure its presence and activity in the upper respiratory tract.

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Table 1. Ingredient composition of 65% concentrate receiving diet

Ingredient	%, DM basis
Steam-flaked corn	46.84
Cottonseed hulls	19.00
Alfalfa hay	19.17
Molasses	3.58
Fat	3.00
Urea	0.71
Cottonseed meal	5.15
Supplement[a]	2.54
Chemical analyses, DM basis[b]	
DM	81.9
ADF, %	22.0
CP, %	13.8
Ash, %	6.3

[a]Supplement contained, DM basis: cottonseed meal (23.36%), Kemin Endox (antioxidant; 0.50%), limestone (42.11%), dicalcium phosphate (1.04%), potassium chloride (8.00%), magnesium oxide (3.56%), ammonium sulfate (6.67%), salt (12.00%), cobalt carbonate (0.0017%), copper sulfate (0.1572%), iron sulfate (0.1333%), EDDI (0.0025%), manganese oxide (0.2667%), selenium premix, 0.2% Se (0.10%), zinc sulfate (0.8450%), vitamin A, 650,000 IU/g (0.0122%), vitamin E, 275 IU/g (0.1260%), Rumensin 80 (0.6750%), and Tylan 40 (0.4500%).

Table 2. Effects of administration of intranasasl lysozyme on performance and health of newly received beef steers

Item	Treatment[a]		SE[b]	OSL[c]
	Lysozyme	Control		
Daily gain, lb				
d 0 to 14	1.47	2.02	0.34	0.28
d 14 to 28	2.10	1.96	0.23	0.67
d 0 to 28	1.79	1.99	0.17	0.46
Dry matter intake, lb/d				
Hay[d]	1.94	1.83	0.08	0.37
d 0 to 14[e]	7.02	7.09	0.25	0.07
d 14 to 28	11.08	12.08	0.45	0.17
d 0 to 28[e]	9.04	9.99	0.32	0.10
Feed:gain				
d 0 to 14[e]	7.49	4.08	2.45	0.38
d 14 to 28	5.42	6.10	0.67	0.51
d 0 to 28[e]	5.26	5.06	0.40	0.74

[a]Lysozyme = intranasal dose of lysozyme/zinc/carbopol (1 mL/nostril); Control = intranasal dose of a glycerol/water solution.

[b]Standard error of treatment means, n = five pens per treatment.

[c]Observed significance level.

[d]Long-stem alfalfa hay was fed for the first 6 d after arrival.

[e]Includes the long-stem alfalfa hay.

Table 3. Effects of administration of intranasal lysozyme on health of newly received beef steers

Item	Treatment[a]		OSL[b]
	Lysozyme	Control	
Morbidity, %			
Single treatment[c]	64.4	47.9	0.11
Multiple treatments[d]	41.4	31.8	0.49
No. of animals treated by day[e]			
Day 0	0	0	-
Day 1	0	0	-
Day 2	12	13	0.86
Day 3	2	2	0.98
Day 4	2	1	0.54
Day 5	12	4	0.02
Day 6	1	1	0.98
Day 7	0	0	-

[a]Lysozyme = intranasal dose of lysozyme/zinc/carbopol (1 mL/nostril); Control = intranasal dose of a glycerol/water solution.

[b]Observed significance level.

[c]Percentage of animals treated at least once for bovine respiratory disease.

[d]Percentage of animals treated more than once for bovine respiratory disease.

[e]Number of cattle treated once for bovine respiratory disease by day after arrival.