Comparison of Bovine Transfer Factor and Tilmicosin Phosphate: Effects on Health and Growth Performance of Newly Arrived Feedlot Heifers

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ABSTRACT

Two experiments were conducted to evaluate bovine transfer factor (TF) for use in receiving cattle. In Experiment 1,665 crossbred beef heifers initially weighing 225 kg were used in a completely randomized design to determine the effects of bovine TF on the health and performance of beef cattle during a 36-day receiving period. During initial processing, heifers received either a subcutaneous injection of tilmicosin phosphate at 10 mg/kg of body weight or an oral drench which provided 700 mg of TF isolated from bovine colostrum. Heifers given bovine TF during initial processing received an additional 700 mg of bovine TF per day in the diet between day 2 and 5. Heifers were monitored for clinical signs of undifferentiated bovine respiratory disease (UBRD), and heifers exhibiting signs of UBRD received antibiotic therapy. In experiment 2, rumen fluid was incubated with casein or with bovine TF and was analyzed for *in vitro* NH_3-N and total amino acid-N concentrations, which were used to calculate the rate of protein degradation. The percentage of heifers treated once, twice, or three times for UBRD was greater for heifers given bovine TF than for heifers given tilmicosin phosphate. During the 36-day receiving period, there were no differences between bovine TF and tilmicosin phosphate with respect to dry-matter intake, average daily gain, or gain efficiency of heifers. The rate of *in vitro* protein degradation was greater for bovine TF than for casein. Orally administering bovine TF as a prophylactic treatment against UBRD in cattle is not as effective as prophylactic medication with tilmicosin phosphate, possibly because of extensive degradation of bovine TF protein by rumen microflora.

INTRODUCTION

Undifferentiated Bovine respiratory disease (UBRD) is the leading cause of morbidity and mortality in feedlot cattle, providing for morbidity and mortality rates of 75 and 50%, respectively.[1] Treatment for UBRD in feedlot cattle generally requires antibiotic

therapy, which fosters public concern regarding the usage of antibiotics in livestock.

Transfer factors are products of T lymphocytes, seem to consist entirely of protein, and are rather small, with a molecular weight of approximately 5,000 Da.[2] Transfer factors are antigen specific and possess the unique ability to transfer delayed-type hypersensitivity and cell-mediated immunity from an individual previously exposed to a specific antigen, to a naive recipient.[3] Transfer factors seem to be highly conserved among species.[4] and bovine transfer factor (TF) has been shown to transfer antigen-specific, cell-mediated immunity to calves against Eimeria bovis.[5] to chickens against laryngotracheitis virus and infectious bursal disease virus.[6] and to humans against intestinal cryptosporidiosis.[7,8] and recurrent Herpes simplex virus infections.[9-11] Currently, bovine TF is being marketed to cattle producers as prevention against bovine respiratory disease. While there is no observable loss of biological activity when transfer factors are administered orally in monogastric species[12,13] suggesting that the chemical structure of transfer factors allow them to resist digestion in the gastrointestinal tract, data is lacking regarding oral administration of TF in functional ruminants.

The objectives of this experiment were to compare oral administration of bovine TF with the antibiotic tilmicosin phosphate as a prophylactic treatment against BRD in receiving cattle and to measure the ruminal degradability of bovine TF protein.

MATERIALS AND METHODS

All experimental procedures used were approved by the Kansas State University Institutional Animal Care and Use Committee, protocol number 1977.

Experiment 1: Animals, Initial Processing, Treatments, and Diet

A total of six hundred and sixty-five crossbred beef heifers initially weighing 225 kg were used in a completely randomized design to determine the effects of bovine TF on the health and performance of beef cattle during a 36-day receiving period. On day 1, heifers were processed within 24 hours of arrival. Initial processing included measurement of body weight; vaccination against bovine respiratory syncytial virus (BRSV), bovine virus diarrhea (BVD), infectious bovine rhinotracheitis (IBR), and parainfluenza (PI3) by using modified live viruses (Bovisheild 4, Pfizer Animal Health, Exton, PA); vaccination against common clostridial diseases by using a clostridial bacterin-toxoid (Fortress 7, Pfizer Animal Health); recording of rectal temperature; and treatment for internal and external parasites (Phoenectin, Pheonix Scientific, St. Joseph, MO). In addition, heifers received either 10 mg of tilmicosin phosphate (Micotil, Elanco Animal Health, Indianapolis, IN) per kg of BW subcutaneously or 700 mg of bovine TF isolated from bovine colostrums delivered orally via dose syringe. Oral administration of bovine TF was accomplished using using 50 mL aliquots of a solution consisting of water and 28 g of a commercial source of bovine TF (Stress Formula, 4Life Research, Sandy, UT). Immediately after initial processing, heifers within each treatment were assigned randomly among 28 pens. Pens contained 21 to 27 heifers each, depending upon pen size, with a total of 14 pens per treatment. Heifers given bovine TF during initial processing received an additional 700 mg of bovine TF per day in the diet as a top dress on days 2 thru 5.

Heifers were offered a diet containing (dry matter basis) 44% steam-flaked corn, 45% alfalfa hay, 6% corn steep liquor, 3.8% soybean meal and 1.2% vitamins and minerals for ad libitum consumption. At the end of the 36-day receiving period, heifers were weighed and final body weight was recorded.

Experiment 1: Health

Heifers were monitored for clinical signs of UBRD including depression, lethargy, anorexia, coughing, rapid breathing, and nasal or ocular discharge and were not showing clinical signs associated with any other organ system at the time of their classification.

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Heifers exhibiting signs of UBRD received antibiotic therapy consisting of tilmicosin phosphate as a first-time and second-time treatment for UBRD, and oxytetracycline (Liquamycin LA200, Pfizer Animal Health, Exton, PA) and dexamethasone as a third-time treatment for UBRD. The number of times a heifer was treated for UBRD was recorded and ranged between 0 and 3. Mortalities were evaluated via gross pathological examination as to cause of death at the Kansas Veterinary Diagnostic Laboratory, Manhattan, KS.

Experiment 1: Statistical Analysis.

Growth performance data were analyzed as a completely randomized design by using Proc Mixed of SAS (SAS Inst. Inc., Cary, NC), with pen serving as the experimental unit and model effects consisting of treatment. Means were separated by the overall F-test. The incidence of treatment for UBRD and mortality data were analyzed as non-parametric data using Proc Freq of SAS to generate a chi square statistic. An alpha level of 0.05 was used for all analyses to decrease the probability of committing a Type 1 error.

Experiment 2: Preparation of Inoculum

In vitro incubations of rumen fluid alone (control), with casein, or with TF were conducted according to procedures described by Broderick.[14] Whole rumen contents were obtained from two ruminally cannulated Jersey steers fed a diet containing 76% steam-flaked corn, 10% alfalfa hay, 3% soybean meal, 1.2% urea, 5% cane molasses, and 4.8% of a mineral vitamin premix (dry-matter basis) offered for ad libitum consumption. Whole rumen contents were strained through two layers of cheesecloth, and the removal of any particle-associated organisms was attempted by washing solid residue remaining on the cheesecloth four times with prepared McDougall's buffer[15] at a total volume equal to that of the original volume of strained rumen fluid. The strained rumen fluid and buffer solution mixture was then filtered through eight layers of cheesecloth and was composited.

The final inoculum contained (per liter) 450 mL of strained rumen fluid, 450 mL of buffer extract from washed solids, 234 mg of 2-Mercaptoethanol, 50 mL of a maltose solution containing 100 mg/mL of maltose, 25 mL of a 60-mM hydrazine sulfate solution, and 25 mL of a chloramphenicol solution containing 1.80 mg/mL of chloramphenicol. Hydrazine sulfate and chloramphenicol were added in an attempt to inhibit microbial uptake and metabolism of NH3 and amino acids.

Experiment 2: Treatments and Incubation

Forty mg of N from either casein or Stress Formula (N concentrations of casein and Stress Formula were predetermined according to analysis of Kjeldahl N, AOAC, 1990) were weighed into 500-mL Erlenmeyer flasks, and 100 mL of McDougall's buffer was added. Flasks containing buffer alone (control), buffer plus casein, or buffer plus Stress Formula were then incubated for 1 hour at 39°C in a temperature-controlled room. A total of twelve flasks were used, providing four replications per treatment.

In vitro incubations were initiated by adding 200 mL of inoculum to each flask while flushing with CO_2. The incu-

Table 1: Effects of prophylactic treatment with either tilmicosin phosphate (TP) or bovine transfer factor (TF) on treatment incidence for undifferentiated bovine respiratory disease (UBRD) and percentage mortality in newly arrived heifers during a 36-day receiving period.

Item	Treatment		
	TP	TF	*P*-value*
Pens, n	14	14	-
Heifers, n	333	332	-
Number of times treated for UBRD			
1, %	47.5	73.2	<0.01
2, %	14.7	31.9	<0.01
3, %	4.8	18.1	<0.01
Mortality, %	1.2	0.9	0.71

*Chi square statistic.

bation was 4 hours in duration, and a 1-mL sample was collected immediately after the addition of inoculum (0 hour) and every 30 minutes thereafter. Upon sampling, the 1-mL samples were placed into disposable microcentrifuge tubes containing 0.25 mL of chilled 25% w/v trichloroacetic acid and were stored at -20° C until subsequent analysis.

Experiment 2: Sample Analysis and Calculation of Rate of Protein Degradation

For analysis, samples were thawed at room temperature and then centrifuged for 15 minutes at $21,000 \times g$, and the resulting supernatant was analyzed for NH_3 and total amino acid concentration according to Broderick and Kang16 (1980) by using a Technicon III AutoAnalyzer (Technicon Instruments Corp., Tarrytown, NY).

Although the *in vitro* incubation was conducted over the course of 4 hours, NH_3 and total amino acid concentrations increased only through 1.5 hours, after which NH_3 and total amino acid concentrations began to decrease, suggesting uptake of NH_3 and total amino acids by microbes. Therefore, only time points between hours 0 and 1.5 were used in calculating the rate of *in vitro* protein degradation. *In vitro* protein degradation at each time point was calculated according to the formula: Percentage protein degraded = blank corrected ([NH-N]) + ([total amino acid-N]) / mg N added to flasks. Percentage undegraded protein at each time point was calculated according to the formula: $100 -$ percentage of undegraded protein.

Experiment 2: Statistical Analysis

Rate of protein degradation was determined by using Proc Reg of SAS to regress the natural logarithms of percentage of undegraded protein against time. The resulting slopes represented the rate of protein degradation in fraction/hour. Slopes representing the rate of protein degradation were analyzed by using Proc Mixed of SAS, with flask serving as the experimental unit and model effects consisting of protein source.

RESULTS

Heifers that received tilmicosin phosphate during initial processing required fewer first-time, second-time, or third-time treatments for UBRD ($P < 0.01$) compared with heifers receiving bovine TF (Table 1). A total of seven animals died during the duration of the study. All were due to UBRD. Mortality percentages among heifers receiving either tilmicosin phosphate or bovine TF as a prophylactic treatment against UBRD were 1.1 and 1.0%, respectively, and were not affected by treatment ($P = 0.88$). Treatment did not affect dry matter intake ($P = 0.73$), average daily gain ($P = 0.92$), or gain efficiency ($P = 0.95$) of heifers during the 36-day receiving period (Table 2). Rate of *in vitro* protein degradation was greater ($P < 0.05$) for bovine TF than for casein (Figure 1).

DISCUSSION

Increased morbidity for heifers receiving bovine TF during initial processing suggests that tilmicosin phosphate was more effective as a prophylactic treatment against UBRD than bovine TF. Although

Table 2: Means and standard error of the mean (SEM) for growth performance of newly arrived heifers during a 36-day receiving period after prophylactic treatment with either tilmicosin phosphate (TP) or bovine transfer factor (TF).

Item	Treatment			
	TP	TF	SEM	*P*-value*
Pens, n	14	14	-	-
Heifers, n	333	332	-	-
Initial body weight, kg	224	225	2.8	0.71
Final body weight, kg	270	271	5.1	0.88
Dry matter intake kg/d	5.7	5.6	0.17	0.73
Dry matter intake, % of body weight	1.59	1.56	0.033	0.50
Average daily gain, kg	1.27	1.26	0.088	0.92
Gain:feed, kg/kg	0.220	0.221	0.0109	0.95

*Probability that treatment differences observed are due to random chance.

Figure 1: Rate of *in vitro* protein degradation of casein and bovine transfer factor. Values are means with standard error of the mean; n = 4. The rate of *in vitro* protein degradation was greater (P < 0.05) for bovine transfer factor than for casein.
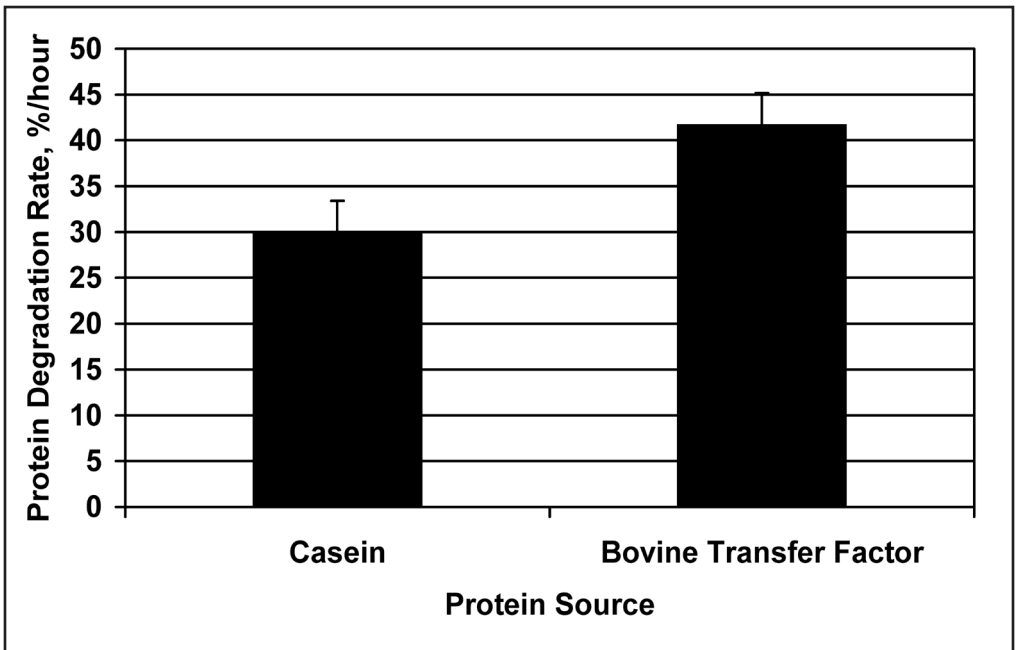
data about bovine TF as a prophylactic treatment against UBRD in cattle is lacking, prophylactic medication with tilmicosin phosphate during initial processing has been shown to be effective in decreasing the incidence of UBRD in newly arrived cattle.[17-19] Although the incidence of UBRD was increased in heifers administered bovine TF during initial processing, the failure of increased incidence of UBRD to affect dry matter intake, average daily gain, and gain efficiency among heifers was unexpected. Bovine respiratory disease has been reported to decrease dry matter intake[20] and average daily gain in cattle.[20,21] However, Galyean et al.[19] reported that during a 28-day receiving period, approximately 46% of control calves were treated for UBRD, compared with 0% of calves that had received tilmicosin phosphate as a prophylactic treatment against UBRD at time of initial processing, yet dry matter intake, average daily gain, and gain efficiency were not affected.

An increased rate of *in vitro* protein degradation for bovine TF, compared with that of casein indicates that bovine TF protein

is rapidly degraded in the rumen. Casein is commonly used as a standard for measuring protein degradability because it is rapidly and extensively degraded by ruminal microflora. The degradation of bovine TF protein by ruminal microflora might have contributed to the failure of bovine TF to protect against UBRD as effectively as tilmicosin phosphate in our experiment. Kirkpatrick4 (2000) sequenced cyanogen bromide digests from both bovine and murine transfer factors and found a conserved amino acid sequence among peptide fragments. Fragments containing the amino acid sequence failed to transfer expression of delayed-type hypersensitivity, indicating that the entire TF molecule is required to transfer cell-mediated immunity.

Results of our experiments indicate that orally administering bovine TF as a prophylactic treatment against UBRD in cattle is not as effective as prophylactic medication with tilmicosin phosphate, possibly because bovine TF protein is readily degraded by rumen microflora. Future research with bovine TF in functional ruminants should be

conducted using ruminally protected bovine TF. However, it is not known how current methods of protecting ingredients from ruminal degradation (i.e. lipid coating) might affect the biological activity of bovine TF.

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