Antimicrobial supplementation alters digestibility and ruminal fermentation in a continuous culture model*

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ABSTRACT
A dual-flow continuous culture system was used to evaluate the effects of laidlomycin propionate and bacitracin zinc on fermentation, nutrient digestibility, and microbial efficiency. Factors were laidlomycin propionate (LP; 2 mg/L of culture volume) and bacitracin zinc (BAC; 1.4 mg/L), and treatments were as follows: (1) no BAC or LP (CON), (2) LP without BAC (LP), (3) BAC without LP (BAC), and (4) LP and BAC (LP/BAC). A fifth treatment was supplemented with monensin sodium (MON; 6 mg/L) to act as a positive control. Both LP and LP/BAC had significantly greater pH than MON (P < 0.05). Antibiotic treatment did not affect NH₃-N concentration (P = 0.62), but did influence total VFA production (P = 0.02). Monensin fermenters had a greater proportion of total VFA than did LP fermenters (P < 0.05), and improved VFA production by 7.2% compared to CON. Laidlomycin suppressed the production of both acetate (P < 0.01) and butyrate (P = 0.05), and acetate was further reduced when LP was fed in combination with BAC (P = 0.01). Laidlomycin numerically reduced the acetate:propionate ratio (P = 0.12). These results suggest that bacitracin may produce ionophore-like effects on pH and VFA production in vitro.

Introduction
Feed-grade antibiotics have been used in beef cattle production for over 60 years. Specifically, ionophores have been widely selected for their ability to modify the ruminal environment through inhibition of gram-positive bacteria. This selectivity results in increased ruminal propionate production, and a corresponding decrease in acetate, butyrate, and methanogenesis (Marounek et al. 2002). A recent survey of feedlot consulting nutritionists reported that more than 95% of clients administered an ionophore in their finishing diets (Samuelson et al. 2016).

Laidlomycin Propionate, marketed as Cattlyst (Zoetis, Parsippany, NJ), was first launched in 1994 as an alternative ionophore to monensin. Its popularity grew due to no reduction in intake commonly seen when monensin sodium is fed. Due to a series of acquisitions within the first few years of its approval, the product failed to capture traction in the market. Furthermore, since it accounts for such a small percentage of cattle on feed, limited studies exist evaluating its effects. Still, based on the results of initial studies and potential for growth in the face of certain industry changes, there has been renewed interest in this compound.

Bacitracin is a polypeptide antibiotic that has been used extensively as an antimicrobial and growth promoter in both swine and poultry (Xie et al. 2013). Literature evaluating bacitracin in cattle is limited; however, several studies have reported improved propionate production and decreased methane in vitro (Russell and Strobel 1988; Spires and Froetschel 1992; Van Nevel and Demeyer 1992). While similar effects have been observed on ruminal fermentation, bacitracin and ionophores differ in their mode of action. Unlike ionophores, which selectively inhibit gram positive bacteria by altering the ion flux of the cell membrane, bacitracin inhibits the synthesis of peptidoglycans necessary for the development of the cell membrane (Stewart and Strominger 1967). The objectives of the current study were to evaluate the combination effects of ionophore and bacitracin supplementation on ruminal fermentation, digestibility, and microbial efficiency in a dual-flow continuous culture model.

Materials and methods
All procedures involving the use of live animals were conducted within the guidelines of and approved by the Texas Tech University Animal Care and Use Committee (Protocol No. 16079-08).

Continuous culture apparatus
Ten 1-L, dual-flow continuous culture fermenters (Bellco Glass, Vineland, NJ) were used to determine the effects of antimicrobial supplementation strategy on fermentation and microbial efficiency in a continuous culture system similar to that described by Wilson et al. (2008). The fermenter flasks were immersed in a water bath equipped with an IsoTemp 2100...
immersion circulating heater pump (Fisher Scientific, Hapton, NH) that maintained the temperature at 39°C. McDougall’s artificial saliva (McDougall 1948) was infused via a Carter-Monostat 12/6 (Barnant Inc., Barrington, IL) peristaltic cassette pump. Flow rate for each flask was adjusted prior to initiation to maintain an infusion of 55 ± 1.7 mL/h. Each fermenter contained a magnetic stir rod that was controlled by an individual stir plate located beneath the flask. An overflow port allowed effluent to flow from the flask into 3.8-L plastic containers that were located beneath the continuous culture system.

**Treatments and experimental design**

Five treatments were used in a randomized complete block design with 2 replicated periods of 7 d. Each period consisted of 4 d of adaptation followed by a 3-d sampling period. The treatment arrangement was a 2 × 2 × 1 factorial design, with 2 levels of bacitracin zinc (BAC; Zoetis, Florham Park, NJ; 0 and 1.4 mg/L of culture volume) and laiddolmycin propionate (LP; Zoetis, Florham Park, NJ; 0 and 2 mg/L of culture volume). A fifth treatment was supplemented with monensin sodium (MON; Sigma-Aldrich Co., St. Louis, MO; 6 mg/L of culture volume), the most commonly utilized ionophore in the U.S. beef cattle production system (Samuelson et al. 2016), in order to act as a positive control. These in vitro doses mimicked actual feeding rates that would be applied in vivo (mg/kg diet DM). Fermenters were blocked (n = 2) within period by location relative to the peristaltic pump used to infuse artificial saliva. Location was determined to be close (n = 5) or far (n = 5) distance from the pump, and the 5 treatments were randomized within block for each period.

Due to equipment failure, 1 fermenter (LP + BAC) was removed from the second period. Additionally, the second collection period was ended on the morning of d 6 due to malfunction of the immersion pump that maintained the temperature of the water bath.

**Treatment application and routine management**

Prior to study initiation, the individual components of the basal diet (Table 1) were dried in a forced air oven at 55°C for approximately 48 h. The diet components were then combined in their appropriate proportions and mixed by tumbling for 3 min in an 18.9-L plastic bucket. The total mixed ration was then ground to pass a 2-mm screen in a Wiley mill, and 20-g portions were individually weighed into 50-mL screw cap tubes to be fed throughout the experiment. Fermenters were fed 40 g DM/d of the basal diet (Table 1) in 2 equal portions at 0830 and 2030 daily for the duration of the study.

Treatments were applied at each feeding. Prior to study initiation, treatment solutions containing LP (2 mg/mL) and MON (6 mg/mL) were prepared in 100% ethanol. Bacitracin zinc was prepared in a 35.7% ethanol solution to achieve a desired concentration of 0.5 mg/mL. All treatment solutions were stored in screw top, smoked-glass flasks and stored at 4°C for the duration of the experiment. Immediately following feed deliveries, 0.5 mL of LP and MON solutions were applied directly to their respective flasks. For BAC treated flasks, 1.4 mL of solution (35.7% ethanol) was added for a total of 0.7 mg of bacitracin zinc per feeding. This was done to ensure equal volumes of ethanol were applied to each flask. Since the combination treatment (LP + BAC) received 0.5 mL of LP and 1.4 mL of BAC for a total of 1-mL of ethanol at each feeding, an additional 0.5 mL of 100% ethanol was added to flasks receiving only BAC, LP, or MON alone. For the treatment receiving 0 mg/mL BAC and 0 mg/mL LP, 1 mL of 100% ethanol was applied at each feeding. In summary, flasks received one of the following concentrations of antimicrobials daily: (1) no antimicrobials, (2) 1.4 mg of bacitracin zinc, (3) 2 mg of laiddolmycin propionate, (4) 1.4 mg of bacitracin zinc plus 2 mg of laiddolmycin propionate, or (5) 6 mg of monensin sodium. Concentrations of ionophores (LP = 0 or 2 mg/L and MON = 6 mg/L of culture volume) and antimicrobials (BAC = 0 or 1.4 mg/L of culture volume) were chosen to equate to approximately 0 or 10 mg/kg of dietary DM for LP, 30 mg/kg of DM for MON, and 0 or 70 mg/(head · d) for BAC in an animal consuming 10 kg of DM/d with a ruminal volume of 50 L. Immediately following application of basal diet and treatment solutions at 08:30 h and 20:30 h daily, flasks were flushed with CO₂ for 30 s to ensure anaerobic conditions were maintained.

**Cattle and ruminal fluid collection**

Two ruminally cannulated Angus heifers (BW = 520 kg) were used as ruminal fluid donors for the experiment. The heifers were housed in an earthen-floor pen and fed once daily at approximately 1000 h to provide an intake of 10 kg of daily DM. Heifers were fed the antibiotic-free basal diet (Table 1) for 3 months prior to collection of inoculum to ensure adequate wash-out and adaptation to the experimental diet.

One day prior to beginning the experiment, a sufficient quantity of ruminal fluid to inoculate all 10 fermenters was collected approximately 4 h after feeding, strained through 2 layers of cheesecloth, placed in a sealed insulated container, and transported 30.5-km to the laboratory. During the first period, a composite of equal amounts of fluid from each

| Table 1. Ingredient and analysed chemical composition (DM basis) of diet. |
|----------------|-------------|
| Item           | %, DM       |
| Steam-flaked corn | 73.61      |
| Cottonseed hulls  | 6.04       |
| Alfalfa hay      | 5.97       |
| Fat (yellow grease)| 5.46     |
| Cottonseed meal  | 4.79       |
| Supplement¹     | 1.99       |
| Calcium Carbonate| 1.25     |
| Urea            | 0.89       |
| Analysed composition (DM basis), %       |
| Dry matter    | 82.00     |
| Crude protein  | 14.35     |
| NDF           | 18.26     |
| ADF           | 9.04      |

¹Supplement composition (DM basis): 69.010% cottonseed meal; 15.000% NaCl; 10.000% KCl; 3.760% urea; 0.986% zinc sulfate; 0.500% Endox (Kemin Industries); 0.167% manganese oxide; 0.196% copper sulfate; 0.157% vitamin E (500 IU/g); 0.125% selenium premix (0.2% Se); 0.083% iron sulfate; 0.010% vitamin A (1,000,000 IU/g); 0.003% ethylenediamine dihydroiodide; and 0.002% cobalt carbonate.
²CP = %N x 6.25 (N measured by LECO analysis); NDF and ADF were determined by the methods of Van Soest et al. (1991).
donor was obtained; however, during the second period, the entire quantity of fluid required for inoculation was collected from 1 heifer due to the expulsion of the ruminal cannula from the second donor animal on the morning of scheduled collection. After returning to the laboratory, the ruminal fluid was bubbled vigorously with CO2 to ensure that the contents remained under anaerobic conditions.

One litre of inoculum was added in random order to each fermenter and was again bubbled vigorously with CO2 prior to experiment initiation. The flasks were immersed in the pre-warmed, circulating water bath. Magnetic stir bars were inserted, the lids were fastened, and the peristaltic pump and stir bars were activated. Upon initiation, 10 g (DM basis) of the basal diet was added to each flask. Thereafter, 20 g of the basal diet and respective treatment solutions were added to each flask at 0830 and 2030h daily.

**Sampling procedures**

Experimental periods were 7 d, with the initial 4 d for equilibration and the final 3 d for sample collection. Beginning on d 5, aliquots (10-mL) of ruminal fluid were collected directly from each flask immediately prior to morning feed delivery at 08:30 h, 10:30 h, 12:30 h, 14:30 h, and before feed was added at 20:30 h daily for the determination of pH (Accumet Basic pH metre, Fisher Scientific, Hampton, NH). Another 1-mL aliquot of ruminal fluid was collected in a 2-mL microcentrifuge tube, 200 μL of a 25% (wt/vol) meta-phosphoric acid solution was added to stop fermentation, and samples were vortexed and frozen for subsequent analysis of volatile fatty acids (VFA). In addition, at 0900 h daily, the 3.8-L effluent from the fermenter contents were retained and preserved at −20°C and two 200-mL samples of effluent were collected and frozen at −20°C, washed with 0.9% NaCl, and re-centrifuged. The bacteria were then separated from the supernatant by centrifuging at 20,000 × g for 20 min and 4°C, washing with 0.9% NaCl, and re-centrifuging. The final pellet was suspended in distilled water, lyophilized, and stored for subsequent analysis. Due to small bacterial yield, lyophilized bacterial isolates were composited by treatment within period prior to analysis. Samples were analysed for N using the Leco analyser previously described, the purine content of the lyophilized bacterial fraction and dried effluent was determined by the procedure of Zinn and Owens (1986) as modified by Ushida et al. (1985), and ash was determined. Diet samples were analysed for DM, ash, ADF, NDF, and N by the methods previously described for fermenter and effluent samples.

**Statistical analysis**

Data were analysed as a randomized complete block design with a 2 × 2 + 1 factorial arrangement of treatments using individual fermenter as the experimental unit. Volatile fatty acid, pH, and NH3-N data were analysed as repeated measures using the MIXED Procedure of SAS (SAS version 9.4; SAS Inst., Cary, NC). Time was used as the repeated measure and compound symmetry was used as the covariance structure based on lowest AIC for all dependent variables as the selection criteria. Model included the effect of treatment, time post-feeding, day of collection, and their interactions. Microbial efficiency and OM digestibility variables were analysed using the GLIMMIX procedure of SAS since these data did not have a normal distribution of the errors. For all data, period and
block within period were considered random effects. The Kenward-Roger adjustment was used to correct the degrees of freedom and a Tukey’s adjustment was applied to correct for pairwise comparisons. Non-orthogonal contrasts were performed to test treatment main effects and interactions of laidlomycin propionate and bacitracin inclusion. Results are reported as least squares means and were separated using the pairwise comparisons, PDIF, option of SAS 9.4. For all analyses, an alpha level ≤ 0.05 was considered significant, and tendencies were declared for values between 0.051 and 0.10.

Results

**Ph, ammonia N, and volatile fatty acids**

Antimicrobial treatment affected pH (P = 0.03) of fermenter flask contents (Table 2), however, no treatment × time interaction was observed (P = 0.22). Monensin had the lowest pH of all antimicrobial treatments, and was lower than that of LP (P = 0.03) and LP/BAC treated flasks (P = 0.05). While pH was numerically lower than all other treatments across all time points, MON supplementation decreased pH compared to LP/BAC (P = 0.03) at 12 h post-feeding (Figure 1). Although treatment had no effect on NH₃-N concentration (P = 0.62), total VFA production was impacted by the addition of the various antimicrobials (P = 0.02). Specifically, MON increased production of VFAs (P < 0.05) when compared to LP, and numerically increased production by 7.2% when compared to negative controls. An LP × BAC combination was observed for molar proportion of acetate, Laidlomycin suppressed the production of both acetate (P < 0.01) and butyrate (P = 0.05), and acetate was further reduced when LP was fed in combination with BAC (P = 0.01). Propionate production was not different between treatments (P = 0.13), but was numerically increased by 14.6% with the addition of MON. While individual treatment did not affect (P = 0.12) the acetate:propionate ratio, the inclusion of laidlomycin propionate showed a significant reduction (P = 0.03) when analysed as a main effect. The inclusion of bacitracin zinc increased valerate production (P = 0.02), and tended to increase the production of isovalerate (P = 0.07). Furthermore, there was a significant LP × BAC interaction for both valerate (P < 0.01) and isovalerate (P = 0.01), as the addition of both LP and BAC showed the greatest improvement in the production of both VFAs.

**OM digestibility and microbial efficiency**

Effects of antimicrobial treatment on measures of OM, NDF, ADF, and N digestibility, microbial N production, and microbial efficiency are presented in Table 3. No significant differences were observed for any of these variables (P ≥ 0.24). When fed alone, both LP and BAC numerically reduced apparent (−10.5 and −4.9%, respectively) and true (−10.1 and −4.5%, respectively) OM digestibility. However, when fed in combination, LP/BAC increased apparent and true OM digestibility by 3.0 and 2.5%, respectively. This resulted in an LP × BAC interaction that approached the threshold for declaring significance (P = 0.15 and P = 0.19 for apparent and true OM digestibility, respectively). No differences in microbial OM synthesis (P = 0.95) or microbial N flow (P = 0.95) were observed.

**Discussion**

The application of antimicrobial feed additives in beef cattle production is believed to be a contributing factor to the development of antibiotic resistance (Van Boeckel et al. 2015). You and Silbergeld (2014) suggested that the repeated exposure to low doses of antibiotics created ideal conditions for the emergence and spread of antibiotic-resistant strains. This mechanism of emergence has been of particular concern in the context of livestock production, as current application generally consists of low doses fed for an extended durations of time. In order to address this issue, the FDA has implemented new policies to curtail the use of medically important antibiotics in the production of livestock. These measures have had implications on the use of antimicrobials in beef production. Therefore, research evaluating the impact of those antibiotics deemed not medically important to humans – including bacitracin zinc, laidlomycin propionate, and

Table 2. Effect of antimicrobial application on pH, VFA, and ammonia in a continuous culture fermentation system.

| Item          | Treatment1               | SEM | P – value2               |
|---------------|--------------------------|-----|--------------------------|
|               | CON          | LP    | BAC    | LP/BAC    | MON    | TRT | Time | TRT × Time | LP        | BAC    | LP × BAC |
| pH            | 4.2779        | 4.85  | 4.76   | 4.85ab    | 5.63b   | 0.050 | 0.03 | < 0.01     | 0.22      | 0.17   | 0.73    | 0.52    |
| NH₃-N, mM     | 0.71          | 0.75  | 0.82   | 0.77ab    | 0.68    | 0.065 | 0.62 | < 0.01     | 0.59      | 0.93   | 0.32    | 0.87    |
| VFA, mM       | 43.34         | 40.48b| 43.08a | 36.63ab   | 41.80ab | 4.188 | 0.03 | < 0.01     | 0.95      | 0.01   | 0.20    | 0.01    |
| Acetate       | 14.50         | 12.11a| 13.04ab| 12.84ab   | 14.74a  | 2.233 | 0.03 | < 0.01     | 0.99      | 0.05   | 0.57    | 0.75    |
| Butyrate      | 62.25         | 64.74 | 66.53  | 66.84     | 71.37   | 4.720 | 0.13 | < 0.01     | 0.34      | 0.56   | 0.20    | 0.70    |
| Valerate      | 4.34         | 3.94ab| 4.36ab | 6.51ab    | 5.19ab  | 0.730 | 0.03 | < 0.01     | 0.74      | 0.10   | 0.02    | < 0.01  |
| Isobutyrate   | 0.74          | 0.76  | 0.72   | 0.79ab    | 0.77    | 0.057 | 0.71 | 0.01       | 0.07      | 0.24   | 0.89    | 0.29    |
| Isovalerate   | 1.55          | 1.52  | 1.55   | 1.74      | 1.58    | 0.119 | 0.12 | < 0.01     | 0.15      | 0.15   | 0.07    | 0.01    |
| Total VFA     | 125.87ab      | 122.46b| 128.89ab| 124.39ab  | 134.94a | 2.587 | 0.02 | < 0.01     | 0.63      | 0.14   | 0.36    | 0.70    |
| Acetate:propionate ratio | 0.72 | 0.62 | 0.67 | 0.55 | 0.59 | 0.121 | 0.12 | < 0.01 | 0.83 | 0.03 | 0.19 | 0.12 |

1. CON = negative control; LP = 2 mg laidlomycin propionate/L culture volume; BAC = 1.4 mg bacitracin zinc/L culture volume; LP/BAC = 2 mg laidlomycin propionate + 1.4 mg bacitracin zinc/L culture volume; MON = 6 mg monensin sodium/L culture volume.
2. Non-orthogonal contrasts were used to evaluate LP = main effect of LP supplementation, BAC = main effect of BAC supplementation, LP × BAC = interaction of LP and BAC treatments.
3. Volatile fatty acid.
4. Acetate to propionate ratio.
Monensin – and investigations into new supplementation strategies of these antimicrobials is warranted.

Monensin is the most widely used ionophore in beef cattle production and is fed in over 90% of finishing diets (Samuelson et al. 2016). Its ability to inhibit the growth of gram-positive ruminal bacteria has been thoroughly elucidated (Russell and Strobel 1989), and it has been consistently reported to increase the production of propionate, decrease the production of both acetate and butyrate (Richardson et al. 1976; Russell 1987), decrease protein degradation to ammonia (Yang and Russell 1993), and increase ruminal pH (Russell and Strobel 1989; Domescik and Martin 1999). Domescik and Martin (1999) reported that 5 ppm monensin supplementation increased propionate production by 32% in a continuous culture study. While others have reported increases of similar magnitude (47% at 5 ppm; Richardson et al. 1976), the current study was only able to realize a 14.7% improvement (6 ppm; \(P < 0.05\)) compared to negative controls. These results are similar to those presented by Smith et al. (2010), who reported a 17% improvement when monensin was included 6 ppm. Previous studies have also reported significant decreases in butyrate production. Domescik and Martin (1999) observed a 76% decrease when in vitro cultures were supplied 5 ppm monensin, and Richardson et al. (1976) reported reductions of nearly 50% when cattle were supplemented with 200 mg/(head · d). In the current study, butyrate production was unaffected by

![Figure 1](image_url). Effect of antimicrobial treatment on pH of continuous culture flask contents. CON = negative control; LP = 2 mg laidlomycin propionate/L culture volume; BAC = 1.4 mg bacitracin zinc/L culture volume; LP/BAC = 2 mg laidlomycin propionate + 1.4 mg bacitracin zinc/L culture volume; MON = 6 mg monensin sodium/L culture volume. (a) At 12 h post-feeding, LP/BAC had significantly greater pH than MON (\(P = 0.03\)).

| Item                      | Treatment 1 | \(P - value^2\) |
|---------------------------|-------------|------------------|
|                          | CON LP BAC LP/BAC MON SEM TRT LP BAC LP x BAC |
| Input, g/d                |             |                  |
| DM                        | 40.0 40.0 40.0 40.0 40.0 | – | – | – | – |
| OM                        | 38.7 38.7 38.7 38.7 38.7 | – | – | – | – |
| N                         | 0.92 0.92 0.92 0.92 0.92 | – | – | – | – |
| True OM flow, g/d         | 14.40 16.95 15.60 13.66 15.45 1.471 | 0.50 0.82 0.44 0.15 |
| Microbial OM synthesis, g/d| 1.00 1.00 1.05 0.91 1.00 | 0.121 | 0.95 0.56 0.85 0.47 |
| Apparent OM digestibility, % | 62.79 56.20 59.70 64.70 60.08 3.800 | 0.50 0.82 0.44 0.15 |
| True OM digestibility, %   | 65.37 58.78 62.41 66.97 62.66 3.800 | 0.55 0.77 0.47 0.19 |
| NDF digestibility, %       | 56.42 49.48 53.98 51.39 49.90 6.800 | 0.92 0.50 0.97 0.97 |
| ADF digestibility, %       | 57.62 51.30 52.80 53.95 49.47 6.600 | 0.90 0.71 0.88 0.85 |
| Total N flow, g/d         | 0.62 0.68 0.69 0.64 0.68 0.023 | 0.24 0.85 0.64 0.16 |
| Ammonia flow, g/d         | 0.02 0.02 0.02 0.02 0.02 0.002 | 0.57 0.53 0.38 0.50 |
| Microbial N flow, g/d     | 0.08 0.08 0.07 0.07 0.08 0.009 | 0.95 0.74 0.51 0.51 |
| Feed N flow, g/d          | 0.53 0.59 0.59 0.55 0.59 0.024 | 0.32 0.74 0.56 0.28 |
| Microbial efficiency^3    | 3.14 3.56 3.28 2.69 3.27 0.464 | 0.56 0.83 0.31 0.13 |
| Total N digestibility,%^4  | 40.48 34.30 33.27 37.94 34.21 2.610 | 0.28 0.78 0.51 0.24 |

1CON = negative control; LP = 2 mg laidlomycin propionate/L culture volume; BAC = 1.4 mg bacitracin zinc/L culture volume; LP/BAC = 2 mg laidlomycin propionate + 1.4 mg bacitracin zinc/L culture volume; MON = 6 mg monensin sodium/L culture volume.
2Non-orthogonal contrasts were used to evaluate LP = main effect of LP supplementation, BAC = main effect of BAC supplementation, LP x BAC = interaction of LP and BAC treatments.
3Expressed as g of N/kg of OM truly fermented.
4Corrected for microbial synthesis.
antimicrobial supplementation, and monensin treated fermenters produced butyrate levels approximately equal to those of controls. Similarly, while other studies have reported reductions in acetate (Richardson et al. 1976; Russell and Strobel 1988; Domescik and Martin 1999), no differences were observed in the current study. A characteristic increase in pH was not observed in this study, but values were not different than negative controls. The lack of effect of monensin on measures of in vitro digestibility in this study are consistent with the results of others (Duff et al. 1994; Smith et al. 2010). Few studies have reported the effects of monensin on microbial cell yield and efficiency, but Van Nevel and Demeyer (1977) did report a significant reduction in microbial growth that was not observed in the current study.

In vitro analyses of laidlomycin propionate have varied. Some studies have reported increased total VFA production (Bauer et al. 1995), while others have concluded that no influence on ruminal VFA concentration exists (Galyean et al. 1992; Domescik and Martin 1999; Quinn et al. 2009). Ruminal lactate production was reported to decrease in a number of evaluations (Spires and Algeo 1983; Bauer et al. 1995), though not to the extent of monensin (Wampler et al. 1998). In the current study, acetate was only numerically reduced with the addition of 2 ppm LP in the presence of bacitracin, however, and we cannot conclude that the reduction was due to the addition of LP. While Domescik and Martin (1999) observed a reduction in the acetate:propionate ratio, it was independent of acetate production which did not differ. This effect was instead a function of increased propionate production when 2 ppm of laidlomycin propionate was combined with mixed rumen contents in vitro, which does not support the results of the current study or previously reported results (Galyean et al. 1992). These inconsistencies were evaluated in more detail by Zinn et al. (1996) who suggested that the variation observed in these measurements was due to dietary magnesium concentrations. He concluded that 76% of the variation observed in feed efficiency could be attributed to the dietary Ca:Mg ratio. Zinn et al. (1996) also concluded that at greater Mg concentrations, LP decreased acetate production (13.2%) and increased propionate production (26.5%). Additional studies confirmed these observations and were able to realize improved propionate production while decreasing the acetate:propionate ratio when laidlomycin was supplemented with adequate levels of magnesium (Chirase et al. 1987). Inconsistent effects have been observed on ruminal pH in LP supplemented cattle (Zinn and Spires 1987; Galyean et al. 1992).

No significant differences were observed on OM or N digestibility in this study, but Zinn et al. (1996) reported decreased degradation of feed N (13.9%) which was similar to the numerical reduction observed in the current study (15.3%).

Research evaluating both monensin and laidlomycin propionate on rumen fermentation is extensive. However, literature surrounding the effects of bacitracin and comparative analyses of these various antimicrobials are less common. Russell and Strobel (1988) first investigated bacitracin in vitro and reported responses that were ‘strikingly similar’ to those of monensin. While bacitracin is clearly not an ionophore, it has been shown to have similar inhibitory effects on the growth and proliferation of gram-positive bacteria. It acts by inhibiting the synthesis of peptidoglycan, which comprises nearly 90% of gram-positive bacterial cell wall, thereby reducing structural integrity and inducing cell death (Stewart and Strominger 1967). Though its mechanism of action has been defined, its effects have been variable when compared to those of monensin.

Russell and Strobel (1988) evaluated the effects of monensin and bacitracin on in vitro fermentation of mixed contents from a nonlactating dairy cow. With corn as the substrate, both antibiotics significantly increased the production of propionate and decreased the accumulation of ammonia. While Marounek et al. (1998) reported significantly greater molar proportions of propionate with bacitracin, the shift in VFA profile was accompanied by a decrease in total VFA, resulting in total production that was similar to controls. This evaluation was conducted in the mixed contents from rabbit caeca, characterized by much greater proportions of bacitracin-resistant gram-negative bacteria than the rumen, which may explain why the results differ from other studies. In a further evaluation, Marounek et al. (2002) reported a significant reduction in total VFA production when starch was incubated with bacitracin in vitro, but observed more than twice the percentage of propionate. In the current study, BAC showed a numerical increase in propionate production of 7% with no effect on total VFA. Both Russell and Strobel (1988) and Van Nevel and Demeyer (1992) reported that bacitracin was less inhibitory to fibre digestion than monensin; however, in the current study, no differences were observed on either OM or N digestibility, however, this is not surprising as the diet fed to the in vitro fermenters was primarily concentrate based as exhibited by a NDF content of less than 20% (DM basis). Thus, there might be potential that bacitracin might offer some ionophore like effects when fed to cattle. Further research in regards to bacitracin in high concentrate diets is warranted, especially using an in vivo model.

Conclusion
The application of gram-positive antibiotics in mixed rumen cultures affects fermentation parameters including production of specific VFA’s and pH. The addition of 2 ppm laidlomycin propionate decreased the production of both acetate and butyrate and improved pH over monensin. Monensin (6 ppm) was able to numerically improve total VFA production and the molar proportion of propionate, but did not reduce the production of acetate or increase pH as reported in previous literature. Bacitracin increased the production of the secondary VFAs valerate and isovalerate, and when combined with laidlomycin propionate significantly reduced the production of acetate in continuous culture. Bacitracin is sparingly used in beef cattle production. However, concerns surrounding antibiotic resistance and new legislation addressing these concerns are likely to impact the application of this antibiotic and the ionophores evaluated in this study. In light of these changes and the results observed in the current study, it is important that research continues on alternative supplementation strategies which utilize feed additives that are not subject to these regulations.
Disclosure statement

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