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Optimising rumen modifier use for feedlot performance and carcase attributes

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Abstract

Inclusion of fermentation modifiers in the diets of feedlot cattle is standard industry practice to increase feed use efficiency and reduce risk of ruminal acidosis. With few new rumen modifiers reaching market, there is an imperative to identify strategies to maximise the performance response to existing modifiers. A series of studies was undertaken to investigate the response to providing Monensin in combination or in daily rotation with alternate rumen modifiers. Small differences in rumen fermentation attributes were observed between cattle supplemented with Monensin alone or Monensin with Lasalocid or Bambermycin in combination or in daily rotation. There were no effects of rumen modifier treatment on bacterial Genera in the rumen that exceeded 2% of the biome. A feedlot study with 450 head then evaluated performance of cattle supplemented with Monensin alone, Lasalocid alone or with Monensin and Lasalocid in daily rotation. Monensin cattle had a faster average daily gain for the first 83 days on-feed (2.57 kg/d) and the lowest Feed:Gain ratio (4.40 kg/kg) over the entire 109 days on feed. Modifier treatment did not affect daily DM intake, carcass weight or any measured aspect of the carcass. Daily rotation of Monensin and Lasalocid is not recommended for feedlots with leading rates of cattle growth and feed-use efficiency, as it offers no advantage over supplementing with Monensin alone.

Executive summary

Rumen modifiers are included in rations for most feedlot cattle to reduce the incidence of lactic acidosis and improve the efficiency of feed-use during finishing. While there are a number of probiotic and non-antibiotic compounds emerging for lactic acid control, there is a need to explore options to get maximum performance from existing rumen modifiers. A series of animal house studies followed by a feedlot finishing study were undertaken to quantify the effects of rotating and or of combining currently used rumen modifiers.

Initial technology evaluation trials were conducted to evaluate the 'eSense' (Allflex Australia) accelerometer eartag as a tool to quantify rumination time of cattle, and the smaXtec intraruminal bolus as a way of monitoring rumen pH. These studies were conducted in rumen cannulated cattle. The 'eSense' eartag provided a useful means of quantifying time cattle spent ruminating, showing a strong correlation with visual assessment. The smaXtec bolus was not found as useful in its application, with some boluses failing to initialise and most showing initial bias and time-associated drift in the pH reported, relative to a manual determination of pH in buffer or in rumen fluid collected *in-situ* from immediately in front of the smaXtec pH sensor membrane.

A set of intensive studies were then conducted addressing rumen fermentation when provided with Monensin alone (M), or in combination (with Lasalocid [L] or Bambermycin [B]), or in daily rotation with L or B during the diet adaptation period, then during the finisher period. The study during adaptation (Starter, two transition diets and commencing on Finisher) identified a clear differentiation between pairing of M with B compared to pairing M with L in fermentation but not in the major bacterial genera. The pairing with L offered significant advantage over pairing with B through a consistent pattern of change. Monensin alone or paired (rotation of combination) with L supported the highest pre- and post-feeding ruminal pH (manual sample) so the least risk of lactic acidosis. They also offered the lowest redox potential, the lowest acetate percentage and lower NH_3 concentration than treatments with B. On these aspects, M alone or in pairing with L warrants further investigation.

With the same (rumen cannulated) cattle these pH responses did not persist during the extended finisher period. Monensin cattle ate significantly more dry matter (DM) than did other treatment groups; there were no effects on redox potential and, while rumen pH did not show treatment effects for the first 9h post-feeding, at 10 and 11h post-feeding Monensin had among the lowest rumen pH values. There was no effect of rumen modifier on daily methane production or methane yield (methane/kg DMI).

Three rumen modifier treatments (M, L and M+L in combination) were then fed in a large scale feedlot trial (15 pens of each treatment, with 10 cattle/pen) with average daily gain (ADG), dry matter intake (DMI), Feed:Gain (F:G) ratio and carcass attributes determined.

Overall ADG and feed efficiency for all treatments were high (mean ADG = 2.57 kg/hd/d; mean F:G = 4.5 on DM basis) and above average for feedlot cattle, but ADG of M cattle still tended ($P<0.10$) to be greater than of L and M/L fed cattle over the entire 109d. Significant advantages ($P<0.05$) were seen in feed conversion efficiency (+2.6%) throughout the entire trial with continuous inclusion of M compared to L or M/L. Monensin-only cattle had improved average liveweight (+1.2%) and average weight gain (+3.1%) over the first 83 days of the feeding period. When assessed on day 83, no treatment differences in the rumen bacterial biome of any consequence were observed, with no differences in the prevalence of genera that exceeded 0.1% of the bacterial biome. There were no differences in carcass characteristics with modifier treatment. Net economic benefit was the same for all treatments, but cost of gain was significantly lower for M cattle. Rotating ionophores, or inclusion of Lasalocid alone continuously, did not improve feedlot performance compared to continuously feeding M alone.

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1 BACKGROUND

This project addressed the key MISP key imperative of increasing feedlot industry productivity by 1% by 2020 and 5% by 2030 so improving the "Production efficiency in farms and feedlots". By concomitantly reducing the incidence and/or severity of lactic acidosis and assessing behavioural change, the project also addressed the priority area of the "welfare of the animals within our care".

A key component in improving enterprise production efficiency is to improve the efficiency of converting feed to gain and this can be achieved by optimising the rate and products of rumen fermentation to support a propionate rich fermentation with low risk of lactic acidosis. Antibiotic rumen modifiers have been routinely included in feedlot rations for this purpose but with few exceptions (e.g. Laidlomycin "Cattlyst" in USA) no new antibiotic type rumen modifiers are being registered. There is thus a need to refine industry use of existing rumen modifiers to improve feedlot efficiency.

The ionophore Monensin is used in most Australian feedlots, increasing efficiency by causing a slight acceleration of animal growth (ADG) while decreasing feed intake. Recently, Shreck et al., (2016) reported a 4.81% advantage in ADG and 250g DMI/d improvement from daily rotation of Monensin and Lasalocid relative to Monensin alone. Similarly, the potential to combine rumen modifiers to improve productivity has only recently been revisited to overcome intake suppression (Potter and Wagner 1986; Lemos et al., 2016; Benatti et al., 2017). There is a lack of data on response to combination of, or short term rotation of rumen modifiers in Australian feedlot diets. This project sought to confirm and quantify feeding, growth and carcass advantages available to the Australian feedlot industry from combining and rotating Monensin with Lasalocid or Bambermycin (glycophospholipol), and to elucidate the underlying rumen fermentation differences and rumination changes associated with these responses.

2 PROJECT OBJECTIVES

1. Determine the effect of Monensin in combination or rotation with Lasalocid and flavophospholipol on rumen fermentation
2. Determine the effect of Monensin in combination or rotation with Lasalocid and flavophospholipol on feedlot performance and carcase characteristics.
3. Determine the effect of Monensin in combination or rotation with Lasalocid and flavophospholipol on the rumen microbiome

3 VALIDATION OF ACCELEROMETER EAR TAGS TO QUANTIFY RUMINATION TIME

3.1 Abstract

Rumination is an essential process to maintain feedlot cattle health and welfare when fed high concentrate diets. Changes in rumination and other feeding behaviours are considered important indicators for early detection of any rumen disorders (e.g. acidosis, heat stress and bloat). For the purposes of research and on-farm health indication, rumination and other behaviour can be monitored continuously using sensors. This study aimed to 1) test the accuracy of rumination time measured by a commercially available ear tag and 2) to quantify how time spent eating, ruminating and other behaviours differ according to amount of grain in the diet.

Commercial RFID tags (eSense, Allflex Australia), each containing an accelerometer, were fitted to the ears of ten lowline Angus steers housed in individual indoor pens and fed three diets containing different levels of tempered barley. Cattle behaviour was video recorded in 52 2-h blocks over 3 weeks to match with the ear tag recorded data. Validation of the ear tag was performed by comparison with rumination time calculated from observation of video recordings. Regression analysis (observed on predicted) and Bland and Altman analyses were performed to test the agreement between the two methods. The slope of the relationship was compared to one (unity). The regression of these two measurements shows a moderate positive association (r = 0.60, $P<0.001$), the slope of the relationship was 1.03 and was not significantly different from 1 ($P = 0.74$). The tags reported approximately an overall mean bias 8.06 ± 14.9 min/2-h greater than observed.

Data generated from video recordings and ear tags were also used to assess the effect of diets of increasing grain content on rumination time and other behaviours. Among the three different diets, there was a significant difference between starter (49% grain) and intermediate (60% grain) and starter and finisher (82% grain; as fed basis) on eating behaviour ($P= 0.02$ and 0.01, respectively). From the video data, time spent eating starter diets was greater than for either intermediate or finisher diets. From the ear tag data, mean rumination time also differed significantly from starter to intermediate and from intermediate to finisher diets ($P=0.0002$ and $P= 0.005$, respectively). Time spent ruminating on the intermediate diet was greater than for either starter or finisher (Table 5). Time spent for social behaviour tended to decrease as the grain content of diets increased ($P=0.06$). Time spent in other behaviours was not affected by diet. These findings indicated that for research and feedlot purposes, accelerometer-based rumination estimates could be used to assess the possibility of metabolic issues as a result of high content of grain within feedlot diets.

3.2 Introduction

To ensure high ADG of cattle in feedlots the cattle are fed a diet with a high proportion of cereal grain. Grains consist of a large proportion of starch that is quickly fermented in the rumen. Increased inclusion of grain in a feedlot ration increases growth rate and improves feed conversion efficiency. A heavy reliance on high grain diets, however, can trigger a series of ruminal problems as a result of the high rate and extent of starch degradation within the rumen (González et al., 2012)**,** which in turn can have economic and welfare implications (Dixon & Stockdale, 1999; González, Manteca, Calsamiglia, Schwartzkopf-Genswein, & Ferret, 2012). The rumen is well developed to digest fibrous feeds through the presence of anaerobic fibre digesting microbes (bacteria, protozoa, fungi and viruses). This eco-system is

specialized and buffered to a narrow optimal range of pH (6.5-6.7) (Millen, Arrigoni, & Pacheco, 2016). The excessive production of Volatile Fatty Acids (VFA) and of lactic acid, from high concentrations of easily fermentable grain carbohydrates, causes a decrease in ruminal pH that can lead to health and welfare issues (RAGFAR 2007). This is known as acidosis, which is a serious risk in feeding high grain diets to feedlot steers, often occurring without presence of clinical signs (subclinical rumen acidosis: SARA). Acidosis results from a microbial metabolic cascade with non-lactate producing microbes inhibited as pH declines, allowing proliferation of *Ruminobacter amilophillus*, *Streptococcus bovis*, *Lactobacillus spp* and excessive quantities of VFA produced in a periodic abundance of starch (Schwartzkopf-Genswein, Beauchemin, Gibb, Crews, et al., 2003). In general, saliva that goes into the rumen via rumination (chewing and re-chewing feed) provides a strong buffer for rumen pH (Mertens, 1997), but as the amount of rumination time decrease with grain content of diets, low saliva flow contributes to an increased risk of ruminal acidosis.

Rumination is one of the most easily visible digestive behaviours of ruminants. Rumination is a direct indicator of cattle health and wellbeing as it facilitates saliva secretion which buffers ruminal acids produced during carbohydrate breakdown. The measurement of rumination time allows monitoring of rumen health status since chewing during rumination releases saliva that in turn buffers ruminal pH (Mertens, 1997). Thus, before SARA progresses to become a clinically apparent condition, early detection can be achieved through the change in rumination time (Ambriz-Vilchis, Jessop, Fawcett, Shaw, & Macrae, 2015). Another important function of rumination is physical breakdown of coarse particles so they can pass into the omasum through chewing and re-chewing (McDonald, 2002). An increase in stereotypies such as tongue rolling or bar biting, are considered as signs of reduced welfare shown by cattle that fail to express rumination behaviour (Lindström & Redbo, 2000). This drives the hypothesis that, irrespective of rumen load, oral manipulation of feed is a behavioural need of cattle (Lindström & Redbo, 2000). However, rumination is sensitive to change of diets, feeding management, physiology status of cattle and environment (Goldhawk, Schwartzkopf-Genswein, & Beauchemin, 2013). Therefore, the interpretation of rumination time as a health indicator should be carefully assessed as part of the management of the health and welfare of cattle.

To provide information on rumination time, direct observation is used as the standard for evaluating proxies. To permit remote monitoring, the development of different sensors for automatic measurement of rumination is required. Different automatic recording algorithms have been developed based on particle size (bolus) (Chap, Milligan, & Kennedy, 1984), jaw movement analysis (Braun, Zürcher, & Hässig, 2015; Rutter, Champion, & Penning, 1997; Ungar & Rutter, 2006) or mastication sound (Wolfger et al., 2015). However the accuracy and reliability of the data from sensors was often affected by physical factors such as noises from collision, friction and shaking among the sensors. The results generated from evaluation of these devices shows they lack the predictive power required for a health indicator. The measurement of jaw movement by electronic sensors to record regurgitation and rumination behaviour requires their differentiation from other jaw movements (Burfeind et al., 2011; Schirmann, von Keyserlingk, Weary, Veira, & Heuwieser, 2009). There is limited software to automatically classify feeding behaviour (Büchel & Sundrum, 2014) and issues such as collars being uncomfortable and practical issues such as battery lifetime and short time data for collection (Schirmann et al., 2009) constrain technology adoption. Ear-tag sensors are used to predict other activities in free range system (Bikker et al., 2014) and some automatic rumination detectors, such as ear tags and collars, perform well in dairy cattle fed a roughage-based diet (Bikker et al., 2014; Burfeind et al., 2011; Schirmann et al., 2009).

To what extent the automatic devices can accurately predict rumination behaviour on beef cattle fed high concentrate diets remains to be defined. Several studies have shown variations in the accuracy of automatic measurement used to measure rumination on beef cattle. Goldhawk et al. (2013) reported a moderate correlation between visual and "Hi-tag" collar measurements, whereas other have shown a low correlation between direct observation and automatic measurement (Wolfger et al., 2015). However, these findings used different devices and methods to measure rumination on beef cattle. No published studies have assessed the use of ear-tag to quantify rumination time by beef cattle fed different type of diets. The objectives of this study were 1) to compare the accuracy of rumination time calculated by a commercially available ear tag with rumination time from visual observation and 2), to quantify how time in eating and ruminating behaviours differed according to proportion of grain in the diet in cattle on a fixed intake.

3.3 Material and methods

An observational study was conducted at the University of New England Beef Unit, Armidale, New South Wales, Australia, within the winter period of August to September 2018 that aimed to 1) validation test ear-tag accelerometer based estimates of rumination time by beef cattle and 2) to test the effect of diets on time cattle spend in rumination and other behaviour.

3.3.1 Animal, diets and housing

Twelve Lowline Angus steers (2 years of age; 198± 39 kg of body weight) were used in a repeated measures design. Prior to the start of this experiment, all procedures were approved by the Animal Ethics Committee of the University of New England (AEC approval number 18-028).

The steers were not tethered but were free to walk around in individual indoor pens (2.5m x 2m). Each pen was fitted with a water trough, 120L feed bunk and rubber floor mat. The pens were cleaned daily before feeding and animals remained in the pen during cleaning. All steers were adapted to the animal house for two weeks before the start of trial. During the pre-experimental period, the steers were fed a cereal chaff mix consisting of 25% and 75% oaten hay and lucerne chaff respectively (as-Fed basis), once per day at approximately 09.00 h one week prior to the start of the experiment, a radio-frequency identification (RFID) tag mounted with an accelerometer (eSense, Allflex, Capalaba, Queensland, Australia) was fitted in the proximal half of the left ear between the two cartilage folds (Fig. 1).

Figure 1. Placement of an Allflex accelerometer ear tag.

During the experimental period, the steers were fed four experimental diets fixed at 2.0 % (DM basis) of the mean liveweight of all steers (adjusted as necessary after weekly weighing), consecutively increasing in grain content (Table 1), once per day, at approximately 09:00 h, for seven days per diet. The rations were formulated to provide sufficient energy, protein, minerals and vitamin to exceed the nutrient requirement of steers gaining 1.0 kg/d (National Academies of Sciences & Medicine, 2016). The ration was fed as a Total Mixed Ration (TMR), prepared on daily basis. Fresh water was available *ad libitum*. The data from daily recording of feed offered and refusals during the entire trial were used to calculate feed intake. Diet DM content was measured by recording orts from individual steers that were collected daily and composited once weekly and similarly composited feed samples.

Table 1. Formulated composition of rations, as fed

Item	Starter	Intermediate I	Intermediate II	Finisher
Ingredient, % As Fed				
Tempered Barley	48.7	59.9	70.9	81.8
Molasses	4.0	3.3	2.6	2.0
Vegetable Oil	-	0.9	1.8	2.8
Dry Supplement	2.6	2.6	2.6	2.5
Mill Run	10.0	6.7	3.4	-
Whole Cottonseed	11.7	10.7	9.7	8.7
Wheat Straw	7.7	5.3	3.0	2.2
Oaten Hay	15.3	10.6	6.0	-
Nutrient Composition				
Dry Matter	100	100	100	100
Ash, % DM	7.61	7.14	6.67	6.09
TDN, % DM	74.28	75.76	77.22	78.53
ME, Mcal/kg DM	2.69	2.82	2.95	3.09
NEm, Mcal/kg DM	1.77	1.88	2.00	2.11
NEg, Mcal/kg DM	1.15	1.25	1.35	1.46
Starch, % DM	30.31	35.85	41.44	47.08
Fat, % DM	4.37	5.18	6.01	6.98
NDF, % DM	38.00	34.46	30.91	27.47
CP, % DM	13.72	13.65	13.57	13.36
DIP, % DM	9.80	9.83	9.85	9.78
UIP, % DM	3.92	3.82	3.72	3.58
Ca, % DM	0.74	0.73	0.72	0.68
P, % DM	0.39	0.37	0.36	0.34
Mg, % DM	0.26	0.25	0.23	0.22
K, % DM	1.04	0.92	0.79	0.66
Na, % DM	0.11	0.09	0.07	0.04
Cl, % DM	0.32	0.24	0.16	0.07
S, % DM	0.23	0.23	0.22	0.21
Co, ppm	0.43	0.36	0.28	0.18
Cu, ppm	19.22	18.52	17.79	16.69
I, ppm	0.57	0.56	0.56	0.53
Fe, ppm	82.99	59.06	34.94	9.81
Mn, ppm	61.67	53.65	45.48	36.22
Mo, ppm	0.75	0.65	0.56	0.46
Se, ppm	0.15	0.14	0.12	0.10
Zn, ppm	92.34	91.81	91.22	88.11
Vitamin A, KIU/kg DM	2.17	2.20	2.23	2.18
Vitamin E, IU/kg DM	24.66	25.01	25.37	24.76
Salt, % DM	0.25	0.25	0.25	0.25
Urea , % DM	0.39	0.40	0.41	0.40
Vitamin D, IU/kg DM	271.22	275.16	279.12	272.40

3.3.2 Data collection

Every minute of the experimental period was monitored continuously using an ear-tag accelerometer and transmitted through radio frequency technology (ZigBee Alliance, San Ramon,CA) via a router and coordinator to a local computer. The working frequencies of accelerometers and RFID tags were 2.4 GHz and 134.2 kHz, respectively. Although recorded by the ear tags, the data transferred and made available for analysis is not raw data, permitting analysis of timing or duration of individual rumination bouts, but rather a summary of total time (minutes) spent for ruminating during a fixed 2-h block.

Steer behaviours were recorded using 3 video cameras (GoPro Hero4 Silver San Mateo, California, U.S, resolution 1080s-30). The cameras were positioned 1 meter away from the feed trough of each steer and fixed at 1 to 1.50 m above the ground, depending on the height of each steer, so that rumination behaviour and posture of all steers could be accurately observed at any given time. The observation area was naturally light during daylight hours. If light intensity decreased, the building's lights were turned on. The time settings of the video and ear-tag systems were synchronised before the trial. Data from two steers was excluded from all analyses due to partial failure of video recording.

For three of the four diets (starter, intermediate II and finisher, Table 1), two days were chosen to record behaviours for the two measured variables (ear tag validation and effect of diets on behaviour). Each camera recorded behaviours for two steers simultaneously (total six steers) from 10 am to 4 pm on one day of each of the diets. The recording period was repeated again on the following day for the remaining steers, so that all steers should have one 6-h block recording for each diet. However, due to equipment failure, not all steers had complete 6-h block recordings for each diet. Then, videos from two 2-h periods (12 to 2 pm and 2 to 4 pm) were selected to match exactly the period reported by the ear tag, for further analysis. These times were chosen because they had complete video data from the most animals on the most diets, and given that feeding occurred at 10 am each day, it was assuming that rumination would tend to occur during this period. Excluding 2-h steer periods where visibility of potential rumination behaviour was impeded, there were 52 recordings of video data available for testing the effect of diets on behaviours, which comprised of 16, 14, and 22 available recordings for the starter, intermediate II and finishing diets respectively. For testing the effect of diets on allocation of time to different activities (behaviour and posture), the 52 recordings, which comprised of 12, 14, and 22 available recordings for the starter, intermediate II and finishing diets respectively, were categorized based on the three different diets (Tables 2, 3). A subset of 48 complete, paired tag and video recordings of rumination time /2-h were used in the method comparison, which was conducted for the entire data sub-dataset, and separately for each diet.

3.3.3 Analysis of behaviours

The video recorded during the measurement periods (52 recordings from two 2-h timeslot of 10 steers, specifically: each of steers 1, 2, 3, had 6 recordings, each of steers 4, 5 had 5 recordings and steers 6, 7, 10, 11, 12 had 10, 3, 4, 3 and 4 recordings respectively) were subsequently encoded on a computer by one trained observer using Behavioural Observation Research Interactive Software (BORIS version 7.4.5, Friard and Gamba, 2017). Seven mutually exclusive behavioural categories (idling, eating, drinking, ruminating, self-grooming, licking, social behaviour, unknown; Table 3) and posture (standing, lying, and walking; Table 2) were recorded according to the ethograms shown in Table 2 and 3.

Table 2. Ethogram definitions of registered postures as assessed by trained observer

Posture	Definition
Standing	Standing up and supported by all 4 legs
Lying	Lying down with whole body against the ground
Walking	Moving around the pen

Table 3. Ethogram definitions of registered behaviours

Behaviour	Definition
Eating	Muzzle in feed trough, taking feed into mouth, or chewing and swallowing feed with head raised over the feed-bunk
Drinking	Muzzle in water trough or swallowing immediately after putting muzzle in water trough
Ruminating	Regurgitation, followed by chewing and re-swallowing a bolus of feed
Self-grooming	Licking of own body or scratching with a hind limb or against a fixture
Social behaviour	Licking or nosing with the muzzle on neighbouring steers
Licking/biting fixtures	Licking or biting on fixtures
Unknown	Observer cannot detect what steers doing due to the direction that the animal was facing and is therefore unable to see the head and rumination behaviour.
Idling	Standing or lying with no other behaviour observed

3.3.4 Statistical Analysis

Video analysis was conducted using BORIS App v0.2.2 based on the ethogram to investigate the behaviours. Because of differences in how the ear-tag and BORIS apps reported activity outputs, raw data from the BORIS App (initially reported in seconds) were converted to minutes of time for each 2-h block to allow the same scale comparison. Initial data processing was implemented in MS Excel 2016, and statistical data analysis was implemented in R (R Core Team, 2013).

3.3.4.1 Validation of ear tag measurement of rumination

To test the intra-observer reliability, a subset of 24 2-h steer-blocks of video were scored twice for rumination time by the same trained observer using BORIS App based on the ethograms in Tables 2 and 3. Linear regression was performed to assess the correlation between each independently scored dataset.

The data generated from video recordings were selected in such way so that each data set matched the timing of data from the ear tags. The relationship between rumination time measured by video analysis (observed) was regressed over the measurement from the ear tag (predicted) in a simple linear regression to determine the coefficient of determination, and tested that the slope of this linear regression was not different from 1 with a t-test, using a cut off of $P \leq 0.05$ for significant difference. Several measures of deviance were calculated (Table 4), including mean proportional bias (MPB), mean absolute error (MAE), mean square prediction error (MSPE), root mean square prediction error (RMSPE) and root mean square error (RMSE). Lin's Concordance Correlation Coefficient (CCC) (Lin, 1989, 2000) was calculated in R using e*piR* (Stevenson, 2019). Definitions of these and other statistical parameters reported are provide in Table 4. To determine mean and linear bias, residuals were regressed on mean-centred predicted values of the tag in the method of St-Pierre (2003). If there was a significant linear bias, then bias at maximum and minimum predicted rumination time were calculated. To quantify the agreement between the rumination measurements from tags and video recordings analysis, Bland and Altman (1986, 2010) limits of agreement (LoA) were calculated and plotted in R using *BlandAltmanLeh* (Lehnert, 2015).

Table 4. Calculations and abbreviations used in statistical analyses

Statistic	Abbreviation	Description
Coefficient of determination	r^2	Proportion of variation of observed rumination time (video) that can be explained by predicted rumination time (tag).
Mean absolute error	MAE	$(\Sigma\|O_i - P_i\|)/n$, where n = number of paired observed (O) and predicted (P) rumination time values being compared.
Mean bias	-	Pi - Oi
Mean proportional bias	MPB	Slope of the simple linear regression of predicted on observed pH with the intercept forced to zero
Mean square prediction error	MSPE	Σ (Oi − Pi)2/n, where n = number of paired observed (O) and predicted (P) rumination time values being compared. The MSPE can be expressed as a sum of the mean bias squared, systematic bias, and random variation components.
Root mean square prediction error	RMSPE	Square root of the MSPE.
Root mean square error	RMSE	Square root of the error mean square of the simple linear regression of observed on predicted rumination time.
Upper Limit of Agreement	Upper LOA	$b_0 + (\sigma \times 1.96)$, where b_0 = mean bias ; σ = standard deviation of differences (Pi - Oi)
Lower Limit of Agreement	Lower LOA	$b_0 - (\sigma \times 1.96)$, where b_0 = mean bias ; σ = standard deviation of differences
Critical difference	-	$2(\sigma)$ = (lower LOA - upper LOA)/2, where b_0 = mean bias ; σ = standard deviation of differences
Bias at minimum rumination time	-	$[b_0 + b_1(R_{MIN} - R_{MEAN})]$, where b_0 = mean bias; b_1 = linear bias; R_{MIN} = minimum predicted rumination time; and R_{MEAN} = mean predicted rumination time. Calculated if linear bias is significant ($P < 0.05$).
Bias at maximum rumination time	-	$[b_0 + b_1(R_{MAX} - R_{MEAN})]$, where b_0 = mean bias; b_1 = linear bias; R_{MAX} = maximum predicted rumination time; and R_{MEAN} = mean predicted rumination time. Calculated if linear bias is significant ($P < 0.05$).

3.3.4.2 Effect of diet on cattle behaviour

Effects of diets on behavioural activities were assessed using available recordings from 12 to 4 pm. The 52 recordings were categorized based on diets. Daily behavioural activities including eating, drinking, ruminating, self -grooming, licking or biting, social behaviour, standing, lying and walking are expressed by calculating the total minutes of each behavioural activities during the time of recording period. To test whether time and diets had an interaction on behaviour analysis, a mixed linear model using either 1) diets and 2) diets plus time as fixed effects with steer as a random effect was applied, then these two models were assessed using ANOVA with significant differences determined using Satterthwaite's method. From these two models, time was excluded from model as there was no significant effect on behaviours. To test which diets had effects on the given behaviours, a one-way ANOVA was run to see the effect of diets on given behaviours. For all analyses, effects were considered significant at a $P \leq 0.05$**.**

3.4 Results

3.4.1 Method comparison for rumination time

The correlation of intra-observer reliability for a 2-h time block was very strong (mean = 15.6 ± 16.4 and 15.7 ±16.1 min/2-h; r = 0.96, P <0.001).

There was a linear relationship between rumination time measured by the tag and the video overall, and for each diet, except Intermediate II (Table 5, Fig. 2). This relationship did not differ significantly from equality for any diet, nor overall, although CCC was only close to 1 for the finisher diet (Table 5). Overall, the mean bias shows an overprediction of 8.06 ± 14.58 min/2-h by the tag. This ranged from 3.00 for the finisher diet to 14.80 min/2-h for the Intermediate II diet (Table 5). A positive linear bias existed for all comparisons of methods (Table 5). The Bland Altman critical difference for the 95% limits of agreement of the two methods was 24.95, 40.89, 15.23 and 28.49 min/2-h for the Starter, Intermediate II and Finisher diets, and overall, respectively (Table 5, Fig. 3).

Table 5. Parameters of fit and agreement of video measurement of rumination time (y) with tag measurements of rumination time (x) for individual diets and over all data points using linear regression of y|x, Bland-Altman limits of agreement and Lin's concordance correlation coefficient.

	Overall	Starter	Intermediate II	Finisher
No. of observations	48	12	14	22
Mean ± s.d. of video rumination time (min/2h)	19.04 ± 17.76	18.67 ± 17.34	15.14 ± 13.25	21.73 ± 20.54
Mean ± s.d. of tag rumination time (min/2h)	27.10 ± 23.48	28.17 ± 25.51	29.93 ± 23.72	24.73 ± 23.08
Variance of video rumination time (min/2h)	309	376	163	402
Variance of tag rumination time (min/2h)	540	596	522	508
r^2 of simple regression of video ~ tag	0.609	0.775	0.168	0.884
P-value (slope difference from zero)	<0.001	<0.001	0.0811	<0.001
P-value (slope difference from equality)	0.558	0.550	0.790	0.409
SD of residuals (min/2h)	14.58	12.79	20.93	7.68
Mean bias (min/2hr)	8.06	9.50	14.80	3.00
P-value	<0.001	<0.001	<0.001	0.036
Lower LOA	-20.34	-15.45	-26.11	-12.23
Upper LOA	36.55	34.45	55.68	18.23
Linear bias	0.41	0.393	0.731	0.154
P-value	<0.001	0.002	<0.001	0.030
Bias at minimum tag value (min/2h)	2.93	1.58	4.90	-0.13
Bias at maximum tag value (min/2h)	-30.73	-28.32	-47.73	12.34
MSPE	274	242	631	65
RMSE	16.6	15.5	25.1	8.09
MAE	10.6	10.9	17.8	5.9
Mean proportional bias	0.657	0.639	0.419	0.861
Concordance correlation coefficient (95% CI)	0.70 (0.55-0.81)	0.75 (0.47- 0.89)	0.31 (-0.04-0.59)	0.93 (0.84- 0.96)

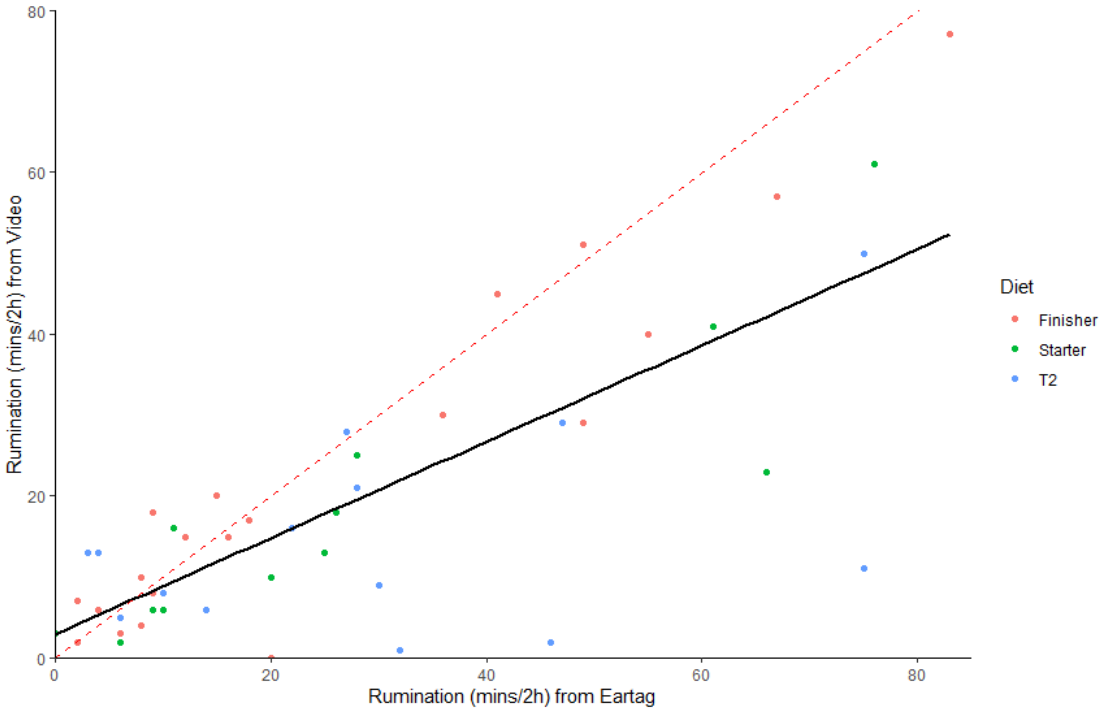

Figure 2. The relationship of minutes ruminating from tag and minutes ruminating from video without steer 3 (min / 2h), solid line, y = 2.930 + 0.594x, r^2 = 0.61; dashed line, y=x.

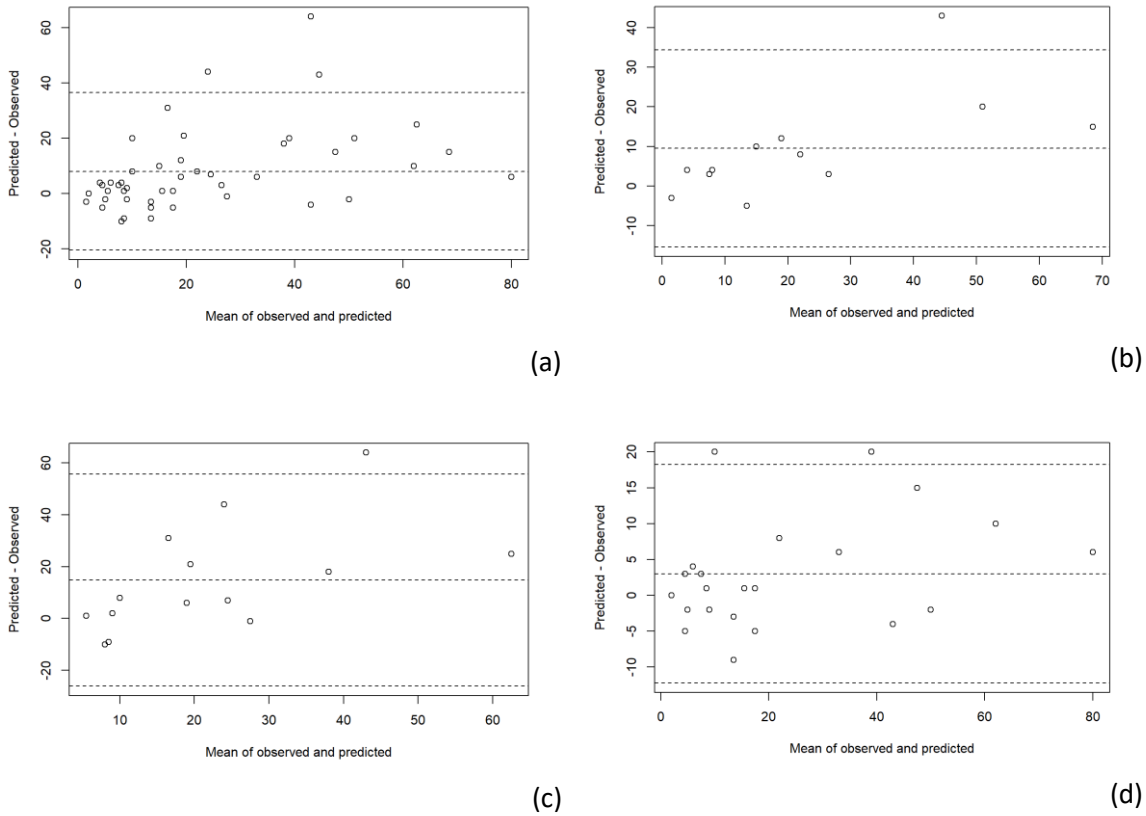

Figure 3. The 95 % limits of agreement between average video (observed) and tag (predicted) rumination time and difference of rumination from tag and video (min / 2h) for (a) overall, (b) Starter diet, (c) Intermediate II diet, and (d) Finisher diet.

3.4.2 Effect of diets on behaviours

Behaviour data from the ethogram showed that amongst measured variables, only eating and ruminating behaviour was significantly affected by diet (Table 6). There was a significant difference on time spent eating starter and finisher diets (*P*= 0.01) and between starter and intermediate diets (*P*=0.02). Ear tag generated data showed a significant difference in mean time spent ruminating on starter and intermediate and intermediate and finisher diets (*P*=0.0002 and *P*= 0.005, respectively). However, an unexpected result was that there was no significant difference between time spent ruminating (*P*=0.6) between starter and finisher diets. Time spent for social behaviour tended to decrease as the grain content of diets increased (*P*=0.06). Other behaviours were not affected by diet (Table 6).

Table 6. Effect of diets of increasing grain content on time (μ ± s.d., minutes) spent by steers exhibiting various behaviours between 12 – 4 pm, from video observation; and total 23 h rumination time as measured by ear tags.

Behaviour	Starter	Intermediate	Finisher	*P* value
Rumination from video	17.72 ± 15.54	16.45 ± 12.56	21.76 ± 20.61	>0.1
Mean rumination*	24.7^{a} ± 20.02	34.87^{b} ± 22.75	26.81^{a} ± 21.88	<0.01
Eating	28.39^{a} ± 27	9.48^{b} ± 11.42	9.56^{b} ± 15.25	<0.05
Drinking	2.06 ± 2.18	3.75± 4.62	2.04 ±4.02	>0.1
Self grooming	6.94 ± 6.36	8.07 ± 6.11	11.78 ± 11.19	>0.1
Social Behaviour	0.89 ± 1.1	0.53 ± 0.75	0.27 ± 0.52	0.06
Licking/biting	2.12 ± 2.91	2.68 ± 3.73	2.45 ± 2.69	>0.1

*Rumination from eartag was calculated as mean of 24 hours of 2-hour blocks. Note: This is not the same data set as presented in Table 5.

3.5 Discussion

3.5.1 The relationship between rumination times from ear tag and video recordings

The interpretation of the relationship between two measurement methods requires care. Commonly used regression and correlation methods can indicate a relationship between two methods, and may be adequate for model evaluation, but do not provide guidance on agreement and the value of expected differences of the two methods, which is the indicator which a clinician or practitioner requires in order to determine whether two methods can be used interchangeably or substituted for each other. The overall coefficient of determination for the two methods of measuring rumination time was moderate (0.61, *P* <0.001). While r^2 gives an indication of the relationship between two methods, by quantifying the proportion of variance that the two methods have in common, it is not interpretable as a measure of the agreement of the methods, since this represents an evaluation of the residuals from any linear relationship, not a line of equality, and two measurements on the same parameter will almost always have a significant relationship (Bland and Altman, 1986). Thus, high correlation does not automatically imply good agreement. Moreover, regression analysis is highly influenced by spread of the sample range, outliers and linear relationship, and presumes that there is no error in the measurement (Bilic-Zulle, 2011). Regression of observed (video, y) on predicted (tag, x) rumination time for the entire dataset yielded a relationship that was not significantly different from equality (slope = 1). The CCC value of 0.70 for the whole dataset indicates that the concordance and precision of the estimate of rumination from the tag is moderate. Analysis of concordance combines two measures (precision and accuracy) to provide

information on the agreement between two methods, more than just the presence of a relationship, but calculates a concordance index value (between 0 and 1) rather than giving a value of agreement in the terms of the measured variable. Analysis of biases in the relationship reveals more specific information about the scale of agreement. The St Pierre model evaluation method uses regression of residuals on mean-centred predicted values to quantify bias (most uniquely, linear (proportional) bias) of the prediction, based on the difference of the slope from zero. The centring of the independent variable at its mean allows estimation of mean bias independently from linear bias. This evaluation of the present dataset demonstrated an overall mean bias of 8.1 min rumination /2-h, an overprediction of rumination time by the ear tag, since the mean proportional bias is less than 1. The linear bias in the entire dataset ranged from 2.93 min/2-h at the minimum tag value to -30.73 min/2-h at the maximum tag value (average 0.41 min rumination /2-h). Since maximum bias exceeded the RMSE in the entire dataset, this bias was substantial. The Bland Altman method comparison analysis quantifies the mean bias similarly to the St Pierre regression analysis, but supplements this by constructing limits of agreement, at ± 2 standard deviations of the mean difference. For normally distributed data, 95% of data points are expected to fall within these limits. This provides a quantification of the agreement between two measurement methods, and the spread over which individual measurements may be expected to fall, rather than just the mean bias. Overall, the critical difference within which 95% of observations can be expected to fall was 24.95 mins of rumination/ 2-h. Although the significance of the bias can be quantified using both regression and Bland Altman analysis, decisions about the acceptability of the range of agreement can only be determined by the practitioner in the context of the application of these methods. For research uses where individual 2-h blocks of time are a key variable of interest, the critical difference of 24.95 mins/2-h would meaningfully affect the interpretation of individual measurement results. However, for practical or long-term research application, this critical difference may be acceptable, depending on how the data is being used. For instance, when averaged over a longer period of measurement, the mean bias will become more relevant than the limits of agreement, and the range of mean biases of 3.00 to 14.80 (overall 8.06) mins/2-h is likely to be more acceptable.

In the present study, although the tag both under- and over-estimated rumination time from the video in individual cases, overall, the tag over-estimated rumination time. Previous validation of ear tags accelerometer sensors (CowManager SenSoor,Agis Automatisering BV, Harmelen, the Netherlands) in dairy cattle also found that they overestimated rumination time (Pereira et al., 2018). Overestimation could be caused by the sensors including pause time between rumination bouts as time spent ruminating, whereas the video observation ethogram only coded for rumination when the steer was regurgitating, chewing and re-swallowing. In contrast, previous studies using ear tags on dairy cows (Bikker et al., 2014) and on beef cattle (Wolfger et al., 2015) or using collars on dairy cattle (Ambriz-Vilchis et al., 2015) found that automatic measurements often underestimate rumination time compare to either visual observation or video recordings.

For datasets using repeated measures on subjects, as in the present study, the standard Bland and Altman analysis is usually modified to account for within- and between-subject variance (Bland and Altman, 2007), as is the calculation of Lin's CCC. The very large size of within-subject variance in the current dataset suggests that rumination time in these steers is not correlated with subsequent measures of rumination time, and therefore we have not made this modification, and instead treated the data pairs as independent of each other.

There was a large variance within the data set, with standard deviations that were almost as high as the mean for each measurement method. The variation among steers could contribute to the wider

spread of the data within the limits of agreement plot area. In the present study steer 3 was considered a major contributor to the large variation of data spread – not only did this steer return a large difference between the two methods, but it was by far the greatest ruminator in the observation period. Inter-animal variation such as variation in skin thickness could have interfered with the sensors, displacement of the sensors due to the movement of animals or self-grooming, licking or biting, individual variation in behaviours when ruminating, or other variable could also have contributed to variation in the performance of sensors (Ambriz-Vilchis et al., 2015; Elischer et al., 2013; Goldhawk et al., 2013; Wolfger et al., 2015).

Most previous work comparing direct and sensor measures of rumination in cattle have relied on indicators of agreement which have limited use for this purpose, including reliance on Pearson's product momentum coefficient (r) and the coefficient of determination (r^2), or by regressing predicted rumination (sensor), on the y-axis on observed (directly measured) rumination on the x-axis. Many of the former group of papers do not specify whether the predicted sensor measurement was correctly regressed as the x-axis against observed on the y-axis, or vis versa, which meaningfully affects the values of slope and intercept coefficients, and thus the interpretation of the relationships (Pineiro et al., 2008). High agreement between direct and indirect observations of rumination have also been reported by other authors on dairy cattle using acoustic sensors (Schirmann et al., 2009) and on 9 month old heifers, r = 0.88 (Burfeind et al., 2011). Bikker et al. (2014) observed a high CCC (0.93) for comparison of an ear tag similar to that tested in the present study, and direct observation in dairy cattle while fed diets similar in composition to the starter diet in the present study. In comparison, using the same technology in beef cattle, minutes of ruminating per 2-h time block between sensor and visual observation had a poor CCC (0.30) (Goldhawk et al., 2013). Variation in breed, sex, age, and ration may be important factors affecting the correlation between measurement methods. Anatomical differences such as musculature structure, dewlaps, diets composition and feeding management (beef cattle typically eat from bunk while dairy cattle eat directly from the ground) could explain differences between how sensors capture rumination time in beef and dairy cattle (Goldhawk et al., 2013).

Diet may be an important factor affecting the use of rumination-detection ear-tags. However, the validation results on individual diets should be interpreted with care due to low sample size. A minimum sample size of 40 is recommended for method comparison studies (Westgard, 2019). In the present study, we had 48 observation pairs overall, but on each individual diet, observation numbers fell well below this threshold. Fit and agreement were greatest on the finisher diet, however this diet had nearly twice as many sample points as the starter diet, and 50% more points than the Intermediate II diet. There was no pattern for improved fit or agreement with increasing concentrate inclusion, since the Intermediate II diet had the worst fit and agreement of the three diets. Similar CCCs were observed between a rumination collar and direct visual observation in tie-stalled cattle on high grain diets (0.29) compare to high forage ration (0.27, Goldhawk et al. 2013). A similar comparison of rumination time between ear tag and visual observation using Hereford-Angus yearling steers fed 100% barley silage in an outdoor dirt floor pen, found a lower relationship (r = 0.44) and a low to moderate sensitivity in rumination (Wolfger et al., 2015). Diet compositions may be an important point of difference between that research and the present study. It has been highlighted by Galyean and Defoor (2003) that in forage based diets, forage intake plays an important role in determining rumination time, however, in grain based diets, intake is primarily altered by metabolic factors rather than bulky factors. Intake increases to compensate energy intake of low dietary energy content (Galyean & Defoor, 2003). Therefore rumination time is more closely correlated with NDF intake than NDF content (Beauchemin, 2018). Unfortunately, intake was not tested in the present study, since all animals were fed a restricted intake as a percentage of their live weight. The

research environment could also affect test. The present study was conducted in indoor pens that were cleaned on a daily basis, while the previous study Wolfger et al. (2015) was conducted in outdoor pens which had a large fly burdens that disturbed rumination time. Head, tails, ears, legs and skin movement were used by cattle to protect their body from flies and could have contributed to misclassification of behaviours by the ear tag in detecting rumination (Wolfger et al., 2015). Therefore it was suggested by Lindgern (2009) to place two ear tags on one steer to evaluate the steers where the discrepancies have occurred or placing the same sensor on different steers.

3.5.2 Effect of diets on behaviours

During the fattening period, beef cattle are fed concentrate-rich diets to maximise daily liveweight gain. To maintain healthy rumen activity while maximizing energy intake, the diets contain a low proportion of forage and a high proportion of grains. However with these diets, feedlot cattle may not be able to meet their behavioural and physiological mastication and rumination needs (Bozkurt, Ozkaya, & Ap Dewi, 2006). Monitoring behaviours of cattle is therefore valuable as an indicator of health and welfare, and can be achieved via automated technologies (Schirmann et al., 2012; Schirmann et al., 2009) or by video (Acatincăi et al., 2010; Shane, White, Larson, Amrine, & Kramer, 2016). The present study assessed to what extent the observed behaviours of feedlot steers were affected by different levels of grain in the diet. In this study, eating behaviour changed with different levels of grain within the diets, while time spent ruminating, drinking, self-grooming, licking/ biting features did not change with the increase of diet grain content.

Although it has a lower nutrient value, the cereal straw in feedlot diets is important in preventing an unhealthy rumen environment, which commonly occurs in intensive rearing systems. The inclusion of roughage, such as straw, promotes saliva secretion through increased rumination activity (Faleiro et al., 2011) due to its NDF content. Ideally, the increased NDF content of diets stimulates rumination activity which leads to increased ruminal pH (Mertens, 1997). However, in the present study, the total time steers spent for rumination was not affected by diet when assessed by video data. These findings could be explained by Dong et al. (2018) who suggested that for accurately estimating rumination behaviour of beef cattle using digital video recording, the minimum recommended sampling frequency should be 3 days with 4-minutes intervals. In the present study, the available duration of video recording was 4 hours. Therefore, to see whether the rumination behaviour changed as the grain content of diets increased, 24 hour rumination time data generated from the ear tags was used. The effect of diets on rumination behaviour was analysed using the one day continuously recorded data from the ear tag. The results showed that time spent for ruminating in finisher diets was lower than intermediate (P= 0.005). However, counter to our hypothesis, rumination time increased as the grain content of the diet increased between starter diets and intermediate diets (P<0.001).

Rumination time is associated with the level of inclusion of roughage within the diet. The time spent ruminating on the finisher diet was lower than for the intermediate diet, reflecting the reduced NDF content of the diet. However, the response of animals to low fibre rations depends not only on the lack of fibre within the ration, but could be attributed to its replacement with nonstructural carbohydrates (Mertens, 1997). A trial to evaluate how feeding behaviour of cattle under high-concentrate diets was affected by dietary non-structural carbohydrate, found that rumination time was higher on barley based diets compared to corn based diets (Rotger et al., 2006). The time spent for rumination was lower for starter diets compared to intermediate diets (P<0.001). This might have been influenced by the high resistance of pericarp and hulls of barley that would require a longer rumination time (Beauchemin &

Rode, 1997). However, further measurement such as dry matter intake and ruminal pH should be taken to confirm this implication. In addition, unexpectedly there was no difference in time spent ruminating between diet starter and finisher (P=0.6). Lindgren (2009) stated that it could be possible there was a temporary disturbance in ear tag due to a longer period of observation.

That eating time was lower than rumination time for each of the diets also showed in the present study and could possibly related to the high amount of barley as non-structural carbohydrate source within the diets. This agrees with previous research, that a greater proportion of time devoted to rumination than eating was commonly observed in cattle on high concentrate diets, which reflecting that grain was easier to form a bolus and to swallow that forage (Beauchemin & Rode, 1997). In this study, behavioural data from videos only consists of 4 hours of data collected in the few hours after feeding time.

The characteristics of the different rations could explain differences in time spent eating in our observation period. Feedlot cattle spend 6-10% of their day eating and this typically lessens as the concentrate level of the diets increases (Hicks et al., 1989). The total time in the observation period spent eating by steers in the present study did not differ between the intermediate and finisher rations. This finding might indicate that steers consumed the two diets at the same rate. However, when steers were fed finisher and intermediate diets, steers spent less time eating than for starter diets. This is probably a result of less time needed to chew diets consisting of greater proportions of concentrate. In agreement to this finding, more time was taken for eating by bulls when fed high amount of wheat straw compared to low level of wheat straw or even with maize silage (Mazzenga, Gianesella, Brscic, & Cozzi, 2009). When cattle were fed wheat straw, the eating time was longer than those fed high proportion of maize silage, and this in turn resulted in the increase of rumen liquid outflow rate (Mazzenga et al., 2009).

It has previously been reported that reduction in eating time was associated with reduced NDF and ADF intake of heifers fed diets without barley straw compared to heifers fed barley straw based rations (Faleiro et al., 2011). The total time spent eating (min/day) also depends on metabolisable energy level of the diets as intake increases to compensate for low dietary energy content (Galyean & Defoor, 2003). A linear decrease in eating time (P<0.0001) was observed when sheep were fed diets with different energy levels (0.96, 1.28, 1.72, 2.18, and 2.62 Mcal/kg DM), which was associated with lower cell wall content and higher starch content, requiring less time to chew it (de Araújo Camilo et al., 2012). Interestingly, as the eating time decreased with reduced dietary forage content, the frequency of self-grooming and licking was greater during eating times than during the time that rumination activities were performed. This might suggest that the drive to chew other objects while ruminating was less important that the drive to chew while eating. Also, visual prompting from neighbouring animals may positively stimulate eating motivation (Faleiro et al., 2011). However, in the present study there was no effect of diets on welfare related behaviours.A high standard deviation in eating time among the three different diets in present study reflects the variability in eating behaviour. This is consistent with other reports (Shane et al., 2016) where differences in time spent eating were attributed to breed, social interactions and temperament, feed quality, type and mode of feeding

Measuring drinking behaviour was challenging. Ruuska et al., (2016) observed that a systematic error occurred when drinking-time data from two observers was compared to that from a sensor (the regression line slope was 1.77; R^2=0.20). Video recording measurement is less accurate than other continuous behavioural sampling using sensors especially for short duration behaviours such as drinking, social behaviour, licking or biting (Mitlöhner et al., 2001). The time spent by animals for drinking was only of short duration, 5.5-6.8 minutes per day (Huzzey et al., 2005). Therefore, the overall drinking

measurement was highly sensitive to the smallest imprecision in classifying drinking behaviour (Ruuska et al., 2016), or from scan sampling of observations. These findings contrast with those of Robles et al. (2007) who reported that there was a statistical tendency for a linear increase in water intake in heifers fed high grain diets as the feeding frequency increased (P= 0.08). Water intake has a positive correlation with DMI, number of meals per day and physiological status of cows (Huzzey et al., 2005). The discrepancy among the published research could be attributed to differences in experimental conditions and methodologies including the number of observation days in an experiment (ranging between 1-5 days).

Social behaviours tended to decrease as the amount of grain within the diets increased. This finding was partly in agreement with previous research, (Faleiro et al., 2011) who observed that even though time spent eating, drinking and self-grooming was not affected whether heifers were fed concentrate with barley straw or not, time spent for social behaviour and licking/biting tend to longer in non-barley straw concentrate diets, whereas ruminating time was longer in barley straw concentrate diets both in experimentally environment and feedlot condition (P=0.02 and <0.001, respectively).

In the present study, there was no difference observed for self-grooming behaviour among the three different diets. It is now widely believed that body care activities such as self-grooming have nutritional, communicative and psychological functions. Self-grooming is associated with licking the neck region , head or other part of body or other animal activities that increase in a more intensive environment such as in feedlot pens, where the cattle are fed high grain diets. When the cattle performed self-grooming due to significant soiling of the coat, it can signify a reduction in their welfare (Phillips, 2002). Increasing self-grooming was considered by Mattiello et al. (2002) to be an abnormal behaviour displayed by calves fed rations lacking in fibre. Calves fed a beet pulp ration displayed more self-grooming and social behaviours compared to those fed wheat straw and control (traditional all-liquid ration; P < 0.01 and P < 0.001, respectively) (Mattiello et al., 2002). In contrast, other research observed that activities such as self-grooming, licking/biting features, social behaviours were observed to be not different between heifers on high concentrate diets based with a different source of non-structural carbohydrates and protein (P =0.8 and 0.2, respectively) (Rotger et al., 2006). Again the short duration of these behaviours could be an explanation for inconsistency among published research (Mitlöhner et al., 2001). The lack of effect of diets on observed behaviours (drinking, self-grooming, social behaviour, and licking/biting features) suggest that the diet composition and the protocol for transition between diets could provide a sufficient adaptive response in the steers, and thus did not contribute to welfare issues.

3.6 Conclusion

The present study showed that the relationship for rumination time generated from ear tag and video was moderate, and analysis of agreement showed that there was a critical difference of ± 24.95 min/ 2-h with a mean bias of ~ 8 minutes between the two measurements. The acceptability of this difference will depend on the application. The limits of the mean and linear bias of the ear tags is likely sufficient for many commercial purposes, and may be sufficient for longer-term research purposes to show a trend, although not specific enough for the absolute values of individual samples of rumination time to be considered reliable. Therefore, accelerometer ear tags are a promising tool for ruminating behaviour measurement. The level of grain in the diet affected the time spent for eating and rumination in the period after feed delivery. As the grain content of diets increases, the time spend for eating and ruminating decreases. Increasing levels of grain content in this study did not affect other behaviours. These findings suggest that the level of grain within the diets did not contribute to health and welfare issues.

4 VALIDATION OF AN INTRARUMINAL BOLUS TO MEASURE RUMEN PH

4.1 Introduction

When cattle first enter a feedlot system, rumen microbiomes are acclimated for the degradation of fibre, not the large amounts of rapidly fermentable starches that are present in feedlot rations (Negara 2019). As rapidly-fermentable starches enter the rumen environment, they are broken down into short chain fatty acids, predominately acetate, propionate, butyrate, and lactate (Owens et al., 1998). Rumen microbiomes in cattle acclimated to grass are associated with fermentations providing higher proportions of acetate in the rumen and a higher acetate-to-propionate ration, relatively stable rumen pH, and only small amounts of lactate (RAGFAR 2007). Greater production of these acidic fermentation end products can result in a decrease in ruminal pH under 5.6, resulting in subacute ruminal acidosis, or SARA (Owens et al. 1998). If rumen pH continues to decline, acute ruminal acidosis (RA) will occur when ruminal pH is less than 5.2. SARA is likely to arise during the transition period in grain-fed cattle, and in unknown frequency throughout the duration of the individuals' time on high-grain rations (Russell and Rychlik, 2001). SARA not only causes an immediate depression in feed intake, but can cause permanent damage to the gastrointestinal tract, resulting in long-term reduction in feed efficiency and productivity (Owens et al., 1998; Nagaraja and Chengappa, 1998). SARA is not a straightforward syndrome but is the product of interactions of large amounts of starch entering the rumen; low fibre intake, changes in feed intake volume or timing, and inclement weather (Owens et al. 1998).

Feedlots present many of the risk factors for SARA: large amounts of rapidly fermentable grain in the ration relative to small amounts of NDF, and feed is provided 1-3 times a day, reducing meal frequency and increasing meal size. Live monitoring of ruminal pH provides a powerful insight to overall gastrointestinal health and individual animal productivity. Historically, continuously monitoring rumen acidity required cannulation to be able to directly connect to the sensor via wires, or removal of the sensor to retrieve a memory chip with data (Antanaitis et al. 2016). Such systems are obviously not applicable to feedlot systems. Currently, there is technology available in the dairy industry that utilizes an orally administered bolus with wireless data transmission and long-life batteries, to provide long-term assistance in heat detection and herd health. Their application to the feedlot industry has not been explored, and accuracy in the rumen has been poorly tested. Thus, the objective of this study was to determine the accuracy of rumen pH reported by a commercially available intraruminal bolus both relative to pH standards and in the rumen of grain-fed cannulated steers, relative to manual pH determination of fluid sampled from the same site.

4.2 Materials and Methods

4.2.1 Animals, Diets, and Experimental Procedures

Lowline Angus steers (n = 12) with established rumen cannulae were used for the validation of pH data provided by the smaXtec® intraruminal bolus (smaXtec® Premium Bolus; smaXtec Animal Care Sales, GMBH, Graz, Austria). The 12 ruminally cannulated Lowline Angus steers were on two occasions adapted from pasture to a feedlot finisher ration (Tables 7, 8), and then maintained on the finisher ration for 24 days. Overall, data was collected across the lifespan of the bolus reporting pH (150 days).

Validation of the initial pH measure was done by comparing the pH from the bolus to that of a pH meter (EcoScan Portable pH/ORP meter with TPS pH Sensor) calibrated daily, by two methods: 1) in warmed pH standards three times (one at bolus initialization, and at the start of each finisher period), and 2) by a comparison of manual pH measures made on samples taken from the immediate proximity to the sensor membrane of the intraruminal pH bolus (Table 8). While all twelve steers were fitted with intraruminal boluses and assemblies, the finisher period utilized only ten steers for sampling. However, during the first finisher period, one steer was removed from the study due to intake concerns prior to validation, so nine steers completed the validation component in the first period. For the second period, the same nine steers were used, plus another steer not utilized in the previous period. Averaged across both periods, steers weighed 237.5 ± 55kg. Boluses were randomly allocated to steers and remained in the same steer throughout the trial.

Table 7. Schedule of validation of ruminal boluses in cannulated steers.

Date	Event	Validation Method	Days Between
23/7/18	Bolus Initialization	Warmed pH standard	--
8/8/18	Boluses administered to steers	--	14
13/9/18	Start of Finisher 1st	Warmed pH standard	36
2/10/18	F1 rumen sampling	Intraruminal assembly	19
18/11/18	Start of Finisher 2nd	Warmed pH standard	39
4/12/18	F2A rumen sampling	Intraruminal assembly	24

Table 8. Ingredients, formulated rations and intake for cannulated cattle during bolus validation.

Item	Starter	INT-1	INT-2	Finisher
Ingredient, % As Fed				
Tempered Barley	48.7	59.9	70.9	81.8
Molasses	4.0	3.3	2.6	2.0
Vegetable Oil	-	0.9	1.8	2.8
Dry Supplement	2.6	2.6	2.6	2.5
Mill Run	10.0	6.7	3.4	-
Whole Cottonseed	11.7	10.7	9.7	8.7
Wheat Straw	7.7	5.3	3.0	2.2
Oaten Hay	15.3	10.6	6.0	-
Nutrient Composition				
Dry Matter	100	100	100	100
Ash, % DM	7.61	7.14	6.67	6.09
TDN, % DM	74.28	75.76	77.22	78.53
ME, Mcal/kg DM	2.69	2.82	2.95	3.09
NEm, Mcal/kg DM	1.77	1.88	2.00	2.11
NEg, Mcal/kg DM	1.15	1.25	1.35	1.46
Starch, % DM	30.31	35.85	41.44	47.08
Fat, % DM	4.37	5.18	6.01	6.98
NDF, % DM	38.00	34.46	30.91	27.47
CP, % DM	13.72	13.65	13.57	13.36
DIP, % DM	9.80	9.83	9.85	9.78
UIP, % DM	3.92	3.82	3.72	3.58
Ca, % DM	0.74	0.73	0.72	0.68
P, % DM	0.39	0.37	0.36	0.34
Mg, % DM	0.26	0.25	0.23	0.22
K, % DM	1.04	0.92	0.79	0.66
Na, % DM	0.11	0.09	0.07	0.04
Cl, % DM	0.32	0.24	0.16	0.07
S, % DM	0.23	0.23	0.22	0.21
Co, ppm	0.43	0.36	0.28	0.18
Cu, ppm	19.22	18.52	17.79	16.69
I, ppm	0.57	0.56	0.56	0.53
Fe, ppm	82.99	59.06	34.94	9.81
Mn, ppm	61.67	53.65	45.48	36.22
Mo, ppm	0.75	0.65	0.56	0.46
Se, ppm	0.15	0.14	0.12	0.10
Zn, ppm	92.34	91.81	91.22	88.11
Vitamin A, KIU/kg DM	2.17	2.20	2.23	2.18
Vitamin E, IU/kg DM	24.66	25.01	25.37	24.76
Salt, % DM	0.25	0.25	0.25	0.25
Urea , % DM	0.39	0.40	0.41	0.40
Vitamin D, IU/kg DM	271.22	275.16	279.12	272.40
Average Daily DMI, kg DM				
F1	-	-	-	3.92
F2	-	-	-	4.36

4.2.2 Boluses and Validation

Boluses were purchased in two lots from smaXtec® Animal Care Sales. The first eleven boluses were initialized (turned on) with a magnet device supplied by the company three weeks prior to the start of the first adaptation period, and placed in the buffer solution provided by smaXtec® for self-calibration, as per manufacturer instruction. Of the eleven, nine successfully initialized. Three more boluses were ordered from the company, but did not arrive until after the adaptation period had commenced. After initialization in smaXtec buffer, the original nine boluses that successfully initialized were placed in newly purchased pH standards of 7 or 4 warmed in a 39°C water bath to validate accuracy of range prior to administration to steers. The pH standards were pre-warmed in the water bath in 50mL plastic containers, containing enough pH standard to fully cover the sensor end of the bolus once the bolus was placed inside (Fig. 4). Once the bolus was placed in the container, in the water bath, water level was maintained at a height to be slightly above that of the pH standard level to maintain constant temperature. Boluses remained in the warmed standard (pH 4 or 7) for a minimum of 3 hours before being removed, rinse with warmed RO water, and placed in the other pH standard. Time was recorded for the bolus entering and

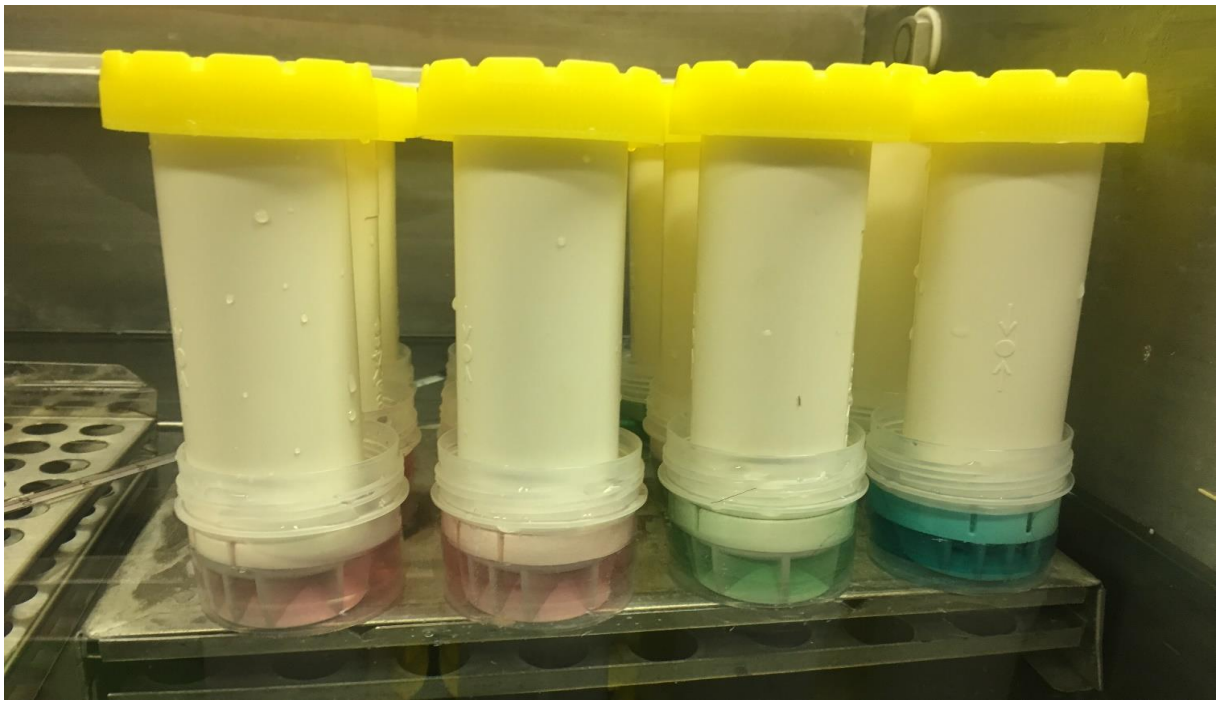

Figure 4. Rumen boluses sitting in containers containing pH standards warmed in a water bath for validation.

being removed from each pH standard. In order to account for actual pH drift of the standard solutions, time and pH of each of the warmed pH standards were measured with a calibrated pH probe (EcoScan Portable pH/ORP meter with TPS pH Sensor) when the bolus was placed into, and removed from the container. This same procedure was repeated twice more, at the beginning of each finisher period.

Keeping boluses in the water bath and not administering to steers within one hour of successful initialization was against the manufacturer's instructions, and they claimed could contribute to some error of reported pH, but how is not clear. Due to some concerns with drift occurring by day 3, bolus administration to the first 9 steers was delayed 14 days after activation, while the boluses stayed in warmed buffer provided by the manufacturer. The original nine boluses were administered to steers 14 days after initialization, despite some concerns during monitoring in buffer.

Boluses were installed into intraruminal assemblies, which were composed of 1m of 0.2mm diameter flexible poly-tubing attached to a weight of metal chain holding the sampling point low in the rumen ventral sac. Also attached to the chain was the bolus. The bolus was contained in a fine porous nylon bag to filter contents, as was the intake end of the rumen fluid sampling tube, with the opening of the sampling tube placed immediately in line with the face of the bolus and the remaining tube fed up through the cannula to the exterior and closed by a 3-way tap (Figure 5 and 6).

Rumen fluid samples were obtained by aspiration through the 0.2mm diameter sampling tube drawing from the face of the rumen bolus via the rumen assembly as described above. For each bolus, spot samples were collected across two periods, in which spot samples were collected 13 times at -2, -1, 0, 1, 2, 5, 6, 7, 8, 9, 10, 11, and 12-hours after feeding (exact times recorded) and pH of the sample was determined immediately using a calibrated, handheld pH meter (total = 13 x 2 = 26 intraruminal comparisons for each bolus). The pH values from the bolus corresponding to each spot sample were obtained by downloading bolus data from smaXtec® messenger website and finding bolus pH value at recorded sample time. Additionally, pH values from the bolus when in pH standards of 4 and 7, at 39°C at the beginning and end of each period were compared.

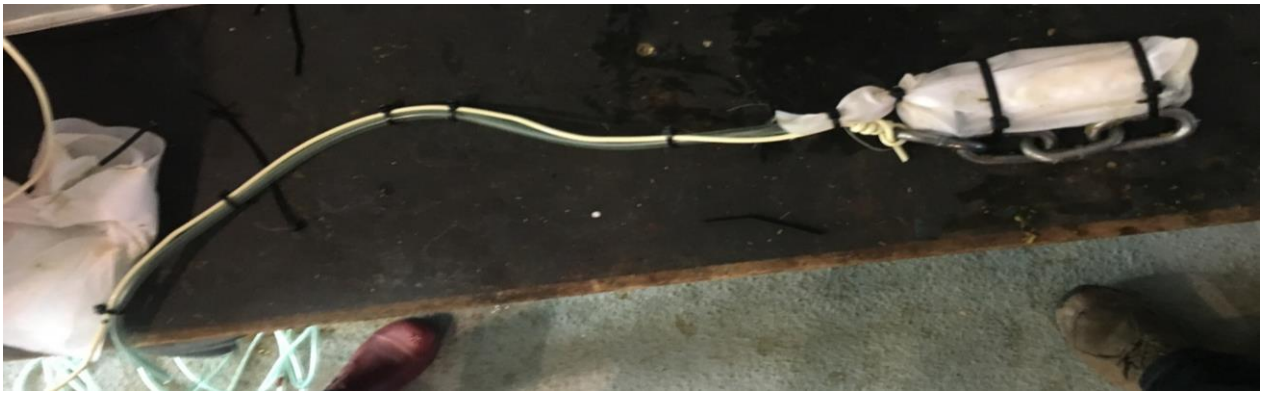

Figure 5. Intraruminal assembly with rumen bolus inside a fine nylon bag, secured to a chain to hold assembly in lower ventral sac. Flexible plastic tubing secured in the immediate vicinity of the bolus sensor head, and leads up to allow for external access.

Figure 6. Close up of bolus sensor head showing end of plastic tubing secured with fishing line to allow for rumen sampling in the same immediate area that the bolus would be measuring.

4.2.3 Statistical Analysis

For the validation of the ruminal boluses ability to accurately report pH, paired comparisons from rumen fluid samples and two pH standards (pH 4 and pH 7) were collected via calibrated probe (observed) and boluses. There were 568 paired observations, with variables that comparison location (intraruminal or pH standard), bolus ID (1-12), bolus age (in days), bolus pH, and probe pH. Statistical data analysis was accomplished by using R (R Core Team, 2013) and MS Excel 2016.

Data was subset by comparison location, age, and location x age. Each subset was analysed separately in attempt to understand the decomposition of bias. Concerning age subsets, the bolus manufacturer states the measurement accuracy of the bolus is equivalent to ±0.2 pH units up to day 90, and ±0.4 up to day 150 (after which the pH recording function of the bolus switches off). Therefore, bolus age was divided into "young" (age in days 0-90) and "old" (age in days 91-150). The pH standard comparisons were further subset into the individual pH standards of 4 and 7, but these were not linearly regressed since the range of pH was less than 1 pH unit. Therefore, only a few of the deviation measures could be calculated for individual standard comparisons. Three paired t-tests were utilized to compare means, once with a hypothesized mean difference of 0, and then with the manufacturers stated accuracy for the age (0.2 for young and 0.4 for old), which was ran twice to test for the absolute value of the mean difference. A two-tailed *P*-value of <0.05 was considered significant.

To evaluate model adequacy, several measures of deviance were calculated (Table 9), including mean absolute error (MAE), mean square prediction error (MSPE), root mean square prediction error (RMSPE) and root mean square error (RMSE). The relationship between bolus and probe pH was assessed using four separate simple linear regressions. The first regressed probe pH on bolus pH to test if the slope was different than 0 (significance determined at *P*-value ≤ 0.05), and gain the r^2 and slope values. Secondly, probe pH was regressed on bolus pH again, but with the addition of the OFFSET term (RStudio), to test whether the slope was different from 1. Third was a regression from St-Pierre (2003), where the residuals (probe pH minus bolus pH) were regressed on mean centred bolus pH. From this regression, the mean bias and linear bias were obtained from the intercept and slope coefficients, respectively. If there was significant linear bias, then bias at maximum and minimum bolus pH were calculated. Finally, bolus pH was regressed on probe pH with the intercept forced to 0 to find the mean proportional bias (slope). Limit biases were also calculated following the procedure of St-Pierre (2003). Additionally, a modification of the standard limit of agreement (LoA) from Bland and Altman (1986, 2010) was adopted to quantify the agreement between the two pH measurements while including effects of varying number of observations per individual bolus. A definition of all statistical parameters is provide below (Table 9).

Table 9. Statistical parameters used in validation of smaXtec intraruminal pH bolus.

Statistic	Abbreviation	Description
Coefficient of determination	r^2	Proportion of variation of observed pH (Probe) that can be explained by predicted pH (bolus).
Bland Altman adjusted standard deviation	σ_a	$\sqrt{\left(\frac{CoV_1}{CoV_2}\right) + MSR}$ where CoV_1 = mean square for boluses minus means square for residuals; $CoV_2 = \frac{(\sum m_i)^2 - \sum {m_i}^2}{(n-1)\sum m_i}$ where *n* = number of boluses; m_i is the number of observations *i* on bolus m_i; MSR = mean square for residuals
Mean absolute error	MAE	$(\Sigma \lvert O_i - P_i \rvert)/n$, where n = number of paired observed (O) and predicted (P) pH values being compared.
Mean bias	-	Observed minus predicted mean pH
Mean proportional bias	MPB	Slope of the simple linear regression of predicted on observed pH with the intercept forced to zero
Mean square prediction error	MSPE	$\Sigma (O_i - P_i)^2/n$, where n = number of paired observed (O) and predicted (P) pH values being compared. The MSPE can be expressed as a sum of the mean bias squared, systematic bias, and random variation components.
Root mean square prediction error	RMSPE	Square root of the MSPE.
Root mean square error	RMSE	Square root of the error mean square of the simple linear regression of observed on predicted pH.
Systematic bias	-	$(1 - b^2) \times s_p^2$, where b = the slope of the regression of observed on predicted pH and s_p^2 = the variance of the predicted pH.
Upper Limit of Agreement	Upper LOA	$b_0 + (\sigma_a \times 1.96)$, where b_0 = mean bias ; σ_a = Bland Altman adjusted standard deviation
Lower Limit of Agreement	Lower LOA	$b_0 - (\sigma_a \times 1.96)$, where b_0 = mean bias ; σ_a = Bland Altman adjusted standard deviation
Bias at minimum bolus pH	-	$[b_0 + b_1(P_{MIN} - P_{MEAN})]$, where b_0 = mean bias; b_1 = linear bias; P_{MIN} = minimum predicted pH; and P_{MEAN} = mean predicted pH. Calculated if linear bias is significant ($P < 0.05$).
Bias at maximum bolus pH	-	$[b_0 + b_1(P_{MAX} - P_{MEAN})]$, where b_0 = mean bias; b_1 = linear bias; P_{MAX} = maximum predicted pH; and P_{MEAN} = mean predicted pH. Calculated if linear bias is significant ($P < 0.05$).

4.3 Results

The correlation between bolus pH and probe pH was not very strong overall ($r^2 = 0.49$; Table 10), and was highest in the standards ($r^2 = 0.81$) but poor in the intraruminal comparisons ($r^2 = 0.34$). However, the value of the correlation in the standards should be carefully considered since these comparisons were only made against two points of reference, pH of 4 and 7, instead of a range of pH values like the intraruminal comparisons. Overall mean bias shows a significant over-prediction of 0.37 ± 0.78 pH units (*P<0.0001)*, which results in a Bland Altman critical difference for the 95% limits of agreement spanning 3.04 pH units (Fig 11). A negative mean bias was significantly present in all life stages and comparison locations, and became more negative with age (Table 10). An example of the difference between bolus and probe pH for one bolus is displayed in Fig. 7.

There was a significant (P<0.000) negative linear relationship of 0.41 between bolus pH and probe pH overall (Fig 8), and was seen across all life stages and comparison locations. Linear bias was numerically more negative in the intraruminal comparisons relative to pH standards (-0.55 vs -0.15, respectively). Linear bias became more negative with age with intraruminal measurements, but when

including pH standard measurements, linear bias was similar between age groups overall. However, the interpretation of results and influence of bias and error measurements in the pH standards should be done with prudence since the comparisons only took place at two pH values and are not a robust indictor of actual relationship. The significant linear bias across all comparison locations and ages was assessed by evaluating the maximum bias over the full range of predicted values relative to RMSE (St-Pierre, 2003). Because the absolute vales of the bias at bolus maximum for each of the regressions was larger than the RMSE, it can be concluded that large amounts of bias were present in these comparisons.

Evaluating the overall biases, while the mean bias itself was within the accuracy limits stated by the manufacturer, the range between the Bland Altman limits of agreement is quite large, suggesting a large variance in the data. This is supported when the data is plotted (Figs 9, 11) and it appears that there is nearly a divergence of two groups of boluses that is not explained by bolus lot number or other distinguishable attributes or treatments. This divergence of one group that over-predicts pH and one that slightly under-predicts pH creates a large confidence interval, therefore while the overall mean bias is -0.37, the confidence interval spans 3.04 pH units (Fig 11). This divergence and its effects on overall bias can again be visually represented when residuals are plotted on mean centered bolus pH and linear regression lines are added. When the overall regression line (linear bias) is plotted on residuals regressed on mean centered bolus pH (Fig 9), the slope is quite negative, but when comparing the individual bolus regression lines plotted (Fig 10), we again see two clusters emerge on either side of the overall regression line. However, individual boluses within the same groups do not all share the slope linear bias, such that some have a negative linear bias (negative slope) while others in the same group have a positive linear bias. Generally, the range between the upper and lower limits around the mean bias is between 2.69-3.28 pH units, while the interval between the minimum and maximum bias for linear bias is between 0.81-2.74 pH units.

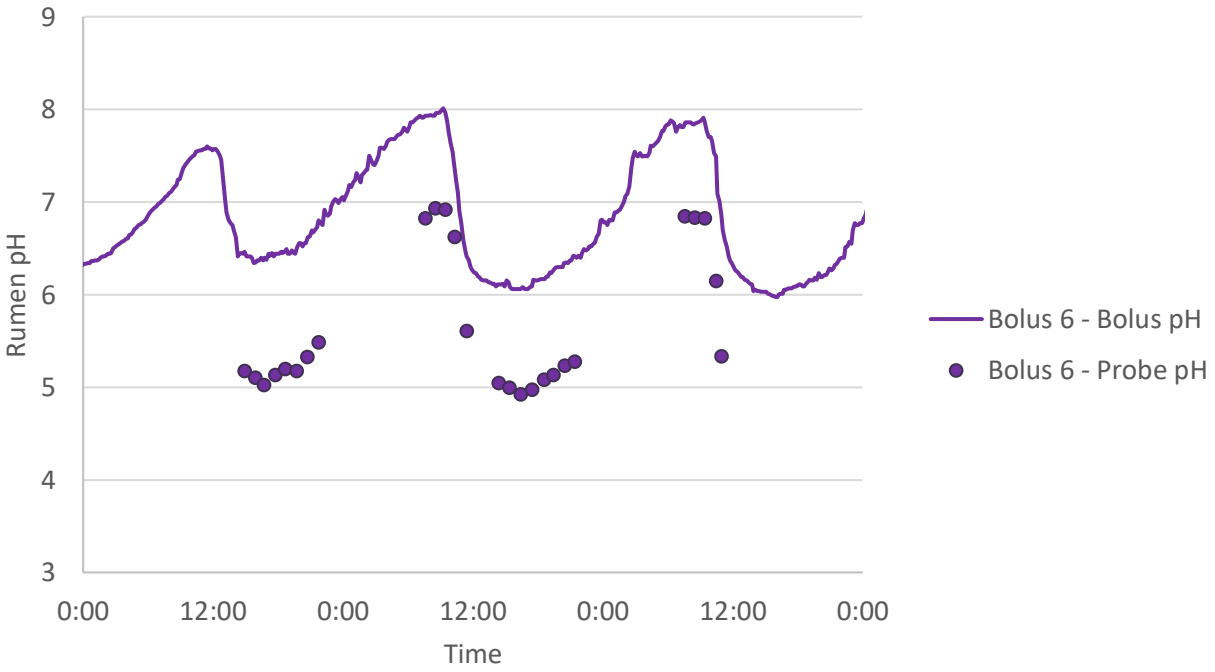

Figure 7. Plot displaying differences in continuous reported pH from bolus and probe spot sampling for one bolus across a 48-hour period. Bolus 6 shows the intraruminal bolus overestimates pH relative to the spot sampling measured by probe

Table 10. Descriptive statistics and statistics from regressions of calibrated probe measurements on bolus measurements and Bland Altman analysis by comparison type.

	All comparisons			**Intra-rumen comparisons only**			**pH standard comparisons only**				
Item[1]	**Life[2]**	**Young[2]**	**Old[2]**	**Life**	**Young**	**Old**	**Life**	**pH 4, Young[3]**	**pH 4, Old[3]**	**pH 7, Young[3]**	**pH 7, Old[3]**
No. of observations	568	290	278	489	229	260	79	27	9	34	9
Mean of probe pH	5.90	5.94	5.85	5.95	6.02	5.88	5.58	3.98	3.93	6.95	6.87
Mean of bolus pH	6.27	6.14	6.40	6.33	6.24	6.40	5.91	4.14	5.04	7.03	7.85
Variance of probe pH	0.75	0.75	0.75	0.49	0.33	0.63	2.22	0.00	0.02	0.00	0.00
Variance of bolus pH	1.06	1.10	0.98	0.81	0.71	0.88	2.48	0.36	0.47	0.27	0.44
Simple Regression of Probe pH ~ Bolus pH											
r^2	0.49	0.54	0.48	0.34	0.24	0.44	0.81	N/A	N/A	N/A	N/A
Bolus slope	0.59	0.61	0.61	0.45	0.33	0.56	0.85	N/A	N/A	N/A	N/A
P-value (different from zero)	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*NS*	*<0.005*	*NS*	*<0.05*
P-value (different from equality)	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.005*	*<0.1*	*<0.05*	*NS*	*<0.05*
SD of residuals[4]	0.74	0.72	0.73	0.75	0.75	0.72	0.69	0.58	0.67	0.53	0.66
Mean bias	-0.37	-0.20	-0.56	-0.38	-0.22	-0.52	-0.33	-0.16	-1.11	-0.08	-0.99
P-value	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*	N/A	N/A	N/A	N/A
Lower LOA[4]	-1.83	-1.60	-1.99	-1.86	-1.70	-1.94	-1.68	-1.29	-2.42	-1.12	-2.28
Upper LOA[4]	1.09	1.21	0.88	1.10	1.26	0.90	1.02	0.97	0.19	0.95	0.30
Linear bias	-0.41	-0.39	-0.39	-0.55	-0.67	-0.44	-0.15	N/A	N/A	N/A	N/A
P-value	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.000*	*<0.005*	*N/A*	*N/A*	*N/A*	*N/A*
Bias at minimum pH	0.87	0.95	0.44	0.65	0.98	0.18	0.07	N/A	N/A	N/A	N/A
Bias at maximum pH	-1.35	-1.14	-1.44	-1.59	-1.75	-1.43	-0.74	N/A	N/A	N/A	N/A
MSPE	0.69	0.55	0.84	0.71	0.61	0.79	0.58	0.35	1.63	0.28	1.36
RMSPE	0.83	0.74	0.92	0.84	0.78	0.89	0.76	0.59	1.28	0.53	1.17
RMSE	0.62	0.59	0.62	0.57	0.50	0.60	0.65	N/A	N/A	N/A	N/A
MAE	0.70	0.61	0.80	0.73	0.67	0.78	0.54	0.42	1.11	0.35	1.03
Mean Proportional Bias	1.06	1.03	1.09	1.06	1.03	1.08	1.05	N/A	N/A	N/A	N/A
Decomposition of MSPE											
Mean Bias, %	19.82	6.88	36.51	20.01	7.54	33.96	18.73	7.36	75.88	2.45	71.60
Systematic Bias, %	25.55	31.01	17.83	33.79	51.49	21.40	9.48	92.17	22.87	96.38	28.29
Random error, %	54.63	62.11	45.66	46.20	40.97	44.64	71.79	0.48	1.26	1.17	0.10

[1]MSPE = mean square prediction error; RSME = root mean square error; MAE = mean absolute error
[2]Life = bolus age 0 to 150 (in days since activation); Young = bolus age 0 to 90; Old = bolus age 91 to 150
[3] Individual pH standard comparisons were not linearly regressed but descriptive statistics of residuals and errors were still assessed
[4]Standard deviation of residuals and limits of agreement (LOA) were adjusted based on number of observations
[5] P-values for individual standard comparisons are two-tailed from paired t-test for means with a hypothesized mean difference of 0
[6] P-values for individual standard comparisons are two-tailed from paired t-test for means with a hypothesized mean difference of 0.2 for young, and 0.4 for old.

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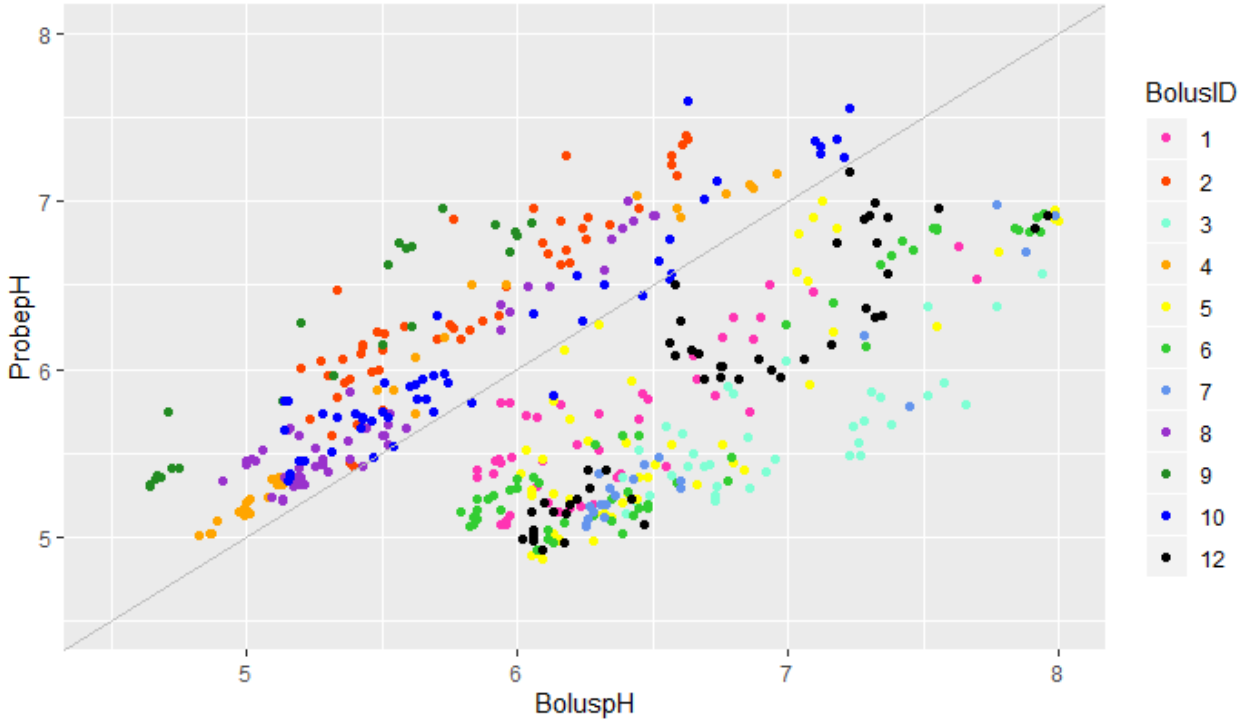

Figure 8. Plot of Probe pH regressed on Bolus pH by bolus ID, with a reference line with a slope of 1.

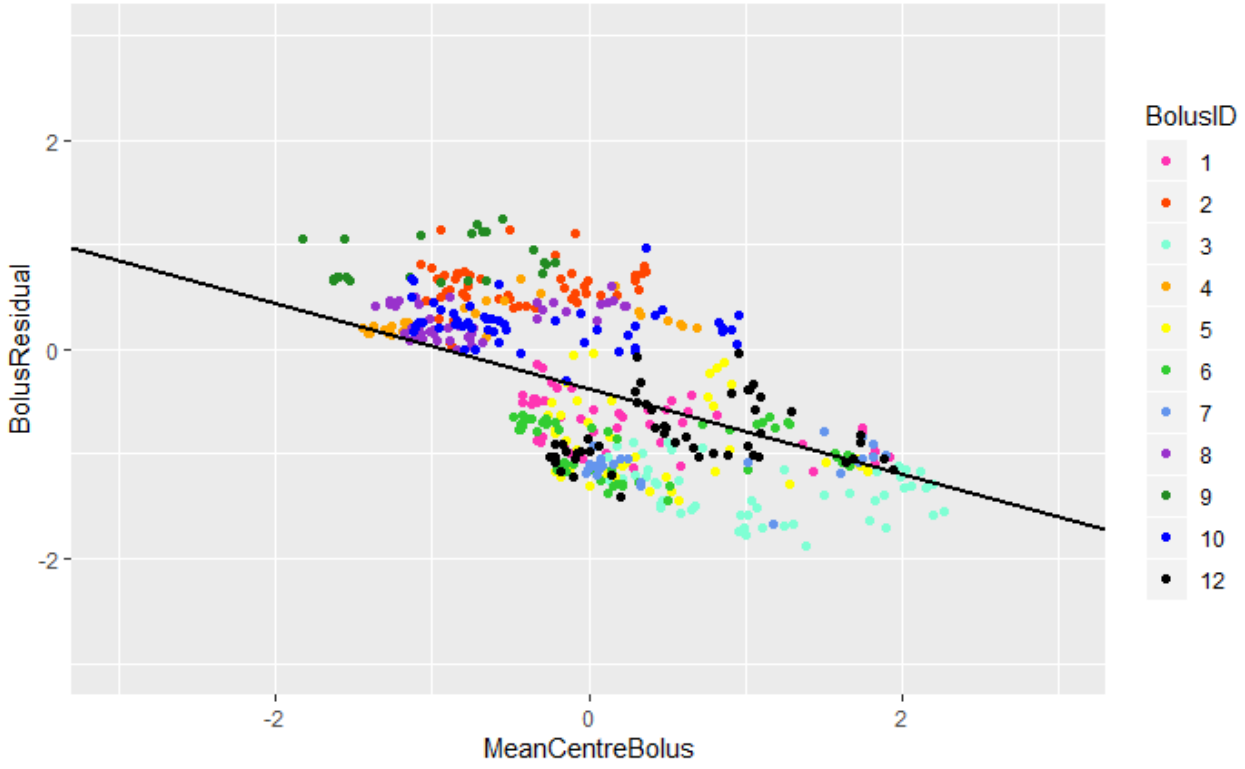

Figure 9. Residuals plotted on mean centered bolus pH with individual boluses separated by color and a black regression line, which represents the overall linear bias across boluses, ages and comparison locations.

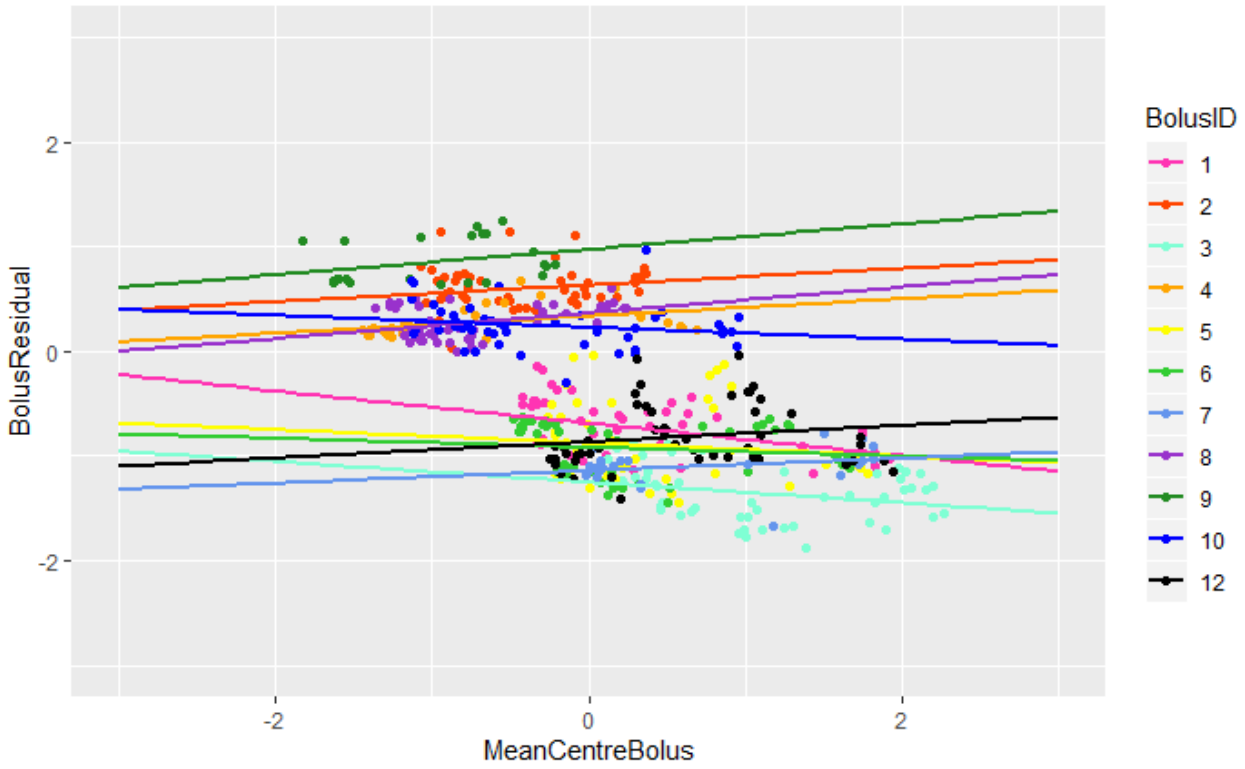

Figure 10. Residuals plotted on mean centered bolus with individual bolus and their respective regression lines (linear bias), separated by color.

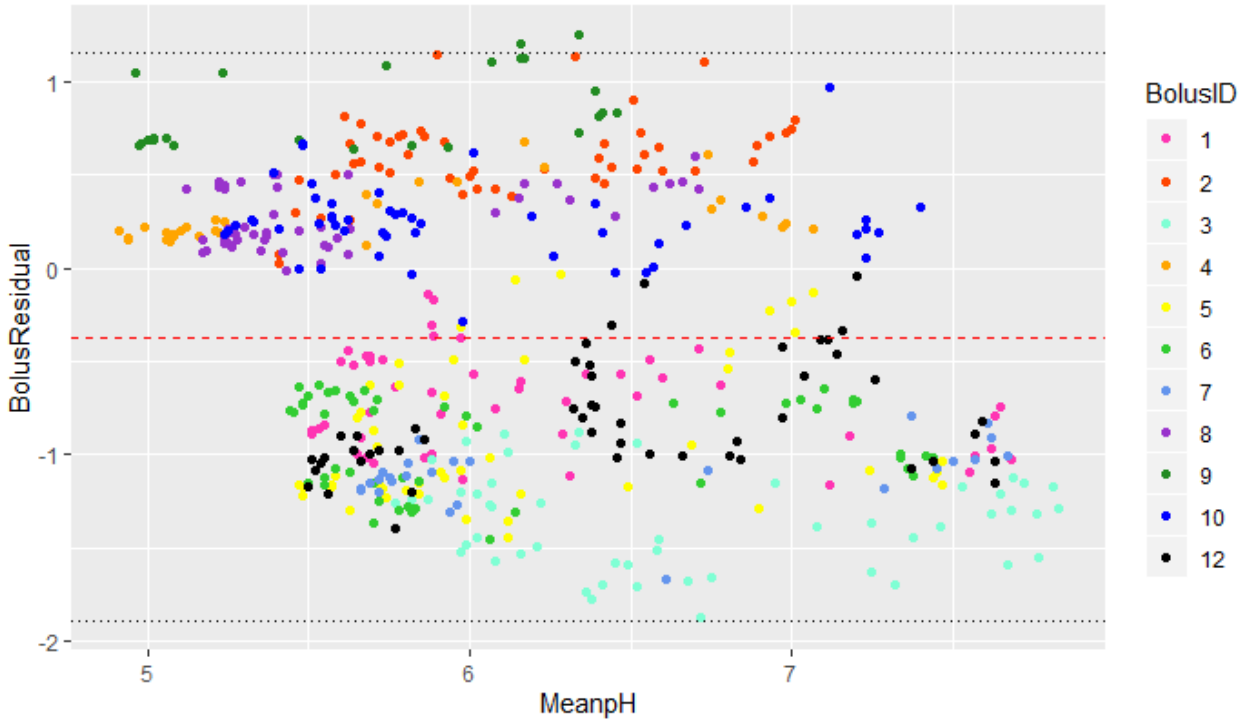

Figure 11. Residuals plotted on Mean pH (probe and bolus pH averaged) with the overall mean bias (red dashed line) and upper and lower Bland Altman limits of agreement (black dotted line) for reference.

In most cases the differential between bolus and manual pH when compared in buffer increased from initial testing out to 150d, appreciating the boluses had been in the rumen for most of this time and were only retrieved and placed in warm buffer when rations were about to be changed (Table10). When the pH was compared within the rumen between bolus value and the pH measured cannually from a sample drawn from immediately in front of the bolus sensor, there was a strong age of bolus effect, indicating drift of the pH value over time but in all cases there was a high correlation between the 2 pH measures.

4.4 Discussion

The smaXtec bolus is an expensive device (~AU$650/unit) that provides data over the internet for approximately 150d, after which the pH data becomes unavailable. Unlike its predecessor, the Khan bolus (no longer manufactured) it cannot be fitted with new membranes and recalibrated for a prolonged working life.

Initial experience with the smaXtec bolus was disappointing with two new boluses failing to initiate. The company replaced these free of charge but were very displeased to find we were testing the boluses in pH buffers beyond that specified after activation. The commented that this could compromise results but how this could be technically possible is unclear. None the less, there is no intention to publish these findings in a fuller form.

As discussed in previous sections, elucidation of relationships between two measurement methods of the same measurement should be done so with caution. Typically, the value of coefficient of correlation is overemphasized since it evaluates the presence of any linear relationship rather than the two measurements being in good agreement. When analysing the equality between bolus pH and probe pH, the St Pierre model evaluation focuses on residuals to avoid overstating the goodness of fit just based on trending data.

Overall, bolus pH deviation from probe pH and error worsened with time, which was expected. When evaluating the plots, it can be concluded that while there is significant deviance of bolus pH from probe pH, one overall general model is not sufficient to correct bolus pH for all boluses.

One of the challenges with the bolus was that some gave more stable accurate pH measures relative to the manual pH measure, others were biased quite soon after activation and drift over time was also a concern. Consequently, most required correction of their pH measure for both mean and linear bias with the increasing age of bolus. Our conclusion was that while the bolus provided an excellent method of quantifying the chronological pattern of pH change in the rumen, and that the overall mean pH is somewhat close to the actual overall mean pH, in this experience, individual bolus pH recording varied greatly and, if the user is not able to quantify correction parameters, which cannot be readily determined in non-cannulated cattle, any individual bolus could not be relied upon to provide an accurate estimate of individual rumen pH.

5 EFFECTS OF RUMEN MODIFIERS ON RUMINAL FERMENTATION OF CANNULATED STEERS DURING ADAPTATION TO A HIGH-GRAIN RATION

5.1 Introduction

At feedlot entry, cattle are transitioned from a roughage-based diet to a high-concentrate diet (NSW DPI 1997). This transition period is a crucial time in beef cattle nutritional management as transition feeding programs attempt to adapt cattle onto a finisher ration as fast as possible but without causing upsets in gastrointestinal function and health, which can cause severe gastrointestinal disorders that permanently impact performance and animal health (Plaizier et al., 2008). The dietary shift results in significant change to the rumen microbiome; with the increase in rapidly fermentable carbohydrates (starches) in high concentrate rations resulting in an increase in amylolytic bacteria populations (Owens et al., 1998). End products of starch fermentation result in an increased acid load in the rumen, decreasing ruminal pH, creating a favourable environment for the proliferation of lactic acid-producing bacteria (Galyean and Rivera, 2003). If the rumen is not transitioned correctly to high-grain diets, these bacteria can bloom, resulting in an accumulation of lactic acid in the rumen, driving rumen pH lower and putting the ruminant at risk of subacute or acute acidosis and the associated health and performance challenges (Owens et al., 1998; RAGFAR 2007). A successful adaptation to high-concentrate rations results in an established, principally amylolytic biome that can outcompete lactic-acid producing bacteria for rapidly fermentable starches, as well as remove lactic acid effectively preventing accumulation (Elam, 1976).

Antibiotic rumen modifiers have been routinely included in feedlot rations to aid the microbial profile shift to the safe fermentation of starches, reduce risks of metabolic upset associated with human error (feed delivery errors), and improve the retention of carbon and nitrogen (Lean et al., 2013). However, with few exceptions (e.g. Laidlomycin “Cattlyst” in the USA), no new antibiotic type rumen modifiers are being registered. The use of antibiotics in animal production continues to be scrutinized by consumers due to the concern of resistances, which lead to the development and implementation of the Veterinary Feed Directive in the USA in 2017 and the antimicrobial stewardship focus in Australia (AMR 2015-2019). The ionophores (including Monensin and Lasalocid) are not used in human medicine and this is recognised in the WHO (2019) listing of critical antibiotics. MLA have recently set in place Antimicrobial Stewardship Guidelines for the Australian Cattle Feedlot Industry for feedlot use (MLA 2018). Thus, there is a need to evolve the current industry approaches to use of existing rumen modifiers to improve feedlot efficiency, and decrease microbiome transition interval during the adaptation period.

Ionophores like Monensin and Lasalocid interfere with the natural ion transport systems of both prokaryotic and eukaryotic cells (Butaye et al., 2003). This disruption results in the elimination of ion gradients, removing the energy source of the cell, and causing death. Ionophores are effective against gram-positive bacteria since the cell wall allows the penetration of large, hydrophobic molecules, while gram-negative cells are resistant to this, thus less susceptible to ionophores (Westley, 1983). By decreasing the activity of gram-positive organisms, the rumen microbial population is shifted away from bacteria responsible for lactic acid production, methanogenesis and ruminal protein degradation, resulting in a decreased risk of acidosis, and less energy wasted in the production of methane and ammonia, respectively (Elsasser, 1984). This shift allows more beneficial bacteria to proliferate and be more efficient by converting feed carbon to propionate rather than methane (Fellner, 2009), resulting in a lower ruminal ratio of acetate to propionate, suggesting a greater efficiency of the use of energy that is supplied by feed.

Monensin is used in most Australian feedlots, increasing feed efficiency and average daily gain while controlling bloat (Lean et al., 2013). While there have been reports of Monensin toxicity, Lasalocid has not been reported to show such effects, most likely due to its lower alteration of ruminal fermentation compared to Monensin (Armstrong and Spears 1988).

Another rumen modifier, Bambermycin, inhibits peptidoglycan synthesis for cell wall formation, specifically that of the protein murien, a principal component of gram-positive bacteria (Butaye et al., 2003). Mammalian cells do not contain murien, and are thus not susceptible to Bambermycin. Bambermycin has been shown to promote fibrolytic bacteria, which could stimulate early intake of higher roughage diets in weaner calves (Loy, 1997). Generally, Bambermycin shows similar effects when compared to Monensin versus no additive (Arana, 1994a; Hammond, Scheffel, Titgemeyer, & Stevenson, 2002; Loy, 1997), but is less likely to have the long term intake stabilization benefits of Monensin (DelCurto et al., 1998). Bambermycin most likely does not have an effect on the production of methane since most methanogens lack murein in their cell wall (König, 1988), but Bambermycin appears to prevent or even reverse antimicrobial resistance to β-lactam antibiotics in Gram-negative enteropathogens (Watanabe et al., 1971, 1977b; Edrington et al., 2003; Pfaller, 2006).

The potential to combine rumen modifiers to improve productivity has only recently been revisited to overcome intake suppression (Benatti, Alves Neto, de Oliveira, de Resende, & Siqueira, 2017; Lemos et al., 2016; Potter et al., 1985), improve rumen fermentation parameters and decrease methane emissions (Crossland et al., 2017), as well as improve energy balance and reduce subsequent risk of ketosis (Erasmus et al., 2008). Possible explanations for effects of rotating or combining rumen modifiers included avoidance of microbial adaptation to modifiers (Callaway et al. 2003), or altering the site and extent of digestion (Galyean and Hubbert, 1989). There is a lack of data on response to combination or daily rotation of rumen modifiers in ruminal fermentation parameters when cattle are being adapted to a finisher diet like those in Australian feedlots. This investigation sought to explore principal rumen fermentation differences associated with combining or rotating Bambermycin or Lasalocid with Monensin, and how these changes in rumen modifier management approaches might make feeding, growth and animal health advantages available to the Australian feedlot industry during the adaptation period.

5.2 Materials and Methods

5.2.1 Animals, Housing, and Experimental Procedures

Cattle used in this study were housed in individual pens with cement floors and thick rubber mats in the Large Animal Handling Facility at the University of New England (Armidale, NSW, AUS) as approved by the University of New England Animal Ethics Committee (AEC 18-028). Twelve ruminally-cannulated Lowline Angus steers were twice studied following periods of grazing on pasture during two 28-day Adaptation periods onto a barley-based feedlot ration. Treatments were randomly allocated to steers within a period and again between periods so no steer would receive the same dietary treatment twice. Cattle were allocated randomly, and treatments were distributed in each period such that one combination and one rotation treatment had three replicates, and the remaining treatments had two replicates. After two periods, each of the rotation and combination treatments had 5 repetitions, and the Monensin treatment had four, due to removal of an animal for inappetance. Averaged across periods, steers weighed 237 ± 59kg.

5.2.2 Diets, Feeding, and Treatments

Steers were fed diets typical of feedlots in Australia. Dietary transition from 45 to 80% tempered barley (DM basis) was accomplished over 21 d Adaptation periods using two intermediate diets, followed by 10 d on a finisher diet (Tables 11, 12). All diets were formulated to meet NRC requirements for minerals and vitamins. Diets were prepared twice weekly in a small free-standing paddle mixer (Bonser Engineering, Merrylands, AUS). All steers were fed 2.0% of the average LW as DM, delivered daily at 1000 h. Tempered barley, wheat straw and cottonseed were sourced from Tullimba Feedlot (University of New England, Kingstown, NSW, AUS). Mill-run and oat chaff were sourced from Armidale GrazeAg, Mineral premix was custom made by DSM (Wagga Wagga, NSW, AUS). Feed samples were collected daily and bulked weekly to determine nutrient composition. Rumen modifiers and doses (100% DM basis) used were 25 ppm of Monensin fed as Rumensin Granular, Elanco Animal Health, Greenwood, Indiana, United States; 30 ppm Lasalocid fed as Bovatec (Zoetis LLC, Salisbury, Maryland, United States); and 2 ppm Bambermycin fed as Flavo 40 (Microflora Management, Huvepharma, Inc, Peachtree City, Georgia, United States). Treatments were Monensin-only (MON), Monensin in combination with Bambermycin (M+B), Monensin in combination with Lasalocid (M+L), Monensin in daily rotation with Bambermycin (M/B), and Monensin in daily rotation with Lasalocid (M/L). Modifiers (MON, LAS, BAM, M+L, M+B) were diluted into wheat flour (24-29%) and mill run (70%). Modifier doses were manually added directly into each individual steers' feed bin and hand mixed thoroughly before offering to cattle.

5.2.3 Rumen Sampling and Analysis

Rumen fluid from all steers was sampled 1-hour pre-feeding and 5 hours post-feeding on day 1, 3 and 7 of each ration, such that each ration was sampled 3 times pre-feeding and 3 times post-feeding. Rumen fluid was sampled via an intraruminal assembly, composed of 1m of small flexible poly-tubing attached to a weight of metal chain holding the sampling point low in the rumen ventral sac. Also attached to the chain was a smaXtec® intraruminal bolus (smaXtec® Premium Bolus; smaXtec Animal Care Sales, GMBH, Graz, Austria) for rumen pH and temperature monitoring. The bolus was contained in a fine porous nylon bag to filter contents, as was the intake end of the rumen fluid sampling tube, with the opening of the sampling tube placed immediately in line with the face of the bolus and the remaining tube fed up through the cannula to the exterior and closed by a 3-way tap. If the rumen assembly was not used, then a 35cm perforated metal tube was used to collect rumen fluid through the cannula. A minimum 15mL of rumen fluid was drawn out with a 60mL syringe to flush the sampling line and discarded, before a minimum 30mL of fresh rumen fluid was collected for subsampling and analysis. Rumen fluid was immediately measured for reduction potential (Mettler Toldeo SevenEasy S20 pH meter with TPS Intermediate Junction Redox Sensor) and pH (EcoScan Portable pH/ORP meter with TPS pH Sensor) calibrated daily, and then subsampled for protozoa and fermentation metabolite analysis. Additional subsampling occurred on day 1 and 7, before feeding, of each ration for microbiome analysis, with samples (1.5mL) being centrifuged directly, supernatant discarded and pellet stored at -80^{o}C.

Rumen fluid (15ml) subsampled for fermentation metabolites was acidified with 5 drops of concentrated sulfuric acid and frozen at -18C until analysis. Volatile fatty acids were determined by gas chromatography (Nolan et al., 2010). Rumen ammonia-N was determined by Skalar methodology, based on the modified Berthelot reaction (de Raphelis et al., 2016). Rumen fluid suspended in 4% formal saline was subsampled and stained with Brilliant Green to visualize protozoa for microscopic counts before counting on a Fuchs-Rosenthal chamber of 0.2mm depth.

Table 11. Metabolism Trial Diet Formulations. All values are expressed on a dry matter basis unless noted.

Item	Starter	INT-1	INT-2	Finisher
Ingredient, % As Fed				
Tempered Barley	48.7	59.9	70.9	81.8
Molasses	4.0	3.3	2.6	2.0
Vegetable Oil	-	0.9	1.8	2.8
Dry Supplement	2.6	2.6	2.6	2.5
Mill Run	10.0	6.7	3.4	-
Whole Cottonseed	11.7	10.7	9.7	8.7
Wheat Straw	7.7	5.3	3.0	2.2
Oaten Hay	15.3	10.6	6.0	-
Nutrient Composition, % DM				
Dry Matter	100	100	100	100
Ash, % DM	7.61	7.14	6.67	6.09
TDN, % DM	74.28	75.76	77.22	78.53
ME, Mcal/kg DM	2.69	2.82	2.95	3.09
NEm, Mcal/kg DM	1.77	1.88	2.00	2.11
NEg, Mcal/kg DM	1.15	1.25	1.35	1.46
Starch, % DM	30.31	35.85	41.44	47.08
Fat, % DM	4.37	5.18	6.01	6.98
NDF, % DM	38.00	34.46	30.91	27.47
CP, % DM	13.72	13.65	13.57	13.36
DIP, % DM	9.80	9.83	9.85	9.78
UIP, % DM	3.92	3.82	3.72	3.58
Ca, % DM	0.74	0.73	0.72	0.68
P, % DM	0.39	0.37	0.36	0.34
Mg, % DM	0.26	0.25	0.23	0.22
K, % DM	1.04	0.92	0.79	0.66
Na, % DM	0.11	0.09	0.07	0.04
Cl, % DM	0.32	0.24	0.16	0.07
S, % DM	0.23	0.23	0.22	0.21
Co, ppm	0.43	0.36	0.28	0.18
Cu, ppm	19.22	18.52	17.79	16.69
I, ppm	0.57	0.56	0.56	0.53
Fe, ppm	82.99	59.06	34.94	9.81
Mn, ppm	61.67	53.65	45.48	36.22
Mo, ppm	0.75	0.65	0.56	0.46
Se, ppm	0.15	0.14	0.12	0.10
Zn, ppm	92.34	91.81	91.22	88.11
Vitamin A, KIU/kg DM	2.17	2.20	2.23	2.18
Vitamin E, IU/kg DM	24.66	25.01	25.37	24.76
Salt, % DM	0.25	0.25	0.25	0.25
Urea , % DM	0.39	0.40	0.41	0.40
Vitamin D, IU/kg DM	271.22	275.16	279.12	272.40

[1]All ingredients and nutrients are displayed in % DM unless noted differently

[#]Rumen modifier treatments were top-dressed and fed at rates of 25ppm, 30ppm and 2ppm for Monensin, Lasalocid and Bambermycin, respectively. Treatment doses (100% DM basis) were as follows: Monensin fed alone continuously at a rate of 25ppm (MON), Monensin fed in daily combination with Lasalocid at rates of 25ppm and 30ppm, respectively (M+L), Monensin fed in daily rotation with Lasalocid at rates of 25ppm and 30ppm, respectively (M/L), Monensin fed in daily combination with Bambermycin at rates of 25ppm and 2ppm, respectively (M+B), and Monensin fed in daily rotation with Bambermycin at rates of 25ppm and 2ppm (M/B)

5.2.4 Sensors: Rumen pH and Rumination Parameters

Each steer was administered a smaXtec® intraruminal bolus (smaXtec® Premium Bolus; smaXtec Animal Care Sales, GMBH, Graz, Austria) that collected pH, temperature and activity every ten minutes, with the

data being wirelessly transferred to an external data capture module and database for display very 10 min by the smaXtec messenger® computer software. Bolus pH values were validated by comparison of recorded bolus pH and measured pH of rumen fluid sampled from immediately in front of the bolus sensor face as previously described, as well as by comparing against freshly purchased pH standards of 4 and 7 maintained in a water bath at 39°C, at the beginning and end of each period. Adjustments were made by multiple linear regression modelled in R statistical package (R Core Team, Boston, MA, USA).

All steers were also fitted with a commercial accelerometer ear tag (eSense Flex ear tag, Allflex, Capalaba Queensland, Australia) to measure rumination. Ear tags were validated by video recordings (see preceding chapter). Total minutes ruminating in 2-hour time blocks were gathered from the eSense ear tag data.

Rumen Bolus correction

Rumen pH data collected from intraruminal boluses was found to require some correction for the purposes of this study (Table 11b).
The full general linear model was expressed as follows:

$$y_{ij} = \mu + B_i + A_j + e_{ij}$$

Where y_{ij} is the corrected pH, μ is the general mean, B_i is the fixed effect of bolus pH, A_j is the fixed effect of Bolus Age, and e_{ij} is the random residual error. Corrected data was tested for adequate correction and used for analysis. To evaluate correction model adequacy, the relationship between corrected bolus and probe pH was assessed using the regression method from St-Pierre (2003), where the residuals (probe pH minus corrected bolus pH) were regressed on mean centred corrected bolus pH. From this regression, the mean bias and linear bias and their significance were obtained from the intercept and slope coefficients and P-values, respectively. If there were no linear or mean bias present, then all significant coefficients were used to correct bolus pH.

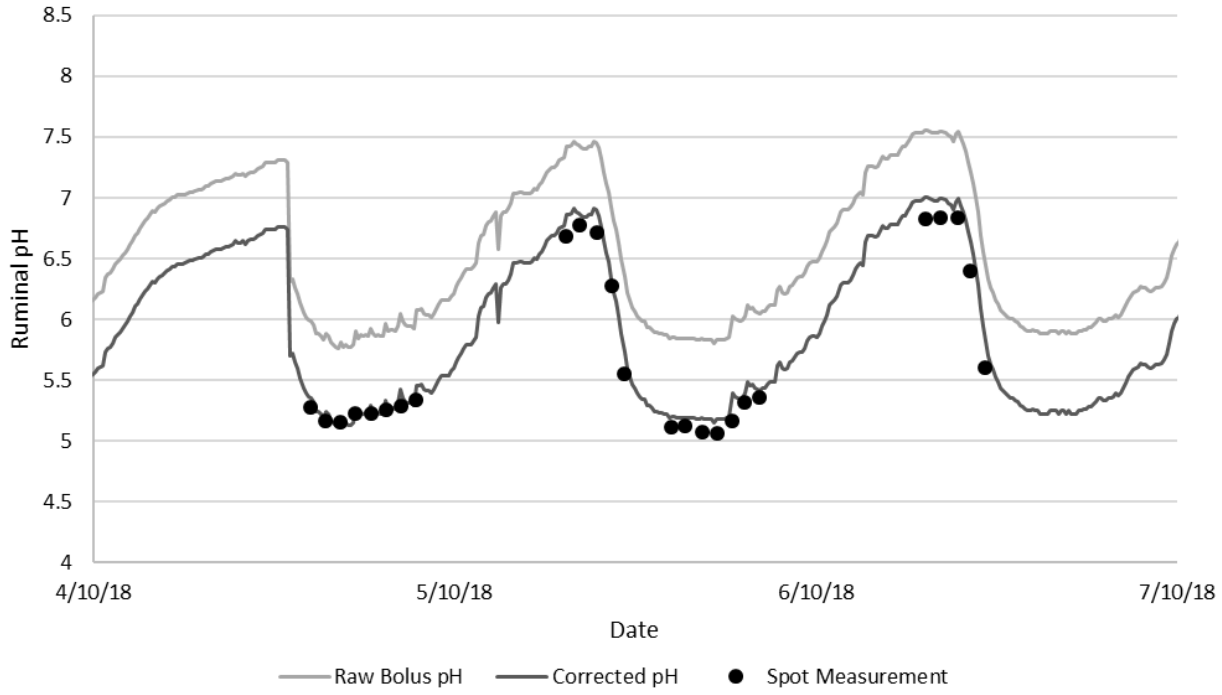

Figure 12. Comparing raw bolus pH, spot measurement, and corrected pH in the rumen of a cannulated steer used for intraruminal validation of a rumen bolus. This is an example of a bolus showing significant drift (~1.0 pH unit) and successful correction using linear regression.

Table 11b. Table of coefficient output from multiple linear regression model used to correct raw bolus pH, regressing the probe pH on the predicted (corrected) values, and results from St Pierre analysis of bias assessment to verify correction of bolus pH.

	Coefficients from multiple regression correction model[1]			Results of Probe pH ~ Corrected pH		Coefficients of St-Pierre bias assessment of corrected pH			
Bolus ID	**Intercept**	**Bolus pH**	**Bolus Age**	**Slope**	**r^2**	**Mean bias**	***P*-value**	**Linear bias**	***P*-value**
1	0.231	0.962	-0.007	1.00	0.947	<0.00	*NS*	<0.00	*NS*
2	0.156	1.043	0.002	1.00	0.898	<0.00	*NS*	<0.00	*NS*
3	-0.020	0.823	NS	1.00	0.799	<0.00	*NS*	<0.00	*NS*
4	0.149	1.048	-0.001	1.00	0.965	<0.00	*NS*	<0.00	*NS*
5	-0.131	1.008	-0.008	1.00	0.885	<0.00	*NS*	<0.00	*NS*
6	-0.383	1.047	-0.008	1.00	0.961	<0.00	*NS*	<0.00	*NS*
7	-0.675	1.040	-0.006	1.00	0.920	<0.00	*NS*	<0.00	*NS*
8	0.298	1.028	-0.003	1.00	0.958	<0.00	*NS*	<0.00	*NS*
9	0.256	1.050	0.008	1.00	0.905	<0.00	*NS*	<0.00	*NS*
10	0.147	0.979	0.002	1.00	0.937	<0.00	*NS*	<0.00	*NS*
11	-0.239	1.017	-0.011	1.00	0.991	<0.00	*NS*	<0.00	*NS*
12	-0.206	1.006	-0.006	1.00	0.905	<0.00	*NS*	<0.00	*NS*

[1] All regression coefficients listed are significant (P<0.05) unless noted; linear regressions were done to test if the slope of the pH was different than 1 by using OFFSET function (RStudio) and the simple LR results were used to determine if the intercept and bolus age are different than 0.

5.2.5 Data Handling and Statistical Analysis

Mean pH values are affected by sampling pattern, and can be over- or under-estimated, depending on meal frequency and magnitude of pH fluctuation (Pitt and Pell, 1997). Pitt and Pell (1997) also suggest a different approach by finding the area under the pH verses time curve and divide by the total time period, especially when meal frequency is less than eight meals/d. Currently, pH means are presented as simple arithmetic means, but the method by Pitt and Pell (1997) to present mean pH and patterns is being explored.

Rumen pH data from the intraruminal bolus were corrected using linear regression in R statistical package (R Core Team, Boston, MA, USA) based on validation results previously reported, and summarized by daily mean, with a day defined as the time between feedings, not midnight to midnight. Proportion of time pH spent under 5.8 and 5.5 were calculated by dividing the number of times the corrected pH dropped below the defined level by the total number of times the pH was measured in the same timeframe.

Rumination was summarised as total minutes ruminating within 2-hour time blocks. Daily totals were summed by adding all the 2-hour time blocks within a day, defined as time between meals, so the daily DMI could be used as a covariate. When comparing rumination and pH, pH was averaged into the same 2-hour time blocks as rumination, but DMI could not be included in the 2-hour interval analysis, since eating rate was not recorded in this study.

All responses were analysed using the linear mixed model procedure (LMER; R Core Team, Boston MA, USA). Each treatment was replicated five times across the two periods, except control (MON), which was

replicated four times. Steer was the experimental unit. The full general linear mixed model was expressed as follows:

$$y_{ghjk} = \mu + T_g + P_h + D_j + R_k + e_{ghjk}$$

Where y_{ghjk} is the response, μ is the general mean, T_g is the fixed effect of treatment, P_h is the fixed effect of period, D_j is the fixed effect of DOF, R_k is the fixed effect of ration, and e_{ghjklm} is the random residual error. Steer was set as the random effect in all models and DMI was used as the covariate. Interactions between treatment and ration, as well as treatment and DOF were explored as well, and included only if significant (P<0.05). For rumen fermentation parameters (total VFAs, VFA proportions, and ammonium-N), the fixed effect of sampling time (pre- or post-feeding) was also included. All responses are presented as raw means ± standard error of the mean. Differences were declared significant as $P \leq 0.05$, and tendencies declared at $0.05 \leq P \leq 0.10$.

5.3 Results

5.3.1 Intake, rumen acidity and redox potential

Despite intake being limited to 2.1% of average liveweight for both periods, DMI was significantly affected by treatment, steer, and period. Over both periods, cattle receiving M+L consumed significantly more than all other treatments until steers were transitioned to the finisher ration, in which they still consumed numerically the most.

Table 12. Nutrient analysis of bulked daily samples of rations for Adaptation periods.

Nutrient, (% in DM basis)	***Starter***	***INT-1***	***INT-2***	***Finisher***
Dry Matter	86.4 ± 1.4	87.2 ± 2	83.8 ± 0.5	83.7 ± 0.4
Moisture	13.6 ± 1.4	12.9 ± 2	16.2 ± 0.5	16.4 ± 0.4
NDF	39 ± 0	31 ± 1	28 ± 1	30 ± 1
ADF	21 ± 1	16.5 ± 0.5	14 ± 1	12.5 ± 0.5
Crude Protein	12.7 ± 0.4	13.1 ± 1	13.2 ± 0.5	14 ± 0.6
DMD	66 ± 1	72.5 ± 0.5	76 ± 1	80.5 ± 0.5
DOMD	65.5 ± 0.5	71.5 ± 0.5	75 ± 1	79.5 ± 0.5
Inorganic Ash	6.5 ± 0.5	6 ± 0	5 ± 0	4 ± 0
Organic Matter	93.5 ± 0.5	94 ± 0	95 ± 0	96 ± 0
ME (MJ/kg DM)	11 ± 0.1	12.2 ± 0.2	14.6 ± 1.6	14 ± 0.1
Crude Fat	4.2 ± 0.1	5.3 ± 0.4	12.4 ± 6.3	7.7 ± 0.4
WSC	6.2 ± 0.9	6.1 ± 0.7	4.9 ± 1.2	3.8 ± 0.5
Starch Total	24.5 ± 0.5	34 ± 1	36 ± 2	43 ± 0

There was no treatment effect on average pH (manual sample) over the entire adaptation period (Table 13), but there was a significant decline in average pH with time as adaptation progressed from starter through T1, T2 and finisher ($P<0.05$). Despite having the highest DMI, M+L cattle had pre-feeding rumen pHs (manually determined) higher than did cattle on other modifier treatments, and even post-feeding, M+L cattle had a significantly higher rumen pH than did all but Monensin cattle (Table 13). The smaXtec bolus pH data did not reflect identical treatment effects, with M/L having high pH similar to M and M+L. These 3 treatments ensured less time was spent with pH<5.8 and <5.5 relative to the Bambermycin treatments. In general there was a downward trend in rumen pH (Fig. 14) through the 21 d adaptation period.

Overall the redox potential (a function of H^+ ion concentration and dissolved H_2 concentration) was significantly more negative in M and M+L cattle, but the overall differences were largely due to differences being greatest in the finisher period (Table 13).

The redox potential of rumen fluid (mV) also became more negative (more reduced) as cattle progressed from starter to finisher diets (Fig. 13; Table 13). MON and M+L had the most negative redox potentials in adaptation overall ($P<0.05$), mainly due to the significant decrease once steers were transitioned on the finisher ration. M+L and MON also appeared to have numerically lower redox potentials as steers were transitioned through the rations, but only showed a significantly more negative redox potential in the finisher ration, alongside M/L.

Table 13. Effect[#] of Treatment and Ration on DMI, ruminal redox potential and pH manually sampled from cannulated steers during adaptation to high-grain diet. Values presented as raw means. Fixed effects are Treatment, Period, and Ration, with Steer as a Random Effect. Rumen sampling parameter models also included DMI as a covariate.

	Treatments						P-values		
Parameter	**MON**	**M/B**	**M/L**	**M+B**	**M+L**	**SEM**	**Treatment**	**Period**	**Ration**
DMI, kgs	**4.39_{ab}**	**4.65_{b}**	**4.20_{a}**	**4.17_{a}**	**5.78_{c}**	**0.05**	***0.000***	***0.000***	***0.000***
Starter	4.50_{a}	3.90_{a}	4.51_{a}	4.04_{a}	4.75_{b}	0.10	*0.001*	*0.000*	
INT-1	4.99_{a}	4.31_{a}	4.73_{a}	3.94_{b}	5.23_{c}	0.11	*0.000*	*0.000*	
INT-2	5.08_{ab}	4.44_{a}	4.93_{b}	4.60_{a}	5.30_{c}	0.10	*0.000*	*0.000*	
Finisher	5.08_{a}	4.40_{b}	4.61_{ab}	5.10_{ab}	5.14_{a}	0.09	*0.000*	*0.000*	
Redox Potential	**-261_{a}**	**-249_{b}**	**-245_{b}**	**-245_{b}**	**-266_{a}**	**1.92**	***0.011***	***0.000***	***0.000***
Starter	-251	-242	-227	-237	-256	3.62	*0.207*	*0.000*	
INT-1	-257	-247	-240	-251	-271	4.56	*0.425*	*0.000*	
INT-2	-257	-248	-251	-241	-265	3.98	*0.504*	*0.000*	
Finisher	-283_{a}	-263_{b}	265_{ab}	-254_{b}	-273_{a}	3.24	*0.082*	*0.000*	
Mean pH	**6.27**	**6.24**	**6.16**	**6.20**	**6.42**	**0.03**	***0.197***	***0.001***	***0.000***
Starter	6.50	6.51	6.36	6.56	6.69	0.06	*0.387*	*0.512*	
INT-1	6.22	6.26	6.19	6.27	6.50	0.07	*0.366*	*0.754*	
INT-2	6.25	6.22	6.16	6.04	6.36	0.07	*0.625*	*0.354*	
Finisher	6.09	5.92	5.88	5.88	6.06	0.07	*0.570*	*0.098*	
Pre-feeding pH	**6.82_{a}**	**6.75_{a}**	**6.84_{a}**	**6.78_{a}**	**7.10_{b}**	**0.03**	***0.096***	***0.179***	***0.001***
Starter	6.85	6.79	6.85	7.02	7.15	0.05	*0.567*	*0.897*	
INT-1	6.84	6.86	6.86	6.79	7.21	0.05	*0.706*	*0.741*	
INT-2	6.70	6.97	6.77	6.56	7.08	0.05	*0.111*	*0.116*	
Finisher	6.68_{a}	6.67_{a}	6.82_{a}	6.65_{a}	7.28_{b}	0.06	*0.013*	*0.001*	
Post-feeding pH	**6.45_{ab}**	**6.37_{b}**	**6.26_{b}**	**6.32_{b}**	**6.51_{a}**	**0.03**	***0.025***	***0.000***	***0.000***
Starter	6.34	6.42	6.09	6.31	6.40	0.07	*0.569*	*0.003*	
INT-1	5.87	5.86	5.84	6.05	6.00	0.06	*0.709*	*0.000*	
INT-2	5.88	5.86	5.66	5.66	5.89	0.05	*0.328*	*0.000*	
Finisher	5.94_{ab}	5.80_{ab}	5.59_{c}	5.68_{bc}	5.85_{ab}	0.05	*0.035*	*0.000*	

[#]: means within a row showing different superscripts show significant effects of Treatment for treatment (P<0.05)

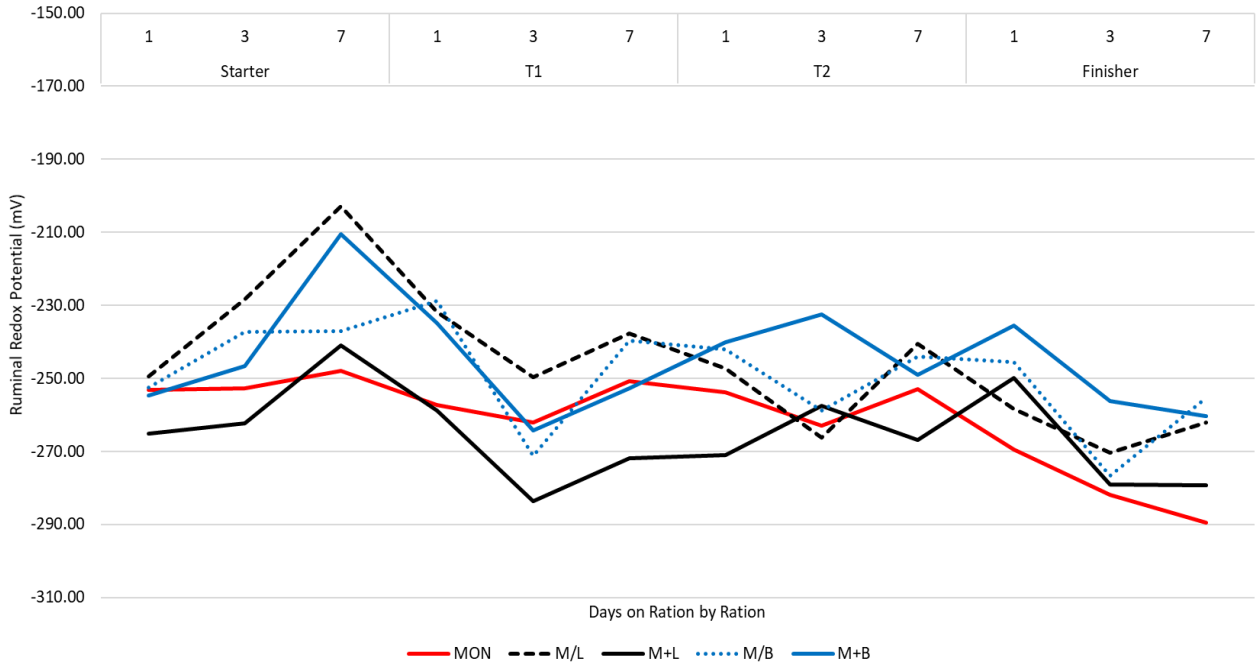

Figure 13. Interaction of treatment and days on ration by ration on average ruminal redox potential in cannulated steers.

While mean pH sampled via manual probe did not change significantly with treatment, there were differences in fasted verses fed state rumen pH. M+L had significantly the highest rumen pH while fasted (P<0.05), and also had the highest pH when in the fed-state alongside MON. As seen in the fasted-state pH, these differences were significant only in the finisher ration, but numerical differences in the other rations most likely contributed to the overall advantage of M+L and MON.

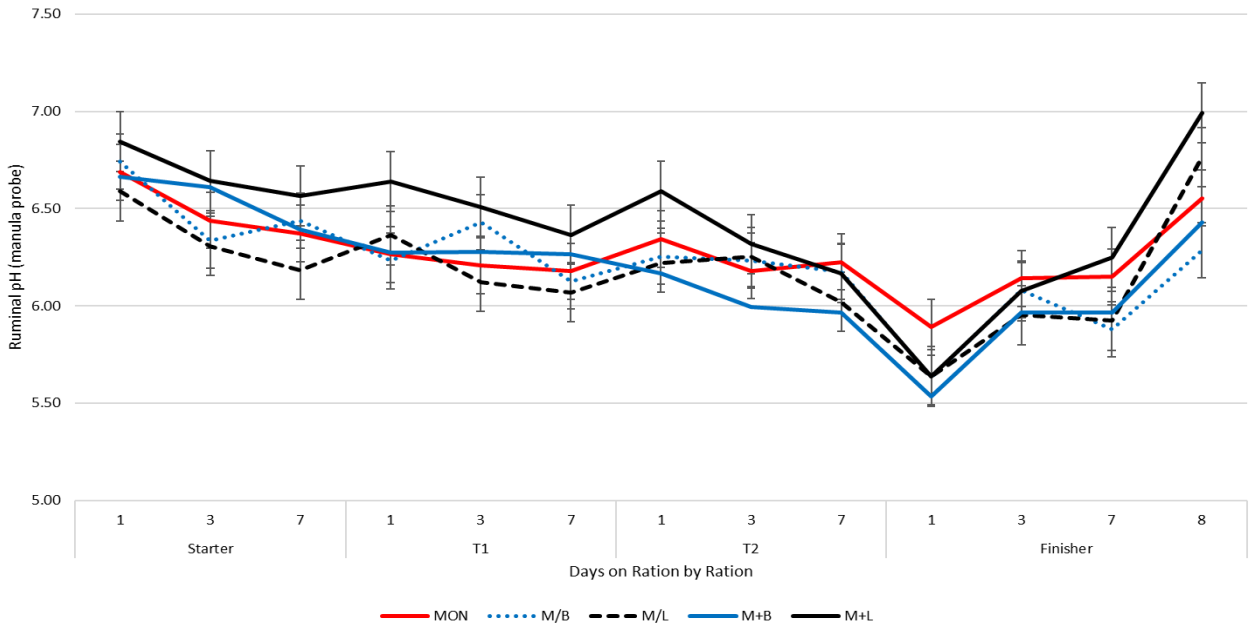

Figure 14. Interaction of treatment and days on ration on average ruminal pH measured by manual probe in cannulated steers.

Ruminal pH patterns were generally lowered with the addition of Bambermycin, resulting in M/B and M+B steers spending more time with a pH below 5.6 and 5.2, increasing the risk of SARA and RA, respectively, compared to MON, M+L and M/L (Table 14). Further analysis needs to be made of bolus pH data to better describe patterns.

Table 14. Diagnostics of corrected bolus pH and the effects of rumen modifiers and ration on average pH, as well as average percent time daily spent in SARA and RA conditions. Model includes Treatment as the fixed effect, with DMI and DOF as covariates and steer as a random effect. Raw means are presented.

Parameter	Treatments					SEM	*P*-value
	MON	M/B	M/L	M+B	M+L		
Average pH (bolus)	6.77_{a}	5.73_{c}	6.40_{ab}	6.08_{bc}	6.43_{ab}	0.03	*<0.05*
Starter	6.94	5.55	6.50	6.24	6.55	0.06	*<0.1*
INT-1	6.71	5.77	6.45	6.07	6.46	0.05	*NS*
INT-2	6.81	5.99	6.42	5.98	6.33	0.06	*NS*
Finisher	6.63_{a}	5.65_{b}	6.30_{a}	6.03_{b}	6.40_{a}	0.05	*<0.05*
Mean Time pH < 5.6, %	0.5_{a}	44.8_{b}	4.8_{a}	19.0_{a}	3.8_{a}	0.15	*<0.01*
Starter	1.8_{a}	48.2_{b}	3.2_{a}	4.8_{a}	4.5_{a}	0.02	*<0.000*
INT-1	0.0_{a}	32.7_{b}	2.2_{a}	16.4_{a}	0.9_{a}	0.02	*<0.005*
INT-2	0.0_{a}	23.2_{b}	5.1_{a}	32.1_{a}	9.2_{a}	0.03	*NS*
Finisher	0.0_{a}	79.9_{b}	8.8_{a}	23.3_{a}	0.0_{a}	0.04	*<0.05*
Mean Time pH < 5.2, %	0.0	13.6	0.0	7.1	0.1	0.01	*NS*
Starter	0.0_{a}	24.4_{b}	0.0_{a}	0.0_{a}	0.3_{a}	0.02	*<0.000*
INT-1	0.0	7.7	0.0	7.4	0.0	0.01	*NS*
INT-2	0.0	5.4	0.0	19.6	0.0	0.02	*NS*
Finisher	0.0	34.0	0.0	1.7	0.0	0.01	*NS*

5.3.2 Rumen fermentation

Modifier treatment did not affect the average total VFA concentration in the rumen (Table 15) but there was a tendency (P<0.1) for Monensin (alone or in combination or rotation with Lasalocid) to have a lower acetate molar percentage than other treatments, while MON and M+L tended to have a correspondingly greater propionate percentage than other treatments (Table 15, Fig. 15).

5.3.3 Microbiome

Data on rumen microbial populations will be made available after 16S RNA analysis and bioinformatics has been completed on these samples for the next milestone.

Table 15. Effect of Treatment and Ration on Volatile Fatty Acid amounts and profile in cannulated steers during adaptation to high-grain diet.

	Treatments						
Parameter	**MON**	**M/B**	**M/L**	**M+B**	**M+L**	**SEM**	***P*-value**
Ammonium-N, ug/mL	**73.28**	**100.21**	**78.53**	**92.87**	**71.50**	**4.5**	*0.60*
Starter	95.75	117.07	105.87	135.50	99.05	7.84	*0.24*
Intermediate-1	77.59	103.49	63.90	98.46	51.64	6.85	*0.42*
Intermediate-2	39.67	63.24	29.26	39.99	29.46	8.80	*0.79*
Finisher	22.30	85.84	34.30	29.65	41.00	11.05	*0.68*
Total VFAs	**117.16**	**113.72**	**118.69**	**112.45**	**106.13**	**2.34**	***0.255***
Starter	107.78	102.71	111.74	92.91	96.20	3.78	*0.791*
Intermediate-1	123.65	118.15	109.70	85.39	104.97	5.26	*0.680*
Intermediate-2	116.68	111.27	123.96	124.30	104.45	4.68	*0.190*
Finisher	121.17	125.70	127.80	141.75	120.61	4.53	*0.844*
Acetate, %	**62.4**	**65.7**	**63.3**	**65.8**	**62.4**	**0.38**	***0.095****
Starter	70.4	70.2	69.6	74.1	70.3	0.81	*0.910*
INT-1	65.7	69.0	65.3	71.8	64.6	0.68	*0.422*
INT-2	58.3	61.8	58.0	61.8	57.6	0.72	*0.190*
Finisher	57.4	64.1	62.4	57.2	62.2	0.71	*0.089**
Propionate, %	**28.3**	**24.3**	**25.2**	**24.3**	**28.7**	**0.41**	***0.075****
Starter	22.4	21.5	22.5	16.9	22.9	0.87	*0.611*
INT-1	26.8	22.1	24.7	19.5	28.4	0.82	*0.259*
INT-2	30.5	27.5	28.8	27.6_{a}	32.6	0.77	*0.655*
Finisher	32.4_{a}	24.2_{bc}	23.1_{bc}	31.5_{ac}	27.1_{bc}	0.81	*0.012*
Butyrate, %	**5.9_{bc}**	**6.8_{ac}**	**7.8_{a}**	**6.7_{ac}**	**5.8_{bc}**	**0.18**	***0.041****
Starter	5.2	6.1	5.4	6.5	4.8	0.26	*0.480*
INT-1	4.8	6.1	6.5	6.0	4.5	0.37	*0.680*
INT-2	6.8	7.1	9.1	6.9	6.4	0.32	*0.064*
Finisher	6.1	7.8	9.8	7.5	6.7	0.42	*0.117*
Acetate:Propionate	**2.50**	**3.01**	**2.84**	**3.33**	**2.46**	**0.07**	***0.116***
Starter	3.62	3.69	3.45	4.98	3.62	0.16	*0.674*
INT-1	2.55	3.28	2.82	4.27	2.32	0.12	*0.164*
INT-2	2.16	2.44	2.22	2.56	1.95	0.11	*0.795*
Finisher	1.83_{a}	2.99_{b}	3.12_{b}	1.89_{a}	2.49_{a}	0.12	*0.245*

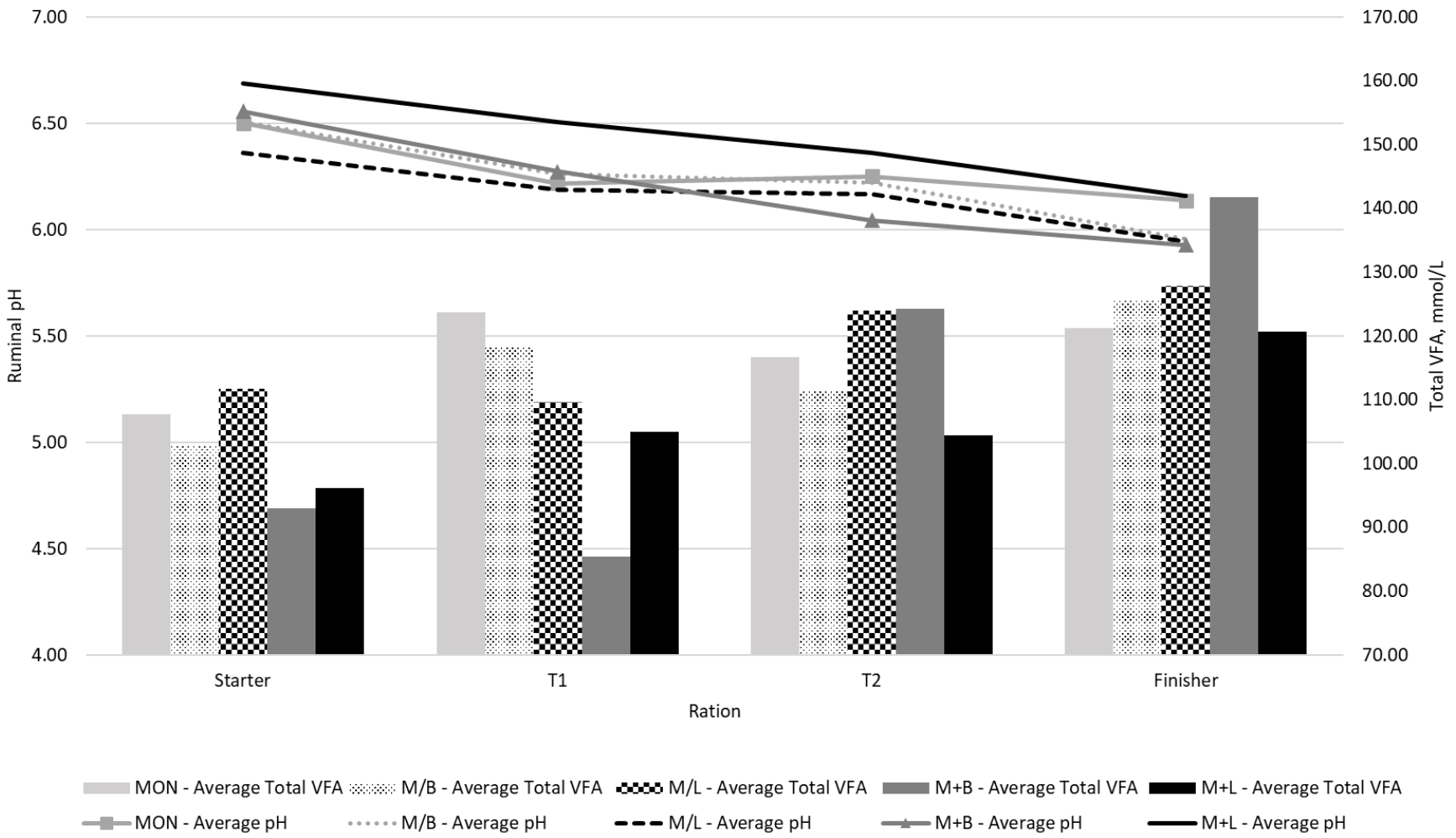

Figure 15. Comparing effects of treatment across the adaptation rations on total volatile fatty acids and pH of rumen fluid. Values are averages. Treatments: MON = Monensin-only; M/B = Monensin in daily rotation with Bambermycin; M/L = Monensin in daily rotation with Lasalocid; M+B = Monensin in combination with Bambermycin; M+L = Monensin in combination with Lasalocid.

5.3.4 Rumination

Cattle on the MON treatment spent significantly less time ruminating each day compared to M+L, and significantly more than did M/L (Table 16; Fig 16). When DMI is used as a covariate, MON was not different than any other treatment except M/L. No effect of ration on rumination, but there was a DMI effect. And there is an effect of treatment on both DMI and rumination (Table 16, Figs. 16, 17).

Table 9. Effect[#] of Treatment and Ration on Total Daily Minutes Ruminating of cannulated steers during adaptation to high-grain diet. Data is adjusted for DMI as a covariate.

	Treatments							
Parameter	**MON**	**M/B**	**M/L**	**M+B**	**M+L**	**Overall**	**SEM**	***P*-value**
Total Rumination, min/day	**415_{ab}**	**401_{b}**	**363_{c}**	**415_{b}**	**468_{a}**	**414**	**7**	**0.000**
Starter	406_{b}	387_{b}	365_{c}	353_{bc}	504_{a}	407	13	0.000
INT-1	454_{abc}	457_{bc}	356_{d}	369_{d}	494_{ab}	430	15	0.000
INT-2	395_{b}	404_{ab}	338_{c}	513_{ab}	420_{a}	414	14	0.000
Finisher	404_{a}	353_{c}	396_{a}	421_{a}	453_{b}	405	15	0.002
Daily Dry Matter Intake, kg	**4.92_{ac}**	**4.27_{ac}**	**4.69_{ac}**	**4.43_{a}**	**5.11_{b}**	**4.67**	**0.05**	**0.000**
Starter	4.50	3.90	4.51	4.04	4.75	4.33_{a}	0.10	0.000
INT-1	4.99	4.31	4.73	3.94	5.23	4.64_{b}	0.11	0.000
INT-2	5.08	4.44	4.93	4.60	5.30	4.85_{b}	0.10	0.000
Finisher	5.08	4.40	4.61	5.10	5.14	4.83_{b}	0.09	0.000

[#]: Means on the same row with different superscript are significantly different (P<0.05)

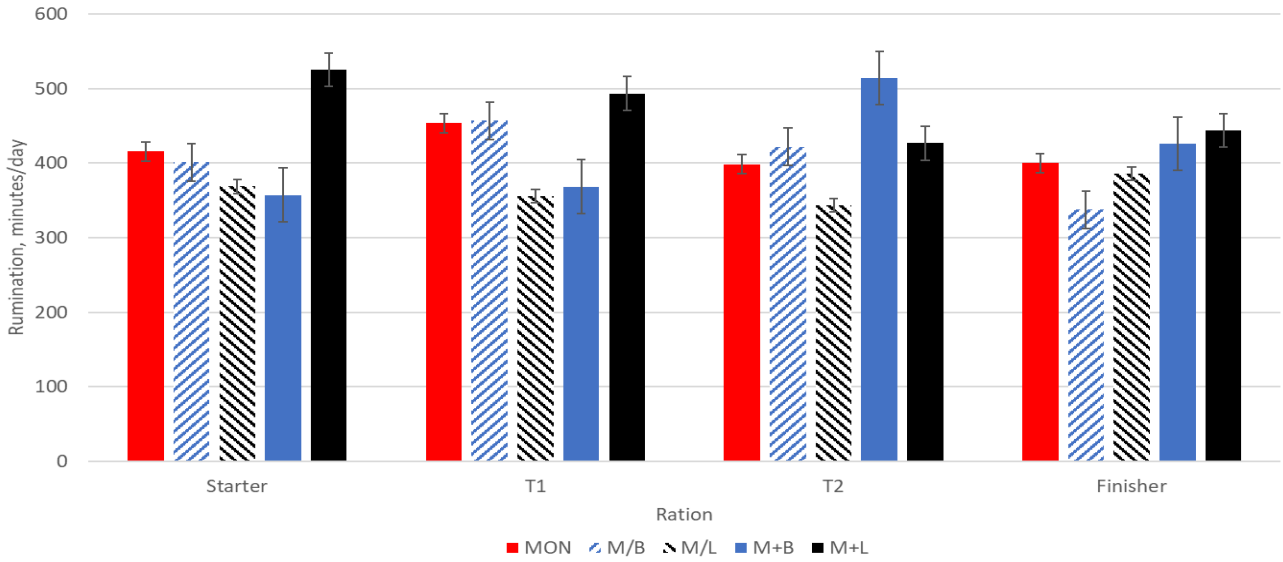

Figure 16. Effect of rumen modifiers on total rumination in minutes per day, while adapting to a high grain finisher ration.

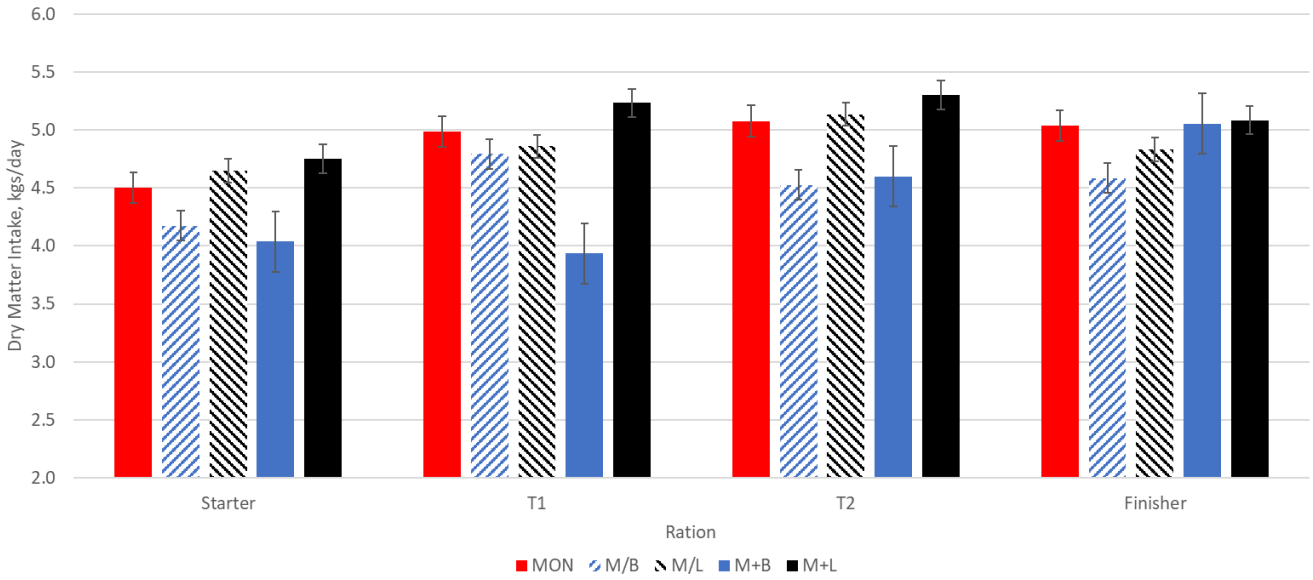

Figure 17. Effect of rumen modifiers on dry matter intake in kgs per day, while adapting to a high grain finisher

5.4 Discussion

There are only limited, full, current reports available for rumen fermentation parameters of cattle fed Bambermycin, especially ones that compare Monensin and/or Lasalocid. This study identified a clear differentiation between pairing of M with B compared to pairing M with L. This pairing with L offered significant advantage over pairing with B through a consistent pattern of change. Monensin alone or paired (rotation of combination) with L supported the highest pre- and post-feeding ruminal pH (manual sample) so the least risk of lactic acidosis. They also offered the lowest redox potential, the lowest acetate percentage and lower NH3 concentration than treatments with B. On these aspects, M alone or in pairing with L warrants further investigation.

The current study did not have a 'no modifier' treatment to test for overall effects of the presence of modifiers, but all treatments ensured the rumen spent only small proportions of time with pH<5.8 and generally less again below pH 5.5 (Table 14). It was apparent that M alone offered greatest protection against SARA with no time below 5.8, but it is surprising that coupling with another modifier in all cases allowed some time with rumen pH<5.8. It is not clear why other modifiers should diminish the efficacy of M by being in combination, but it may become apparent when the rumen microbial biome is assessed.

5.4.1 Rumen acidity and redox potential (pH and patterns)

DelCurto et al. (1998), found that while the inclusion of modifiers in a high grain ration significantly decreased rumen pH (due to increased total fermentation acid concentrations), BAM delivered a significantly lower pH compared to MON and LAS. Meanwhile, modifiers had no effect of pH on high-forage diets. Contrastingly, other authors found no differences in pH between BAM vs CON in dairy diets (Arana, 1994), or BAM vs MON or rotations in moderate-forage diets in beef steers (Crossland et al., 2017).

5.4.2 Rumen fermentation (VFAs, ammonium-N)

DelCurto et al. (1998) found that BAM, MON and LAS fed individually in high concentrate diets lower ruminal pH and increased VFA concentrations compared to no modifier. Additionally, they found that BAM decreased pH further than MON and LAS. However, BAM, compared to LAS, increased total VFAs as well as shifted individual VFA proportions away from butyrate and isobutyrate, and toward propionate. As in the current study, MON and BAM did not differentially affect total VFA and proportions, except DelCurto et al., (1998) found BAM had significantly more isovalerate proportionally than MON.

Lemos et al. (2016), found neither MON or BAM provided individually or in combination, had any advantages compared to each other on ruminal pH, rumen ammonium-N, or VFAs when provided to zebu cattle on a no-roughage finishing diet after 14 days on the modifier. However, these cattle were used in 7x7 Latin square design with no washout between periods, potentially allowing the manifestation of carry-over effects between modifier treatments. Crossland et al (2017) also found no difference in pH, or TVFA with rotation of MON and BAM in steers moderate forage diets overall, but there was a treatment x week interaction, such that BAM and rotation steers did not have reduced A:P like M did compared to control. The Crossland study found that MON, and short rotations between MON and BAM decreased A:P compared to BAM and a 21-day rotation of the two in the first 3 weeks, indicating the duration of MON inclusion was affecting A:P more than BAM. But no information of what was happening in at the end of week 1 or 2.

However, there are several authors who have found significant benefits with the addition of BAM, including increased protein supply (Kraszwski et al., 1991; Behrens et al., 1993). This could be explained by a decrease of proteolytic activity by bacteria in the rumen (Poppe et al., 1993; Corpet et al., 2000), thus increasing ruminal protein outflow (Behrens et al., 1993). Additionally, Fallon et al. (1986) and Murray et al. (1990) found inclusion of BAM increased molar proportions of propionate, and Van Nevel (1991) found BAM was successful at stabilizing rumen pH in dairy cattle.

6 EFFECTS OF RUMEN MODIFIERS ON RUMINAL FERMENTATION PARAMETERS OF CANNULATED STEERS WHILE MAINTAINING ON A HIGH-GRAIN FINISHER RATION

6.1 Introduction

Cattle enterprise profitability is largely driven by the efficiency of converting feed to gain by optimizing the rate and products of starch fermentation in the rumen to support a propionate rich fermentation with low risk of lactic acidosis. Antibiotic rumen modifiers have been routinely included in feedlot rations for this purpose but with few exceptions (e.g. Laidlomycin "Cattlyst" in the USA), no new antibiotic type rumen modifiers are being registered. Thus, there is a need to evolve the current industry approaches to existing rumen modifiers to improve feedlot efficiency.

The ionophore Monensin is widely used in most Australian cattle feedlots, increasing efficiency by causing a slight acceleration of animal growth (ADG) while decreasing feed intake. Recently, Shreck et al., (2016) reported a 4.81% advantage in ADG and 250g DMI/d improvement from daily rotation of Monensin and Lasalocid relative to Monensin alone. Similarly, the potential to combine rumen modifiers to improve productivity has only recently been revisited to overcome intake suppression (Potter and Wagner 1986; Lemos et al., 2016; Benatti et al., 2017). Where daily rotation was found to be beneficial, most authors could not attribute the improvement in performance to any alteration in rumen fermentation, site or extent of nutrient digestion. There is a lack of data on response to combination of, or short term rotation of rumen modifiers in ruminal fermentation parameters when cattle are maintained on a finisher diet similar to that fed in Australian feedlots. This project seeks to quantify differences in rumen fermentation associated with combining or rotating Bambermycin or Lasalocid with Monensin, and how these changes in rumen modifier management approaches might lead to feeding, growth and carcass advantages available to the Australian feedlot industry.

6.2 Materials and Methods

6.2.1 Animals, Housing, and Experimental Procedures

Cattle used in this study were housed in individual pens with cement floors and thick rubber mats in the Large Animal Handling Facility at the University of New England (Armidale, NSW, AUS) as approved by the University of New England Animal Ethics Committee (AEC18-028). Ruminally-cannulated Lowline Angus steers (n=10) were used during two 24-day Finisher periods. Steers weighed, on average, 236 ± 49kgs. Treatments were randomly allocated to steers within a period and again between periods with no steer receiving the same treatment twice.

During the Adaptation period prior to Finisher period, all steers were on treatments and sampled regularly for rumen fermentation parameters (previous chapter). After cattle were successfully transitioned to the finisher ration and completed adaptation sampling, cattle went through a 10-day washout period to equalize any significant microbial shifts by the different treatments from the Adaptation. At commencement of the

washout period 500mL of rumen fluid was collected from each animal and pooled and mixed at 39^{o}C under a blanket of CO_2 before 300ml of mixed rumen fluid was reintroduced to each animal via the ruminal cannula. During the 10d washout period all cattle received 25ppm of Monensin in a finisher ration. Samples of strained rumen fluid were collected centrifuged immediately at 10,000RPM, the supernatant poured off, and pellet frozen at -18 until all were collected and then transferred to -80°C prior to inoculation, and on day 1 of each Finisher period to verify the starting microbial diversity.

After the first Finisher period concluded, cattle underwent the same Washout period process before starting the next Finisher period. However, before sampling began during the second finisher period, feed intake decreased sharply for all cattle, so steers underwent a washout and recovery period where they were restarted at an intermediate ration and brought back up to finisher, while maintaining on MON, to improve intake and rumen health, across ten days (five days on intermediate, five days on finisher).

6.2.2 Diets, Feeding, and Treatments

Cannulated steers (n=10) were adapted to a high-grain finisher ration on two occasions. Steers were fed diets typical of feedlots in Australia. Dietary transition from 45 to 80% tempered barley (DM basis) was accomplished over 21 d using two intermediate diets (Table 17). All diets were formulated to meet NRC requirements for minerals and vitamins. Diets were prepared twice weekly in a small free-standing ribbon mixer (Bonser Engineering, Merrylands, AUS). All steers were fed 2.0% of the average LW as DM, delivered daily at 1000 h. Tempered barley, wheat straw and cottonseed were sourced from Tullimba Feedlot (University of New England, Kingstown, NSW, AUS). Mill-run and oat chaff were sourced from Armidale GrazeAg, Mineral premix was made by DSM (Wagga Wagga, NSW, AUS). Feed samples were collected daily and bulked weekly to determine nutrient composition.

Rumen modifiers and doses (100% DM basis) used were 25 ppm of Monensin fed as Rumensin Granular, Elanco Animal Health, Greenwood, Indiana, United States; 30 ppm Lasalocid fed as Bovatec, Zoetis LLC, Salisbury, Maryland, United States; and 2 ppm Bambermycin fed as Flavo 40 Microflora Manangement, Huvepharma, Inc, Peachtree City, Georgia, United States. The 5 treatments were Monensin-only (MON), Monensin in combination with Bambermycin (M+B), Monensin in combination with Lasalocid (M+L), Monensin in daily rotation with Bambermycin (M/B), and Monensin in daily rotation with Lasalocid (M/L). Modifiers were purchased as raw materials from DSM Nutritional Products Australia Pty Limited, Wagga Wagga, NSW, Australia, and diluted into wheat flour (24-29%) and mill run (70%) such that the final dilution contained the respective ppm of modifier(s) in a single 20g dose based on the target DMI.

Cattle were maintained on the finisher ration and their allocated rumen modifier treatments for 14 days before sampling began. Treatment allocations were re-randomized between both finisher periods, and no steer received the same treatment between both periods, providing an incomplete cross-over design.

Table 10. Metabolism Trial Diet Compositions as formulated[1].

Item	Starter	INT-1	INT-2	Finisher
Ingredient, % As Fed				
Tempered Barley	48.7	59.9	70.9	81.8
Molasses	4.0	3.3	2.6	2.0
Vegetable Oil	-	0.9	1.8	2.8
Dry Supplement	2.6	2.6	2.6	2.5
Mill Run	10.0	6.7	3.4	-
Whole Cottonseed	11.7	10.7	9.7	8.7
Wheat Straw	7.7	5.3	3.0	2.2
Oaten Hay	15.3	10.6	6.0	-
Nutrient Composition, % DM				
Dry Matter	100	100	100	100
Ash, % DM	7.61	7.14	6.67	6.09
TDN, % DM	74.28	75.76	77.22	78.53
ME, Mcal/kg DM	2.69	2.82	2.95	3.09
NEm, Mcal/kg DM	1.77	1.88	2.00	2.11
NEg, Mcal/kg DM	1.15	1.25	1.35	1.46
Starch, % DM	30.31	35.85	41.44	47.08
Fat, % DM	4.37	5.18	6.01	6.98
NDF, % DM	38.00	34.46	30.91	27.47
CP, % DM	13.72	13.65	13.57	13.36
DIP, % DM	9.80	9.83	9.85	9.78
UIP, % DM	3.92	3.82	3.72	3.58
Ca, % DM	0.74	0.73	0.72	0.68
P, % DM	0.39	0.37	0.36	0.34
Mg, % DM	0.26	0.25	0.23	0.22
K, % DM	1.04	0.92	0.79	0.66
Na, % DM	0.11	0.09	0.07	0.04
Cl, % DM	0.32	0.24	0.16	0.07
S, % DM	0.23	0.23	0.22	0.21
Co, ppm	0.43	0.36	0.28	0.18
Cu, ppm	19.22	18.52	17.79	16.69
I, ppm	0.57	0.56	0.56	0.53
Fe, ppm	82.99	59.06	34.94	9.81
Mn, ppm	61.67	53.65	45.48	36.22
Mo, ppm	0.75	0.65	0.56	0.46
Se, ppm	0.15	0.14	0.12	0.10
Zn, ppm	92.34	91.81	91.22	88.11
Vitamin A, KIU/kg DM	2.17	2.20	2.23	2.18
Vitamin E, IU/kg DM	24.66	25.01	25.37	24.76
Salt, % DM	0.25	0.25	0.25	0.25
Urea , % DM	0.39	0.40	0.41	0.40
Vitamin D, IU/kg DM	271.22	275.16	279.12	272.40

[1]Rumen modifier treatments were top-dressed and fed at rates of 25ppm, 30ppm and 2ppm for Monensin, Lasalocid and Bambermycin. Treatments were as follows: Monensin fed alone continuously at a rate of 25ppm (MON), Monensin fed in daily combination with Lasalocid at rates of 25ppm and 30ppm, respectively (M+L), Monensin fed in daily rotation with Lasalocid at rates of 25ppm and 30ppm, respectively (M/L), Monensin fed in daily combination with Bambermycin at rates of 25ppm and 2ppm, respectively (M+B), and Monensin fed in daily rotation with Bambermycin at rates of 25ppm and 2ppm (M/B)

6.2.3 Methane Emissions

On d15, cattle were placed into individual, fully-enclosed respiration chambers for two 24-hour periods to collect methane emissions (Hegarty et al., 2012). Two 24-hour periods were implemented to capture potential day differences between individual modifiers of the rotation treatments. In the morning of d15 before feeding, cattle were randomly allocated to a chamber, where each steer was kept and fed individually while methane data was collected. Once all cattle were secured in the chambers, chambers were sealed and respiration collection began. After 24hrs, chambers were opened to collect orts, provide new feed, and start the next 24-hour methane monitoring period.

6.2.4 Rumen Sampling

On d17, cattle were moved into individual metabolism crates and were intensively rumen sampled for 48 consecutive hours. To determine sampling timing, all pH bolus data was plotted and time frames were identified to capture each steers ruminal pH apex and nadir twice consecutively. Hourly sampling began 3hrs before feeding and continued until 2hrs after feeding to captured apex form. Similarly, hourly sampling to capture nadir shape commenced at 4hrs after feeding until 12hrs after feeding.

Sampling was done via intraruminal assembly or 35cm metal probe. A minimum of 15mL of rumen fluid was drawn out with a 60mL syringe to flush the sampling line and then discarded. A minimum 30mL of fresh rumen fluid was collected for subsampling and analysis. Rumen fluid was immediately measured for reduction potential (Mettler Toldeo SevenEasy S20 pH meter with TPS Intermediate Junction Redox Sensor) and pH (EcoScan Portable pH/ORP meter with TPS pH Sensor) calibrated daily, and then subsampled for protozoa and fermentation metabolite analysis. Additional subsampling occurred on day 1 and 25, before feeding, for microbiome analysis, with samples (1.5mL) being centrifuged immediately at 10,000RPM for 10 minutes, supernatant discarded and pellet stored at -80^{o}C.

Rumen fluid (15ml) subsampled for fermentation metabolites was acidified with 5 drops of concentrated sulphuric acid and frozen at -18C until analysis. Volatile fatty acids were determined by gas chromatography (Nolan et al., 2010). Rumen ammonia-N was determined by Skalar methodology, based on the modified Berthelot reaction (de Raphelis et al., 2016). Rumen fluid suspended in 4% formal saline was subsampled and stained with Brilliant Green to visualize protozoa for microscopic counts before counting on a Fuchs – Rosenthal chamber of 0.2mm depth.

6.2.5 Total Collection

On d19-25, cattle remained in the metabolism crates so total daily faeces and urine could be collected for 6 consecutive days. Total daily faecal output was collected into containers, weighed, subsampled (2% of total) and frozen at -18C° until analysis for DM and starch analysis. Total urine output was collected into pre-acidified containers with 100ml of 10% H_2SO_4 (Makkar and Chen 1995) to ensure pH< 3.5 to prevent degradation of purine derivatives. Total daily urine output was weighed and brought to a uniform volume (typically 20 L) before subsampling 80mL into a bulked container. Urine subsamples were bulked by period for each steer and kept frozen at -18C° until analysis for purine-derivative determination.

6.2.6 Data Handling and Statistical Analysis

Methane emissions were measured as daily methane production (DMP; g CH_4 produced per day), and the 2-day average of DMI to calculate methane yield (MY; g CH4/kg DMI) CH_4. Recoveries of introduced methane gas were completed on each respiration chamber the day before cattle went in for emission measurement, to allow adjust for any loss of gas through the system.

Rumen pH data from the intraruminal bolus were corrected using linear regression in R statistical package (R Core Team, Boston, MA, USA) based on validation results (previous chapter) and summarized by daily mean, with a day defined as the time between feedings, not midnight to midnight. Percentage of time the rumen pH spent under 5.8 and 5.5 were calculated by dividing the number of times the corrected pH dropped below the defined level by the total number of times the pH was measured in the same timeframe.

Rumination was gathered by total minutes ruminating in 2-hour time blocks. Daily totals were summed by adding all the 2-hour time blocks within a day, defined as time between meals, so the daily DMI could be used as a covariate. When comparing rumination and pH, pH was averaged into the same 2-hour time blocks as rumination, but DMI could not be included in the 2-hour interval analysis, since eating rate was not recorded in this study.

All responses were analysed using the linear mixed model procedure (LMER; R Core Team, Boston MA, USA). Each treatment was replicated five times across the two periods, except control (MON), which was replicated four times. Steer was the experimental unit. The full general linear mixed model was expressed as follows:

$$y_{ghj} = \mu + T_g + P_h + D_j + e_{ghj}$$

Where y_{ghjk} is the response, μ is the general mean, T_g is the fixed effect of treatment (describe), P_h is the fixed effect of period, D_j is the fixed effect of DOF, and e_{ghj} is the random residual error. Steer was set as the random effect in all models and DMI was used as the covariate. Interactions between treatment and ration, as well as treatment and DOF were explored as well, and included only if significant (P<0.05). For rumen fermentation parameters (total VFAs, VFA proportions, and ammonium-N), the fixed effect of sampling time (hours relative to feeding) was also included. To determine separate effects of combination method (combination vs rotation) and additional modifier (Bambermycin vs Lasalocid) and interactions between combination method and modifier, orthogonal contrasts were used to evaluate differences. All responses are presented as means ± standard error of the mean. Differences were declared significant as $P \leq 0.05$, and tendencies declared at $0.05 \leq P \leq 0.10$.

6.3 Results

Table 18. Nutrient composition of metabolism test diet as chemically analysed (100% DM basis)

Item	Units	Starter	INT-1	INT-2	Finisher
Dry Matter	%	86.4	87.15	83.8	82.85
Moisture	%	13.6	12.85	16.2	17.15
NDF	%	39.0	31.0	28.0	27.5
ADF	%	21.0	16.5	14.0	10.0
Crude Protein	%	12.7	13.05	13.2	12.8
DMD	%	66.0	72.5	76	82.5
DOMD	%	65.5	71.5	75	81.5
Inorganic Ash	%	6.5	6.0	5.0	4.0
Organic Matter	%	93.5	94.0	95.0	96.0
ME	MJ/kg DM	11	12.15	14.55	14.05
Crude Fat	%	4.15	5.25	12.35	7.05
Water Soluble Carbohydrates	%	6.2	6.1	4.9	3.4
Starch Total	%	24.5	34	36	44.5

6.3.1 Dry Matter Intake and Digestibility

Table 19. Effect[#] of rumen modifier treatment on Dry Matter Digestibility, Starch Digestibility, and Purine Outflow in cannulated steers maintained on a finisher ration.

	Treatments						
Parameter	**MON**	**M/B**	**M/L**	**M+B**	**M+L**	**SEM**	***P-value***
Dry Matter Intake, kg DM	4.37_a	3.98_b	3.84_b	3.18_c	3.98_b	0.04	*0.000*
Dry Matter Digestibility, %	76.1	77.7	77.1	73.9	78.1	4.32	*0.638*

[#]: Means on the same row with different superscript are significantly different (P<0.05)

Modifiers significantly affected DMI while cattle were maintained on a finisher ration (Table 16). Cattle on MON treatments had the highest intake compared to all other treatments, while M+B cattle had an intake significantly lower than did all other treatments. There was no effect of modifier treatment on dry matter digestibility.

6.3.2 Rumen Parameters

All cattle showed a post-feeding decline in rumen pH (Fig. 18), with pH minimum being achieved 6-7 h post feeding.

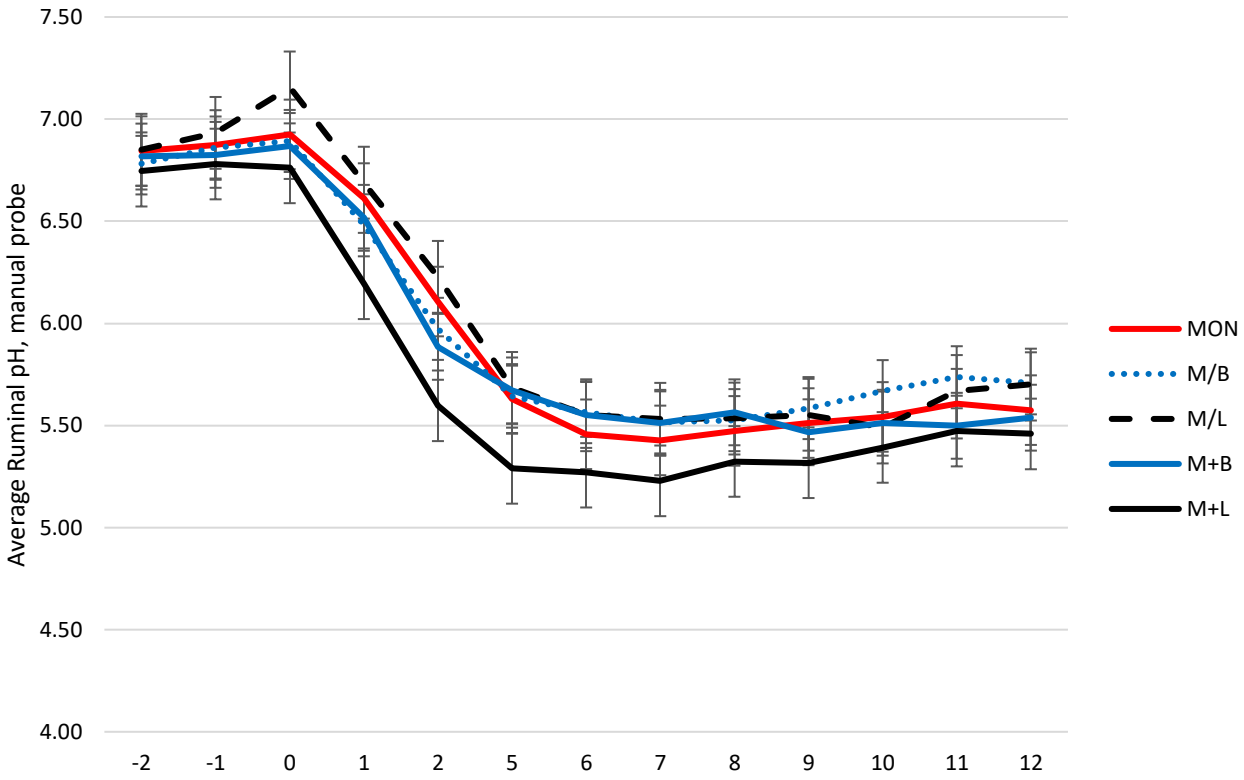

Figure 18. Interaction of treatment and hours relative to feeding on ruminal pH (spot sampling) in cannulated steers. Values are averages +/- SE. Treatments: MON = Monensin-only; M/B = Monensin in daily rotation with Bambermycin; M/L = Monensin in daily rotation with Lasalocid; M+B = Monensin fed continuously with Bambermycin; M+L = Monensin fed continuously with Lasalocid.

Table 20. Effect[#] of rumen modifier treatment on rumen acidity and fermentation parameters -2 to 12hrs relative to feeding in cannulated steers maintained on a finisher ration. Numerical means are displayed. Significant covariates include Treatment, Period, DMI, Day and Hour.

	Treatments						
Parameter	**MON**	**M/B**	**M/L**	**M+B**	**M+L**	**SEM**	***P-value***
pH relative to feeding	**5.98_{cd}**	**6.00_{a}**	**6.05_{cd}**	**5.93_{bc}**	**5.76_{bcd}**	0.03	*0.000*
-2hrs	6.84	6.78	6.85	6.82	6.75	0.06	*0.948*
-1hrs	6.87	6.86	6.93	6.83	6.78	0.06	*0.931*
0hrs	6.93	6.89	7.16	6.87	6.76	0.06	*0.423*
1hrs	6.61	6.48	6.69	6.52	6.19	0.07	*0.168*
2hrs	6.11	5.97	6.23	5.89	5.60	0.08	*0.227*
5hrs	5.63	5.64	5.68	5.67	5.29	0.08	*0.246*
6hrs	5.46	5.57	5.55	5.55	5.27	0.07	*0.115*
7hrs	5.43	5.52	5.53	5.51	5.23	0.07	*0.217*
8hrs	5.47	5.53	5.53	5.56	5.32	0.07	*0.407*
9hrs	5.51	5.59	5.55	5.47	5.32	0.06	*0.235*
10hrs	5.73_{bc}	6.04_{a}	5.65_{bc}	5.92_{ab}	5.75_{bc}	0.05	*0.033*
11hrs	5.32_{c}	5.91_{a}	5.40_{c}	5.66_{abc}	5.67_{b}	0.05	*0.007*
12hrs	5.07	5.59	5.18	5.49	5.44	0.06	*0.102*
Redox relative to feeding	**-263**	**-267**	**-272**	**-261**	**-267**	**2**	***0.680***
-2hrs	-283	-269	-289	-288	-283	5	*0.433*
-1hrs	-291	-283	-296	-301	-301	5	*0.495*
0hrs	-314	-319	-341	-323	-333	6	*0.870*
1hrs	-336	-333	-356	-323	-336	6	*0.960*
2hrs	-291	-297	-293	-283	-282	5	*0.790*
5hrs	-223	-239	-240	-210	-229	7	*0.670*
6hrs	-233	-232	-257	-227	-226	7	*0.730*
7hrs	-220	-231	-230	-244	-235	6	*0.606*
8hrs	-234	-234	-237	-223	-244	6	*0.903*
9hrs	-233	-249	-245	-233	-239	6	*0.617*
10hrs	-246	-270	-242	-250	-255	6	*0.401*
11hrs	-247	-251	-253	-249	-249	6	*1.000*
12hrs	-263	-266	-259	-268	-260	6	*0.980*

[#]: Means on the same row with different subscript are significantly different (P<0.05)

There was no effect of rumen modifier treatment on redox potential in the rumen of cattle once established on finisher ration, at any stage during the feeding cycle (*P*>0.05; Table 20)

6.3.3 Methane Emissions

Table 21. Effect[#] of rumen modifier treatment on methane emissions in cannulated steers while maintaining on a finisher ration. Methane per kg of DM model used Treatment, Period, and Day used as a covariates, with Steer as the random effect.

Parameter	Treatments					SEM	*P*-values
	MON	M/B	M/L	M+B	M+L		
DMI kgs/d[1]	4.76_{a}	4.61_{ab}	4.45_{b}	2.91_{c}	4.44_{ab}	0.18	0.001
GE, MJ	92.30_{a}	89.43_{ab}	86.42_{b}	56.51_{c}	86.10_{ab}	3.4	0.001
CH_4 / kg of DM[2]	10.77	12.66	13.63	16.52	10.56	1.27	0.371
CH_4 g/day [3]	61.56	51.65	50.53	31.26	48.02	3.85	*0.984*
Day 1, g CH_4	59.16	57.29 (M)	61.30 (M)	32.61	44.13	4.90	*0.320*
Day 2, g CH_4	63.50	49.25 (B)	54.89 (L)	25.49	46.46	5.31	*0.320*

[1]DMI value is an average of DMI of the two days in the chambers

[2] Model for CH4 per kg DM included Treatment, Period and Day as covariates, and Steer as the random effect.

[3]Model for CH_4 g per day included Treatment, Period, DMI and Day as covariates, and Steer as the random effect.

There was a significant modifier effect on DMI on the few days of methane measurement (Table 21), so DMP (g/d) was adjusted for DMI. There was no significant different in DMP between treatments although DMI (included as a covariate) had significant effect on DMP. There was no difference in methane yield (grams of methane per kilogram of DM intake).

6.3.4 Rumination

Considering the duration of the finisher period, modifier treatment significantly affected the average daily DMI and total rumination ($P<0.05$). Monensin alone significantly improved daily DMI compared to all other treatments, while M/B, M/L and M+L cattle ate significantly more than did M+B (Fig. 19). Despite eating the least, M+B cattle spent more time (mins) ruminating compared to MON, M/B and M+L cattle. Interestingly, the treatments with numerically the lowest DMI had numerically the highest rumination (M+B and M/L).

Despite not being quickly evident with the numerical means of pH displayed, when the full model is used, M/B cattle have a significantly higher average rumen pH compared to all other treatments. Monensin alone had the lowest average rumen pH numerically, and was significantly lower than treatments containing Bambermycin. Combination treatments performed in the median together, but M+B was significantly higher than MON and M/L. Overall, there appeared to be an advantage with the addition of Bambermycin, especially in daily rotation with Monensin, on average rumen pH.

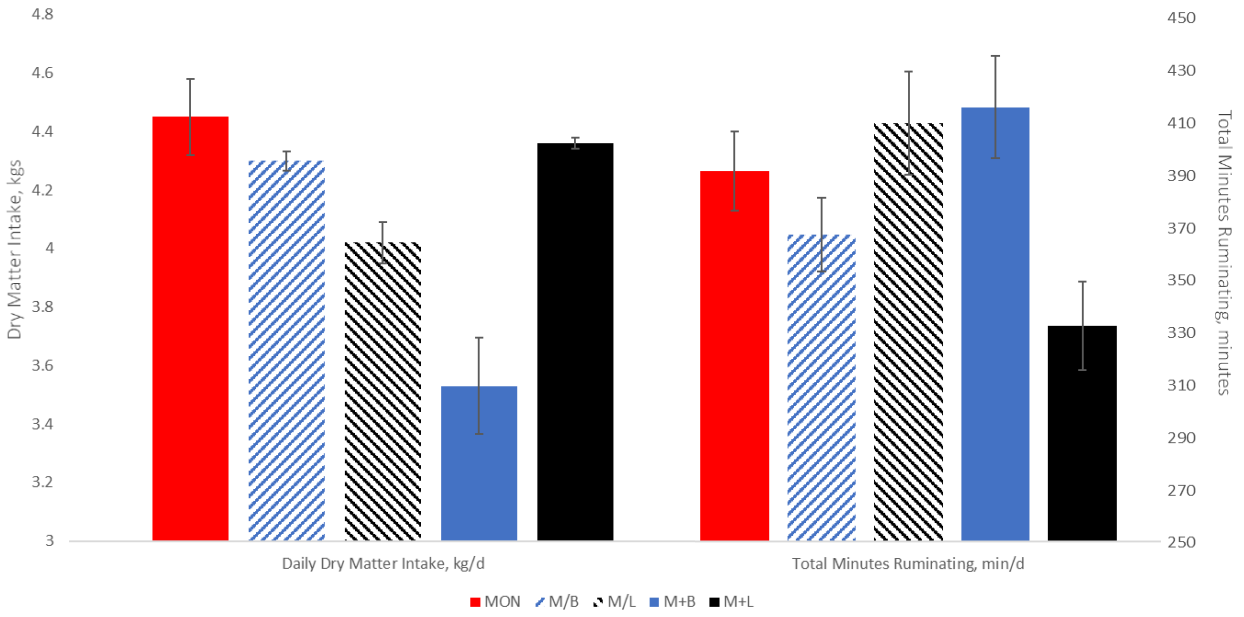

Figure 19. Effect of rumen modifiers on daily total minutes ruminating and dry matter intake in cannulated steers maintained on a finisher ration. Error bars represent the SEM for the treatment.

6.3.5 Predicting pH from Rumination, Intake and Ration Parameters

Feedlots present many risk factors for SARA; being large amounts of rapidly fermentable grain in the ration relative to small amounts of NDF, and feed is provided 1-3 times a day, reducing meal frequency and increasing meal size. Real-time monitoring of ruminal pH provides a powerful insight to overall gastrointestinal health and individual animal productivity. However, such systems require either a device that can withstand the harsh rumen environment (intraruminal bolus) or continuous, direct access to the rumen (cannulation), that are often expensive, moderately to highly invasive, and, most importantly, not economically applicable to feedlot systems.

The measurement of rumination time by an eartag accelerometer allows monitoring of rumen health status since mastication during rumination releases saliva that buffers the rumen (Mertens, 1997; Bailey, 1961). An increase in chewing behaviour can be associated with increases of the forage and effective fibre content of the ration (Cassida & Stokes, 1986) and previous studies have observed correlations of ruminal pH with NDF (Kolver & De Veth, 2002; Pitt et al., 1996), ruminal temperature (AlZahal, Steele, Valdes, & McBride, 2009), meal frequency, and the interactions between meal frequency, effective fibre and carbohydrate digestion rate (Pitt & Pell, 1997). Thus, before SARA progresses and becomes a clinically apparent concern, early detection can be monitored through the change in rumination time (Ambriz-Vilchis, Jessop, Fawcett, Shaw, & Macrae, 2015).

Quantifying rumination through sensors has been explored by an internal bolus sensing particle size (Chap, Milligan, & Kennedy, 1984), external accelerometer fitted to a halter recording and analysing jaw movement (Braun, Zürcher, & Hässig, 2015; Ungar & Rutter, 2006) or microphones analysing mastication sound

(Wolfger et al., 2015). Recently, the application of accelerometer ear tags to automatically record rumination were validated (Ngun, 2019).

Initial analysis of using adjusted bolus data to predict total daily minutes ruminating as well as intake and ration information, across all periods from this experimental data, is very promising (Table 19). Daily totals for minutes ruminating, starch intake, NDF intake, their interaction, and hours relative to feeding significantly predicted daily average ruminal pH. As predicted, starch intake, hours relative to feeding, as well as the interaction of starch intake and NDF intake all had negative effects on ruminal pH. Surprisingly, total minutes ruminating also had a negative correlation with ruminal pH. NDF intake was the only parameter that had a positive correlation with ruminal pH. This study was limited in its analysis of the full power of predicting ruminal pH since cattle were not fed *ad libitum*, and feeding rate and behaviour throughout the day was not recorded.

Table 22. Coefficient results of predicting ruminal pH from rumination and intake parameters.

Fixed Effect	Estimate	P-value
Total Daily Minutes Ruminating	-8.08e-05	0.009
Daily Starch Intake, kg	-0.345	0.000
NDF Intake, kg	0.828	0.000
Hours rel. to feeding	-0.034	0.000
Starch:NDFI interaction	-0.180	0.000
(Intercept)	6.14	0.000

6.4 Discussion

Modifier treatments had no effect on methane emissions or dry matter digestibility while cattle were maintained on a finisher ration. Monensin-only cattle displayed the greatest DMI compared to all other treatments, which most likely was a key driver in lowering MON cattle rumen pH significantly lower than treatments containing Bambermycin, but equal to treatments with Lasalocid. This contrasts to the previous (animal house) trial in which Monensin cattle had high rumen pHs pre- and post-feeding. Conversely, while M+B had the second most stable rumen pH, this treatment consumed a significantly lower DMI compared to other treatments, which questions if the advantage in rumen pH is worth the compromise in DMI. Balancing rumen pH and DMI, rotation treatments or M+L appear to be advantageous compared to MON or M+B.

6.4.1 Methane emissions

Since intake has been previously proven to have a large influence on methane emissions (Blaxter and Clapperton, 1965; McAllister et al., 1996; Johnson and Johnson, 1995,; Charmley et al., 2016), it is possible any effects of rumen modifiers are concealed in the large fluctuations of intake, despite being included as a covariate in the statistical model. However, the lack of response in anti-methanogenic effect of rumen

modifiers agrees with Crossland et al., (2017), who also found no effect of treatment on potential activity for methane production. The small effect of modifiers on methanogenesis has long been a source of caution in using this strategy for methane mitigation (Van Nevel and Demeyer 1996), and longer term studies have often seen methane inhibition decline over time (eg for Monensin; Waghorn et al., 2008).

6.4.2 Rumen sampling (VFAs, ruminal ammonium-N, pH, redox)

Lemos et al. (2016), found neither MON or BAM provided individually or in combination, had any advantages compared to each other on ruminal pH, rumen ammonium-N, or VFAs when provided to zebu cattle on a no-roughage finishing diet after 14 days on the modifier. However, these cattle were used in 7x7 Latin square design with no washout between periods, potentially allowing the manifestation of carry-over effects between modifier treatments.

6.4.3 Total Collection (digestibility)

Crossland et al. (2017) found that rumen fluid collected from steers on modifiers used to ferment forage in vitro, BAM and rotation treatments had a significant initial advantage over MON and control for forage diets, but this disappeared over time, even with rotation. Similarly, Lemo et al. (2016) found no statistical differences between MON or BAM fed separately or in combination on apparent total tract digestibility in no-roughage finishing diets fed to zebu cattle. As all animals shared the same basic diet and differed only in modifier(s), and since whole tract DMD does not differentiate any compensatory change in hindgut DMD if rumen DMD is affected, it is not surprising that whole tract DDM was not affected in this study.

6.4.4 Microbiome

Crossland et al., (2017) found no treatment effect on archaeal genera (focusing on Methanobacter). But there were differences in relative abundances of Gram-positive bacteria with treatment, such that control and BAM had significantly the lowest relative abundance of Gram-positive bacteria, then MON, and rotation between the two had the highest abundance, which contradicts previous studies which suggest that Gram-positive bacteria are more susceptible to MON and B compared to Gram-negative bacteria (Chen and Russell, 1988; Russell and Strobel, 1989; Butaye et al., 2003; Pfaller, 2006), thus decreasing the relative abundance of Gram-positive bacteria.

6.5 Conclusion

Rumen modifier treatment did not show the same pH responses on the finisher ration as had been observed during adaptation, showing no advantage of continuous Monensin over other treatments. Modifier treatment did not affect methane production (g/d) or methane yield (g/kg DMI) or DM digestibility. In conclusion, with a restricted feed offering, there was very small effect of modifier choice on rumen fermentation.

7 EFFECTS OF RUMEN MODIFIERS ON FEEDLOT PERFORMANCE AND CARCASS ATTRIBUTES OF STEERS – Tullimba study

7.1 Abstract

Advantages in average daily gain and Feed:Gain of 4.8% and 2.7%, respectively, have been previously found with the daily rotation of Monensin and Lasalocid in Canadian feedlot steers. With diets similar to that in Australia, improvements such as these would be economically advantageous to Australian lot feeders. Hence, yearling *Bos taurus X* steers (n = 450) with an initial LW of 353kg, were used to evaluate Monensin (25mg/kg DM; MON), Lasalocid (30mg/kg DM; LAS), and a daily rotation of the two (M/L) on feedlot performance and carcass attributes on cattle fed for 109 days. All cattle were adapted to an 82% tempered-barley finisher ration across 21 days and 3 transition rations. Steers were blocked by body weight and allocated into 45 pens, with each pen of ten head being an experimental unit. Overall ADG and feed efficiency for all treatments were high (mean ADG = 2.5kg/hd/d; mean Feed to Gain = 4.5 on DM basis) and above average for feedlot cattle, F:G of MON cattle were both still significantly greater ($P<0.05$) than of LAS and M/L cattle over the entire 109d. Significant advantages ($P<0.05$) were seen in feed conversion efficiency (+2.6%) throughout the entire trial with continuous inclusion of MON compared to LAS or M/L. Monensin-only cattle also had a significantly ($P<0.05$) improved ADG, average liveweight (+1.2%) and average weight gain (+3.1%) over the first 83 days of the feeding period. There were no differences in carcass characteristics with modifier treatment. Net economic benefit was the same for all treatments, but cost of gain was significantly lower for MON cattle. Rotating ionophores, or inclusion of Lasalocid alone continuously, did not improve feedlot performance compared to continuously feeding Monensin alone, but the advantages of MON were not reflected in carcass attributes.

7.2 Introduction

Ionophore-antibiotics have been extensively used in the beef cattle industry to improve feed efficiency and average daily gain since their approval by the Food and Drug Administration in the mid 1970's, with Monensin being the principal modifier of choice among Australian feedlot operators (Lean et al, 2017). Like other feed additives, ionophores can be fed in any segment of the cattle industry, and have been effective in reducing the energy and protein losses in the rumen.

Classified as carboxylic polyether antibiotics, ionophores, as a general class, disrupt the ion concentration gradient across the membrane of microorganism in the rumen, causing an ineffective cycle to right the ion concentration (Bergen and Bates 1984). This futile cycle causes the microorganism to waste energy on pumping ions and prevents normal metabolism. Ionophores target gram-positive ruminal bacteria and protozoa, which are usually those that are responsible for decreasing the efficiency of rumen digestive processes. By decreasing the activity of these protozoa and bacteria, the rumen microbial population is shifted away from microbiota responsible for methanogenesis and ruminal protein degradation, resulting in less energy wasted in production of methane and ammonia, respectively (Elsasser 1984). This shift allows more beneficial bacteria to proliferate and be more efficient by converting feed carbon to propionate rather than methane (Fellner 2009), resulting in a lower ratio of acetate to propionate, suggesting a greater efficiency of capture of the energy that is supplied by feed. However, this shift in microbial population

towards beneficial bacteria, reduces overall ruminal microbial activity, and increases intestinal digestion (Bogaert, Gomez et al. 1989).

While there have been incidents where cattle have been oversupplied with Monensin causing health problems and death with Monensin (Geor and Robinson 1985), the ionophore Lasalocid has not been reported to show such effects, most likely due to its lower alteration on ruminal fermentation compared to Monensin (Armstrong and Spears 1988). Similar to Monensin, Lasalocid transports monovalent alkali metals, like Na^+ and K^+, as well as divalent cations such as Ca^{2+} and Mg^{2+}, but has different affinities and binding selectivity (Elsasser 1984), although the exact physiological significance of this is not well understood.

Antibiotic rumen modifiers have been routinely included in feedlot rations to improve carbon and nitrogen retention, but, with few exceptions (e.g. Laidlomycin "Cattlyst" in the USA), no new antibiotic type rumen modifiers are being registered. Thus, there is a need to evolve the current industry approaches to existing rumen modifiers to improve feedlot efficiency. Recently, Shreck et al., (2016) reported a 4.81% advantage in ADG and 250g DMI/d improvement from daily rotation of Monensin and Lasalocid relative to Monensin alone. Similarly, the potential to combine rumen modifiers to improve productivity has only recently been revisited to overcome intake suppression (Potter and Wagner 1986; Lemos et al., 2016; Benatti et al., 2017). There is a lack of data on response to short term rotation of rumen modifiers in ruminal fermentation parameters when cattle are maintained on a finisher diet similar to that fed in Australian feedlots. This study sought to explore how these changes in rumen modifier management might provide feeding, growth and carcass advantages available to the Australian feedlot industry.

7.3 Materials and Methods

7.3.1 Cattle Management and Housing

Steers (n=475) were sourced from multiple commercial suppliers and combined while grazing on pasture at 'Tullimba' prior to feedlot entry. Steers with 0-2 teeth and primarily purebred British breed were sourced but some British x Euro steers were accepted. On d -14, cattle (n=475) were individually inducted and weighed to provide a body weight for allocating to pens. At induction all cattle were fitted with a visual eartag, vaccinated with 5 in 1 (Ultravac 5 in1; Zoetis and Bovilis MH+IBR vaccine (Coopers) implanted with Component TE-200 (20mg Estradiol 17β, 200mg TBA; Elanco), and treated with the pour-on anthelmintic ("Cydectin plus Fluke": Virbac, Milperra NSW Austr.) Four hundred fifty steers were selected for the experiment to be stratified by body weight into sort pens. Booster vaccines of Bovilis MH+IBR and 5 in 1 were given on d 28 and d 67 respectively.

The experimental design was a Randomised Complete Block Design with 3 treatments and 15 replicates (pens) per treatment. Pens were blocked by weight in 15 blocks consisting of 3 pens/block, with 10 steers per pen. Blocks were allocated lightest to heaviest in a single line of pens along the feedlot. Cattle (n =25) were excluded from the experiment based on either body weight, breed type, implant status, dentition, demeanour, injury or health problems. In the morning on d-4, the selected 450 head of cattle were sorted into 15 weight-block sort pens (n=30), ensuring distribution of breed. On d-2, cattle in each weight block were randomly allocated to three treatments, housed in one of 3 contiguous pens of identical dimensions (6.25 m x 20 m; slope 3˚ West to East (from front of pen to back), and 1° North to South, along the row of

pens; 12.5 m2 /hd, 31.25 cm/hd bunk space; 1 fence line water trough shared between two pens = 3.0 x 0.7 m), and fed good quality cereal hay *ad-libitum*. Each pen had a concrete apron 3 m deep from the back of the feed bunk. The remainder of the pen surface was a manure interface above a compacted soil base of clay and natural rock

Cattle that were pulled to hospital pens for health treatments during the study were noted with their diagnosis and treatment. Cattle that had recovered within 4 days were returned to their experimental pens. Cattle that had not recovered after 4 days were removed from the experiment. If a cull or death occurred within the first 27 d of the experiment, cattle were replaced on d 27 with a spare animal to maintain head count in the pen. Cattle culled or deceased after the first 27 d of the experiment were not replaced and their data was excluded from analysis. Cattle were weighed individually on d 27, 54, 83 and 108. Liveweight and performance parameters will be presented and analysed in intervals from d0 until each weigh day, therefore the first interval is d0-27, the second interval is d0-54, and so on.

7.3.2 Diets, Feeding, and Treatments

The starter, transition and finisher diets to which modifiers were added were high energy diets at the top end of those likely to be used in commercial feedlots in Australia. Dietary transition from 40 to 80% tempered barley (DM basis) was accomplished over 21 d using two intermediate diets (Table 23a, 23b). All diets were formulated (Table 23a) to meet NRC requirements for minerals and vitamins and subsequently analysed for composition after preparation (Table 23b). Barley was tempered for 16h at approximately 20% moisture prior to rolling in an 18” x 36” mill (R & R Machine Works, Dalhart, Texas). Rations were mixed and delivered every morning using a mixing wagon (274-12-Roto-Mix feed mixer trailer; Roto-Mix, LLS, Dodge City) mixer. Capacity for mixes of finisher ration were typically 2.5 t/mix. Bunks were read at 0630hrs and feed calls were made at the bunk. Pens were fed in the same order each day, and a single flush with 200kg of wheat straw was made between rations to remove any residues. Cattle were fed once a day and all feed delivered was automatically recorded via the Digistar computer system in the tractor and Bunk Management Software (Elynx Pty Ltd., Toowoomba Australia).

During adaptation, cattle were fed to target multiple of maintenance energy values for specific days on feed. Once cattle had successfully progressed through the starter chart and adapted to the finisher ration, cattle were fed to meet the intake of the previous day’s amount, plus a small amount of ration left in the bunk (crumbs) at the time the bunk was read. Every day, 6-8 grab samples were collected from each mixer load and bulked by treatment and day for dry matter analysis. Daily ration samples were further bulked into weekly samples per treatment which were analysed for nutrient composition by wet chemistry.

Treatments were applied by using one of two different mineral premixes produced by BEC (Brisbane, QLD, AUS), and formulated to include either 25 ppm (DM basis) of Monensin (Rumensin Granular, Elanco Animal Health, Greenwood, Indiana, United States) or 30 ppm (DM basis) Lasalocid (Bovatec, Zoetis LLC, Salisbury, Maryland, United States). Treatments were Monensin fed continuously (MON), Lasalocid fed continuously (LAS), or Monensin fed in daily rotation with Lasalocid (M/L). Samples of each premix were collected on d 27, 36, 45, 54, 83 and 108 to analyse for confirmation of composition and modifier inclusion rates.

Table 23a. Diet compositions and nutrient profiles as formulated for Feedlot Performance Trial

Item	Starter	INT-1	INT-2	Finisher
Ingredient				
Dry Supplement, % DM	2.5	2.4	2.5	2.5
Mill Run Wheat, % DM	10.5	7.2	3.6	0.0
Oaten Hay, % DM	21.0	14.6	8.3	0.0
Molasses, % DM	3.5	2.9	2.4	1.8
Tempered Barley, % DM	39.7	53.0	66.3	80.0
Vegetable Oil, % DM	0.0	1.0	2.2	3.5
Whole Cottonseed, % DM	12.4	11.5	10.6	9.6
Wheat Straw, % DM	10.5	7.4	4.1	2.6
Nutrient Composition				
Dry Matter, %	86.53	85.06	83.64	82.25
Ash, % DM	4.94	4.35	3.74	3.13
TDN, % DM	72.79	74.92	76.89	78.71
Organic Matter, % DM	81.90	85.94	90.10	94.35
ME, Mcal/kg DM	2.63	2.79	2.95	3.11
NEm, Mcal/kg DM	1.72	1.85	1.99	2.13
NEg, Mcal/kg DM	1.11	1.22	1.35	1.47
Starch, % DM	27.20	33.92	40.60	47.43
Fat, % DM	4.27	5.10	6.06	7.04
NDF, % DM	37.11	33.88	30.45	27.44
CP, % DM	13.27	13.27	13.28	13.15
DIP, % DM	9.42	9.49	9.60	9.61
UIP, % DM	3.85	3.77	3.68	3.54
Ca, % DM	0.77	0.73	0.71	0.69
P, % DM	0.38	0.37	0.35	0.34
Mg, % DM	0.26	0.25	0.23	0.22
K, % DM	1.10	0.96	0.82	0.66
Na, % DM	0.13	0.10	0.08	0.04
Cl, % DM	0.38	0.28	0.18	0.07
S, % DM	0.23	0.22	0.22	0.21
Co, ppm	0.59	0.48	0.39	0.29
Cu, ppm	19.47	18.33	17.66	16.89
I, ppm	0.93	0.89	0.90	0.90
Fe, ppm	98.59	69.84	40.71	10.13
Mn, ppm	65.35	55.48	46.19	36.69
Mo, ppm	0.80	0.69	0.58	0.46
Se, ppm	0.16	0.14	0.12	0.11
Zn, ppm	92.86	90.00	89.70	89.34
Vitamin A, KIU/kg DM	2.21	2.15	2.18	2.22
Vitamin E, IU/kg DM	25.14	24.41	24.82	25.24
Salt, % DM	0.25	0.24	0.25	0.25
Urea , % DM	0.40	0.39	0.40	0.40

[1] All units are in %, except ME, which is in MJ/kg DM. [2] Neutral Detergent Fibre. [3] Acid Detergent Fibre
[4] Dry Matter Digestibility. [5] Dry Organic Matter Digestibility. [6] Metabolisable Energy. [7]Finisher rations were bulked by month and therefore numbers reported are average ± standard deviation

Table 11b. Nutrient composition of feedlot performance trial rations obtained from laboratory analysis.

Nutrient Analysis[1]	Starter		INT-1		INT-2		Finisher[7]	
	MON	LAS	MON	LAS	MON	LAS	MON	LAS
Dry Matter	87.2	86.5	84.8	84.7	83.1	83.7	82.3 ± 0.6	83.0 ± 0.6
Moisture	12.8	13.5	15.2	15.3	16.9	16.3	17.7 ± 0.6	17.1 ± 0.7
NDF[2]	37.0	37.0	29.0	31.0	30.0	30.0	24.8 ± 3.8	25.8 ± 1.9
ADF[3]	20.0	20.0	15.0	17.0	15.0	14.0	9.8 ± 1.8	10.5 ± 0.9
Crude Protein	15.4	14.2	13.8	13.9	13.5	13.8	13.4 ± 1.3	13.6 ± 1.0
DMD[4]	68.0	69.0	71.0	69.0	70.0	75.0	80.0 ± 2.1	79.8 ± 1.1
DOMD[5]	67.0	68.0	70.0	69.0	69.0	74.0	78.8 ± 1.8	78.8 ± 1.1
Inorganic Ash	7.0	7.0	7.0	8.0	6.0	7.0	4.3 ± 0.4	4.3 ±0.4
Organic Matter	93.0	93.0	93.0	92.0	94.0	93.0	95.8 ± 0.4	95.8 ± 0.4
ME[6], MJ/kg DM	11.3	11.5	11.8	11.7	12.1	12.7	13.7 ± 0.3	13.7 ± 0.2
Crude Fat	4.3	4.5	4.9	5.0	6.3	6.0	7.4 ± 0.4	7.3 ± 0.4
Starch	28.0	26.0	37.0	31.0	35.0	38.0	48.5 ± 4.4	47.5 ± 2.7

[1] All units are in % DM, except ME, which is in MJ/kg DM. [2] Neutral Detergent Fibre. [3] Acid Detergent Fibre. [4] Dry Matter Digestibility [5] Dry Organic Matter Digestibility. [6] Metabolisable Energy. [7] Finisher rations were bulked by month and therefore numbers reported are average ± standard deviation

7.3.3 Weighing, Sampling and Analysis

All cattle were weighed on d 0, 27, 54, 83 and 108, using a weigh chute fitted with Ruddweigh 600mm Weigh Beam (2000kg weighing capacity; Ruddweigh, Guyra, NSW, Australia) and Gallagher Weigh Scale readout (W310 v2 to 2kg increments; Gallagher Australia, Epping, Vic, Australia). The scales were validated by placing 20kg x 33 (660kg total) certified test weights onto the scale prior to each weighing. Weighing took place between 9:00 am and 4:00 pm, and pens were weighed in the same order, each weigh day. Cattle were not held off-feed before weighing to minimize risk of gastrointestinal upset. Due to logistical constraints, cattle were weighed the day before feedlot exit (d109), since cattle were loaded on trucks in the early morning. Refusals were collected, weighed, subsampled for DM, and discarded d 1 to 27, 54, 83, 108, and 109.

Rumen fluid was collected by intubation on 1 steer per pen on d 0, 27, 56, and 84. These samples were used for determination of ruminal pH (EcoScan Portable pH/ORP meter with TPS pH Sensor) calibrated daily, volatile fatty acid concentrations, rumen protozoal populations and a further sample stored on days 0, 27 and 84 for future16S analysis of microbial diversity. Rumen fluid (15ml) subsampled for fermentation metabolites was acidified with concentrated sulphuric acid and frozen at -18C° until analysis. Volatile fatty acids were determined by gas chromatography (Nolan et al., 2010). Rumen ammonia-N was determined by Skalar methodology, based on the modified Berthelot reaction (de Raphelis et al., 2016). Rumen fluid suspended in 4% formal saline was subsampled and stained with Brilliant Green to visualize protozoa for microscopic counts before counting on a Fuchs-Rosenthal chamber of 0.2mm depth.

Bulked faecal samples were collected from each pen (5 fresh manure pads/pen) on days 13, 27, 41, 55 and 108 and frozen at -18^{o}C prior to determination of faecal starch concentration using an amylogucosidase and α-amylase enzymic assay kit (Megazyme Pty Ltd; Warriewood NSW). Bulking was done by bulking 5 pens from each treatment together, so that each sample day resulted in three bulked samples for each treatment.

7.3.4 Carcass Attributes

At the end of the trial, after weighing in the early morning prior to feeding, cattle were returned to their home pens and fed ad-libitum until feedlot dispatch to slaughter the following morning. Cattle were dispatched at 1100 h, travelled 450 km and slaughtered at 0600 h the following morning. Cattle were slaughtered, HSCW recorded, and a full MSA carcass assessment received (Sex, Grader, GradeDate,GradeTimeLeft, GradeTimeRight, BodyNo, HangDent, LeftSideHSCW, RightSideHSCW, CWT, Oper, Dest, Lot, EPBI, Hump, EMA, OSS, AUSMB, USMB, MC, FC, RFT, PH, LoinTEMP, RIB, GradeCode) 22-hours after slaughter. Dressing percentage was calculated from final liveweight (d108, pre-shrunk 4%) divided by hot carcase weight provided by the abattoir for each animal.

7.3.5 Data Handling and Statistical Analysis

Feed intake and animal performance were reported over the entire 109d and over d0-27, d0-54, d0-83, d0-109. Total DMI for each time interval was calculated by summing feed dry matter offered for that interval, minus the refusals weighed on each weigh day before weighing (refusals subsampled for DM), and divided by the Head*Days (Table 24). Since cattle were not removed from feed on weigh days to reduce the risk of rumen upset and cattle were weighed after feed delivery, weigh-day feed on offer was summed into the next interval. Because cattle were fed on the morning of dispatch to slaughter, intake and F:G were calculated using 108 days of liveweight gain, and 109 days of feeding, with the extra feeding day included at the end of the feeding period. All performance data, apart from dressing percentage were calculated based on unshrunk weight.

Table 24. List of equations used to calculate production parameters of feedlot performance.

Production Parameter		Formula
Average Liveweight (ALW)	=	Sum of individual weights of all cattle in pen / head in pen
Average Weight Gain	=	Average Liveweight – ALW from previous interval
Pen Total Dry Matter Intake	=	(Total as-fed feed delivered to pen for interval * average dry matter of ration for interval) – (refusals from pen for interval * DM of refusals for interval)
Adjusted Head*Days	=	Head in pen for interval * days in interval[1]
Average Daily Dry Matter Intake	=	Pen Total Dry Matter Intake/Adjusted Head*Days
Average Total Dry Matter Intake	=	Average Daily Dry Matter Intake * Days in interval
Average Daily Gain	=	Average Weight Gain / Days in interval
Feed : Gain	=	Average Total Dry Matter Intake / Average Weight Gain
Net Benefit	=	Pen Average Carcase Value – Pen Average Feed Cost
Cost of Gain[2]	=	Pen Average Total Feed Cost/ Pen Average Total Weight Gain
Cost of Gain[3]	=	Pen Total Feed Cost / Pen Total Weight Gain
Cost of Production	=	Pen Total Feed Cost / Pen Total Hot Carcase kgs

[1]Adjusted Head*Days can account for changes in head count (i.e., a pull or death) within the interval.

[2] Method dilutes effect of pulls/removals. [3] Method takes pulls into account.

All production and slaughter statistical analyses were conducted in R statistical package (R Core Team, 2013). Least squared means for each treatment were separated using pairwise comparisons with an F-protected Least Significant Difference (LSD) using Satterwhaite's method, and significance was declared at $P \leq 0.05$, and tendencies declared at $0.05 \leq P \leq 0.10$. A linear mixed effects model (*lme4* (Bates et al 2015)) was fitted to

Table 25. Ruminal pH of steers fed Monensin alone, Lasalocid alone, or a daily rotation of the two[#].

	Treatments[1]					
Item	**MON**	**LAS**	**M/L**	**Overall**	**SEM**	***P*-value**
Ruminal pH	6.51	6.26	6.34	6.38	0.05	*0.28*
Day 0	7.40	7.40	7.40	7.40_a	0.00	*0.99*
Day 27	5.64	5.73	5.88	5.75_b	0.06	*0.12*
Day 54	6.22	6.27	6.39	6.29_c	0.07	*0.56*
Day 83	6.76	6.77	6.77	6.77_d	0.07	*0.99*
Day 83 Rumen Sampling						
Total VFAs	116	105	109	110	4	*0.312*
Acetate, %	59.2	56.1	57.9	57.7	1.1	*0.560*
Propionate, %	35.9	37.1	36.6	36.5	1.0	*0.908*
Butyrate, %	2.8	4.2	3.4	3.5	0.3	*0.091*
ACE:PRO	1.67	1.59	1.72	1.66	0.25	*0.729*
Total Protozoa	229	203	297	243	24	*0.127*
Faecal Starch, %	2.56	2.10	2.35	2.34	0.18	*0.370*
d27	3.48	2.66	2.86	3.00_a	0.24	*0.25*
d109	1.64	1.54	1.84	1.67_b	0.11	*0.54*

[1]MON = continuous feeding of 25mg/kg of diet DM Monensin (Rumensin, Elanco Animal Health, Greenfield, Indiana, USA); LAS = continuous feeding of 30mg/kg of diet DM Lasalocid; M/L = daily rotation of 25 mg/kg of diet DM Monensin or 30 mg/kg of diet DM Lasalocid.

production and slaughter data. Pen was the experimental unit, and each treatment had 15 replicates. The full general linear mixed model was expressed as follows:

$$y_i = \mu + T_i + e_i$$

Where y_i is the response, μ is the general mean, T_i is the fixed effect of treatment (describe), and e_i is the random residual error. Block was set as the random effect in all models. All responses are presented as means ± standard error of the mean. Data from steer deaths (n = 4) and pulls (n=3) were excluded from analysis. Two pulls occurred before d27 and those individuals were replaced with spares on d27. For calculating average dry matter intake, Head*Days were adjusted to take into account differences in head count for changes in feed-related parameters. Any pulls or deaths after this day were not replaced.

7.4 Results

7.4.1 Rumen acidity, redox potential and ecology

Ruminal pH was not significantly affected by treatment, but pH did significantly decrease over the starter period (d0 - d27) as cattle transferred from hay to the starter ration (Table 22). Thereafter, mean rumen pH increased to d 54 and again to d 83, with no rumen sampling taken on d 108 immediately prior to trucking for slaughter. There was no effect of rumen modifier treatment on faecal starch content on d27 or d 109.

7.4.2 Feedlot Performance

Monensin-only cattle tended to weigh more than Lasalocid-only and Rotation cattle after the first 27 days of the finisher diet and over the entire trial (4.0 and 8.5 kg more, respectively; P = 0.056), but weighed significantly heavier (P<0.05) than the other treatments by 0.9 % and 1.1% (4.5 and 6.5 kg) on d 54 and 83, respectively (Table 26; Fig 20). Monensin-only cattle also gained significantly more weight than both other treatments across all periods, weighing on average, 2.1% (8.55kgs) heavier at d 108 compared to the other treatments. Similarly, compared to the average of LAS and M/L cattle, the ADG of Monensin-only cattle was significantly greater by 5.2%, 3.5% and 3.4% on d 27, 54 and 83, and tended to have an increase by 3.2% overall. There were no significant effects of treatment on average daily dry matter intake for any intervals. Monensin-only cattle ate 70g DM more on a daily basis than the other treatments, but this response was not significant. However, there were significant effects of treatment on feed conversion, such that the feed to gain of Monensin-only cattle was 8.7%, 3.5%, 3.3% and 3.2% (-0.26, -0.13, -0.14, and -0.14 kg/kg) better than other treatments from d0 to d 27, d54, d83 and d109, respectively.

Table 26. Feedlot performance of steers fed a daily rotation of Lasalocid and Monensin. Treatment was a fixed effect, and weight block was the random effect.

	Treatments[1#]					
Item	**MON**	**LAS**	**M/L**	**Overall**	**SEM**	***P*-value**
Pens, No.	15	15	15	45		
Steers, Start No.	150	150	150	450		
Mortality, Clostridial[2]	1	2	1	4		*0.78*[3]
Morbidity, Respiratory	1	1*	0	2		*0.85*[3]
Morbidity, Pinkeye[4]	7	9	3	19		*0.23*[3]
Morbidity, Other[5]	0	1*	0	1		*0.63*[3]
Steers, End No.	148	148	149	445		
Liveweight, kg						
d0	352.7	353.8	353.3	353.3	4.0	*0.740*
d27	440.8	438.1	436.4	438.4	4.7	*0.056*
d54	513.9_a	509.9_b	508.8_b	510.9	5.0	*0.030*
d83	580.8_a	574.3_b	574.2_b	576.4	5.2	*0.038*
d109	632.8	624.8	625.4	627.7	5.2	*0.097*
Average Weight Gain, kg						
d0-d27	88.1	84.3	83.1	85.2	2.2	*0.055*
d0-d54	161.2_a	156.1_b	155.5_b	157.6	1.9	*0.009*
d0-d83	228.1_a	220.5_b	220.9_b	223.2	2.1	*0.013*
d0-d109	280.1	271.0	272.1	274.4	2.4	*0.062*
Average Daily Gain, kg						
d0-d27	3.26	3.12	3.08	3.2	0.08	*0.052*
d0-d54	2.93_a	2.84_b	2.83_b	2.9	0.04	*0.009*
d0-d83	2.75_a	2.66_a	2.66_b	2.7	0.03	*0.013*
d0-d109	2.57	2.49	2.50	2.5	0.02	*0.062*
Average Daily DMI, kg						
d0-d27	9.57	9.73	9.79	9.7	0.07	*0.50*
d0-d54	10.79	10.72	10.79	10.8	0.07	*0.74*
d0-d83	11.14	11.06	11.12	11.1	0.08	*0.77*
d0-d109	11.30	11.24	11.24	11.3	0.09	*0.91*
Feed Conversion Ratio						
d0-d27	3.02_a	3.21_b	3.25_b	3.2	0.08	*0.040*
d0-d54	3.70_a	3.79_b	3.83_b	3.8	0.04	*0.031*
d0-d83	4.06_a	4.17_b	4.18_b	4.1	0.03	*0.026*
d0-d109	4.40_a	4.53_b	4.51_b	4.5	0.03	*0.036*

[1]MON = continuous feeding of 25mg/kg of diet DM Monensin (Rumensin, Elanco Animal Health, Greenfield, Indiana, USA); LAS = continuous feeding of 30mg/kg of diet DM Lasalocid; M/L = daily rotation of 25 mg/kg of diet DM Monensin or 30 mg/kg of diet DM Lasalocid.

[2]All mortalities were determined to be clostridial disease from necropsy by a veterinarian

[3]Distribution of morbidity and mortality analysed by Chi Square

[4] Cattle with pinkeye were treated with topical antibiotics and returned to pen

[5] Other morbidity was removed due to emesis

* Pulled before d27, and replaced with spare for remaining DOF

[#]: Means on the same row with different superscript are significantly different (P<0.05)

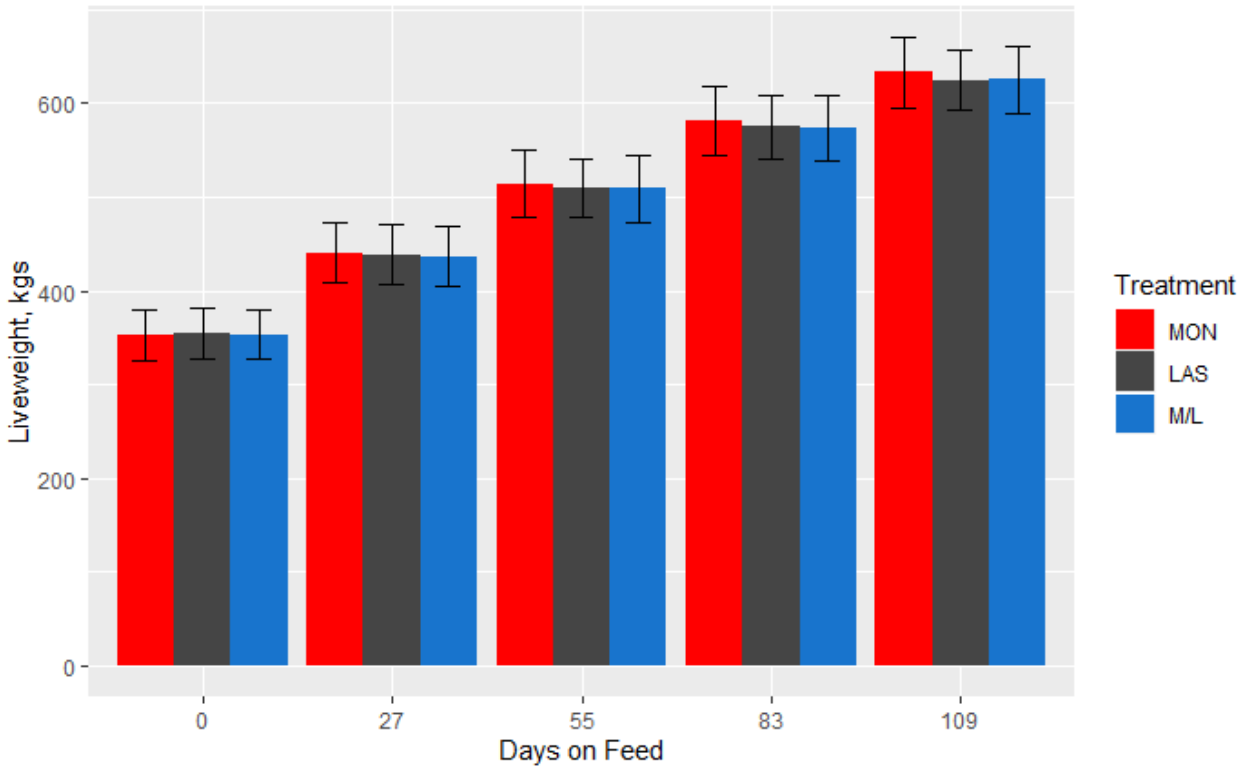

Figure 20. Progressive average liveweight of steers during feedlot finishing with Monensin or Lasalocid or a daily rotation of the 2 modifiers.

7.4.3 Carcass Attributes

There were no significant effects of treatment on any carcass attributes (Table 27). Dressing percentage was slightly lower than expected across all treatments, and no difference occurred between treatments. Monensin cattle had three dark cutters while the other treatments had none. Monensin-only cattle numerically had the best MSA marbling and indexes of the treatments but treatment differences were not significant.

Distribution of marbling scores (MSA and AUS-MEAT) and fat colour score did not differ across treatments. There was a significant effect of treatment on number of high (>4) meat colour scores, such that Monensin-only cattle had significantly more dark cutters compared to the other two treatments.

Table 27. Carcass attributes of feedlot steers fed Monensin alone, Lasalocid alone, or a daily rotation of the two. Treatment was the fixed effect, and weight block was the random effect.

	Treatments[1]				
Item	**MON**	**LAS**	**M/L**	**SEM**	***P*-value**
Dressing percentage %	55.0	55.1	54.9	0.09	*0.902*
Hot Carcase Weight, kg	334.0	330.5	329.7	2.76	*0.270*
Hump Height, mm	74.7	74.5	73.3	0.65	*0.432*
Ossification Score	165	163	168	1.60	*0.604*
P8 Fat Depth, mm	16.8	15.7	16.4	0.27	*0.179*
Eye Muscle Area, cm^2	84.89	86.42	84.69	0.69	*0.401*
Eye Muscle Ultimate pH	5.44	5.37	5.46	0.02	*0.159*
Rib Fat Depth, mm	8.89	8.13	8.16	0.28	*0.155*
Eye Muscle Temp, °C	15.0	11.4	11.1	2.06	*0.797*
MSA Marbling Score	355	344	352	3.67	*0.443*
200-449, no.	132	128	134		*0.931*[2]
450-699, no.	15	16	15		*0.978*[2]
700+, no.	0	1	0		*0.368*[2]
AUS-MEAT Marbling Score	1.2	1.1	1.2	0.04	*0.347*
0 to 1, no.	112	123	115		*0.760*[2]
2 to 3, no.	34	19	29		*0.120*[2]
4+, no.	1	3	5		*0.260*[2]
Meat Colour Score	1.7	1.5	1.6	0.04	*0.530*
<1C, no.	63	68	65		*0.910*[2]
2 to 4, no.	81	77	84		*0.860*[2]
5+, no.	3	0	0		*0.050*[2]
Fat Colour Score	0.6	0.6	0.6	0.04	*0.872*
0, no.	58	61	63		*0.900*[2]
1, no.	88	83	86		*0.930*[2]
2, no.	1	2	0		*0.610*[2]
Accepted MSA, no.	144	146	149		*0.958*[2]
Rejected, no.	4	2	0		*0.112*[2]
MSA Index	53.64	54.08	54.89	0.29	*0.541*
Grid Price, $/kg	5.73	5.73	5.73	0.002	*0.930*
Carcase Value, $/hd	1915.18	1894.09	1889.31	16.25	*0.201*

[1]MON = continuous feeding of 25mg/kg of diet DM Monensin (Rumensin, Elanco Animal Health, Greenfield, Indiana, USA); LAS = continuous feeding of 30mg/kg of diet DM Lasalocid; M/L = daily rotation of 25 mg/kg of diet DM Monensin or 30 mg/kg of diet

7.4.4 Net Benefit

While there was no difference in net financial benefit between treatments, there was a treatment effect on cost of gain when pen averages were used (conceals effect of steers removed; Table 28). When removals were taken into account and pen totals were used to analyse differences, there was still a treatment effect, but M/L is elevated due to the lower number of removals compared to the other treatments (n = 1 vs 2 and 2, M/L vs MON and LAS, respectively). It is important to note that morbidity and

mortality were not significantly affected by treatment and only numerical differences were present. However, one less steer removed from the M/L group resulted the treatment progressing from being numerically at the bottom, to statistically between MON and LAS.

Regardless of whether pen averages or totals were used, MON cattle still reduced cost of gain by 2.6% compared to the other two treatments. When total carcass weight and total feed cost were used to visualize cost of gain, there were no statistical differences.

Table 28. Effect of Monensin and Lasalocid fed alone or in daily rotation for 109-day-fed cattle on feed costs and benefits to feedlot producers.

	Treatments[1]				
Item	**MON**	**LAS**	**M/L**	**SEM**	***P*-value**
Carcase value, $/hd	1915.18	1894.09	1889.31	16.25	*0.201*
Grid price, $/kg HSCW	5.73	5.73	5.73	0.00	*0.933*
Total Feed Cost[2], $/hd	692.34	690.35	689.07	5.20	*0.92*
Net benefit[3], $/hd	1245.68	1228.48	1223.44	12.29	*0.19*
Cost of Gain[4], $/kg LW	2.47_a	2.55_b	2.54_b	0.02	*0.031*
Cost of Gain[5], $/kg LW	2.50	2.58	2.54	0.01	*0.097*
Cost of Production[6], $/kg CW	1.71	1.74	1.71	0.01	*0.489*

[1]MON = continuous feeding of 25mg/kg of diet DM Monensin (Rumensin, Elanco Animal Health, Greenfield, Indiana, USA); LAS = continuous feeding of 30mg/kg of diet DM Lasalocid; M/L = daily rotation of 25 mg/kg of diet DM Monensin or 30 mg/kg of diet DM Lasalocid.
[2]Feed cost was calculated based on $460.66/t for MON, $460.91/t LAS, and $460.79/t for M/L
[3]Net benefit was calculated for individual pens and analysed comparing treatment means to each other
[4]Cost of gain was calculated by Pen Average Total Feed Cost/ Pen Average Total Weight Gain; dilutes effect of pulls/removals
[5]Cost of Gain calculated by Pen Total Feed Cost / Pen Total Weight Gain; takes pulls into account. There was no statistical difference in mortality and morbidity between treatments.
[6]Cost of Production calculated by Pen Total Feed Cost / Pen Total Hot Carcase kgs; takes pulls into account and dressing percentage.

7.5 Discussion

Comparing overall performance averages to these other trials, this trial resulted in cattle that were equal to, or often surpassed the other trials that studied various applications of Monensin and Lasalocid in all feedlot performance parameters (Table 26). In contrast, a recent study done at the same feedlot as the current trial, while not studying rumen modifier use, fed a similar ration which included Monensin, had similar final liveweight, average daily gain, DMI, and F:G (Cowley, et al., 2019). Cattle in the current study had a much lower feed to gain (4.45 ± 0.05 kg), and higher ADG, across the entire 109 days compared to several other trials done with rumen modifiers (Shreck et al. , 2016; Brandt, 1988; Malcolm-Callis et al., 1995; Morris et al., 1990; McKinnon et al., 1991; Table 26). Steers fed Monensin alone out-performed steers fed Lasalocid alone or daily rotation of the two across all intervals in liveweight, average weight gain, and feed efficiency. Cattle fed diets that included Lasalocid alone or in rotation performed the same in all performance parameters measured. Monensin-only cattle also had significantly superior average daily gain in all intervals, and tended to gain more on a daily basis for the entire 109 days. While all cattle consumed the same amount of feed, Monensin-only cattle showed a significant advantage with feed efficiency across all intervals, driven by the improved average daily gain.

It is interesting that in the M/L rotation, providing M every 2nd day was less efficacious than providing M every day. The lack of response when rotating Monensin and Lasalocid agrees with those reported by McKinnon, Cohen, Kowalenko, and Janzen (1992), however results derived comparing the two modifiers fed alone continuously, such that they found no differences in feedlot performance beyond d 28, while this study found significant differences between Monensin-only and Lasalocid-only throughout the entire trial interval. This trials findings contradicts other reports of improved feedlot performance of Lasalocid- only or daily rotation of Monensin and Lasalocid compared to Monensin-only (Shreck et al., 2016) or Monensin plus tylan (Johnson, Hubbert, Ferguson, and Peterson, 1988; Morris, Branine, Galyean, Hubbert, Freeman and Lofgreen, 1990). Improved feed efficiency from Monensin-alone compared to Lasalocid alone is supported by findings of Brandt (1988), which were attributed to the significant decrease in DMI of Monensin-only cattle, rather than an increase in ADG, the latter of which is the case in this trial.

There were no significant effects of treatments on any carcass attributes measured. However, the Monensin cattle did have the only cattle that were MSA rejected due to meat colour. Other authors have found no significant effects of continuously feeding Monensin or Lasalocid compared to daily rotation in carcass attributes despite some performance effects seen leading to slaughter (Shreck, et al., 2016; Brandt, 1988; Morris et al., 1990). However, McKinnon et al. (1992) found that while there was found no effect of rotation or continuously feeding Monensin or Lasalocid, on ribeye area, back fat, marbling, or carcass weight, Lasalocid-only cattle did have significantly higher dressing percentage compared to Monensin-only cattle.

Net benefit was not effected by treatment, however Monensin cattle had the best cost of gain compared to the other two treatments. While there is conflicting responses reported to ionophore management strategies on feedlot performance, which is seems to be largely dependent on feedlot system type and management, there is a general consensus that any advantages seen at the bunk and in the yards have minimal impact on carcass attributes of grain-fed cattle.

While rumen parameters measured in this experiment did not appear to be affected by rumen modifiers, metabolism trials using cannulated steers executed immediately prior to this feedlot study did provide some insight to potential metabolic advantages of combination and rotation of rumen modifiers. During adaptation from low-to-high grain rations, Monensin in daily combination with Lasalocid showed advantages in DMI, rumen redox potential, rumen pH patterns, increases in molar proportions of propionate and rumination, indicating a rumen stabilization effect and increase in feed efficiency. While cattle were maintained on finisher ration, inclusion of Bambermycin improved rumen pH, but this tended to be accompanied by a decrease in DMI, questioning the value of improved rumen health considering the compromise of intake. Contrastingly, inclusion of Lasalocid appeared to increase intake between that of Bambermycin and Monensin alone, but with an equally median rumen pH, suggesting the addition of Lasalocid, in combination or rotation, could have advantages in improving intake over Bambermycin, with less risk of acidosis compared to Monensin.

Other researchers have also investigated the effects of combining or rotating rumen modifiers on adaptation to monensin. While some have found little to no synergistic effects on production parameters when rotating or combining rumen modifiers (Brant, 1988; Branine et al. 1989; Casey, Wessels and Meisnner, 1994; McKinnon et al., 1992

Guan (2005) found that ciliated protozoa populations, which are a significant factor in methane production, while initially susceptible, can adapt to ionophores within six weeks, even overcoming 14-day rotation program. Other authors have also found indications that neither rotation (Crossland et al., 2017) or combination of rumen modifiers delays rumen microbial better than continuous feeding, and have suggested pulse programs, with or without non-antibiotic rumen modifiers, could retard or prevent microbial adaptation.

7.6 Conclusion

This study had been designed to detect a potential 5% change in cattle performance parameters resulting from modifier rotation relative to continuous Monensin treatment However no advantage of rotation or of Lasalocid alone was apparent in ADG, Feed:Gain or carcass weight, carcass attributes or economics. It is concluded that when a high energy ration is offered delivering very high rates of ADG and very low Feed:Gain ratio in steers, that there is no advantage in providing another modifier in rotation with Monensin.

Table 29. Comparison of overall average feedlot performance responses in various trials examining rotation and combination of Monensin and Lasalocid in beef cattle, or performance in NSW, AUS, feedlots with similar rations. Data is presented as overall means ± SEM, if reported. Some data has been converted from U.S. to metric units, so potential for conversion errors that do not match original data, therefore data presented is just for visual comparison and not statistically analysed for differences.

Parameter	This Study, 2019	Cowley et al., 2019[1]	Cafe et al., 2011 [1]	Brandt, 1988	Shreck et al., 2016	Malcolm-Callis et al., 1995	Morris et al, 1990	McKinnon et al, 1991
State (Country)	NSW (AUS)	NSW (AUS)	NSW (AUS)	KS (USA)	AL (CAN)	NM (USA)	NM (USA)	SK (CAN)
Number of head	450	320	49	165	10,012	360	200	275
Trial length, days	109	109	117	92-105	112	112	134	150
Grain type	Tempered Barley	Tempered Barley	DR Barley	HM and DR Maize	Tempered Barley	SF Milo	SF Milo	Barley Concentrate
Treatments	MON LAS M/L			M+T LAS DM+T/L WM+T/L	MON M/L	CON L+O M+T	CON LAS M+T DM+T/L WM+T/L	MON M16+L M25+L LAS M/L
Start LW, kg	352 ± 7	377	319	373 ± 1	439 ± 8	335 ± 1	296 ± 1	338 ± 2
End LW, kg	630 ± 4	638	438	550 ± 3	627 ± 9	560 ± 3	519 ± 5	542 ± 6
ADG, kg/d	2.50 ± 0.04	2.38	1.17	1.77 ± 0.03	1.69 ± 0.04	1.70 ± 0.02	1.69 ± 0.04	1.37 ± 0.02
DMI, kg	11.25 ± 0.13	11.52	8.1	10.47 ± 0.09	11.48 ± 0.17	9.45 ± 0.08	10.60 ± 0.16	8.93 ± 0.23
F:G	4.45 ± 0.05	5.0[2]	7.3	5.94 ± 0.08	6.81	5.57 ± 0.05	6.29 ± 0.08	6.52 ± 0.17
HSCW, kg	331.6 ± 2.5	335.5	245	352.1 ± 2.3	377.3 ± 5.0	344.9 ± 1.7	322 ± 4.5	311.0 ± 3.4
Dressing Percentage, %	55.0 ± 0.2	52.5	55.9[3]	64.1 ± 0.2	60.2 ± 0.3	61.6 ± 0.2	63.3 ±0.3	57.8 ± 0.2
Rib fat Depth, mm	8.4 ± 0.4	8.83	6.0	--	9.5 ± 0.5	--	12.0 ± 0.8	--
Ribeye Area, cm^2	85.3 ± 1.4	79.7	60	85.1 ± 1.2	87.8 ± 1.2	87.5 ± 1.0	77.9 ± 1.5	84.9 ± 1.4

MON = Monensin fed continuously; LAS = Lasalocid fed continuously; M/L = Monensin and Lasalocid in daily rotation; M+T = Monensin plus Tylan fed continuously; M+T/L = Daily rotation of Monensin plus tylan and Lasalocid; WM/L = Weekly rotation of Monensin plus tylan and Lasalocid; CON = No ionophore; L+O = Lasalocid plus oxytetracycline fed continuously; M+T/L+O = Daily rotation of Monensin plus tylan and Lasalocid and oxytetracycline; M16+L= Monensin (16ppm) plus Lasalocid (18ppm) fed in combination; M25+L = Monensin (25ppm) plus Lasalocid (27ppm) fed in combination; HM = high moisture; DR = dry rolled; SF = steam flaked; Start LW = starting or feedlot entrance liveweight; End LW = final or feedlot exit liveweight; DMI = dry matter intake; F:G = feed to gain, or feed conversion ratio; HSCW = hot carcass weight;

[1]Cowley et al., 2019 and Cafe et al., 2011 did not study rumen modifier use, but are placed in the table to show feedlot performance in a NSW Australia feedlot with a similar finisher ration. Cowley et al., 2019, did use Monensin in the rations for the trial, while Cafe et al., 2011 did not report any ionophore use.

[2] Reported originally as gain to feed. Converted to feed to gain by calculating the reciprocal.

8 IMPACTS OF RUMEN MODIFIERS ON THE RUMEN MICROBIOME DURING TRANSITION TO AND MAINTENANCE ON FINISHER DIETS

8.1 ABSTRACT

The rumen bacterial biome of cattle during transition from forage to grain-based feeding, as well as during feedlot finishing was assessed. Adaption to diet (from 0 - 74% tempered barley, DM basis) was assessed in 12 rumen cannulated steers over 2 separate adaptation periods (Experiment 1). During adaptation, the effects of rumen modifiers (Monensin, alone, Monensin and Lasalocid in rotation, Monensin and Lasalocid in combination, Monensin and Bambermycin in rotation or Monensin and Bambermycin in combination) on the biome were assessed. The biome of cattle finished on Monensin, Lasalocid or Monensin and Lasalocid in rotation for 84 days was then assessed (Experiment 2). Transitioning to the tempered barley finisher diet increased the number of major bacterial genera in the rumen from 22 to 65 (>0.1% of bacterial biome). This transition was stable across all modifier treatments evaluated in Experiment 1, implying the type of modifier was of far less importance than was diet in regard to determining the proportions of predominant bacterial populations. In cattle established on finisher ration for 84d, there was no treatment effect on the major Phyla present (>0.1% of biome) or the major Orders and the balance of the Bacteroidales (24%), Clostridiales (28%) and Aeromonadales (39%) were similar to cattle from Experiment 1 on day 29 after adaptation to the finisher ration (36.5%, 17% 39.7% respectively).

8.2 Introduction

A core ruminal microbiota has been observed in ruminants globally (Henderson et al., 2014) but animal species, genome (Golder et la., 2018), diet and diet adaptation (Zhang et al., 2019) as well as residual effects of the dam (Abecia et al., 2013; Clemmons et al., 2018) have all been shown to affect biota residing in the rumen. Substantial difference in rumen biota between grain-fed and roughage feed ruminants are apparent (eg. Petri et al., 2012) and it is the activity of the increasing prevalence of lactic acid producing bacteria in cattle transitioning from a high roughage to a high starch/cereal grain diet that are responsible for the greatest risk of digestive disturbance in feedlot cattle (Tajima et al., 2000; Fernando et al., 2010). Both clinical and subclinical rumen acidosis contribute to lost productivity in grain-based production systems (Beauchemin et al., 2000). Rumen modifiers have proved effective in mitigating this risk (Coe et al., 1999) but as few modifiers are under development, efforts are being made to optimise the use of existing rumen modifiers including rotation and combination of modifiers (Lemos et al., 2016; Rigueiro et al., 2020; Neumann et al., 2020). This study was undertaken to assess the impact of combining or rotating rumen modifiers widely used in the Australian feedlot industry, on the composition of the rumen biota as cattle transition from a starter to finisher diet.

8.3 Materials and methods

Biome samples were collected from 2 cattle experiments as described below. All samples from experiment 1 were collected via rumen cannulae, while samples from experiment 2 were collected by oesophageal intubation (50-100ml volume). Subsamples (1.2ml) were transferred into an

Eppendorf tube and frozen immediately in liquid nitrogen. Samples were stored at -80^{o}C until transferred to the microbiome analysis laboratory in a dry-shipper at liquid nitrogen temperature.

8.3.1 Rumen modifier treatments

Experiment 1.

In this experiment the development of the rumen biome through adaptation to a feedlot finisher diet was assessed in the presence of each of 5 rumen modifier treatments. The study was approved by the Animal Ethics Committee of the University of New England (AEC approval number 18-028). Twelve rumen cannulated LowLine Angus cattle (198 ± 39 kg initial LW) were individually penned and progressively adapted to a feedlot finisher diet on two occasions (adaption periods 1 and 2), with a period of grazing on pasture interspersed between periods. Steers were fed 2.0% (DM basis) of the average LW, adjusted as necessary after weekly weighing, and delivered daily at 1000 h. Each adaption period consisted of 28d in which cattle were progressively adapted through starter, intermediate1, intemediate2 and finisher diets with 1 week on each diet (45%, 56%, 67% and 79% tempered barley grain respectively, DM basis) & sampling at the end of that week. Cattle (2-3/treatment/period) were randomly assigned to treatment in each period, with the treatments being either Monensin alone (MON), or Monensin in daily rotation with Lasalocid (M/L) or with bambermycin (M/B), or Monensin in combination with Lasalocid (ML) or with Bambermycin (MB). The modifiers supplier were 25 ppm of Monensin fed as Rumensin Granular, (Elanco Animal Health, Greenwood, Indiana, United States); 30 ppm Lasalocid fed as Bovatec (Zoetis LLC, Salisbury, Maryland, United States); and 2 ppm Bambermycin fed as Flavo 40 (Microflora Management, Huvepharma, Inc, Peachtree City, Georgia, United States). Modifier treatments were diluted into wheat flour (24-29%) and mill run (70%) and manually added directly into each individual steers' ration and hand mixed thoroughly before offering to cattle. Full details of the experiment are provided in Nortrup et al., (2021b).

Experiment 2.

Following Experiment 1, a feedlot trial involving 450 steers (10 steers/pen, 45 pens) was conducted at "Tullimba" research feedlot. Three rumen modifier treatments (Monensin, 25 ppm in DM; Lasalocid 30 ppm in DM and daily rotation of Monensin (25 ppm) and Lasalocid (30ppm) were fed from commencement with 15 pens of steers receiving each treatment according to a randomised block design with animals blocked on starting liveweight and location in the feedlot. Steer liveweight, feed intake and ultimately carcass attributes were monitored as described by Nortrup et al (2021a). On d83 of feeding, a sample of rumen fluid was collected post-feeding as described above from one animal chosen at random from within each pen with the sample stored at -80^{o}C prior to biome analysis.

8.3.2 DNA extraction, sequencing and bioinformatics

All DNA extraction, purification, amplification and sequencing was conducted by the Australian Genome Research Facility. DNA from the sample as provided was extracted from the samples using

the Qiagen DNeasy PowerSoil Pro kit (https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/dneasy-powersoil-pro-kit/#resources).

PCR amplification and sequencing was performed by PCR amplicons were generated using the forward primer CCTAYGGGRBGCASCAG and reverse primer GGACTACNNGGGTATCTAAT and conditions outlined in Table 1. Thermocycling was completed with an Applied Biosystem 384 Veriti and using Platinum SuperFi mastermix (Life Technologies, Australia) for the primary PCR. The first stage PCR was cleaned using magnetic beads, and samples were visualised on 2% Sybr Egel (Thermo-Fisher). A secondary PCR to index the amplicons was performed with Platinum SuperFi II mastermix (Life Technologies, Australia). The resulting amplicons were cleaned again using magnetic beads, quantified by fluorometry (Promega Quantifluor) and normalised. The equimolar pool was cleaned a final time using magnetic beads to concentrate the pool and then measured using a High-Sensitivity D1000 Tape on an Agilent 2200 TapeStation. The pool was diluted to 5nM and molarity was confirmed again using a Qubit High Sensitivity dsDNA assay (ThermoFisher). This was followed by sequencing on an Illumina MiSeq (San Diego, CA, USA) with a V3, 600 cycle kit (2 x 300 base pairs paired-end) and a 25% PhiX spike-in to improve nucleotide diversity.

Paired-end reads were assembled by aligning the forward and reverse reads using PEAR (version 0.9.5) [1]. Primers were identified & trimmed. Trimmed sequences were processed using Quantitative Insights into Microbial Ecology (QIIME 1.8) [2] USEARCH (version 7.1.1090) [3,4] and UPARSE [5] software. Using usearch sequences were quality filtered, full length duplicate sequences were removed and sorted by abundance. Singletons or unique reads in the data set were discarded. Sequences were clustered followed by chimera filtered using “rdp gold” database as the reference. To obtain the number of reads in each OTU, reads were mapped back to OTUs with a minimum identity of 97%. Using Qiime taxonomy was assigned using Greengenes database (version 13_8, Aug 2013) [6] or Silva database. Relative abundance of microbial groupings from Phyla to order were graphed in Python 2.7 using the Matplotlib library and analysed for treatment effects using Minitab 19.

Table 12 PCR amplification conditions used prior to sequence determination of rumen biome samples.

Target	Cycle	Initial	Disassociate	Anneal	Extension	Finish
16S: V3 - V4	29	95 *C for 7 min	94*C for 30s	50*C for 60S	72*C for 60S	72*C for 7 min

Statistical analyses were conducted in Minitab (V19). The primary variables analysed were the proportions of Phylum and of Genera present in the rumen biota. This required considerable reduction in data from the 13GB of data received back from AGRF. Biota proportions were assessed for Experiment one using a Generalised Linear Model in which period (of adjustment) day of sample (coinciding with cattle being on starter, T1, T2, Finisher for 1 week) and treatment (n=5) were included as fixed effects and the interaction of day x modifier treatment was tested as a first order interaction. For Experiment 2 where only a single sample from an animal in each pen (day 84) was analysed, a simple GLM model testing for effects of Block (n=15) and modifier treatment (n=3) was applied.

8.4 Results

For both Experiment 1 and Experiment 2, the biome was described first at the Phyla level, then the Order level. Only groupings whose median population comprised >0.01% of the total OTUs were considered for statistical analysis and inclusion in graphical depiction.

8.4.1 Experiment 1: Modifier effects on biome during diet adaption

Twenty six phyla of bacteria and Archaea were recorded in the rumen of cattle during experiment 1 diet adaption, but only eight phyla had a median population of 0.1% of the total. When the effects of period (1st or 2nd adaptation), modifier treatment and days on feed were assessed, the effects were attributable to days on feed reflecting whether animals had been on starter (d7), intermediate 1 (d15), intermediate 2 (d21) or finisher (d29).predominant (figure 1).

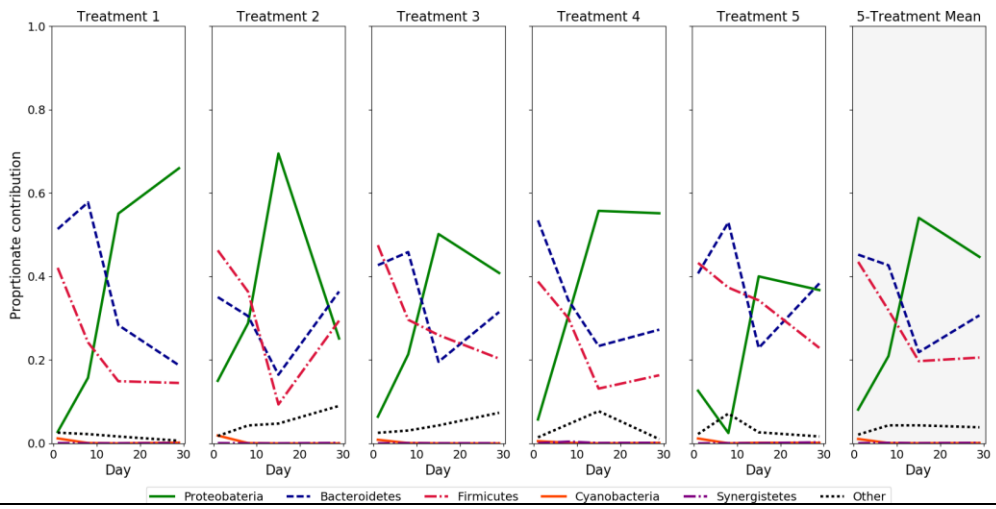

Figure 1. Change in balance of Phyla as animals were progressively adapted to starter (d7), intermediate 1 (d8), intermediate 2 (d21), or finisher (d29) (Raw data in Table 2 in Appendix)

There was a significant reduction in Bacteroidetes and Firmicutes over the feeding period and corresponding rise in the proportion of Proteobacteria in the rumen biome (<0.001). None of these dominant groups showed an effect of Period (1st or 2nd adaptation) on their proportion in the biome ($P > 0.05$). There was no effect of modifier treatment on the proportion of any bacteria constituting >0.1% of the biome ($P > 0.05$) and no treatment x day interaction, identifying that the effect of progressive diet changes was consistent across all modifier treatments.

Neither were there effects of modifier treatment on the proportion of the major orders (>0.1% of total Orders) within the major Phyla (>0.1% of total Phyla). However, again there was a major influence of days on feed on Orders present, There was a significant ($P < 0.001$) progressive decline in Bacteroidales and Clostridiales during diet transitions, a corresponding rise in the major grouping of Aeromonadales

(35% of bacteria) and a less clear rise in the smaller Selenomonadales and Erysipelotrichales (P = 0.004, 6% of bacteria and = 0.027, 5% of bacteria respectively).

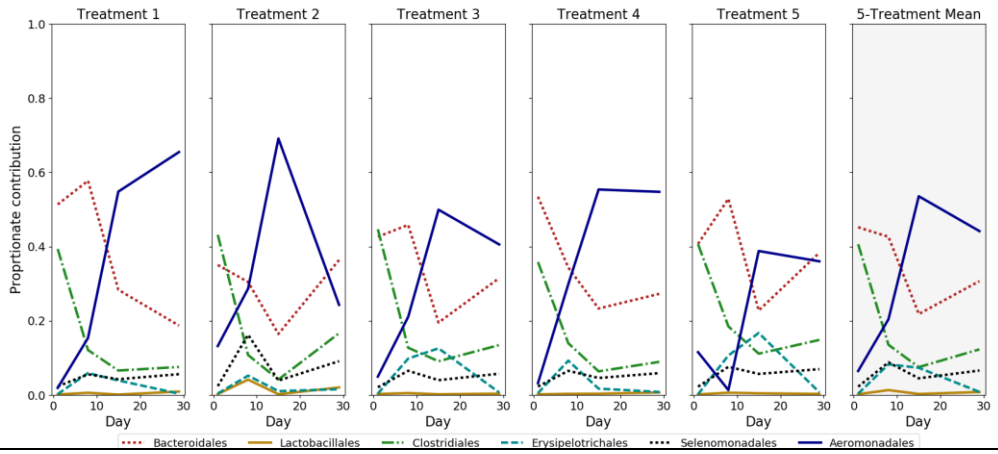

Figure 2. Change in balance of Order as animals were progressively adapted to starter (d7), Intermediate 21 (d8), intermediate 2 (d21) or finisher (d29) (Raw data in Table 3 in Appendix)

Only 24 genera contributed more than 0.1% of the total bacterial biome (range 3-96% of biome; average 66% across all cattle in both periods). In order to get an average of over 80% of the biome explained by included genera, 3 genera with a median prevalence less than 0.1% of biome were included Prevotella 1, Christensenellaceae R-7 group and Veillonellaceae;D_5__uncultured) to give a range 34-98% and average of 82% of the biome explained by listed genera. Again, there were major changes associated with progressive adaption from starter to finisher diet over time, with a very large decline in Prevotella 1 prevalence (from 32.9% to 9.5%) with Selenomonas and Christensenellaceae R-7 groups also declining over time among others. There was a major rise in Succinivibrionaceae UCG-001 (from 0% to 28.2%) and in Succinivibrionaceae UCG-002 and significant rises in some Prevotellaceae and in Syntrophococcus but no change in Megasphaera or Lactobacillus.

Overall, the number of genera contributing more than 1% of the biome increased as the cattle adapted to increasing grain content, with 22 genera on d1, 40 genera on d8, 50 genera on d15 and 65 genera on d29.

8.4.2 Experiment 2: Modifier rotation effects on biome of cattle during feedlot finishing cattle

As for Experiment 1, the Proteobacteria, Firmicutes and Bacteroidetes were the dominant Phyla in cattle established on the finisher ration, specifically the Orders Aeromonadales and Clostridiales. There was no significant effect of treatment (Mon, Las or Mon/Las daily rotation) on the proportion of bacteria in each of the major Phyla (>0.1% of population; P>0.05; Figure 3). In keeping with this high level uniformity, there was also no difference in the proportions of the major Orders (>0.1% of population; P>0.05).

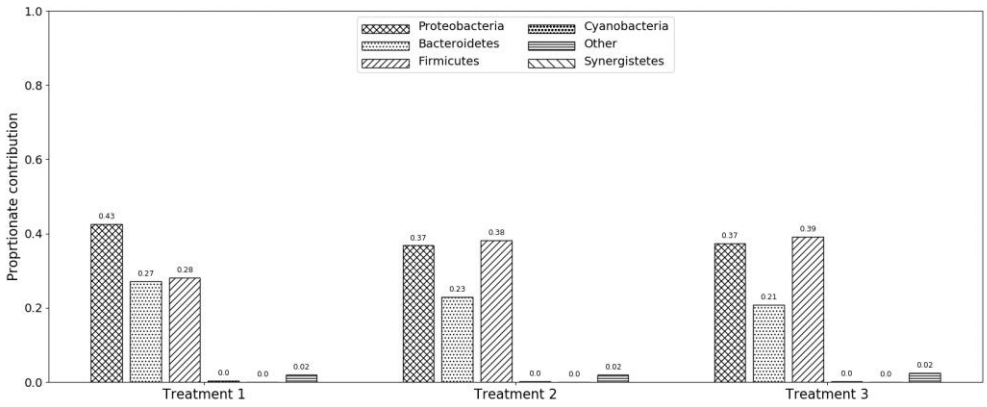

Figure 3. Proportions of bacteria from, each of the major Phyla that constituted more than 0.1% of total organisms in feedlot cattle after 84d supplementation with Monensin (Treatment 1 Mon), Lasalocid (Treatment 2 Las) or Monensin and Lasalocid in daily rotation(Treatment 3). (Raw data in Table 4 in Appendix)

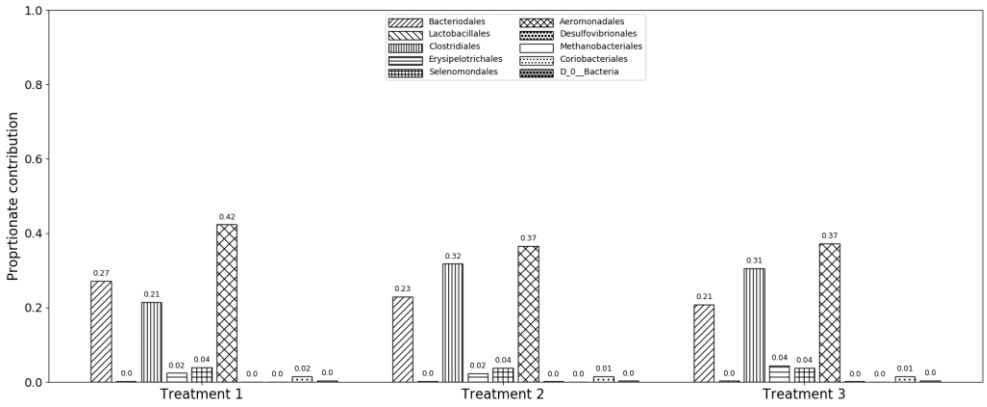

Figure 4. Proportions of bacteria from, each of the major Orders that constituted more than 0.1% of total organisms in feedlot cattle after 84d supplementation with Monensin (Treatment 1 Mon), Lasalocid (Treatment 2 Las) or Monensin and Lasalocid in daily rotation(Treatment 3). (Raw data in Table 5 in Appendix)

The data thus shows no high level effect of differential modifier treatment on the biome.

8.5 Discussion

The microbial balance in the digestive tract is thought to be primarily a product of its immediate environment, principally the quantity and composition of substrates ingested, as well as overarching impacts of the host digestive tract and digesta kinetics. Microbial transition in the rumen biota is rapid for some species when ruminants change diets, both between pastures (Gilbert et al., 2019) and from

roughage to grain-based diets, such as the 5 fold increase in *Ruminococcus bromii* in just 4 days observed by Klieve et al., (2007). However, there is a slower sequential change in the gut biota for 3 weeks and this protracted change may be associated with the rumen volume taking approximately this long to change when cattle move onto a grain based ration.

The dominance of Prevotella on day 1 in Experiment 1 was consistent with them being the dominant group in the rumen of cattle on non-starch diets (Ogunade et al., 2020) and the rise in the Succinovibriacaeae (UCG-001 and UCG-002) as well as Succinovibrio over time in Experiment 1 during diet transition is consistent with these organisms fermenting starch to succinate as a precursor to propionate production. Xue et al., (2018) found a strong association between the prevalence of Succinovibriacaeae and the proportion of propionate in the rumen when studying the core biome of dairy cattle. Overall, the rise in genera diversity with diet change from 22 genera >0.1% of biome on day 1 to 65 genera contributing >0.1% of biome on day 29 in Experiment 1 shows a greater microbial diversity on a grain based diet.

In considering the few effects of rumen modifier on the biome it must be remembered that all treatments in both experiments had at least one modifier so there was no modifier-free control. Consequently, a consistent change in biome structure over time during diet adaption results from all rumen modifiers tested, whether fed alone or in combination or rotation, as identified by no significant 'treatment' effect for any phylum over 0.2% of the bacterial biome. This stability of biome across different modifier treatments was reinforced in the larger feedlot trial where there was no difference in prevalence of major phyla (>0.1% of biome) in which the Proteobacteria, Firmicutes and Bacteroidetes together constituted 96.6 % of the bacteria. This consistency is further evident in there being no treatment effect on Orders present in the rumen biome of feedlot finished steers either. We will interrogate this data further to better define the diversity indices and key species

8.6 Conclusion

The key finding is the large magnitude of effect of progressive transition from pasture to grain based rations on the major bacterial groupings present with a rise in the succinate producing groups underpinning a rise in propionate production. In contrast, whether a single modifier or a rotation or a combination of modifiers was used, all modifier treatments led to very similar progressive change in the balance of bacteria during transition to a feedlot finisher diet (Experiment 1). Once established on the feedlot diet (Experiment 2), whether the rumen was managed by Monensin, Lasalocid or a rotation of the two did not affect the prevalence of the dominant orders of bacteria present.

8.7 References

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APPENDIX: Table 2. Summary data and statistical assessment of Phylum of bacteria in the rumen fluid of cattle in experiment 1 where treatments were 5 different rumen modifier strategies. Only phyla constituting 0.1% of the OTUS (biome) are considered with Eucharouta (Archael) levels included for interest

Variable	N*	Mean	StDev	Minimum	Median	Maximum	period	day	treat	Treat x day
Euryarchaeota	0	0.000573	0.001268	0.000000	0.000259	0.009800	ns	ns	ns	ns
Actinobacteria	0	0.02844	0.04523	0.00013	0.00794	0.24889	ns	ns	ns	ns
Armatimonadetes	0	0.000002	0.000013	0.000000	0.000000	0.000100	ns	<0.001	0.004	0.002
Bacteroidetes	0	0.3347	0.1729	0.0162	0.3202	0.6811	ns	<0.001	ns	ns
Chloroflexi	0	0.000127	0.000332	0.000000	0.000000	0.002147	ns	0.039	ns	ns
Cyanobacteria	0	0.002119	0.004522	0.000000	0.000084	0.024835	ns	<0.001	0.023	0.002
Einococcus-Thermus	0	0.000007	0.000043	0.000000	0.000000	0.000367	ns	ns	ns	0.046
Elusimicrobia	0	0.000066	0.000164	0.000000	0.000000	0.000841	ns	0.075	0.04	ns
Epsilonbacteraeota	0	0.000015	0.000039	0.000000	0.000000	0.000181	0.017	ns	ns	ns
Fibrobacteres	0	0.000570	0.001291	0.000000	0.000000	0.006138	ns	<0.001	ns	ns
Firmicutes	0	0.2679	0.1724	0.0296	0.2441	0.8173	ns	<0.001	ns	ns
Fusobacteria	0	0.000003	0.000020	0.000000	0.000000	0.000166	ns	ns	ns	ns
Hydrogenedentes	0	0.000000	0.000004	0.000000	0.000000	0.000033	ns	ns	ns	ns
Kiritimatiellaeota	0	0.000003	0.000015	0.000000	0.000000	0.000094	ns	<0.001	ns	ns
Lentisphaerae	0	0.000004	0.000018	0.000000	0.000000	0.000122	ns	<0.001	ns	ns
Patescibacteria	0	0.000010	0.000043	0.000000	0.000000	0.000291	ns	ns	0.086	ns
Proteobacteria	0	0.3558	0.2873	0.0005	0.3479	0.9298	ns	<0.001	ns	ns
Spirochaetes	0	0.001541	0.002832	0.000000	0.000316	0.015591	ns	<0.001	<0.001	<0.001
Synergistetes	0	0.000870	0.002350	0.000000	0.000260	0.018980	0.047	ns	ns	ns
Tenericutes	0	0.000708	0.001580	0.000000	0.000000	0.007193	0.022	<0.001	ns	ns
Verrucomicrobia	0	0.000023	0.000074	0.000000	0.000000	0.000450	ns	<0.001	0.081	0.083
WPS-2	0	0.000141	0.000584	0.000000	0.000000	0.004920	ns	ns	ns	ns
D_0__Bacteria;__	0	0.00635	0.02809	0.00000	0.00093	0.18168	ns	ns	ns	ns

Table 3. Summary data and statistical assessment of Genus of bacteria in the rumen fluid of cattle in experiment 1 where treatments were 5 different rumen modifier strategies. Only phyla constituting 0.1% of the OTUS (biome) are considered but 3 genera were found to make substantial contribution to small number of anisl so were also included, despite their median contribution being <0.1% of OTUs. Tabulated effects are based on period, day, treatment, day x treatment interaction There were only 2 interactions and these are noted by a superscript star by the treatment effect

Genus	N	Mean	SE Mean	Minimum	Median	Maximum	Period	Day	Treat
Olsenella	81	.01267	.00323	.00000	.00300	21381	ns	0.038	ns
Prevotella 1	81	.1823	.0184	.0000	.1287	5751	<0.001	<0.001	ns
Prevotella 7	81	.04365	.00692	.00000	.01353	33690	0.003	<0.001	ns
Prevotellaceae UCG-001	81	.003646	.000849	.000000	.001719	052807	ns	<0.001	0.027*
Prevotellaceae;__	81	.04747	.00678	.00000	.02215	29539	0.003	<0.001	ns
Rikenellaceae RC9 gut group	81	.01231	.00222	.00000	.00371	13767	ns	<0.014	0.033*
Lactobacillus	81	.00542	.00228	.00000	.00109	17830	ns	ns	ns
Christensenellaceae R-7 group	81	.01452	.00332	.00000	.00060	13321	ns	<0.001	ns
Acetitomaculum	81	.003703	.000720	.000000	.002008	048598	ns	ns	ns
Lachnospiraceae NK3A20 grp	81	.01213	.00123	.00000	.00906	05118	0.002	ns	ns
Oribacterium	81	.00721	.00138	.00000	.00290	06909	ns	ns	ns
Roseburia	81	.00946	.00223	.00000	.00186	11953	0.012	0.066	ns
Syntrophococcus	81	.002630	.000681	.000000	.001186	045814	0.012	0.043	ns
[Ruminococcus] gauvreauii grp	81	.01163	.00211	.00000	.00478	10168	ns	0.03	ns
Lachnospiraceae;__	81	.01458	.00230	.00000	.00775	15080	ns	<0.001	ns
Ruminococcaceae NK4A214 grp	81	.005418	.000766	.000000	.001839	027628	ns	<0.001	0.037
Ruminococcus 1	81	.00941	.00226	.00000	.00171	10006	ns	<0.001	ns
[Eubacterium] coprostanoligenes	81	.01119	.00139	.00000	.00797	07652	ns	0.01	0.076
Sharpea	81	.02392	.00775	.00000	.00152	41833	ns	0.027	ns
Succiniclasticum	81	.01387	.00137	.00000	.01018	07196	0.012	0.045	0.079
Megasphaera	81	.01309	.00600	.00000	.00191	45914	ns	ns	ns
Selenomonas 1	81	.01019	.00144	.00000	.00475	04799	ns	<0.001	ns
Veillonellaceae;D_5__uncultured	81	.005009	.000929	.000000	.000844	035971	ns	<0.001	ns
Veillonellaceae;__	81	.00806	.00146	.00000	.00204	05958	<0.001	0.001	ns
Succinivibrio	81	.0607	.0139	.0000	.0050	6498	0.033	0.005	ns
Succinivibrionaceae UCG-001	81	.1366	.0304	.0000	.0000	9140	<0.001	<0.001	ns
Succinivibrionaceae UCG-002	81	.1389	.0250	.0000	.0052	8734	<0.001	ns	ns

Appendix Table 4. Summary data and statistical assessment of Phylum of bacteria in the rumen fluid of cattle in Experiment 2 where treatments were cattle were supplemented with Monensin, Lasalocid or a daily rotation of Monensin and Lasalocid. Only phyla constituting 0.1% of the OTUS (biome) are considered with Euryarchaeota (Archaeal) levels included for interest

Variable	N	Mean	SE Mean	Minimum	Median	Maximum	Block	Treat
Euryarchaeota	45	0.000519	0.000128	0.000000	0.000283	0.005067	ns	ns
Acidobacteria	45	0.000000	0.000000	0.000000	0.000000	0.000000	ns	ns
Actinobacteria	45	0.01729	0.00354	0.00166	0.01118	0.13995	ns	ns
Armatimonadetes	45	0.000000	0.000000	0.000000	0.000000	0.000000	ns	ns
Bacteroidetes	45	0.2362	0.0137	0.0371	0.2378	0.4376	ns	ns
Chloroflexi	45	0.000111	0.000097	0.000000	0.000000	0.004336	ns	ns
Cyanobacteria	45	0.002467	0.000430	0.000000	0.001041	0.012157	ns	ns
einococcus-Thermus	45	0.000000	0.000000	0.000000	0.000000	0.000000	ns	ns
Elusimicrobia	45	0.000040	0.000012	0.000000	0.000000	0.000378	ns	ns
Epsilonbacteraeota	45	0.000022	0.000008	0.000000	0.000000	0.000278	ns	ns
_Fibrobacteres	45	0.000001	0.000001	0.000000	0.000000	0.000031	ns	ns
Firmicutes	45	0.3509	0.0286	0.0615	0.3346	0.8204	ns	ns
Fusobacteria	45	0.000030	0.000020	0.000000	0.000000	0.000772	ns	ns
Gemmatimonadetes	45	0.000000	0.000000	0.000000	0.000000	0.000000	ns	ns
Hydrogenedentes	45	0.000000	0.000000	0.000000	0.000000	0.000000	ns	ns
Kiritimatiellaeota	45	0.000000	0.000000	0.000000	0.000000	0.000000	ns	ns
Lentisphaerae	45	0.000000	0.000000	0.000000	0.000000	0.000000	ns	ns
Patescibacteria	45	0.000012	0.000007	0.000000	0.000000	0.000309	ns	ns
Proteobacteria	45	0.3890	0.0357	0.0014	0.3596	0.8514	ns	ns
Spirochaetes	45	0.000121	0.000050	0.000000	0.000000	0.001686	ns	ns
Synergistetes	45	0.000259	0.000057	0.000000	0.000111	0.002060	ns	ns
Tenericutes	45	0.000016	0.000007	0.000000	0.000000	0.000188	ns	ns
Verrucomicrobia	45	0.000000	0.000000	0.000000	0.000000	0.000000	ns	ns
WPS-2	45	0.000003	0.000003	0.000000	0.000000	0.000151	ns	ns
D_0__Bacteria;__	45	0.003064	0.000275	0.000000	0.002469	0.007698	ns	ns

Appendix Table 5. Summary data and statistical assessment of Orders of bacteria in the rumen fluid of cattle in Experiment 2 where treatments were cattle were supplemented with Monensin, Lasalocid or a daily rotation of Monensin and Lasalocid. Only phyla constituting 0.1% of the OTUS (biome) are considered with Euryarchaeota (Archael) levels included for interest

Variable	N	Mean	SE Mean	Minimum	Median	Maximum	Block	Treatment
Bifidobacteriales	45	0.00230	0.00226	0.00000	0.00000	0.10169	ns	ns
Coriobacteriales	45	0.01457	0.00222	0.00166	0.01026	0.07496	ns	ns
Bacteroidales	45	0.2362	0.0137	0.0371	0.2378	0.4376	ns	ns
Gastranaerophilales	45	0.002379	0.000432	0.000000	0.000933	0.012157	ns	ns
Lactobacillales	45	0.002416	0.000274	0.000082	0.001891	0.007345	ns	ns
Clostridiales	45	0.2792	0.0259	0.0416	0.2310	0.7663	ns	ns
Erysipelotrichales	45	0.03026	0.00573	0.00125	0.01837	0.17525	ns	ns
Selenomonadales	45	0.03786	0.00412	0.01359	0.02697	0.11974	ns	ns
Aeromonadales	45	0.3866	0.0358	0.0011	0.3539	0.8505	ns	ns
D_0__Bacteria;__;__;__	45	0.003064	0.000275	0.000000	0.002469	0.007698	ns	ns

9 SUCCESS IN MEETING THE MILESTONE

This milestone report conveys all the findings from Experiments 1 and 2, although further chemical and statistical analysis are likely to be made as these draft manuscripts are tidied up for publication.

10 OVERALL PROGRESS OF THE PROJECT

This report summarises all of the research contracted to be conducted in B.FLT.1002.

11 GENERAL DISCUSSION

11.1 Project findings and results

Prior to the feedlot evaluation of modifiers, the validation of 2 measurement innovations was required; being the accelerometer-derived estimates of time sent ruminating using the eSense eartag (Allflex, Capalaba, Queensland, Australia), and long term *in-situ* monitoring of ruminal pH using the SmaXtec intraruminal bolus (SmaXtec Premium Bolus; smaXtec Animal Care Sales, GMBH, Graz, Austria).

The estimates of rumination time (h/d) were sufficiently closely related to observed time spent ruminating (using camera based monitoring) to strongly endorse the eSense tag as a useful research tool for quantifying rumination time of cattle on feedlot diets.

While some smaXtec boluses performed within the specification of the manufacturer, even from the time activated, some devices were biased, so it is not possible to recommend a general endorsement of these devices for 'off the shelf' use. Certainly if any individual device can provide a substantially biased estimate of rumen pH, their use in identifying individual cattle expressing SARA must be questioned. The manufacturers were not pleased when we sought to apply testing to provide quality assurance of data collected, but we would consider it unwise to assume the accuracy of reported pH without such validation, and recognise the need to quantify bias and drift based on incubations in known pH buffers before and after employing the devices.

The principle focus of the study was to quantify potential advantages of using existing rumen modifiers in combination or rotation, instead of using a single modifier alone. Previous studies have shown some production advantages for both rotation (Morris et al., 1990) and combination (Benatti et al., 2017) of modifiers over providing a single modifier.

In the intensive ruminal studies in the current project, during adaptation modifier treatment had no effect on total VFA concentration in the rumen or on mean ruminal pH, but Bambermycin alone and in combination had substantially showed substantially more time was spent at pH <5.8. Monensin alone or paired (rotation of combination) with Lasalocid supported the highest pre- and post-feeding ruminal pH (manual sample) so the least risk of lactic acidosis. They also offered the lowest redox potential, the lowest acetate percentage and lower NH_3 concentration than treatments with B. On these aspects, M alone or in pairing with L warrants further investigation and so was studied in the feedlot performance trial.

Previous study of daily rotation of Monensin and Lasalocid in feedlot rations has shown improved ADG and Feed:Gain of 4.8 and 2.7% respectively (Shreck et al., 2016) from daily rotation of Monensin and Lasalocid, but McKinnon et al (1992) found no advantage of either daily rotation or combination of these 2 modifiers. The current study extended the results of McKinnon, with the rotation of M and L not affecting ruminal pH, VFA concertation or faecal starch percentage. Where performance differences were observed in the current trial, such as in FCR and ADG, these differences favoured Monensin over L or over daily rotation. No differences at all were present between treatments in carcass attributes from carcass weight to carcass fat colour, so there was no treatment difference in carcass value. Consequently the Tullimba study does not recommend daily rotation of modifiers as a route to either reduce costs (DMI, FCR) or improve the quantity or quality of carcasses generated. The only caveat on this is that ADG in Tullimba cattle was high (mean 2.57 kg/d over 109d), which is higher than previous studies of modifiers used in rotation (Table 29) & may reflect nutrient supply being sufficient to meet animals genetic potential for growth on all treatments.

In hindsight, there was little opportunity to conduct the feedlot trial differently than was done. The primary constraint was the level of replication required to detect differences anticipated to be 5% between treatments. With a few more pens we would have considered introducing a 4th treatment chosen to be very different to the others, to increase power in detecting differences. The major area for change would have been in the intensive studies, as replication over time proved a very demanding task and gave opportunity for cattle to become fussy eaters. We could well have gained similar data by using a larger number of non-cannulated cattle (that could have been

stomach tubed on occasion) across the 5 diets, and just used a similar number of cannulated cattle over a single period scattered among them for a small number of traits.

The close relationship with MLA (Joe McMeniman) worked well in ensuring diets and feedlot operation were of a high standard and this contributed to the growth performance and FCR achieved.

11.2 Meeting of Objectives

The following three objectives listed in the project schedule were addressed in this experimental program:

1. Determine the effect of Monensin in combination or rotation with Lasalocid and flavophospholipol on rumen fermentation.

The design for the intensive study was chosen to allow effects of combination and effects of rotation on fermentation to be assessed relative to Monensin fed alone, then further assessed to see if the effects were consistent across M and B as well as M and L. The studies did not find startling differences resulting from combination or rotation with M but in general the weight of evidence supported Lasalocid in preference to Bambermycin as the preferred partner for Monensin. These assessments addressed mean pH, diurnal variation in pH and time spent below pH thresholds, as well as total and proportions of VFA in the rumen, protozoal populations and methane production.

2. Determine the effect of Monensin in combination or rotation with Lasalocid and flavophospholipol on feedlot performance and carcase characteristics.

The original thinking had been the feedlot trial would evaluate M, L, and M and L in combination and in daily rotation (4 treatments as per stated objective). Scientifically this was not strong as with no 'L' treatment, there would have been no way of knowing if any desirable rotation or combination effects observed were a direct result of Lasalocid and could have been achieved by simply using L alone. For this reason and in association with MLA, the three final treatments were agreed (M, L, M and L in rotation). These treatments were compared in a strong study (15 pens x 10 head/pen for each of the 3 treatments). The lack of advantage in daily rotation of M and L over M alone did not reflect simply a study of insufficient power, rather it gave significant evidence of Monensin's advantage over L or the rotation in a number of growth traits (overall FCR, ADG over first 83 d and a tendency to have greater ADG over the entire feeding period (P=0.062). Similarly, the carcass data showed no significant difference between any treatment and no treatment effect on the monetary value of the carcass. This lack of difference was despite a powerful statistical test and even so, indicated the numerically most desirable attributes were in Monensin-fed not rotation-fed cattle. So we are satisfied with the assessment but recognise that on higher fibre lower energy diets supporting lower animal performance, different results could have occurred which may explain advantages in modifier rotation occasionally reported by other authors.

4. *Determine the effect of Monensin in combination or rotation with Lasalocid and flavophospholipol on the rumen microbiome*

This was done in partnership with Australian Genome Research Facility rather than CSIRO and Dr David Yanis Ruiz as had been intended due to Covid and staff changes. There were very major changes in the biome associated with diet transition from forage to feedlot finisher; specifically a shift from a Bacteroidetes dominance (principally Prevotella 1) and rise in Proteobacteria, notably those that produce succinate, one of the precursors of propionate. However, while these were major biotic shifts with diet transition, the only difference in the biome between one rumen modifier and another was in the minor genera, typically less than 0.1% of the biome. In consequence it can be concluded that the effects of treatment on rumen biome were similar across all treatments in both experiments and the lack of rumen biota differences is consistent with the modest effect of modifier treatment on other parameters.

12 Conclusions/recommendations

- The principle industry objective of this research was to assess whether there was growth, efficiency or carcass advantage in rotating Monensin with an alternate rumen modifier. After assessment of the Tullimba study it is concluded that when industry best practice is followed in ration formulation and delivery, there is no ADG, efficiency, carcass quality or economic value advantages associated with rotation of Monensin with Lasalocid relative to continuous feeding of Monensin.
- There would be merit in assessing modifier rotation response in a commercial feedlot where cattle Feed:Gain and ADG are not as good as those in this trial (4.48 kg/kg and 2.57 kg ADG/d).
- Given the lack of performance response and the strong *in-vitro* and *in-vivo* literature on ruminal effects of rumen modifiers, it is not advantageous in conducting more intensive ruminal studies unless in combination or as comparisons with, new modifiers such as 3-NOP or future compounds.
- While the feedlot industry needs to be informed of these findings, there are not promotional messages to go forth leading to nutritional practice change.
- eSense eartags can be recommended for estimation of rumination time. While 'time spent ruminating' is currently only a research metric, work with these tags may lead to understanding of rumination time as an indicator of nutritional distress (such as lactic acidosis or inappetance) and could be readily implemented in commercial feedlots as an early alert for nutritional or welfare problems.
- smaXtec intraruminal pH monitoring boluses should be used with caution, and while additional checking against pH standards is not currently recommended by the manufacturer, we would recommend development and application of Quality Assurance procedures for research users so results can be endorsed for publication. This is a recommendation for use of any intraruminal pH bolus, not just smaXtec devices. It is recognised that the reticulo-rumen environment is very hostile to artificial membranes and to obtain reliable readings over 150 d or beyond is a major challenge where technology interfaces with biology…. and pebbles and grit that accumulate in the reticulum only magnify this challenge.

13 Key Messages

- There is no productivity, efficiency or economic gain to be achieved from moving to a daily rotation of Monensin and Lacsalocid.in feedlots that already have high ADG and low Feed:Gain ratios. These producers should not be adopting daily rotation of these rumen modifiers
- Combination or rotation of Monensin with either Lasalocid or bambermycin at commercial inclusion rates creates no biologically important changes in rumen biome, fermentation parameters relative to feeding Monensin alone (eg. DMD%, methane/kg feed), although minor differences in pH and VFA proportions were observed.

14 Budget summary (23/12/2020)

The project is currently overspent as we await final invoicing for biome, bioinformatic and rumen metabolite studies and final payment from MLA.

	Pending Expenditure	Pending income	
Current balance @ UNE	-$46,744		
Outstanding Expenditure			
-Biome and bioinformatics	-$15,000		
-Rumen fluid assessment	-$12,000		
-Gas standard replacement	-$3,484		
MLA final payment		$77,228	
Expected balance 31/01/2021			$0.00

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