Evaluation of LactiproFLX in an Acidosis Challenge Model

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Summary with Implications

An acidosis challenge study was conducted comparing different administration techniques for LactiproFLX, a direct fed microbial product containing Megasphaera elsdenii (a lactate-utilizing bacteria) for the prevention of acidosis. Four treatments were utilized in a randomized block design with 24 ruminally cannulated steers. Treatments consisted of a control group which did not receive the product, a group which received the commercial dose of the product four days before the acidosis challenge, one which received the commercial dose of the product one day before the challenge, and one which received ten times the commercial dose one day before the challenge. No differences were detected for rumination time or dry matter intake. Similarly, no differences were detected in the millimolar (mM) concentrations of propionate, valerate, or isovalerate. Several differences, however, were detected for total volatile fatty acids (VFA), acetate, isobutyrate, and butyrate during different periods of the study. Additionally, several differences were detected for ruminal pH parameters with the treatment dosed 4 days before the challenge having the greatest minimum and maximum pH when compared to the other treatments. The group dosed with ten times the commercial dose displayed lower pH variance and magnitude of change when compared to the other treatments. Therefore, if using exogenous Megasphaera elsdenii as an acidosis mitigation strategy, giving the bacteria time to establish in the rumen before an acidotic event could increase the effectiveness of the treatment. If giving the treatment closer to a possibly acidotic event, giving a higher dose could be beneficial.

Introduction

With acidosis being a risk to cattle fed high concentrate diets, considerable efforts have been made to reduce the incidence and severity of acidotic events in the feedlot industry. One of those efforts has been the development of LactiproFLX, a direct-fed microbial product containing a specific strain of lactic acid-utilizing bacteria (*Megasphaera elsdenii* NCIMB 41125; MS Biotech). The dose and administration type used depends on the production system and reason for use, but the product can provide from 5.0×10^9 to 1.0×10^{10} CFU of this bacterium.

Although lactic acid is not generally present in high amounts in the rumen under normal circumstances, certain events such as the step-up period, severe weather events, and illness can cause increased potential for lactic acid accumulation during acidosis in feedlot cattle. Because *Megasphaera elsdenii* utilizes lactic acid in the rumen, much of the interest in the research community has been focused on using it for acidosis mitigation. The theory behind the use of this bacteria for acidosis mitigation relates to the control of lactic acid concentrations in the rumen post-feeding, thereby slowing the decline of rumen pH.

The objective of this study was to evaluate the effects of 3 administration techniques of LactiproFLX compared to a control group in an acidosis challenge model.

Procedure

An acidosis challenge study was conducted using 24 ruminally cannulated steers in a randomized block design containing four treatments with 6 steers per treatment. Steers were blocked by weight, stratified by average intake within weight block, and assigned randomly to treatment. Three blocks of 8 steers were used with weight blocks being light, medium, and heavy. Treatments consisted of a control group which received no LactiproFLX (CON), a group which received the commercial dose of LactiproFLX (1.0×10^{10} CFU) 4 days prior to the acidosis challenge (COMM-4), a group which received the commercial dose of LactiproFLX one day prior to the challenge (COMM), and a group which received 10 times the commercial dose of LactiproFLX (1.0×10^{11} CFU) one day prior to the challenge (10X). All LactiproFLX capsules were dosed via the rumen cannula. Each block consisted of an experimental period which was 20 days in length and each treatment was represented by two animals in each block.

All animals were stepped up onto the finishing diet and fed for at least 32 days prior to the initiation of the experimental period. Diet and supplement composition are presented in Table 1. The supplement used was formulated to include 30 g/ton monensin (Rumensin, Elanco Animal Health) and provide 90 mg/steer daily of tylosin (Tylan, Elanco Animal Health). Steers were fed to target *ad libitum* intake

Table 1. Dietary composition for all treatments

Ingredient	DM Inclusion (%)
Steam-flaked corn	68
Modified distillers' grains	18
Alfalfa hay	9
Supplement[1]	
Fine ground corn	2.32
Limestone	1.67
Tallow	0.125
Urea	0.5
Salt	0.3
Vitamin A-D-E Premix	0.05
Beef Trace Premix	0.015
Rumensin Premix[2]	0.017
Tylan Premix[3]	0.003

[1]Supplement included in the diet at 5% DM

[2]Formulated to supply 30 g/ton DM of Rumensin

[3]Formulated to provide 90 mg per steer daily of Tylosin

Table 2. Dry matter intake of diet by treatment

	Treatment					*P*-value		
Item	CON	COMM	COMM-4	10X	SEM	TRT	Day	TRT*Day
DMI								
Overall, lb[1]	21.2	21.4	21.5	22.0	0.44	0.62	<0.01	0.82
Pre-challenge, lb[2]	20.5	21.8	21.2	21.1	0.52	0.34	<0.01	0.73
Challenge, lb[3]	32.5	31.4	33.8	35.1	1.77	0.50	-	-
Day 1, lb	15.2	13.6	16.4	16.3	2.14	0.77	-	-
Recovery, lb[4]	21.1	20.4	20.0	21.3	1.16	0.52	<0.01	0.64

[1]Values represent average intake over entire period from day -6 through 5 (excluding -1)

[2]Values represent average intake for days -6 through -2

[3]Values represent average intake for the acidosis challenge day (Day 0) only

[4]Values represent average intake for days 2 through 5

Table 3. Time spent ruminating

	Treatment					*P*-value		
Item	CON	COMM	COMM-4	10X	SEM	TRT	Day	TRT*Day
Rumination, min/day								
Pre-challenge[1]	297	270	232	266	35.8	0.66	0.01	0.38
Restriction[2]	228	261	207	256	45.5	0.82	-	-
Challenge[3]	114	121	114	147	17.3	0.48	-	-
Recovery[4]	187	202	140	182	28.2	0.47	<0.01	0.06
Overall[5]	238	229	175	219	29.8	0.21	<0.01	0.29

[1]Prechallenge period consisted of days -6 through -2

[2]Restriction period consisted of day -1

[3]Challenge period consisted of day 0

[4]Recovery period consisted of days 1 through 5

[5]Overall data included days -6 through 5

and fed twice daily at 0700 h and 1300 h. Unlimited access to water was provided. Feed refusals were collected and weighed daily to calculate daily DMI. Cattle were housed in a temperature-controlled room in individual pens equipped with rubber slatted floors. Rumen pH was measured continuously during the experimental period using SmaXtec wireless pH probes and averaged by hour. Minimum, maximum, average, magnitude of change, and pH variance were calculated by day. The number of minutes spent ruminating was continuously measured using CowManager sensor eartags and summarized by day.

During the experimental period, animals had *ad libitum* access to feed until 1900 h on day -2 (two days prior to the challenge) when feed was removed from the bunk to create a 36-h feed restriction period. Animals were only offered 50% of their 7-day average intake on day -1 (restriction day). On d 0, or the challenge day, steers were offered 175% of their average intake. On day 1, animals were offered their previous average intake, and on day 2, normal bunk reading protocols resumed. Rumen fluid samples were taken at 0700 h and 1100 h on day -2 and at 0700, 1100, and 1700 h on days 0, 1, and 2. Rumen contents were collected through the rumen cannula, strained through cheesecloth, and flash frozen in liquid nitrogen for analysis of volatile fatty acid (VFA) concentrations using gas chromatography.

All data were analyzed using a mixed procedure of SAS as a randomized block design with animal as the experimental unit. For DMI and rumination, data were summarized as overall (days -6 through 5), pre-challenge (days -6 through -2), challenge (day 0), day 1, and recovery (days 2 through 5). The periods containing multiple days (overall, pre-challenge and recovery) were analyzed with treatment, day, and treatment by day interaction included in the model, with day considered a repeated measure. All periods for the pH parameters used a similar model in SAS, as all periods were multiple days. For volatile fatty acid concentration, all periods but the restriction period, used a similar model as above but utilized time of collection as the repeated measure instead of day. Each period (except restriction) contained multiple samples taken at different times. Interactions and treatment differences were declared significant at $P \leq 0.05$ and a tendency was considered at $0.05 \leq P \leq 0.10$.

Results

Dry matter intake and rumination

No significant treatment differences or interactions were detected for overall dry matter intake (DMI) ($P = 0.74$) or for intake during any periods of the experiment ($P \geq 0.34$; Table 2). This was unexpected as it was hypothesized that intake would recover more rapidly for Lactipro treated cattle after the challenge. Time spent ruminating

Table 4. Minimum, maximum, average, standard deviation, and magnitude of change of ruminal pH

	Treatment					*P*-value		
Item	CON	COMM	COMM-4	10X	SEM	TRT	Day	TRT*Day
Minimum pH								
Pre-challenge[1]	5.70[b]	5.64[b]	5.99[a]	5.76[ab]	0.100	0.09	0.23	0.11
Challenge[2]	5.09[bc]	5.04[c]	5.47[a]	5.41[ab]	0.145	0.10	<0.01	0.56
Recovery[3]	5.64	5.49	5.85	5.53	0.127	0.17	0.38	0.46
Overall[4]	5.57[b]	5.50[b]	5.89[a]	5.64[b]	0.078	<0.01	<0.01	0.14
Maximum pH								
Pre-challenge	6.72	6.67	6.93	6.62	0.136	0.37	0.02	0.89
Challenge	6.42	6.44	6.68	6.48	0.164	0.66	0.71	0.15
Recovery	6.30[b]	6.54[b]	6.90[a]	6.25[b]	0.156	0.02	<0.01	0.11
Overall	6.57[b]	6.61[b]	6.97[a]	6.38[b]	0.116	0.01	<0.01	0.38
Average pH								
Pre-challenge	6.15	6.24	6.41	6.24	0.132	0.57	<0.01	0.05
Challenge	5.55	5.56	5.87	5.79	0.142	0.30	0.02	0.50
Recovery	6.01	5.93	6.21	5.93	0.141	0.44	0.20	0.78
Overall	6.06	5.99	6.31	6.04	0.097	0.11	<0.01	0.18
pH Variance								
Pre-challenge	0.31	0.27	0.27	0.25	0.021	0.32	0.54	0.51
Challenge	0.36[ab]	0.41[a]	0.35[b]	0.30[b]	0.027	0.05	<0.01	0.26
Recovery	0.20[b]	0.25[ab]	0.28[a]	0.19[b]	0.025	0.05	<0.01	0.16
Overall	0.28	0.29	0.28	0.24	0.014	0.12	<0.01	0.14
pH Magnitude								
Pre-challenge	1.09	0.98	0.89	0.89	0.068	0.16	0.24	0.48
Challenge	1.27[bc]	1.45[ab]	1.23[bc]	1.06[c]	0.092	0.06	<0.01	0.38
Recovery	0.82[b]	0.97[ab]	1.02[a]	0.66[b]	0.084	0.03	0.14	0.33
Overall	1.04[a]	1.06[a]	0.97[ab]	0.86[b]	0.049	0.04	<0.01	0.26

[a-d]Means in a row with different superscripts are different ($P < 0.10$)

[1]Pre-challenge period consists of days -6 through -2

[2]Challenge period consists of days 0 and 1

[3]Recovery period consists of days 2 through 5

[4]Overall includes all days

was not different among treatments during any period of the experiment ($P > 0.20$; Table 3). There was a treatment by time interaction for the recovery period where the COMM-4 treatment did not increase rumination at the same rate as all other treatments following the challenge ($P = 0.06$; Table 3).

Rumen pH

The effects of treatment on ruminal pH are presented in Table 4. No significant treatment effects were detected for the pre-challenge period for maximum, average, variance, or magnitude of change in rumen pH ($P \geq 0.16$). There were tendencies for treatments to differ for minimum pH during the pre-challenge ($P = 0.09$) and challenge periods ($P = 0.10$). For the pre-challenge period the COMM-4 treatment had the greatest minimum pH, CON and COMM groups had lowest pH, and 10X group was intermediate. The challenge period displayed similar results. The overall minimum pH was also significantly different between treatments with the COMM-4 group having the greatest pH when compared to all other treatments ($P < 0.01$). No statistical difference was detected for pH due to treatment during the recovery period ($P = 0.17$).

Maximum pH was also impacted by treatment for the recovery ($P = 0.02$) and overall periods ($P = 0.01$). During the recovery and overall periods, the COMM-4 treatment group had a greater maximum pH than all other treatments. For average pH, the only statistical difference was a significant treatment by time interaction for the pre-challenge period ($P = 0.05$). This was due to an increasing average pH for the CON and 10X groups, and a decrease for COMM-4 and CON groups as the days of the experiment progressed (days -6 through -2).

Significant differences were also detected for pH variance during the challenge ($P = 0.05$) and recovery periods ($P = 0.05$) only. For the challenge period, the COMM group had the greatest variance, 10X and COMM-4 the lowest variance, and CON was intermediate. During the recovery period, COMM-4 had the largest variance,

with CON and 10X had the least, and COMM was intermediate. Several significant differences were also detected between treatments for magnitude of change in pH for all periods except pre-challenge. During the challenge period, the COMM group had the largest magnitude of change, 10X the least, and CON and COMM-4 were intermediate ($P = 0.06$). In the recovery period, the COMM-4 group had the greatest magnitude of change, CON and 10X the lowest, and the COMM group remained intermediate ($P = 0.03$). Finally, the overall magnitude of change was greatest for the CON and COMM treatments, lowest for the 10X group, and intermediate for the COMM-4 treatment ($P = 0.04$).

Volatile fatty acid concentration

No significant interactions or treatment differences were found for propionate, valerate, or isovalerate during any of the periods ($P \geq 0.10$; Table 5). Similarly, no interactions or treatment differences were detected for the pre-challenge or restriction periods for total volatile fatty acids (VFA), acetate, or isobutyrate. There was a tendency for treatment to affect total VFA concentration with the COMM group having the greatest concentration, COMM-4 being intermediate, and CON and 10X having the lowest ($P = 0.10$). No other treatment effects or interactions were detected for total VFA. For butyrate, a significant difference for treatment was found during the pre-challenge period, with the COMM-4 group being statistically greater than the other 3 treatment groups ($P = 0.02$; Table 5). This was to be expected as one product of lactic acid fermentation by *Megasphaera elsdenii* is butyrate. The COMM-4 group was dosed 4 days prior to the challenge, which means theoretically, that group should have contained a larger population of *Megasphaera elsdenii* in the rumen at these time points than the other treatments. There was also a tendency for a treatment by time interaction for butyrate during the challenge period ($P = 0.08$). No significant differences were detected during the other periods for butyrate. There was also a tendency for the COMM group to have a lower acetate concentration than the 10X or CON groups with COMM-4 being intermediate ($P = 0.07$; Table 5). A tendency for an interaction for isobutyrate during the challenge period was also detected ($P = 0.08$; Table 5). The CON group had a slower decline in isobutyrate across time than the other 3 treatments with the COMM group having the lowest average concentration of isobutyrate, the 10X and CON groups having the highest concentrations, and COMM-4 being intermediate (Table 5). Interestingly, there was also a significant treatment effect for isobutyrate during the recovery period with the COMM-4 group having a lower concentration compared to the other three treatments ($P = 0.04$; Table 5). No significant differences were found for the pre-challenge or restriction phases for isobutyrate ($P \geq 0.51$).

Conclusion

Results from this study suggest that LactiproFLX administered using the techniques above had no impact on dry matter intake or rumination. Several differences were observed for ruminal pH parameters. The COMM-4 group was able to maintain greater minimum and maximum pH for the overall period analysis, indicating this administration method could help prevent acidosis. The statistical differences for magnitude of change for pH and pH variance were more complex. However, the 10X group appeared to have lower variance and magnitude of change during the challenge and recovery periods, suggesting this treatment administration method could lessen the variation in daily pH.

The product affected the concentration of some VFAs in the rumen fluid with isobutyrate, butyrate, and acetate being altered by treatment. Notably, the COMM-4 treatment had a much greater butyrate concentration during the pre-challenge period, suggesting that the *Megasphaera elsdenii* dosed 4 days prior to the challenge was able to survive in the rumen and establish a population before the challenge period.

Overall, LactiproFLX had no effect on the intake and rumination parameters measured in this experiment, although the experimental design may not allow for significant power to detect differences in DMI. However, several ruminal pH measurements in this experiment were different among treatments with the COMM-4 group having the greatest minimum and maximum pH, and the 10X group having the lowest pH variance and magnitude of change suggesting these two administration methods could be the most effective at preventing acidosis.

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Table 5. The mM concentration of volatile fatty acids from collected rumen fluid

	Treatment					*P*-value		
Item	CON	COMM	COMM-4	10X	SEM	TRT	Time	TRT*Time
Acetate								
Pre-challenge[1]	51.88	55.42	56.37	52.68	3.876	0.82	<0.01	0.82
Restriction[2]	30.88	29.16	30.34	26.40	2.942	0.71	-	-
Challenge[3]	76.75	81.09	80.12	71.10	3.760	0.26	<0.01	0.23
Recovery[4]	73.08^{b}	64.38^{a}	68.42^{ab}	73.48^{b}	2.765	0.07	<0.01	0.87
Propionate								
Pre-challenge	53.32	57.44	56.06	55.23	5.552	0.96	<0.01	0.53
Restriction	15.99	16.56	13.81	10.78	2.794	0.47	-	-
Challenge	71.09	85.84	71.83	68.84	5.876	0.17	<0.01	0.18
Recovery	93.89	86.88	87.00	94.38	5.274	0.60	<0.01	0.78
Isobutyrate								
Pre-challenge	1.40	1.32	1.30	1.16	0.115	0.51	0.06	0.48
Restriction	1.06	0.94	1.02	0.95	0.090	0.74	-	-
Challenge	0.89^{b}	0.63^{a}	0.79^{ab}	0.88^{b}	0.075	0.08	0.89	0.08
Recovery	1.11^{b}	1.04^{b}	0.89^{a}	1.09^{b}	0.064	0.04	<0.01	0.15
Butyrate								
Pre-challenge	5.60^{a}	6.40^{a}	9.60^{b}	6.17^{a}	0.859	0.02	<0.01	0.33
Restriction	2.16	2.46	3.13	2.86	0.401	0.36	-	-
Challenge	9.72	11.50	13.42	11.55	1.559	0.44	<0.01	0.08
Recovery	8.71	9.60	8.84	9.58	1.107	0.87	0.43	0.48
Isovalerate								
Pre-challenge	1.14	1.15	1.05	1.02	0.182	0.94	0.45	0.71
Restriction	1.44	0.95	1.82	1.94	0.408	0.34	-	-
Challenge	1.34	0.95	1.47	1.52	0.348	0.65	0.36	0.02
Recovery	1.09	0.95	1.09	0.84	0.172	0.68	<0.01	0.56
Valerate								
Pre-challenge	2.17	2.62	2.86	1.78	0.643	0.65	<0.01	0.11
Restriction	0.41	0.56	0.60	0.51	0.177	0.88	-	-
Challenge	4.13	4.88	4.81	4.24	0.512	0.53	<0.01	0.19
Recovery	4.56	4.81	5.03	4.62	0.666	0.94	0.48	0.78
Total								
Pre-challenge	115.50	124.29	127.24	118.05	8.859	0.77	<0.01	0.31
Restriction	51.93	50.63	50.52	43.46	5.763	0.73	-	-
Challenge	164.80^{a}	185.32^{b}	172.53^{a}	156.61^{a}	7.935	0.10	<0.01	0.12
Recovery	181.47	168.10	170.04	185.73	8.296	0.34	<0.01	0.84

[a-d]Means in a row with different superscripts are different ($P < 0.10$)

[1]Pre-challenge period consisted of samples taken at 0700 h and 1100 h on day -2

[2]Restriction period consisted of one sample per animal taken at 0700 h on day 0 (before feeding).

[3]Challenge period consisted of samples taken at 1100 h and 1700 h on day 0 and 0700 h on day 1.

[4]Recovery period consisted of samples taken at 1100 h and 1700 h on day 1 and 0700h, 1100 h, and 1700 h on day 2