Effects of Cultures of *Lactobacillus acidophilus* (BG2FO4) and *Propionibacterium freudenreichii* P-63 with or without Levucell SB (*Saccharomyces cerevisiae*) on Feedlot Performance, Carcass Merit, and *Escherichia coli* O157:H7 Shedding by Finishing Beef Steers

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Story in Brief

The objective of this experiment was to evaluate the effects of *Lactobacillus acidophilus* (BG2FO4) and *Propionibacterium freudenreichii* P-63 with or without Levucell SB (*Saccharomyces cerevisiae*) on feedlot performance, carcass merit, and *Escherichia coli* O157:H7 shedding by finishing beef steers fed a high-grain diet. Compared with control and *P. freudenreichii* without Levucell SB during the last 35 days on feed, feeding *L. acidophilus* resulted in a 2.5% increase in ADG, a 3.3% increase in dry matter intake, and a 10 lb increase in HCW. Carcass quality was improved by feeding *L. acidophilus*. In addition, feeding Levucell SB during the last 35 days on feed improved performance in steers fed *P. freudenreichii* P-63 compared with steers not fed Levucell SB. In the present experiment, the low incidence of *E. coli* shedding during interim periods might be associated with the season of year in which the study was conducted. However, the increase in *E. coli* shedding late in the feeding period is in contrast with previous results.

Key Words: Cattle, Direct-Fed Microbials, *Escherichia coli* O157:H7, Feedlot

Introduction

Direct-fed microbials (DFM) are receiving increased attention from the feedlot industry. Increased interest in DFM has resulted from increasing concerns about antibiotic use in production agriculture, and the need for producers to implement preventive measures against pathogen outbreaks in the food supply. Recent research has shown that bacterial DFM can decrease fecal shedding of *Escherichia coli* O157:H7 by feedlot cattle (Krehbiel et al., 2003). Therefore, a possible application for DFM might be to reduce shedding of this pathogen. In addition, bacterial DFM have been shown to increase daily gain and feed efficiency by feedlot cattle. In several experiments, supplementing feedlot steers with lactate-utilizing and/or lactate-producing bacteria has been shown to improve feed efficiency and daily gain (approximately 2.5%) with little change in DMI (Krehbiel et al., 2003). Few attempts have been made to determine the mechanisms responsible for the beneficial effects of DFM, but the potential for a decrease in subacute acidosis has been suggested. Responses to bacterial DFM have included a decrease in area below subacute ruminal pH, increases in ruminal propionate concentrations, increased protozoal numbers, and changes in viable bacterial counts. Effects on some blood variables (lower CO2 and LDH) also suggest a reduced risk of metabolic acidosis. Overall, data indicate that DFM have the potential to improve production efficiency by feedlot cattle, alter ruminal fermentation processes and products, and decrease fecal shedding of harmful pathogens in inoculated animals. More research is needed to describe the mode of action, and thereby improve the efficiency of DFM use.

The objective of this experiment was to evaluate the effects of *Lactobacillus acidophilus* (BG2FO4; lactic acid producer) and *Propionibacterium freudenreichii* P-63 (lactic acid utilizer) with or without Levucell SB (*Saccharomyces cerevisiae*; yeast) on feedlot performance, carcass merit, and *Escherichia coli* O157:H7 shedding by finishing beef steers fed a high-grain diet.

Materials and Methods

Cattle. One hundred eighty steers (avg initial BW = 814 ± 57 lbs) were delivered to the Willard Sparks Beef Research Center, Stillwater, OK on July 17, 2003. On arrival, steers were individually weighed and a uniquely numbered ear tag was placed in the left ear of each steer. On July 22, steers were individually weighed, horn tipped as needed, vaccinated with IBR-PI3-BVD-BRSV (Titanium 5, Intervet, Millsboro, DE), treated for control of external and internal parasites (Ivomec-Plus injectable, Merial, Duluth, GA), and implanted with Revalor-S (Hoechst Roussel Vet, Clinton, NJ). In addition, an individual fecal sample was collected from each steer to test for shedding of *E. coli* strain O157. Because fecal samples were difficult to obtain from all steers, 143 fecal samples were analyzed.

Treatment and Pen Assignments. Fifty-three steers were found to be shedding *E coli* O157. Of the original 180 steers, 144 steers were sorted by body weight (BW) and into *E. coli* shedding and non-shedding groups. Steers were then blocked by BW into six weight blocks. An equal number of steers shedding and not shedding *E. coli* were assigned to each weight block. Steers were then assigned randomly to four pens/weight block, with two or three *E. coli* shedding steers/pen. The number of observations for each treatment was six.

Treatments included: 1) control (no DFM; Gray); 2) *Lactobacillus acidophilus* (BG2FO4) fed from d 1 through finish (Blue); 3) *Lactobacillus acidophilus* (BG2FO4) fed from d 1 through 27 and *Propionibacterium freudenreichii* P-63 fed from d 28 through finish (Red); or 4) *Lactobacillus acidophilus* (BG2FO4) fed from d 1 through 27, *Propionibacterium freudenreichii* P-63 fed from d 28 through finish, and Levucell SB (*Saccharomyces cerevisiae*) fed from d 105 through finish (Green). Steers were fed for a total of 140 d. The finishing diet was formulated to meet or exceed NRC (1996) nutrient requirements (Table 1). Monensin (30 g/ton of diet) and tylosin (10 g/ton of diet) were fed. Steers were gradually adapted to their final treatment diet by offering 65, 75, and 85% concentrate diets for 5, 5, and 6 days each, respectively. Steers were fed twice daily at 0730 and 1330. Each DFM treatment was color coded and stored in a freezer in individual packets marked with colored dots that correspond to the specific treatment. Each day, the contents of one packet/treatment was reconstituted with 3,600 mL of tap water in an individual container that was labeled with color markings that corresponded with each color-coded treatment. The 3,600 mL of water was equally divided among six containers (600 mL/container) corresponding to the six pens/treatment. Contents of the appropriate container were poured directly onto the feed during the morning feeding after feed was delivered to the bunk in pens of cattle assigned to that treatment. Pens of steers on the control treatment received an equal volume of water with no DFM.

Feed refused was weighed at 28-d intervals and as needed (e.g., following inclement weather). In addition, diet samples were collected, and DM content of diets and dietary ingredients were determined weekly. Diet and ingredient samples were composited by 28-d periods, allowed to air dry, and ground in a Wiley mill to pass a 1-mm screen. Diet samples were analyzed for N,

ash (AOAC, 1996), NDF, and ADF (Goering and Van Soest, 1970). Interim unshrunk BW were taken after 27, 55 and 83 days on feed. Fecal grab samples were collected from three steers/pen on d 27 and 104 and from all steers on d 134 to assess shedding of *E. coli* O157:H7. Steers shedding *E. coli* O157 on arrival were sampled, and in pens with only two shedding steers, the third steer was randomly selected on d 27 and 104.

Microbiological Analyses. Fecal samples for determining prevalence of *E. coli* shedding were immediately placed on ice and transported overnight to Agtech Products, Inc., Waukesha, WI.

Statistical Analyses. Data for BW, dry matter intake, average daily gain feed efficiency, hot carcass weight (HCW), carcass-adjusted variables (calculated using carcass-adjusted final weight, which was calculated as HCW/average dressing percent), and normally distributed carcass characteristics were analyzed as a randomized complete block design using the Proc Mixed procedure of SAS Release 8.02 (SAS Institute Inc., Cary, NC). Non-parametric USDA quality grade data were transformed using Friedman's test by listing the percentage of Choice and Select for each pen within a block and then analyzed as normally distributed data as above (Elam et al., 2003). Pen was the experimental unit. The model statement will include treatment, and the random statement included block. When the overall F-value for treatment was significant ($P \leq 0.10$), Least squares means were separated using the least significant difference. In addition, two preplanned contrasts were used to test treatment effects of: 1) Control vs the average of the DFM treatments; and 2) *Lactobacillus acidophilus* (Blue) vs *L. acidophilus* followed by *Propionibacterium freudenreichii* P-63 (Red and Green).

The *E. coli* shedding data were non-normally distributed and were analyzed as a randomized complete block design with the Genmod procedure of SAS. Block and treatment were included in the model statement, and a binomial distribution specified. The same contrasts noted previously were used to test treatment effects.

Results

Performance. Effects of *L. acidophilus* (BG2FO4) and *P. freudenreichii* P-63 on feedlot cattle performance are shown in Table 2. Body weight did not differ among treatments on d 27, 55, or 83. However, steers fed *L. acidophilus* had greater ($P = 0.08$) BW on d 104 compared with steers fed *P. freudenreichii* (avg = 1222 lb). In addition, BW on d 140 was greater ($P<0.10$) for steers fed *L. acidophilus* than for control steers; steers fed *P. freudenreichii* with or without Levucell SB were intermediate. Carcass adjusted final BW did not differ among treatments.

Daily gain from d 1 to 83 did not differ among treatments. From d 84 to 104, ADG was greater ($P=0.04$) for steers fed *L. acidophilus* compared with steers fed *P. freudenreichii*. Interestingly, steers fed *P. freudenreichii* with Levucell SB from d 105 to 140 had greater ($P<0.10$) ADG than control steers or steers fed *P. freudenreichii* without Levucell SB. Overall (d 1 to 140) and carcass adjusted ADG did not differ among treatments.

Dry matter intake tended ($P=0.14$) to be greater for steers fed *L. acidophilus* from d 0 to 27 compared with steers fed *P. freudenreichii*. Dry matter intake did not differ among treatments from d 28 to 83. However, steers fed *L. acidophilus* consumed more ($P<0.10$) feed from d 105 to 140 than control steers or steers fed *P. freudenreichii*. Overall, steers fed *L. acidophilus* had

greater (P<0.10) dry matter intake than control steers, and tended (P=0.11) to have greater dry matter intake than steers fed *P. freudenreichii* with or without Levucell SB. Feed:gain did not differ among treatments from d 1 to 55. From d 84 to 104, feed:gain was lower (more desirable; P=0.07) for steers fed *L. acidophilus* than for steers fed *P. freudenreichii*. Similar to ADG, feeding Levucell SB with *P. freudenreichii* from d 105 to 140 improved (P<0.10) feed efficiency compared with feeding *P. freudenreichii* without Levucell SB. Overall and carcass adjusted feed efficiency did not differ among treatments.

Carcass Characteristics. Effects of *L. acidophilus* (BG2FO4) and *P. freudenreichii* P-63 on carcass characteristics are shown in Table 3. Hot carcass weight, dressing %, longissimus muscle area, 12th-rib fat depth, % kidney, pelvic and heart fat, and USDA Yield Grade did not differ among treatments. Interestingly, steers fed *L. acidophilus* had 6.7% greater (P=0.09) marbling scores than steers fed *P. freudenreichii* with or without Levucell SB. In addition, the distribution of steers grading Choice was greater (P=0.09) for steers fed *L. acidophilus*.

Fecal Shedding of Escherichia coli O157. On d 28 and 104, the number of steers shedding *E. coli* O157 was generally low and did not differ among treatments (Table 4). At the end of finishing (d 134), 38% of all steers were shedding *E. coli* O157, but no differences were observed among treatments. On d 134, all steers tested were negative for the presence of the H7 antigen.

Table 1. Dry matter and nutrient composition of basal finishing diet

Item	% of diet DM
Dry rolled corn	58.5
Dry rolled wheat	19.5
Ground alfalfa	10.0
Cane Molasses	4.0
Yellow grease	2.0
Supplement	6.0
Nutrients	
Dry matter, % as fed	88.8
Crude protein, % of DM	12.6
NDF, % of DM	17.1
ADF, % of DM	7.0
Calcium, % of DM[a]	.66

Phosphorus, % of DM[a]	.40
Potassium, % of DM[a]	.74
Magnesium, % of DM[a]	.18
Sulfur, % of DM[a]	.18
Manganese, ppm[a]	43.3
Cobalt, ppm[a]	.14
Iron, ppm[a]	131.3
Copper, ppm[a]	10.0
Selenium, ppm[a]	.18
Zinc, ppm[a]	90.1

[a]Values are estimated (NRC, 1996).

Table 2. Effects of *Lactobacillus acidophilus* (BG2FO4) and *Propionibacterium freudenreichii* P-63 on feedlot cattle performance

Item	Gray	Direct-Fed Microbials (DFM)[a]			SEM[b]	Contrasts	
		Blue	Red	Green		Control vs DFM	Blue vs Red and Green
BW, lb							
Initial	809	818	816	811	19.5	.24	.43
d 27	926	935	930	927	18.7	.49	.52
d 55	1037	1052	1047	1042	18.5	.27	.44
d 83	1146	1164	1164	1153	18.7	.14	.52
d 104	1221[cd]	1243[c]	1230[cd]	1213[d]	20.8	.47	.08
d 140	1321[c]	1355[d]	1322[cd]	1333[cd]	21.3	.31	.12
Carcass adj final BW	1321	1339	1326	1335	21.0	.54	.70
Daily gain, lb							

d 1 – 27	4.32	4.34	4.26	4.30	.26	.94	.85
d 28 – 55	3.96	4.19	4.10	4.12	.33	.63	.84
d 56 – 83	3.91	4.00	4.18	3.94	.12	.25	.63
d 84 – 104	3.54[c]	3.75[c]	3.14[cd]	2.88[d]	.29	.38	.04
d 105 – 140	2.86[c]	3.19[cd]	2.67[c]	3.43[d]	.24	.36	.61
d 1 – 140	3.68	3.86	3.64	3.76	.10	.50	.16
Carcass adj ADG	3.68	3.75	3.64	3.77	.12	.77	.75
DM intake, lb/d							
d 1 – 27	22.9[cd]	23.9[c]	23.2[cd]	22.6[d]	.58	.59	.14
d 28 – 55	24.1	24.6	24.7	24.4	.47	.29	.95
d 56 – 83	25.3	26.5	25.8	25.8	.55	.22	.28
d 84 – 104	24.7	25.4	24.5	24.2	.60	.99	.13
d 105 – 140	24.5[c]	26.0[d]	24.6[c]	25.3[cd]	.45	.10	.05
d 1 – 140	24.3[c]	25.3[d]	24.6[cd]	24.5[cd]	.41	.24	.11
Feed:Gain, lb/lb							
d 1 – 27	5.36	5.57	5.53	5.31	.31	.73	.67
d 28 – 55	6.10	6.14	6.37	6.00	.54	.91	.94
d 56 – 83	6.49[cd]	6.64[c]	6.21[d]	6.59[cd]	.19	.94	.26
d 84 – 104	7.12[cd]	7.00[c]	8.15[cd]	8.46[d]	.61	.26	.07
d 105 – 140	8.62[cd]	8.34[cd]	9.50[c]	7.68[d]	.70	.88	.75
d 1 – 140	6.61	6.57	6.76	6.56	.18	.91	.65
Carcass adj F:G	6.61	6.77	6.76	6.54	.21	.67	.57

[a]Gray = control; Blue = *Lactobacillus acidophilus* (BG2FO4) fed from d 0 through finish; Red = *Lactobacillus acidophilus* (BG2FO4) fed from d 0 through 28 and *Propionibacterium freudenreichii* P-63 fed from d 29 through finish; and Green = *Lactobacillus acidophilus* (BG2FO4) fed from d 0 through 28, *Propionibacterium freudenreichii* P-63 fed from d 29 through finish, and Levucell SB (*Saccharomyces cerevisiae*) fed for the final 28 d on feed.
[b]Standard error of the Least squares means; n = 6.
[c,d]Within a row, means that do not have a common superscript letter differ (P<0.10).

Table 3. Effects of *Lactobacillus acidophilus* (BG2FO4) and *Propionibacterium freudenreichii* P-63 on carcass characteristics

Item	Gray	Direct-Fed Microbials (DFM)[a]			SEM[b]	Contrasts	
		Blue	Red	Green		Control vs DFM	Blue vs Red and Green
HCW, lb	836	848	840	845	13.3	.53	.69
Dressing, %	63.3	62.6	63.3	63.4	.62	.74	.31
Longissimus area, in^2	13.2	13.3	13.0	13.1	.20	.85	.22
12th-rib fat, in	.60	.62	.63	.61	.03	.47	.87
Marbling score	382[cd]	399[c]	377[cd]	371[d]	12.2	.96	.09
KPH, %	2.72	2.76	2.90	2.95	.15	.39	.35
Yield grade	3.01	2.98	3.11	3.17	.15	.65	.35
Choice, %	36.1	44.4	36.1	19.4	-	.47	.09
Select, %	63.9	55.6	64.9	80.6	-	.47	.09

[a]Gray = control; Blue = *Lactobacillus acidophilus* (BG2FO4) fed from d 0 through finish; Red = *Lactobacillus acidophilus* (BG2FO4) fed from d 0 through 28 and *Propionibacterium freudenreichii* P-63 fed from d 29 through finish; and Green = *Lactobacillus acidophilus* (BG2FO4) fed from d 0 through 28, *Propionibacterium freudenreichii* P-63 fed from d 29 through finish, and Levucell SB (*Saccharomyces cerevisiae*) fed for the final 28 d on feed.
[b]Standard error of the Least squares means; n = 6.
[c,d]Within a row, means that do not have a common superscript letter differ (P<0.10).

Table 4. Effects of *Lactobacillus acidophilus* (BG2FO4) and *Propionibacterium freudenreichii* P-63 on fecal shedding of *Escherichia coli* O157

Item	Gray	Direct-Fed Microbials (DFM)[a]			Contrasts	
		Blue	Red	Green	Control vs DFM	Blue vs Red and Green
No. of pens 0157 +						
d 27	2	3	4	2	.44	.94
d 104	4	2	4	2	.31	.46
d 134	6	5	6	6	1.00	1.00

No. of steers 0157 +						
d 27	2	3	5	3	.38	.64
d 104	5	3	6	3	.48	.50
d 134	13	13	16	13	.45	.59

[a]Gray = control; Blue = *Lactobacillus acidophilus* (BG2FO4) fed from d 0 through finish; Red = *Lactobacillus acidophilus* (BG2FO4) fed from d 0 through 28 and *Propionibacterium freudenreichii* P-63 fed from d 29 through finish; and Green = Lact*obacillus acidophilus* (BG2FO4) fed from d 0 through 28, *Propionibacterium freudenreichii* P-63 fed from d 29 through finish, and Levucell SB (*Saccharomyces cerevisiae*) fed for the final 28 d on feed.

Implications

Similar to data summarized by Krehbiel et al. (2003), these results suggest that cattle fed *L. acidophilus* have an approximately 2.5% advantage in average daily gain over control cattle, resulting in increased hot carcass weight.

Literature Cited

Association of Analytical Chemists. 1996. 16th ed. Assoc. of Offic. Anal. Chem., Arlington, VA.

Elam, N.A. et al. 2003. J. Anim. Sci. 81:2686.

Goering, H.K., and P.J. Van Soest. 1970. Agric. Handbook No. 379, ARS, USDA.

Krehbiel, C.R., et al. 2003. J. Anim. Sci. 81:(E. Suppl. 2):E120-E132.

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