

# Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action<sup>1,2</sup>

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**ABSTRACT:** Direct-fed microbials (DFM) have been shown to increase daily gain and feed efficiency in feedlot cattle, enhance milk production in dairy cows, and improve health and performance of young calves. However, their effects on performance have been mixed, and the mode of action remains unclear. Bacteria used as DFM have been defined as single or mixed cultures of live organisms, which, when fed to animals, beneficially affect the host. The original concept of feeding DFM to man and livestock was based primarily on the potential for beneficial intestinal effects, including the establishment of a desirable gut microflora and/or prevention of the establishment of pathogenic organisms. More recently, however, there has been some indication that certain bacterial DFM might have beneficial effects in the rumen, such as decreasing the potential for ruminal acidosis. In several experiments, supplementing feedlot cattle 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. In addition, increased milk yield (0.75 to 2.0 kg/

d) has been reported in studies using dairy cows fed DFM, with little change in milk composition. 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 evaluated. Responses to bacterial DFM have included a decrease in the 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 CO<sub>2</sub> and LDH) also suggest a reduced risk of metabolic acidosis. Recent research has shown that DFM decreased fecal shedding of *Escherichia coli* O157:H7 from infected calves. Therefore, a possible application for DFM might be to reduce shedding of this pathogen from cattle. Overall, data indicate that DFM have the potential to decrease ruminal acidosis in feedlot cattle and dairy cows, and improve immune response in stressed calves. More research is needed to describe the mode of action, and thereby improve the efficiency of DFM use.

Key Words: Feed Additives, Probiotics, Ruminants, Rumen Fermentation

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J. Anim. Sci. 81(E. Suppl. 2):E120–E132

## Introduction

The term “probiotic” has been defined as “a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance” (Fuller, 1989) and has been used to describe viable microbial cultures, culture extracts, enzyme preparations,

or various combinations of the above (Yoon and Stern, 1995). Therefore, the U.S. FDA has required feed manufacturers to use the term “direct-fed microbial” (DFM) instead of probiotic (Miles and Bootwalla, 1989) and has narrowed the definition to “a source of live, naturally occurring microorganisms” (Yoon and Stern, 1995). Microorganisms used as DFM for ruminants include viable cultures of fungi and bacteria.

Concern regarding the use of antibiotics and other growth stimulants in the animal feed industry has increased in recent years. There has been increasing emphasis placed on disease prevention as a means of reducing the use of antibiotics and also public concern about pathogens in meat and meat products. As result, interest in the effects of DFM on animal health and performance has increased. For ruminants, microbial cultures have been used to potentially replace or reduce the use of antibiotics in neonatal and stressed calves, to enhance milk production in dairy cows, and to im-

<sup>1</sup>Approved for publication by the Director of the Oklahoma Agric. Exp. Stn. The authors wish to express their gratitude to the Oklahoma Agric. Exp. Stn. for financial support of this paper.

<sup>2</sup>The authors express their gratitude to C. S. Abney, S.-W. Kim, C. A. McPeake, E. M. Ungerfeld, and M. T. Yokoyama for their important contributions to this manuscript.

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Received August 6, 2002.

Accepted January 2, 2003.

prove feed efficiency and daily gain in beef cattle. Most recently, cultures of *Lactobacillus acidophilus* have been shown to reduce fecal shedding of *Escherichia coli* O157:H7 by feedlot cattle. Although responses to DFM have been positive in many experiments, basic mechanisms are not well defined and are not clearly understood. Enhancing our understanding of the mode of action of DFM would improve our ability to select and apply appropriate DFM to ruminant diets. This review summarizes the literature pertaining to bacterial DFM and their influence on health and performance of ruminant animals. Moreover, information on underlying mechanisms is discussed.

## History

Historical information pertaining to the use of bacterial DFM has been reviewed (Stern and Storrs, 1975; Newman and Jacques, 1995; Yoon and Stern, 1995). In his book, *The Prolongation of Life*, Metchnikoff (1908) first proposed that consuming lactobacilli capable of living in the intestinal tract was desirable (Yoon and Stern, 1995). He suggested that longevity of the Bulgarians was partly due to their consumption of a fermented milk product and that lactobacilli present in the fermented product prevented disease caused by enteropathogens. Metchnikoff's (1908) postulation led to several studies on the efficacy of the *Lactobacillus* species during the 1920's (Stern and Storrs, 1975). Stern and Storrs (1975) reported that the early popularity of *L. acidophilus* therapy in the United States reached its peak by about the mid-1930s, and then faded. Following World War II, antibiotics came into use and were often so efficient that they destroyed all the intestinal bacteria (Mannheim, 1951). The net effect was an increase in the incidence of "antibiotic diarrhea" and related side effects, and interest in acidophilus therapy for restoration of normal intestinal microorganisms began to be renewed. Since then (mid-1950s), there has been a slow but steady increase in the study of bacterial DFM for humans and animals. However, production responses of growing and lactating ruminants and interest in the corresponding mode of action of bacterial DFM have not occurred until more recently (Yoon and Stern, 1995).

### *Bacterial Direct-Fed Microbials in Dairy Production*

*Preruminant Calves.* In terms of ruminant production systems, the efficacy of bacterial DFM has been studied most extensively in the neonatal dairy calf. Bacterial DFM, such as species of *Lactobacillus*, *Enterococcus*, *Streptococcus*, and *Bifidobacterium*, have been studied in young calves, and the data have been reviewed (Newman and Jacques, 1995). In general, the importance of bacterial DFM (primarily *Lactobacillus* species) fed to young and/or stressed calves has been to establish and maintain "normal" intestinal microorganisms rather than as a production (i.e., gain and efficiency) stimulant. For dairy calves, rapid adaptation to solid feed by

accelerating the establishment of ruminal and intestinal microorganisms and avoiding the establishment of enteropathogens, which often results in diarrhea, is the primary goal. In the neonate and in stressed calves, the microbial population is in transition and extremely sensitive; abrupt changes in diet or the environment can cause alterations in microbial populations in the gastrointestinal tract (GIT; Savage, 1977). For example, Tannock (1983) reported that stress often leads to an increased incidence of diarrhea in neonates, which is associated with decreases in the population of *Lactobacillus* in the gut. Moreover, Sandine (1979) reported that fecal counts of lactobacilli normally are higher than coliforms in healthy animals and reversed in those suffering from diarrhea.

Feeding calves viable cultures of species of *Lactobacillus* and *Streptococcus* has been reported to decrease the incidence of diarrhea (Bechman et al., 1977; Maeng et al., 1987; Fox, 1988). In a more recent experiment by Abu-Tarboush et al. (1996), calves fed *L. acidophilus* 27SC had a significantly lower scour index during wk 5, 7, and 8 compared with calves fed the control diet, which confirmed the beneficial effect of lactobacilli in reducing the incidence of diarrhea in dairy calves suggested by earlier research. The decreased incidence of diarrhea might be associated with a consistently increased shedding of *Lactobacillus* (Gilliland et al., 1980; Jenny et al., 1991; Abu-Tarboush et al., 1996) and an inconsistent decreased shedding of coliforms (Bruce et al., 1979) in feces in response to supplements of *Lactobacillus*. Previous researchers (Ellinger et al., 1980; Gilliland et al., 1980; Abu-Tarboush et al., 1996) have suggested that animals experiencing normal stools are less likely to be shedding coliforms in feces. Fecal shedding of coliforms has generally not increased when calves were not experiencing diarrhea (Ellinger et al., 1980; Gilliland et al., 1980; Abu-Tarboush et al., 1996), and authors have suggested that this could be related to the fact that animals were not experiencing intestinal disorders (e.g., diarrhea). Interestingly, in experiments where there has been no advantage to feeding bacterial DFM (Morrill et al., 1977; Jenny et al., 1991), calves were generally experiencing no health problems.

Rapid adaptation to solid feed by neonatal calves also depends on the development of the ruminal epithelium and ruminal capacity. In one experiment (Nakanishi et al., 1993), lactic acid bacteria added to starter diets were suggested to affect ruminal function in the young animal. Holstein calves supplemented with yogurt containing *L. acidophilus* tended to ruminate more at 30 d than untreated calves, indicating that *L. acidophilus* may promote ruminal development. There were no performance benefits associated with the treated calves in this experiment and any possible microbial changes were not determined (Nakanishi et al., 1993).

Performance results for neonatal calves consuming bacterial DFM have been variable. Morrill et al. (1977), Ellinger et al. (1978), and Abu-Tarboush (1996) reported no improvement in daily gain as a result of feed-

ing lactobacilli. In contrast, Bechman et al. (1977) reported improved (17%) rates of gain when  $2.5 \times 10^{11}$  cfu/d of *L. acidophilus* species was added to milk or milk replacer. Feed efficiency is generally not altered by feeding DFM to young calves (Jenny et al., 1991; Abu-Tarboush et al., 1996). Beeman (1985) used 52 Holstein male calves that had a history of diarrhea and antibiotic therapy to evaluate the effects of feeding a culture of *Lactobacillus* on weight gain of calves convalescing from neonatal diarrhea. All animals were treated with antibiotics for 3 d before the study was initiated. At the 2-wk evaluation, calves treated with lactobacilli gained an average of 8.0 kg, whereas control calves gained an average of 3.5 kg. By d 56 of the experiment, average BW gains were 47.3 and 37.8 kg for treated and control groups, respectively. These benefits were hypothesized to result from improvement of intestinal conditions because of lower fecal scores (i.e., less scouring) in calves fed DFM.

Performance response is likely not important early in the preruminant's life when enteric disease is most prevalent. Improved health and reduction in the incidence or severity of diarrhea, though difficult to measure for statistical analysis, is most likely a more important response. As suggested by Newman and Jacques (1995), more experiments that include detailed information about the microbial supplement, and fecal culture data from scouring experimental animals are needed to determine the usefulness of microbial supplements in neonatal calves.

**Milk Yield and Composition in Dairy Cows.** Limited research has evaluated the efficacy of bacterial DFM for lactating dairy cows. Table 1 summarizes five experiments in which bacterial DFM, or combinations of bacterial and fungal DFM, have been fed to lactating cows. It should be cautioned that all experiments were published as abstracts, and therefore information was limited and not peer reviewed. In general, increased milk yield has been a consistent response, whereas changes in milk composition have been variable. Jaquette et al. (1988) and Ware et al. (1988a) reported that milk yield was 1.8 kg/d greater for cows fed a diet containing  $2.0 \times 10^9$  cfu of *L. acidophilus* (BT1386) per day compared with those fed a control diet. Dry matter intake and milk fat and milk protein percentage were not affected by *L. acidophilus* (Table 1). In a more recent experiment, Gomez-Basauri et al. (2001) evaluated the effect of a supplement containing *L. acidophilus*, *L. casei*, *Enterococcus (Streptococcus) faecium* (total lactic bacteria =  $10^9$  cfu/g) and mannanoligosaccharide on DMI, milk yield, and milk component concentration. Cows fed lactic acid bacteria and mannanoligosaccharide consumed 0.42 kg less DM and produced 0.73 kg/d more milk. The authors reported that milk yields increased over time for DFM- and mannanoligosaccharide-fed cows, whereas control cows maintained constant milk yields.

Other experiments have been conducted with combinations of fungal cultures and lactic-acid bacteria (Ko-

**Table 1.** Effects of bacterial direct-fed microbials on dry matter intake, milk yield, and composition in lactating dairy cows

Treatment	n	DMI, kg/d	Yield, kg/d	Milk		Diet	Reference
				Fat, %	Protein, %		
Control	16	—	29.1 <sup>a</sup>	3.81	3.34	Corn silage, alfalfa, pelleted grain	Jaquette et al. (1988)
<i>L. acidophilus</i> (BT1386)	16	—	30.9 <sup>b</sup>	3.75	3.36		
Control	550	21.2	31.8 <sup>a</sup>	3.64	—	Alfalfa hay, silage, whole cottonseed, grain concentrate, protein	Ware et al. (1988)
<i>L. acidophilus</i> (BT1386), $2 \times 10^9$ cfu/d	550	21.4	33.6 <sup>b</sup>	3.63	—		
Control	6	—	8.20 <sup>a</sup>	3.30 <sup>a</sup>	3.09	Tropical feeding conditions	Komari et al. (1999)
<i>S. cerevisiae</i> (yeast culture)	6	—	9.34 <sup>b</sup>	3.96 <sup>b</sup>	3.15		
<i>S. cerevisiae</i> and <i>L. acidophilus</i>	6	—	9.28 <sup>b</sup>	3.57 <sup>b</sup>	3.13		
Control	32	24.6	48.2 <sup>c</sup>	—	3.01 <sup>a</sup>	—	Block et al. (2000)
$5 \times 10^9$ cfu of yeast plus $5 \times 10^9$ cfu of <i>L. plantarum</i> / <i>E. faecium</i>	32	25.1	49.1 <sup>d</sup>	—	3.27 <sup>b</sup>		
Control	100	25.0 <sup>a</sup>	38.8 <sup>c</sup>	4.24 <sup>c</sup>	3.02	Corn silage, alfalfa/grass hay, crop silage, whey, commercial feed blend	Gomez-Basauri et al. (2001)
<i>L. acidophilus</i> , <i>L. casei</i> , <i>E. faecium</i> ( $10^9$ cfu/g) and mannanoligosaccharide	100	24.6 <sup>b</sup>	39.6 <sup>d</sup>	4.34 <sup>d</sup>	3.04		

<sup>ab</sup>Means in a column with different superscripts differ ( $P < 0.05$ ).

<sup>cd</sup>Means in a column with different superscripts differ ( $P < 0.10$ ).

mari et al., 1999; Block et al., 2000). Milk yields were increased by 1.08 and 0.90 kg/d, respectively, when lactating cows were fed *S. cerevisiae* in combination with *L. acidophilus* or  $5 \times 10^9$  cfu of yeast in combination with  $5 \times 10^9$  cfu of *L. plantarum*/*E. faecium*.

In contrast to feeding bacterial DFM directly, Colenbrander et al. (1988) found that treatment of alfalfa silage with *L. acidophilus* did not improve DMI, milk yield, or milk composition in dairy cows, but efficiency (kg of fat-corrected milk/kg of feed) of milk production was improved by 7.1%. The *L. acidophilus* may not have survived in the silage; thus few, if any, viable cells would have been consumed.

These studies suggest that bacterial DFM fed alone or in combination with fungal cultures might be efficacious for increasing milk production by lactating dairy cows. However, studies have been minimal, and more research is needed before recommendations to dairy producers should be made.

### Bacterial Direct-Fed Microbials in Beef Production

*Stressed Calves.* Newly received beef calves entering the feedlot undergo a variety of stresses, such as recent weaning, transport, fasting, assembly, vaccination, castration, and dehorning. Such stresses can alter microorganisms in the rumen and lower gut (Williams and Mahoney, 1984), resulting in decreased performance and increased morbidity and death loss. Administration of bacterial DFM to repopulate the gut might reduce these changes in the microbial population. In the early to mid-1980s, several research trials (Crawford et al., 1980; Hutcheson et al., 1980; Kiesling and Lofgreen, 1981; Davis, 1982; Kiesling et al., 1982; Hicks et al., 1986) were conducted at different locations to evaluate the efficacy of a combination bacterial DFM containing live cultures of *L. acidophilus*, *L. plantarum*, *L. casei*, and *S. faecium*. Averaged across all trials, feeding the DFM at processing, throughout the receiving period (average = 30 d), or both resulted in a 13.2% increase in daily gain, 2.5% increase in feed consumption, and a 6.3% improvement in feed:gain (Fox, 1988). The greatest performance response to the bacterial DFM generally occurred within the first 14 d of the receiving period (Crawford et al., 1980; Hutcheson et al., 1980). Morbidity was reduced by 27.7% in cattle receiving the bacterial DFM compared with control cattle. However, it should be pointed out that morbidity was generally low. Similarly, Gill et al. (1987) fed a bacterial DFM during a 28-d receiving period and reported a 9.3% increase in daily gain, 9.5% improvement in feed efficiency, and a 10.9% reduction in morbidity. In contrast, other research has shown no performance response to feeding bacterial DFM to newly weaned (Dew and Thomas, 1981; Kercher et al., 1985; 1986) or newly received (Kiesling and Lofgreen, 1981; Krehbiel et al., 2001) feedlot calves.

In a more recent experiment (Krehbiel et al., 2001), 466 newly received calves from southern Oklahoma and northern Texas auction barns were received and used to study the effects of administering  $5 \times 10^9$  cfu lactic acid-producing bacteria (*E. faecium*, *L. acidophilus*, *Bifidobacterium thermophilum*, and *B. longum*) on health and performance. Daily gain did not differ among calves receiving DFM vs. no DFM. However, calves treated with DFM during their first antimicrobial treatment were less likely to be treated a second time within 96 h. In addition, the number of calves treated twice tended to be lower for calves administered DFM compared with calves not receiving DFM. These data suggested that DFM might improve recovery of morbid newly received feedlot calves.

Dose titration studies for bacterial DFM fed to newly received calves are limited and more are needed. Orr et al. (1988) showed a quadratic relationship for daily gain when lightweight (185 kg) steer calves were fed 0,  $2.2 \times 10^6$ ,  $2.2 \times 10^8$ , or  $2.2 \times 10^{10}$  cfu of *L. acidophilus* daily. Daily gain was significantly greater for calves fed  $2.2 \times 10^6$  or  $2.2 \times 10^8$  cfu of *L. acidophilus* than when control or  $2.2 \times 10^{10}$  cfu was fed. Feed intake and feed efficiency did not differ among treatments (Orr et al., 1988). In contrast, Lee and Botts (1988) showed a similar improvement in performance over control animals when  $2.2 \times 10^8$ ,  $2.2 \times 10^9$ , or  $2.2 \times 10^{10}$  cfu of *S. faecium* was fed.

Although studies are limited, these results suggest that the addition of bacterial DFM to the diet can improve health and performance of stressed stocker calves. Similar to the neonatal calf, response to bacterial DFM might be greater when newly weaned and/or received beef calves are more prone to health problems. However, Gill et al. (1987) suggested that extremely healthy calves and extremely sick calves might be less likely to respond to DFM treatment.

*Feedlot Cattle.* Supplementing diets on a daily basis with lactate-producing and/or lactate-utilizing bacteria has recently been shown to improve feed efficiency and daily gain of feedlot cattle (Swinney-Floyd et al., 1999; Galyean et al., 2000; Rust et al., 2000a,b). Ware et al. (1988b) was one of the first to report that *L. acidophilus* BT1386 increased daily gain and improved feed efficiency in yearling steers fed a high-concentrate diet compared with controls. However, *L. acidophilus* did not affect DMI, USDA yield grade, USDA quality grade, dressing percentage, marbling score, or incidence of liver abscesses. More recent experiments have evaluated the efficacy of *Propionibacteria* species fed alone or in combination with *Lactobacillus* species (Swinney-Floyd et al., 1999; Galyean et al., 2000; Rust et al., 2000a,b). Swinney-Floyd et al. (1999) showed improvements in feed efficiency when feedlot steers were supplemented with a combination of *L. acidophilus* 53545 and *P. freudenreichii* P-63. During the first 10 d of high-concentrate feeding, daily gains were 0.93, 1.11, and 1.63 kg/d, and feed efficiencies were 5.17, 5.32, and 4.50 kg daily DMI/kg ADG for control, *P. freudenreichii*

**Table 2.** Distribution of treatment groups by location

State		Treatments <sup>a</sup>						
		CON	TRT2	TRT3	TRT4	TRT5	TRT6	TRT7
CO	No. of pens	8	8	—	—	—	—	—
	No. of animals/pen	9	9	—	—	—	—	—
IA	No. of pens	8	—	8	8	—	—	—
	No. of animals/pen	6	—	6	6	—	—	—
MI 2000	No. of pens	10	10	10	10	—	—	—
	No. of animals/pen	7	7	7	7	—	—	—
MI 1999	No. of pens	10	—	10	—	10	10	—
	No. of animals/pen	7	—	7	—	7	7	—
MI 1998	No. of pens	10	10	10	—	—	—	10
	No. of animals/pen	8	8	8	—	—	—	8
TX	No. of pens	12	12	12	12	—	—	—
	No. of animals/pen	5	5	5	5	—	—	—
Total	No. of pens	58	40	50	30	10	10	10
	No. of animals/pen	400	282	328	178	70	70	80

<sup>a</sup>Treatments are as follows: CON = control; TRT 2 =  $10^9$  *P. freudenreichii* (PF24) +  $10^6$  *L. acidophilus* (LA45) cfu·animal<sup>-1</sup>·d<sup>-1</sup>; TRT 3 =  $10^9$  PF24 +  $10^6$  LA45 +  $10^6$  LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup>; TRT 4 =  $10^9$  PF24 +  $10^4$  LA45 +  $10^4$  LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup>; TRT 5 =  $10^9$  PF24 +  $2 \times 10^6$  LA 51 cfu·animal<sup>-1</sup>·d<sup>-1</sup>; TRT 6 =  $10^8$  PF24 +  $10^6$  LA45 +  $10^6$  LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup>; and TRT 7 =  $10^9$  PF24 +  $10^8$  LA45 +  $10^8$  LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup>.

alone, and the combination of *P. freudenreichii* and *L. acidophilus*, respectively. Feed efficiencies for the 120-d experiment were 5.17, 5.32, and 4.97 kg daily DMI/kg ADG, and liver abscesses at harvest were 8, 8, and 0% for the respective treatments.

Data from six research trials (n = 1,249; 184 pens) conducted in four states (CO, IA, MI, and TX) were assembled to summarize the effects of varying concentrations and strains of *L. acidophilus* (LA45 and LA51) and *P. freudenreichii* (PF24) on feedlot performance and carcass characteristics of feedlot steers (McPeake et al., 2002). Treatments represented and their distribution across locations are shown in Table 2. Data were analyzed using the mixed model procedure for repeated measures (SAS Inst., Inc., Cary, NC). Because of unequal replication of treatments at each location, the year and location were compressed to allow for data analysis across experiments. Feedlot data were analyzed using initial weight as a covariate to account for location differences in starting weight. Least squares means were separated using the Tukey adjustment factor for selected treatments. Orthogonal contrasts included control (CON) vs. all DFM treatments; CON vs.  $10^9$  PF24,  $10^6$  LA45, and  $10^6$  LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup> (TRT3);  $10^9$  PF24 and  $10^6$  LA45 cfu·animal<sup>-1</sup>·d<sup>-1</sup> (TRT2) vs. TRT3; and the linear relationship between  $10^9$  PF24,  $10^4$  LA45, and  $10^4$  LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup> (TRT4), TRT3, and  $10^9$  PF24,  $10^8$  LA45, and  $10^8$  LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup> (TRT7), respectively.

From d 0 to 28, cattle fed TRT2 had greater ( $P < 0.05$ ) DMI than cattle fed TRT3, TRT4, or  $10^9$  PF24 and  $2 \times 10^6$  LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup> (TRT5; Table 3). Dry matter intakes were greater ( $P < 0.05$ ) for steers fed TRT5 or  $10^8$  PF24,  $10^6$  LA45 and  $10^6$  LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup> (TRT6) vs. CON, TRT2, TRT3, or TRT4 from d 57 through 84. Cattle fed treatments CON through TRT6

were more efficient than cattle consuming TRT7 from d 57 through 84. Dressing percentage ( $P = 0.62$ ), quality grade ( $P = 0.59$ ), and percentage USDA Choice ( $P = 0.73$ ) were not influenced by bacterial DFM (data not shown). However, hot carcass weight (HCW, kg) ( $P = 0.04$ ) and carcass ADG (kg/d) ( $P = 0.12$ ) were 346, 349, 350, 350, 349, 344, and 350, and 1.58, 1.61, 1.63, 1.62, 1.62, 1.62, 1.56, and 1.62 for CON through TRT7, respectively.

Contrasts performed to estimate differences between CON steers and steers receiving diets inoculated with DFM revealed greater ( $P = 0.007$ ) final live weight, overall ADG ( $P = 0.02$ ), overall DMI ( $P = 0.07$ ), HCW ( $P = 0.02$ ), and carcass ADG ( $P = 0.05$ ) for treated steers (Table 4). In addition, contrasts for steers receiving TRT3 compared with CON revealed greater ( $P = 0.007$ ) final live weight, overall ADG ( $P = 0.02$ ), HCW ( $P = 0.008$ ), and carcass ADG ( $P = 0.01$ ) for steers inoculated with the DFM. Calculated feed energy values (NE<sub>m</sub>,  $P = 0.12$ ; NE<sub>g</sub>,  $P = 0.07$ ; ME,  $P = 0.08$ ) tended to be greater for diets containing DFM. There were no effects ( $P > 0.10$ ) of feeding LA45 compared with LA45 and LA51. Interestingly, a positive linear effect ( $P = 0.05$ ) was observed for DMI with increasing *L. acidophilus*. However, this resulted in a trend ( $P = 0.12$ ) for a linear increase in DMI:ADG. Diet NE<sub>g</sub> ( $P = 0.14$ ) and ME ( $P = 0.16$ ) tended to increase with increasing *L. acidophilus*. Results of these analyses suggest that feeding combinations of lactic acid- and propionic acid-producing bacteria in diets of growing/finishing cattle might improve growth rate (2.6%) and carcass weight (6 kg) in feedlot steers.

Huck et al. (2000) studied the effects of phase feeding of bacterial DFM on growth performance and carcass characteristics of finishing heifers. *Lactobacillus acidophilus* BG2FO4 and *P. freudenreichii* P-63 were fed

**Table 3.** Least squares means and standard errors for the effects of bacterial direct-fed microbials on feedlot performance of crossbred feedlot steers

Item	Treatments <sup>a</sup>						
	CON	TRT 2	TRT 3	TRT 4	TRT 5	TRT 6	TRT 7
Final wt., kg	568 ± 2.99	574 ± 3.05	575 ± 3.01	573 ± 3.31	575 ± 5.19	572 ± 5.51	574 ± 4.61
DMI, kg/d							
d 0 to 28	7.99 ± 0.14 <sup>bc</sup>	8.41 ± 0.16 <sup>b</sup>	7.67 ± 0.15 <sup>c</sup>	7.63 ± 0.17 <sup>c</sup>	7.39 ± 0.27 <sup>c</sup>	7.74 ± 0.25 <sup>bc</sup>	8.01 ± 0.26 <sup>bc</sup>
d 29 to 56	9.09 ± 0.14	9.38 ± 0.16	9.05 ± 0.15	8.94 ± 0.17	8.61 ± 0.27	8.84 ± 0.25	9.15 ± 0.26
d 57 to 84	9.86 ± 0.14 <sup>c</sup>	9.83 ± 0.16 <sup>c</sup>	10.19 ± 0.15 <sup>c</sup>	9.82 ± 0.17 <sup>c</sup>	11.40 ± 0.27 <sup>b</sup>	11.43 ± 0.25 <sup>b</sup>	10.32 ± 0.26 <sup>bc</sup>
d 85 to harvest	9.66 ± 0.14	9.60 ± 0.16	10.08 ± 0.15	10.11 ± 0.17	9.54 ± 0.27	9.47 ± 0.25	10.63 ± 0.26
d 0 to harvest	9.22 ± 0.06	9.38 ± 0.08	9.32 ± 0.07	9.21 ± 0.10	9.30 ± 0.18	9.43 ± 0.18	9.59 ± 0.16
ADG, kg							
d 0 to 28	1.90 ± 0.04	2.03 ± 0.05	1.90 ± 0.05	1.81 ± 0.07	1.98 ± 0.07	1.79 ± 0.06	1.99 ± 0.09
d 29 to 56	1.79 ± 0.04	1.80 ± 0.05	1.87 ± 0.05	1.86 ± 0.07	1.81 ± 0.07	1.94 ± 0.06	1.84 ± 0.09
d 57 to 84	1.62 ± 0.04	1.54 ± 0.05	1.62 ± 0.05	1.71 ± 0.07	1.68 ± 0.07	1.66 ± 0.06	1.38 ± 0.09
d 85 to harvest	1.21 ± 0.04	1.30 ± 0.05	1.32 ± 0.05	1.32 ± 0.07	1.31 ± 0.07	1.25 ± 0.06	1.47 ± 0.09
d 0 to harvest	1.56 ± 0.02	1.59 ± 0.02	1.61 ± 0.06	1.58 ± 0.06	1.61 ± 0.06	1.60 ± 0.06	1.60 ± 0.06
Feed:gain							
d 0 to 28	4.32 ± 0.17	4.26 ± 0.19	4.11 ± 0.18	4.47 ± 0.24	3.75 ± 0.37	4.40 ± 0.38	3.83 ± 0.35
d 29 to 56	5.17 ± 0.17	5.26 ± 0.19	4.97 ± 0.18	5.04 ± 0.24	4.83 ± 0.37	4.56 ± 0.38	4.84 ± 0.35
d 57 to 84	6.45 ± 0.17 <sup>b</sup>	6.76 ± 0.19 <sup>b</sup>	6.48 ± 0.18 <sup>b</sup>	5.90 ± 0.24 <sup>b</sup>	7.07 ± 0.37 <sup>b</sup>	7.01 ± 0.38 <sup>b</sup>	8.29 ± 0.35 <sup>c</sup>
d 85 to harvest	8.18 ± 0.17	7.53 ± 0.19	7.98 ± 0.18	8.02 ± 0.24	7.75 ± 0.37	7.98 ± 0.38	7.37 ± 0.35
d 0 to harvest	6.02 ± 0.04	6.01 ± 0.05	5.91 ± 0.05	5.89 ± 0.07	5.87 ± 0.12	5.98 ± 0.12	6.09 ± 0.11
Feed NE <sub>m</sub> , Mcal/kg <sup>d</sup>	2.39 ± 0.02	2.40 ± 0.03	2.45 ± 0.03	2.44 ± 0.04	2.47 ± 0.06	2.39 ± 0.06	2.35 ± 0.06
Feed NE <sub>g</sub> , Mcal/kg <sup>d</sup>	1.52 ± 0.01	1.52 ± 0.01	1.54 ± 0.01	1.54 ± 0.01	1.55 ± 0.02	1.52 ± 0.02	1.50 ± 0.02
Feed ME, Mcal/kg <sup>d</sup>	3.38 ± 0.02	3.39 ± 0.02	3.42 ± 0.02	3.41 ± 0.03	3.44 ± 0.05	3.38 ± 0.05	3.34 ± 0.04

<sup>a</sup>Treatments are as follows: CON = control; TRT 2 =  $10^9$  *P. freudenreichii* (PF24) +  $10^6$  *L. acidophilus* (LA45) cfu·animal<sup>-1</sup>·d<sup>-1</sup>; TRT 3 =  $10^9$  PF24 +  $10^6$  LA45 +  $10^6$  LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup>; TRT 4 =  $10^9$  PF24 +  $10^4$  LA45 +  $10^4$  LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup>; TRT 5 =  $10^9$  PF24 +  $2 \times 10^6$  LA 51 cfu·animal<sup>-1</sup>·d<sup>-1</sup>; TRT 6 =  $10^8$  PF24 +  $10^6$  LA45 +  $10^6$  LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup>; and TRT 7 =  $10^9$  PF24 +  $10^8$  LA45 +  $10^8$  LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup>.

<sup>b,c</sup>Means in a row with different superscripts differ ( $P < 0.05$ ).

<sup>d</sup>Values for feed NE<sub>m</sub>, NE<sub>g</sub>, and ME (DM basis) were calculated from performance data.

alone or in sequence across a 126-d finishing experiment. Treatments included: 1) no bacterial DFM; 2)  $5 \times 10^8$  cfu·animal<sup>-1</sup>·d<sup>-1</sup> of *L. acidophilus* fed during the entire experiment; 3)  $1 \times 10^9$  cfu·animal<sup>-1</sup>·d<sup>-1</sup> of *P. freudenreichii* fed during the entire experiment; 4) *L. acidophilus* fed for 28 d, then *P. freudenreichii* for the remainder; or 5) *P. freudenreichii* fed for 28 d, then *L. acidophilus* for the remainder. Feeding either *L. acidophilus* BG2FO4 or *P. freudenreichii* P-63 throughout the entire experiment did not affect daily gain, DMI, or feed efficiency. Feeding *P. freudenreichii* for 28 d followed by *L. acidophilus* improved daily gain, but not feed efficiency, compared with controls. Heifers fed *L. acidophilus* for 28 d followed by *P. freudenreichii* had greater gain (5.0%) and improved feed efficiency (5.1%) compared with controls. These authors suggested that growth performance of finishing cattle could be improved by targeting the appropriate DFM to a particular phase of production.

In summary, these results suggest that feeding bacterial DFM to feedlot cattle results in a 2.5 to 5% increase in daily gain and an approximately 2% improvement in feed efficiency, whereas DMI is inconsistent. In studies reviewed, carcass weight was generally increased by 6 to 7 kg.

**Reduction of *E. coli* O157:H7 in Feedlot Cattle.** Feedlot cattle have been recognized as a host for *E. coli* O157:H7. This organism appears to be confined to the GIT and is shed in feces. Ohya et al. (2000) developed

and studied the effect of a DFM containing lactic-acid producing *Streptococcus bovis* LCB6 and *L. gallinarum* LCB 12 isolated from adult cattle on the elimination of *E. coli* O157:H7 from experimentally infected Holstein calves. An increase in VFA, especially acetate, correlated with the diminution of *E. coli* O157:H7. These authors suggested the possible application of bacterial DFM to reduce fecal shedding of *E. coli* O157:H7 from cattle. Similarly, results by Zhao et al. (1998) suggest that bacteria inhibitory to *E. coli* O157:H7 can be isolated from feces and intestinal tissue samples of cattle, grown in culture, and fed to reduce the carriage of *E. coli* O157:H7 in feedlot cattle. In a recent experiment (M. Brashears and M. Galyean, personal communication), supplementing feed with certain strains of *L. acidophilus* (NPC 747 and NPC 750) was shown to decrease the incidence of *E. coli* O157:H7 in the feces of finishing beef cattle. On d 14, 28, and 42 of the experiment, steers consuming both cultures (NPC 747 and NPC 750) had significant reductions in the incidence of cattle shedding *E. coli* compared with controls. At slaughter, strain NPC 747 was the most effective at decreasing the incidence of shedding of *E. coli* O157:H7. Based on these results, supplementing feed for cattle with certain DFM might decrease the incidence of *E. coli* O157:H7 in feedlot cattle. However, the incidence of *E. coli* shedding at slaughter was small (only 10% of control animals were positive) in the latter study, and more data are needed to validate the results.

**Table 4.** Contrast means and probability levels (parenthesis) for differences in overall feedlot performance and carcass characteristics by steers fed or not fed (CON) bacterial direct-fed microbials (DFM)

Item	Treatment contrasts <sup>a</sup>			Linear dose response <sup>b</sup>
	CON vs. DFM	CON vs. 10 <sup>6</sup> LA45 + LA51	LA45 vs. LA45 + LA51	10 <sup>4</sup> 10 <sup>6</sup> 10 <sup>8</sup>
Final BW, kg	568 574 ( <i>P</i> = 0.01)	568 575 ( <i>P</i> = 0.01)	574 575 ( <i>P</i> = 0.75)	573 575 574 ( <i>P</i> = 0.88)
DMI, kg/d	9.22 9.37 ( <i>P</i> = 0.07)	9.22 9.32 ( <i>P</i> = 0.28)	9.38 9.32 ( <i>P</i> = 0.61)	9.21 9.32 9.59 ( <i>P</i> = 0.05)
ADG, kg	1.56 1.60 ( <i>P</i> = 0.02)	1.56 1.61 ( <i>P</i> = 0.02)	1.59 1.61 ( <i>P</i> = 0.59)	1.58 1.61 1.60 ( <i>P</i> = 0.57)
Feed:gain	6.02 5.92 ( <i>P</i> = 0.29)	6.02 5.92 ( <i>P</i> = 0.10)	6.01 5.92 ( <i>P</i> = 0.21)	5.89 5.92 6.09 ( <i>P</i> = 0.12)
Hot carcass weight, kg	345.6 348.9 ( <i>P</i> = 0.02)	345.6 350.0 ( <i>P</i> = 0.01)	349.2 350.0 ( <i>P</i> = 0.67)	350.0 350.4 350.3 ( <i>P</i> = 0.98)
Dressing percentage	61.49 61.34 ( <i>P</i> = 0.32)	61.49 61.49 ( <i>P</i> = 0.99)	61.34 61.49 ( <i>P</i> = 0.44)	61.49 61.53 61.52 ( <i>P</i> = 0.99)
Quality grade <sup>c</sup>	18.64 18.72 ( <i>P</i> = 0.61)	18.64 18.75 ( <i>P</i> = 0.43)	18.64 18.75 ( <i>P</i> = 0.50)	18.74 18.83 18.81 ( <i>P</i> = 0.91)
Choice, %	59.92 61.94 ( <i>P</i> = 0.56)	59.92 62.45 ( <i>P</i> = 0.53)	59.25 62.45 ( <i>P</i> = 0.49)	63.42 62.45 65.83 ( <i>P</i> = 0.77)
Carcass ADG, kg	1.58 1.61 ( <i>P</i> = 0.05)	1.58 1.63 ( <i>P</i> = 0.01)	1.61 1.63 ( <i>P</i> = 0.46)	1.62 1.63 1.62 ( <i>P</i> = 0.94)
Feed NE <sub>m</sub> , Mcal/kg <sup>d</sup>	2.39 2.42 ( <i>P</i> = 0.39)	2.39 2.45 ( <i>P</i> = 0.12)	2.41 2.45 ( <i>P</i> = 0.32)	2.44 2.45 2.35 ( <i>P</i> = 0.21)
Feed NE <sub>g</sub> , Mcal/kg <sup>d</sup>	1.52 1.53 ( <i>P</i> = 0.28)	1.52 1.54 ( <i>P</i> = 0.07)	1.52 1.54 ( <i>P</i> = 0.26)	1.54 1.54 1.50 ( <i>P</i> = 0.14)
Feed ME, Mcal/kg <sup>d</sup>	3.38 3.40 ( <i>P</i> = 0.33)	3.38 3.42 ( <i>P</i> = 0.08)	3.39 3.42 ( <i>P</i> = 0.27)	3.41 3.42 3.34 ( <i>P</i> = 0.16)

<sup>a</sup>Treatment contrasts were CON (control) vs. all DFM treatments; CON vs. 10<sup>9</sup> *P. freudenreichii* (PF24), 10<sup>6</sup> *L. acidophilus* (LA45) LA45, and 10<sup>6</sup> LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup> (TRT3); 10<sup>9</sup> PF24 and 10<sup>6</sup> LA45 cfu·animal<sup>-1</sup>·d<sup>-1</sup> vs TRT3, respectively.

<sup>b</sup>Linear relationship between 10<sup>9</sup> PF24, 10<sup>4</sup> LA45, and 10<sup>4</sup> LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup>, TRT3, and 10<sup>9</sup> PF24, 10<sup>8</sup> LA45, and 10<sup>8</sup> LA51 cfu·animal<sup>-1</sup>·d<sup>-1</sup>, respectively.

<sup>c</sup>23 = Prime; 20 = Choice; 17 = Select; 14 = No roll.

<sup>d</sup>Values for feed NE<sub>m</sub>, NE<sub>g</sub>, and ME (DM basis) were calculated from performance data.

## Mode of Action

Variable response to feeding bacterial DFM in ruminant production systems emphasizes the need for greater understanding of underlying mechanisms. Research conducted to determine the potential mode of action of bacterial DFM has most often used the human or rodent model. Holzapfel et al. (1998) reviewed the literature and outlined several general microbiological criteria as keys for DFM to be efficacious. These included nonpathogenicity (i.e., safety), survival through regions of the gut (saliva, gastric, and bile), specificity to the host, and genetic stability. Upon reviewing the literature, it is apparent that in many animal studies, the use of nonhost-specific species and/or strains might be the reason why there was no response to bacterial DFM. Assuming criteria are met, bacterial DFM have been reported to modify the balance of intestinal microorganisms, adhere to intestinal mucosa and prevent pathogen adherence or activation, influence gut permeability, and modulate immune function (Salimen et al., 1996; Holzapfel et al., 1998). It has also been observed that certain lactic acid bacteria showed adjuvant properties by stimulation of a specific antibody response

after injection with pathogenic microorganisms. Data supporting the occurrence of these mechanisms of DFM fed to livestock species are discussed.

### Bacterial Direct-Fed Microbials and the Gut

**Competitive Attachment.** Early research (Jones and Rutter, 1972) suggested that attachment to the intestinal wall was important for enterotoxin-producing strains of *E. coli* to induce diarrhea. Therefore, it seems logical that bacterial DFM could compete with pathogens for sites of adherence on the intestinal surface. Attachment is believed to support proliferation and reduce peristaltic removal of organisms (Salimen et al., 1996). In support, Muralidhara et al. (1977) found that homogenates of washed intestinal tissue collected from piglets dosed with *L. lactis* had markedly higher numbers of attached *Lactobacilli* and lower *E. coli* counts than scouring or normal control pigs. Similarly, in the study of Abu-Tarboush et al. (1996), the adherence of *L. acidophilus* 27SC to the GIT was confirmed in young calves; the organisms used were apparently compatible with the GIT.

Adhesion is thought to be mediated either nonspecifically by physicochemical factors, or specifically by

adhesive bacterial surface molecules and epithelial receptor molecules (Holzapfel et al., 1998). Nonspecifically, the ability of bacteria to adhere to epithelial cells appears to depend on the interaction between an acidic mucopolysaccharide forming the outer layer of the bacterial cell wall and the similar mucopolysaccharide layer on the intestinal cells (Fuller and Brooker, 1974). Fibrils are often found on adhering bacteria and might reinforce attachment (Fuller and Brooker, 1980).

**Antibacterial Effect.** Many species of lactobacilli have demonstrated inhibitory activity against pathogens. *Lactobacillus acidophilus* has been shown to be antagonistic toward enteropathogenic *E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Clostridium perfringens* (Gilliland and Speck, 1977). Mann et al. (1980) showed that a strain of *E. coli*, which causes illness and death when it is the sole microbial species in young lambs, could be tolerated in the presence of lactobacilli. Lactic acid has been shown to decrease counts of coliforms throughout the GIT of piglets (Ratcliffe et al., 1986). This might result from a reduction in pH, which can prevent growth of many pathogens (Fuller, 1977).

Hydrogen peroxide produced by lactobacilli appears to be partially responsible for the antagonistic interaction (Gilliland and Speck, 1977). Hydrogen peroxide has been demonstrated to have bacteriocidal activity in vitro (Reiter et al., 1980); however, it might not have much involvement in the gut since oxygen is necessary for its formation by lactobacilli. A number of reports suggest that antimicrobial proteins and/or bacteriocins either mediate or facilitate antagonism by *L. acidophilus* (Hamdan and Mikolajcik, 1974; Gilliland and Speck, 1977; Barefoot and Klaenhammer, 1983). However, because of the presence of proteolytic enzymes, their importance might be limited.

**Immune Response.** Modulation of host immunity may represent another mechanism by which DFM promote intestinal health and overall well-being of the host (Erickson and Hubbard, 2000; Isolauri et al., 2001). The animal host immune system is capable of mounting both nonspecific (innate) and specific (adaptive) immune responses against a variety of pathogens when encountered. In addition to its role in the digestion and absorption of nutrients, the GIT provides its host a protective defense against a constant presence of antigens from food and microorganisms in the gut lumen. Besides epithelial cells, immune cells in the GIT consist of natural killer cells, macrophages, neutrophils, dendritic cells, and T and B lymphocytes that are aggregated in Peyer's patches, lamina propria, and intraepithelial regions. Upon infection by an antigen via the oral route, immune cells are rapidly activated, leading to enhanced phagocytosis as well as the production of a vast array of humoral mediators (Zhang and Ghosh, 2001). Interleukin (IL)-1, IL-6, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interferons (IFN), reactive oxygen/nitrogen intermediates, and antimicrobial peptides are among the first humoral mediators produced in response to

pathogenic bacteria, and they collectively either provide immediate protection for the host or help induce the development of specific immune responses. Cytokines produced later during microbial infection direct responses toward either cell-mediated T-helper type-1 (Th1) or humoral Th type-2 (Th2) immunity. Interleukin-2 and IL-12 promote development of Th1 cells from naïve T cells, whereas IL-4, IL-10, and transforming growth factor- $\beta$  (TGF- $\beta$ ) inhibit the production of TNF- $\alpha$ , IL-1, IL-6, IL-12, and IFN- $\gamma$ , and thus enhance Th2 immune responses (Infante-Duarte and Kamradt, 1999).

Bacterial DFM have been shown to affect the innate, humoral, and cellular arms of the immune system. Oral administration of lactobacilli generally resulted in an augmentation of innate immune responses (i.e., enhanced phagocytosis and natural killer cell activity), as well as an elevated production of immunoglobulin (Ig) A and a decreased IgE production in both humans and animals (Erickson and Hubbard, 2000; Isolauri et al., 2001). However, influence of DFM on cytokine production and T and B cell responses show mixed results depending on the strain, dose, and duration of feeding DFM, as well as the type of tissues and cells analyzed. *Lactobacillus rhamnosus* and *L. bulgaricus* strongly induced production of IL-2, IL-6, IL-10, TNF- $\alpha$ , and Th1-promoting cytokines (IL-12, IL-18, and IFN- $\gamma$ ), but not Th2-promoting cytokine, IL-4, in peripheral blood mononuclear cells (Miettinen et al., 1998). In contrast, *L. acidophilus*, *L. bulgaricus*, *L. casei*, and *S. thermophilus* did not alter gene expression of IL-6, TNF- $\alpha$ , and IFN- $\gamma$  in Peyer's patches, spleen, or lymph nodes of mice after 14 d of oral exposure (Tejada-Simon et al., 1999). *Lactobacillus johnsonii* had a very low potential to induce proinflammatory responses, but rather favored the induction of TGF- $\beta$  in an intestinal epithelial cell line (Haller et al., 2000b). Furthermore, some species of probiotics appear to be capable of altering the immunomodulatory effects exerted by other species. For example, *L. reuteri* DSM12246 was shown to potentially suppress *L. casei*-induced production of IL-6, IL-12, and TNF- $\alpha$  in dendritic cells (Christensen et al., 2002), suggesting that the composition of bacterial DFM administered should be considered. Increased populations of helper (CD4+) and activated (CD25+) T cells were observed in the blood of elderly people after 3-wk consumptions of *B. lactis* HN019 (Gill et al., 2001), but this did not happen in mice fed daily with *L. acidophilus*, *L. rhamnosus*, or *B. lactis* for 4 wk (Gill et al., 2000) or in human peripheral blood mononuclear cells stimulated in vitro for 3 to 5 d with *L. johnsonii* or *L. sakei* (Haller et al., 2000a).

These data provide evidence that bacterial DFM have the potential to protect animals and humans against pathogenic organisms. Several mechanisms are likely involved, but an ability to adhere to and colonize the GIT is most likely important. Bacterial DFM also show promise as immune modulators, although more re-

search is needed to determine the underlying mechanisms.

### *Bacterial Direct-Fed Microbials and Ruminal Fermentation*

The original concept of feeding bacterial DFM to livestock was based primarily on potential beneficial post-ruminal effects; however, there has been some indication that certain bacterial DFM also might have beneficial effects in the rumen, in particular, helping to prevent ruminal acidosis. Ruminal acidosis can be characterized by low ruminal pH (below 5.6) and high ruminal concentrations of total VFA (subacute) or lactic acid (acute). Lactate-producing bacteria (*Lactobacillus* and *Enterococcus*) might help prevent ruminal acidosis in dairy cows (Nocek et al., 2002), potentially because the presence of these bacteria cause the ruminal microorganisms to adapt to the presence of lactic acid in the rumen (Yoon and Stern, 1995). Inoculation of in vitro fermentation with lactate-utilizing bacterium *Megasphaera elsdenii* has been shown to prevent lactate accumulation when a highly fermentable substrate was used (Kung and Hession, 1995).

*Megasphaera elsdenii*. *Megasphaera elsdenii* inoculation has modified ruminal fermentation and prevented the accumulation of lactate during the transition from low- to high- concentrate diets in both in vitro and in vivo studies (Greening et al., 1991; Kung and Hession, 1995). In the study of Kung and Hession (1995), the pH of cultures treated with *M. elsdenii* ( $8.7 \times 10^6$  cfu/mL of culture fluid) was decreased below 5.5 at 4 h and remained at approximately 5.3 (24-h culture), whereas the control was decreased to 4.8. Lactate concentration peaked at more than 40 mM in control after 8 h and remained fairly constant thereafter, but in the *M. elsdenii* treatment, it was less than 5 mM through incubation. Total VFA concentration of cultures treated with *M. elsdenii* was more than twice that of control (131.4 vs. 63.3 mM). Acetate concentration was not significantly different after 2 h. The concentration of propionate, butyrate, valerate, isobutyrate, and isovalerate for control and *M. elsdenii* inoculation at 6 h were 38:47, 2:35, 1:15, 1:11, and 1:2 (mM, control:*M. elsdenii*), respectively. Therefore, most differences in VFA concentration between treatments resulted from increased butyrate, valerate, and branched-chain fatty acids.

Greening et al. (1991) reported that inoculation with *M. elsdenii* significantly decreased minimal pH and lactate concentration in acidosis induced beef cattle. Minimal pH for control, inoculation prior to acidosis induction, and 0 h, or 2 h after acidosis induction were 4.65, 4.73, 5.51 and 5.26 and maximal lactate concentrations were 124, 121, 49.9, and 45.9 mM, respectively. Accumulated total VFA were 472, 507, 910, and 870 mM·h for respective treatments. Robinson et al. (1992) reported the effects of inoculation with *M. elsdenii* on feed intake, ruminal pH, osmolarity, lactate, and VFA concentration in acute acidosis-induced steers fed a 90%

concentrate diet. In that study, the interaction between inoculation and day of diet switch moderated pH, lactate, VFA, and feed intake significantly. Steers inoculated with *M. elsdenii* ate 24% more DM.

*Propionibacteria*. Kung and Hession (1995) discussed the choice of *M. elsdenii* for inoculating ruminal fermentations that have not been adapted to readily degradable carbohydrates. During the feeding of readily degradable and soluble carbohydrates, *M. elsdenii* seems to be the major ruminal lactate utilizer (Counotte et al., 1981) because *S. ruminantium* undergoes catabolite repression (Russell and Baldwin, 1978) and is relatively acid-intolerant (Mackie and Gilchrist, 1979). Furthermore, *M. elsdenii* simultaneously uses lactate, glucose, and maltose (Russell and Baldwin, 1978) and would compete with lactate-producing organisms for substrate. Although *Propionibacterium* is a lactate utilizer, it has been focused on propionate production rather than lactate fermentation for use as a DFM. Propionate is quantitatively the most important single precursor of glucose synthesis among VFA, and therefore has a major impact on hormonal release and tissue distribution of nutrients (Nagaraja et al., 1997). For growing ruminants and lactating cows, propionate has been estimated to account for 61 (Reynolds et al., 1994) to 67% (Huntington, 2000) of glucose release. Propionate spares glucogenic amino acids in gluconeogenesis, and consequently reduces the maintenance cost of metabolizable protein and possible heat increment (Van Soest, 1994). Nutrient intake lags nutrient demand during early lactation, especially in dairy cows, and therefore ruminal supply of propionate might not be sufficient (Overton et al., 1999). Also, decreased acetate:propionate has been accompanied with a decrease in methane production according to the stoichiometric laws of chemical balance and its equation (Van Soest, 1994). When acetate:propionate decreases, CH<sub>4</sub> production declines, and energy retention by cattle would theoretically increase (Wolin, 1960).

Volatile fatty acid proportion depends on species of microorganisms and culture conditions. Propionate production by *Propionibacterium* is usually accompanied by the formation of acetate and CO<sub>2</sub>. This occurs for stoichiometric reasons and to maintain hydrogen and redox balance. Also, product ratios are controlled for thermodynamic reasons, such as ATP production and entropy generation (Lewis et al., 1996). However, *Propionibacterium* seems to produce propionate more efficiently compared with *M. elsdenii*. Although the culture condition was very different, *P. shermanii* fermented 1.3 mM of glucose resulting in final concentrations of 0.8 mM acetate and 2.3 mM propionate, whereas incubations with 6 mM lactate resulted in final concentrations of 1.72 mM acetate and 3.38 mM propionate during a 14-d incubation (Johns, 1951). The highest rate of decarboxylation of succinate occurred at about pH 5.0. *Megasphaera elsdenii* fermented 8 mM of glucose to 6.8 mM acetate and 4.3 mM butyrate, and fermented 40 mM of lactate to 13.5 mM acetate, 8.4 mM propio-

nate, and 8.5 mM butyrate during a 12-h incubation (Hino et al., 1994). Mackie et al. (1978) and Mackie and Gilchrist (1979) reported that in the rumen of sheep, during stepwise adaptation to a high-concentrate diet, *Propionibacterium* accounted for 40 to 50% of the lactate utilizers on occasion; however, the population of *Propionibacterium* usually seemed very low. Therefore, the concept of daily or periodic supplementation of *Propionibacterium* may be on the basis of the increment of propionate production when cattle are fed a high-concentrate diet.

The effect of increasing dosage levels (none,  $10^7$ ,  $10^8$ ,  $10^9$ , and  $10^{10}$  cfu) of *P. acidipropionici* on ruminal fermentation in steers fed a high-concentrate diet was recently studied (Kim et al., 2000). When supplemented with *P. acidipropionici*, all dosage levels and the post-test period had lower levels of acetate, but only the  $10^7$  and post-test period were significantly greater than the pretest period. Propionate levels were greater for all dosage levels. Numerically, propionate increased as the dosage level increased and tended to decrease in the post-test period. Consequently, acetate:propionate decreased at all dosages except  $10^8$ . It would appear the *P. acidipropionici* altered ruminal metabolism toward less acetate and more propionate. Butyrate concentration decreased as the dose of *P. acidipropionici* increased. When *P. acidipropionici* was removed, butyrate concentration returned to near pretest levels. This suggests that *P. acidipropionici* did effectively reduce butyrate concentration in the rumen. There was no effect on pH, lactate, or branched-chain fatty acids with supplementation of *P. acidipropionici* (Kim et al., 2000). In contrast, Ghorbani et al. (2002) fed *Propionibacterium* or *Propionibacterium* and *E. faecium* and found no effect on ruminal concentrations of L-lactate, total VFA, propionate, isobutyrate, and isovalerate, or the ratio of acetate:propionate. Acetate concentration in ruminal fluid was greater for steers receiving *Propionibacterium* and *E. faecium* than for steers receiving *Propionibacterium* alone or control. In contrast to Kim et al. (2000), steers fed *Propionibacterium* alone had greater concentrations of ruminal butyrate (Ghorbani et al., 2002). Other researchers (Slyter et al., 1992; Kung and Hession, 1995) have reported accumulation of butyrate when *M. elsdenii* is grown in pure culture.

Ghorbani et al. (2002) found no effect of *Propionibacterium* P15 or a combination of *Propionibacterium* P15 and *E. faecium* EF212 on ruminal pH. Mean ruminal pH of steers fed steam-rolled barley was 5.71. In contrast, results from studies with *Lactobacillus* species have shown lower area under the pH curve (Huffman et al., 1992; Nocek et al., 2000), suggesting reduced risk of subacute ruminal acidosis. Similar to ruminal pH, blood pH was not affected by bacterial DFM supplementation in the study of Ghorbani et al. (2002). However, steers fed *Propionibacterium* and *E. faecium* tended to have lower concentrations of blood CO<sub>2</sub> and had lower concentrations of LDH than control steers. The authors suggested that lower blood CO<sub>2</sub> and LDH indicated that

feeding a lactate-producing bacteria along with a lactate-utilizing bacteria reduced the risk of metabolic acidosis.

Aviles (1999) conducted an experiment with six ruminally-cannulated steers in an acidosis challenge study to evaluate the effects of a lactate utilizer, *P. acidipropionici*, strain DH42, on ruminal acidosis. In that study, *P. acidipropionici* significantly lowered ruminal and blood pH 2 h after feed engorgement. However, ruminal VFA and lactate levels were unaffected by treatment.

Huffman et al. (1992) suggested that *L. acidophilus* might modify subacute ruminal acidosis. Ruminally fistulated steers were fed a 50% concentrate diet for 12 d. On d 13, steers were dosed with a 100% concentrate diet via a ruminal cannula to induce subacute acidosis. Feeding *L. acidophilus* ( $5 \times 10$  cfu/d) reduced the amount of time that ruminal pH was below 6.0 compared with control. Recently, Van Koeveering et al. (1994) reported that ruminal concentrations of D-lactate and total lactate were lower in steers fed *L. acidophilus*. These data suggested that *L. acidophilus* alone might decrease the severity of subacute acidosis.

Feeding *Propionibacterium* increased protozoa (especially *Entodinium*) and decreased amylolytic bacteria in the rumen of feedlot steers (Ghorbani et al., 2002). Similarly, Van Koeveering et al. (1994) reported that including cultures of lactobacilli in the diet prolonged retention of protozoa. The mechanism by which bacterial DFM stimulate protozoa remains unclear (Ghorbani et al., 2002).

In summary, DFM might reduce the risk for subacute acidosis by reducing the time ruminal pH remains below 5.6. Lower blood CO<sub>2</sub> and LDH also suggest a lower risk for metabolic acidosis. However, these responses seem to depend on the species of DFM fed. The concept of supplementation of a combination of *Propionibacterium* and *Lactobacillus* might be developed with the aforementioned characteristics of microorganisms with the ruminal ecosystem in mind. In particular, the inhibition of methane production with lactate production by *Lactobacillus* may promote propionate production by *Propionibacterium* and improve the energy efficiency in the rumen, and consequently animal performance.

## Implications

Bacterial direct-fed microbials fed to ruminant livestock have been shown to decrease scours in neonatal calves, increase milk yield in dairy cows, decrease morbidity in newly weaned calves and/or calves newly received in the feedlot, and increase daily gain and carcass weight in feedlot cattle. Moreover, strains of *Lactobacillus acidophilus* were shown to reduce fecal shedding of *Escherichia coli* O157:H7 by feedlot cattle at harvest. Although the mode of action is not fully understood, it seems that adhesion, colonization, inhibitory action, and stimulation of immune function are all important for direct-fed microbials to improve health. Some bacterial direct-fed microbials also seem able to

function in the rumen. Depending on the species or combination of species, they can increase ruminal propionate concentration and decrease area below subacute ruminal pH, suggesting the potential for more efficient energy utilization and a reduction in acidosis exists.

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