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Direct-fed Microbials for Ruminant Animals*

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ABSTRACT : Direct-fed microbials (DFM) are dietary supplements that inhibit gastrointestinal infection and provide optimally regulated microbial environments in the digestive tract. As the use of antibiotics in ruminant feeds has been banned, DFM have been emphasized as antimicrobial replacements. Microorganisms that are used in DFM for ruminants may be classified as lactic acid producing bacteria (LAB), lactic acid utilizing bacteria (LUB), or other microorganisms including species of *Lactobacillus, Bifidobacterium, Enterococcus*, *Streptococcus*, *Bacillus* and *Propionibacterium*, strains of *Megasphaera elsdenii* and *Prevotella bryantii* and yeast products containing *Saccharomyces* and *Aspergillus*. LAB may have beneficial effects in the intestinal tract and rumen. Both LAB and LUB potentially moderate rumen conditions and improve feed efficiency. Yeast DFM may reduce harmful oxygen, prevent excess lactate production, increase feed digestibility, and improve fermentation in the rumen. DFM may also compete with and inhibit the growth of pathogens, stimulate immune function, and modulate microbial balance in the gastrointestinal tract. LAB may regulate the incidence of diarrhea, and improve weight gain and feed efficiency. LUB improved weight gain in calves. DFM has been reported to improve dry matter intake, milk yield, fat corrected milk yield and milk fat content in mature animals. However, contradictory reports about the effects of DFM, dosages, feeding times and frequencies, strains of DFM, and effects on different animal conditions are available. Cultivation and preparation of ready-to-use strict anaerobes as DFM may be cost-prohibitive, and dosing methods, such as drenching, that are required for anaerobic DFM are unlikely to be acceptable as general on-farm practice. Aero-tolerant rumen microorganisms are limited to only few species, although the potential isolation and utilization of aero-tolerant ruminal strains as DFM has been reported. Spore forming bacteria are characterized by convenience of preparation and effectiveness of DFM delivery to target organs and therefore have been proposed as DFM strains. Recent studies have supported the positive effects of DFM on ruminant performance. (**Key Words :** DFM, Probiotics, Mode of Action, Ruminants)

INTRODUCTION

Improvements in feed utilization, animal production and health, and animal food safety are the goals of rumen microbial studies. These goals may be achieved by facilitating desirable fermentation, minimizing ruminal disorders, and excluding pathogens. Several feed additives have been used to improve animal performance and feed efficiency and to prevent disease. Antibiotics, probiotics (direct-fed microbials, DFM) and prebiotics (microbial growth promoters) have been studied to manipulate the microbial ecosystem and fermentation characteristics in the rumens and intestinal tracts of livestock animals.

The use of growth promoting antibiotics in animal feeds is banned in Europe due to potential risks such as the spread of antibiotic resistance genes (Hong et al., 2005) or the contamination of milk or meat with antibiotic residues. As a result, many livestock producers have explored alternative strategies to enhance animal performance and health. Recently, DFM have been increasingly evaluated to replace or facilitate reductions in the use of antibiotics.

The term "probiotics" is defined as "a live microbial feed supplement that may beneficially affect the host animal upon ingestion by improving its intestinal microbial balance" (Fuller, 1989). This term has been used to describe viable microbial cultures, culture extracts, enzyme preparations, or various combinations of those products (Yoon and Stern, 1995). DFM has a narrower definition

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relative to probiotics, and are defined as microbial based feed additives.

Practical issues related to the effects of DFM include dosage, timing, strains of DFM, and animal conditions. DFM that target the rumen must be active in the rumen and remain viable during delivery, therefore studies of DFM are limited to few species. In this review, we will survey microorganisms that have been studied as DFM and their modes of action as well as their effects in host animals. Convenience of delivery, aero-tolerance of strains and advantages of using spore-forming bacteria as DFM will also be discussed. Species of bacilli were found to be the best DFM candidates for ruminant animals.

MICROORGANISMS USED IN DFM PRODUCTS

Microorganisms used in DFM for ruminants include species of *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, *Bacillus* and *Propionibacterium*, all of which are commonly used in probiotics for human and mono-gastric animals or as inocula for dairy product processing (Table 1). Other distinctive bacterial species such as *Megasphaera elsdenii* and *Prevotella bryantii* have also been used as DFM to stabilize or improve rumen function. These bacterial DFM strains may be classified as lactic acid producing, lactic acid utilizing, or other microorganisms. In ruminant animals, the rumen is the first organ that DFM reach upon ingestion. DFM grow in the rumen and beneficially modify its microbial ecosystem and (or) fermentation characteristics. The intestinal tract may also provide a habitat for DFM. Lactic acid production and utilization in the rumen is closely related to feed efficiency and animal health. Although bacterial DFM are emphasized, fungal DFM are also common feed additives to ruminant diets (Kung Jr, 2001). Most commercial yeast products contain species of *Saccharomyces* and *Aspergillus*.

Table 1. Microorganisms used as DFM for ruminants

Genus	Species
Lactic acid producing bacteria	
Lactobacillus	*Lactobacillus acidophilus*
	Lactobacillus plantarum
	Lactobacillus casei
	Lactobacillus gallinarum
	Lactobacillus salivarius
	Lactobacillus reuteri
	Lactobacillus bulgaricus
Bifidobacterium	*Bifidobacterium pseudolongum*
	Bifidobacterium thermophilium
	Bifidobacterium longum
	Bifidobacterium lactis
Streptococcus	*Streptococcus bovis*
	Streptococcus faecium
Enterococcus	*Enterococcus faecium*
	Enterococcus faecalis
Lactic acid utilizing bacteria	
Megasphaera	*Megasphaera elsdenii*
Propionibacterium	*Propionibacterium shermanii*
	Propionibacterium freudenreichii
	Propionibacterium acidipropionici
	Propionibacterium jensenii
Other bacteria	
Prevotella	*Prevotella bryantii*
Bacillus	*Bacillus subtilis*
	Bacillus licheniformis
	Bacillus coagulans
Yeast	
Saccharomyces	*Saccharomyces cerevisiae*
	Saccharomyces boulardii
Fungi	
Aspergillus	*Aspergillus oryzae*
	Aspergillus niger

MODES OF DFM ACTION

Mode of action of DFM in the rumen

Bacterial DFMs have potential beneficial effects on the post-ruminal gastrointestinal tract, but certain bacterial DFMs were recently found to play a beneficial role in the rumen itself. The modes of action of different DFM sources in the rumen are summarized in Table 2. Lactic acid producing bacteria (LAB) have been proposed to have beneficial effects in the intestinal tract. However, some researchers have suggested that LAB might also have positive effects in the rumen. LAB such as lactobacilli and enterococci might prevent ruminal acidosis in dairy cows (Nocek et al., 2002) by facilitating the growth of ruminal microorganisms adapted to the presence of lactic acid in the rumen (Yoon and Stern, 1995) and by stimulating lactic acid utilizing bacteria (LUB).

LUB have also been proposed as DFM and have been used successfully to decrease concentrations of lactate and maintain ruminal pH. *Megasphaera elsdenii* may utilize lactate and prevent drastic pH drops caused by accumulation of lactate in the rumen when fed a highly fermentable diet (Kung and Hession, 1995), and the supplementation of *M. elsdenii* was proposed as a means of preventing acute acidosis in transition animals.

Propionibacteria ferments lactate to propionate. Since propionate is the major precursor for gluconeogenesis in early lactation dairy cows (Reynolds et al., 2003), increments of propionate production in the rumen result in increases of hepatic glucose production (Stein et al., 2006), providing more substrates for lactose synthesis, improving

Table 2. Modes of action of DFM in the rumen

Proposed mechanisms
Lactic acid producing bacteria
1. Provision of a constant lactic acid supply
2. Adaptation of overall microflora to the lactic acid accumulation
3. Stimulation of lactate utilizing bacteria
4. Stabilization of ruminal pH
Lactic acid utilizing bacteria
1. Conversion of lactate to VFA (e.g., *Megasphaera elsdenii*)
2. Production of propionic acid rather than lactic acid (e.g., *Propionibacterium spp.*)
3. Increase of feed efficiency
4. Decrease of methane production
5. Increase of ruminal pH
Fungal DFM
1. Reduction of oxygen in the rumen
2. Prevention of excess lactic acid in the rumen
3. Provision of growth factors such as organic acid and vitamin B
4. Increase of rumen microbial activity and numbers
5. Improvement of ruminal end products (e.g., VFA, rumen microbial protein)
6. Increase of ruminal digestibility

energetic efficiency and reducing ketosis (Weiss et al., 2008). In addition, increased propionate may reduce hydrogen available for methane production in the rumen. Certain species of propionibacteria were reported to modify rumen fermentation and increase the molar portion of ruminal propionate (Stein et al., 2006).

Fungal DFM have been extensively used in ruminants for improving performance and normalizing rumen fermentation. Increases in bacterial numbers recovered from the rumen are the most reproducible effects of dietary yeast supplementation. Rose (1987) suggested that yeasts remove oxygen in the rumen. Yeast cells in the rumen use available oxygen on the surfaces of freshly ingested feed to maintain metabolic activity. Jouany et al. (1999) observed a significant decrease in redox potential, up to -20 mV, in the rumen with yeast supplementation. This change creates better conditions for the growth of strict anaerobic cellulolytic bacteria, stimulates their attachment to forage particles (Roger et al., 1990), and increases the initial rate of cellulolysis. In addition, *S. cerevisiae* was able to compete with other starch utilizing bacteria for fermentation of starch (Lynch and Martin, 2002) leading to the prevention of lactate accumulation in the rumen (Chaucheyras et al., 1995). Chaucheyras et al. (1995) also reported that *S. cerevisiae* had the ability to provide growth factors, such as organic acids or vitamins, thereby stimulating ruminal populations of cellulolytic bacteria and LUB.

Mode of action of DFM in the post-ruminal GIT

As noted, previous inquiries regarding feeding bacterial DFM to ruminant animals focused on its potential beneficial effects on the post ruminal GIT. Some suggested mechanisms are summarized in Table 3. Proposed roles of beneficial DFM are to:

i) attach to the intestinal mucosa and prevents potential pathogen establishment
ii) maintain lower pH in the GIT thereby inhibiting growth of pathogens
iii) produce antibacterial compounds such as bacteriocin and hydrogen peroxide
iv) modulate immune cells and stimulate immune function
v) modulate microbial balance in the GIT
vi) prevent illness caused by intestinal pathogens or stress

Table 3. Modes of action of DFM in the post-rumen GIT

Proposed mechanisms
1. Production of antibacterial compounds (acids, bacteriocins, antibiotics)
2. Competition with pathogens for colonization of mucosa and/or for nutrients
3. Production and/or stimulation of enzymes
4. Stimulation of immune response by host
5. Metabolism and detoxification of desirable compounds

Enterotoxin-producing strains of *E. coli* attach to intestinal epithelial cells and mucus to induce diarrhea (Jones and Rutter, 1972). Lee et al. (2003) discovered that *L. rhamonsus* GG could attach to epithelial cells via hydrophobic interactions and limit pathogens from attaching to the enterocytic receptor. Steric hindrance displaces pathogens, which eventually detach from the enterocytic receptor. In addition, *L. rhamonsus* (Lcr35) decreases adhesion of enteropathogenic and enterotoxigenic *E. coli* and *Klebsiella pneumonia* (Forestier et al., 2001). In other experiments, LAB was able to adhere to the intestinal tracts of mice, protecting animals against *Salmonella Dublin* DSPV 595T (Frizzo et al., 2010). LAB produces lactate and acetate as main metabolic end products. These acids have critical roles in penetrating microbial cells and interfering with essential cell function to reduce intracellular pH (Holzapfel et al., 1995).

Hydrogen peroxide and several bacteriocins produced by LAB are also important compounds due to their competitive exclusion and probiotic characteristics. Hydrogen peroxide can oxidize on the bacterial cell, on sulfhydryl groups of cell proteins and on membrane lipids (Dicks and Botes, 2010), thereby blocking glycolysis due to the oxidation of sulfhydryl groups in metabolic enzymes such as glucose transport enzymes, hexokinase, and glycerol aldehyde-3-phosphate dehydrogenase (Carlsson et al., 1983). Holzapfel et al. (1995) suggested that LAB produced hydrogen peroxide, which effectively inhibited *S. aureus* and *Pseudomonas spp.*.

LAB bacteriocins were well documented by Cotter et al. (2005). Reuterin, produced by *L. reuteri* when grown anaerobically in the presence of glucose and glycerol (Dicks and Botes, 2010), inhibits the binding of substrates to the subunit of ribonucleotide reductase so that interfering with DNA-synthesis of target microorganisms (Dobrogosz et al., 1989). *Lactobacillus* GG, isolated from humans, was able to produce unidentified antimicrobial compounds that limited the growth of *Staphylococcus spp.*, *Streptococcus spp.*, and *Pseudomonas spp.* in *in vitro* (Silva et al., 1987).

Modulation of host immune function is another mode of action identified by DFM. In the GIT, various immune cells exist such as dendritic cells, natural killer cells, macrophages, neutrophils, and T and B lymphocytes that are aggregated in Peyer's patches, lamina propria, and intraepithelial regions (Krebiel et al., 2003). After DFM are administered to the GIT, they are directly taken up by intestinal epithelial cells via transcytosis. Antigen presenting cells, macrophages or dendritic cells engulf them, finally stimulating an immune response (Dicks and Botes, 2010). Various strains of LAB activate macrophages to produce cytokines that stimulate immune response. Matsuguchi et al. (2003) suggested that *L. casei* Shirota and *L. rhamnosus* Lr23 stimulated macrophages to secrete TNF-α or promote development of regulatory dendritic cells. Miettinen et al. (1996) also reported that LAB could induce the production of proinflammatory cytokines, TNF-α, and interleukin-6 from human peripheral blood mononuclear cells (PBMC), thereby stimulating non-specific immunity.

EFFECTS OF DFM ON PERFORMANCES

Young calves

Since young calves have to digest a significant amount of ration nutrients in their intestines, they may be at risk of intestinal proliferation of detrimental organisms. Neonatal calves are often stressed in new environments, such as transport, weaning, vaccination, and dehorning (Krehbiel et al., 2003). In intensive farm systems, calves are rapidly separated from cows before their intestinal microbiota have completely colonized. This situation might increase the possibility of diarrhea and weight loss. The administration of large amounts of beneficial microorganisms may allow stressed intestinal environments to be colonized and return GIT function to normal more quickly in scouring calves (Kung Jr, 2001). Therefore many studies have been conducted to evaluate the effects of DFM on young calves (Table 4).

Many studies indicated that LAB could regulate diarrhea incidence as well as improve weight gain and feed efficiency when used as a DFM source. Holstein calves supplemented with *L. acidophilus* 27SC had significantly higher colony counts in feces compared to calves fed a control diet. As a result, calves fed *L. acidophilus* 27SC showed significant differences in scour index during weeks 5, 7 and 8 compared with calves fed a control diet and, during weeks 7 and 8 compared with calves fed a mixed lactobacilli diet (Abu-Tarboush et al., 1996). Abe et al. (1995) investigated the effects of oral administration of *Bifidobacterium pseudolongum* or *L. acidophilus* on newborn calves. Oral administration of the two types of LAB improved BW gain and feed efficiency, and reduced frequencies of diarrhea occurrence compared calves that did not receive LAB. The body weight gain was different ($p< 0.05$) between treated and control groups, but not between groups fed bifidobacteria and lactobacilli. Dicks and Botes (2010) suggested that bifidobacteria produce acetic and lactic acids at a ratio of 3:2, and that these acids may be more effective for the control of gram-negative pathogens and yeasts in the GIT than *Lactobacillus spp.* because acetate is more effective against gram-negative bacteria, moulds and yeasts (Gilliland, 1989).

In recent experiments, LAB were also inoculated into young calves to improve growth performance (Frizzo et al., 2010). Young calves were fed milk replacer and a large quantity of spray-dried whey powder to generate an

Table 4. The effects of various DFM on calf performance

Strains	Dose	Effects	References
Aspergillus oryzae	5×10^7 cfu/ml	Higher total VFA, propionate, and acetate concentrations in the rumen. Cellulolytic bacterial counts tended to be higher than controls.	(Beharka et al., 1991)
Lactobacillus acidophilus	5×10^7 cfu/ml	Calves receiving *L. acidophilus* maintained initial BW, and control calves lost BW until 2 wk of age.	(Cruywagen et al., 1996)
Bifidobacterium pseudolongum *Lactobacillus acidophilus*	3×10^9 cfu/ml	Both strains improved ADG, feed efficiency and reduced diarrhea incidence.	(Abe et al., 1995)
Lactobacillus acidophilus *Lactobacillus plantarum* *Lactobacillus acidophilus 27SC*	Not noted Not noted 1.85×10^7 cfu/ml	Incidence of diarrhea decreased after week 1 in calves fed DFM containing *Lactobacillus.* Lactobacilli increased in feces of calves fed a liquid diet treated with *L. acidophilus* 27SC.	(Abu-Tarboush et al., 1996)
Lactobacillusa acidophilus *Propionibacterium freudenreichii*	from 1×10^6 cfu/ml to 1×10^9 cfu/ml	Calves fed DFM showed lower fecal shedding of *E.coli.*	(Elam et al., 2003)
Propionibacterium jensenii 702 (PJ702)	1.1×10^8 cfu/ml 1.2×10^9 cfu/ml	Calves fed PJ 702 exhibited successful gastrointestinal transit of the bacterium.	(Adams et al., 2008)
Lactobacillus acidophilus *Saccharomyces cerevisiae*	1×10^9 3×10^9 cfu/flask/kg	ADG and feed efficiency were higher in calves receiving probiotics plus enzyme supplements.	(Malik and Bandla, 2010)
Lactobacillus casei DSPV 318T *Lactobacillus salivarius* DSPV 315T *Pediococcus acidilactici* DSPV 006T	3×10^9 cfu/kg live weight	Inocula stimulated earlier consumption of starter and earlier development of the rumen.	(Frizzo et al., 2010)

intestinal imbalance. Under these conditions, calves fed probiotics had higher daily gain, total feed intake, and starter diet intake as well as lower fecal consistency index, indicating that diarrhea incidence was reduced (Frizzo et al., 2010).

Adams et al. (2008) examined the effect of a novel bacterial strain, *Propionibacterium jensenii* 702, isolated in Australia on growth performance. Most bacterial DFM for young calves contain LAB, whereas dairy propionibacteria are rarely used. Propionibacteria can increase propionate and butyrate concentration in the rumen thereby stimulating rumen development. Faecal recovery of *P. jensenii* 702 from the treatment groups from week 2 indicated successful gastrointestinal transit of the bacterium and these calves exhibited higher weight gain during preweaning and postweaning periods.

Adult ruminants

During transition periods, defined as 3 weeks prior to calving to 3 weeks after calving (Grummer, 1995), dairy cows are stressed due to calving, changing diets to rapidly fermented carbohydrate sources, and lactation. Sudden changes that occur during this time may cause metabolic disorders such as subacute acidosis in dairy cows (Oetzel et al., 2007; Chiquette et al., 2008). In finishing beef cattle, it is also very important to prevent ruminal acidosis caused by highly fermentable feeds. Both dairy and beef cattle fed DFM showed improved growth performance, milk and meat production, and feed efficiency in many experiments (Ghorbani et al., 2002; Nocek et al., 2002; Krehbiel et al., 2003; Stein et al., 2006).

LAB with yeast or LUB has been used as DFM to improve performance of dairy cows. *Enterococcus faecium* with yeast was top dressed in a supplement during both pre- and postpartum periods. DFM increased dry matter intake, milk yield, and milk protein content during the postpartum period. Blood glucose and insulin levels were higher and NEFA levels were lower for cows receiving DFM during the postpartum period (Nocek et al., 2003). In another study (Nocek and Kautz, 2006), cows supplemented with *E.*

Table 5. The effects of various strains of DFM on adult ruminant performance

Strains	Dose	Effects	References
Enterococcus faecium *Lactobacillus plantarum*, *Sacchromyces cerevisiae*	from 1×10^5 cfu/ml to 1×10^7 cfu/ml	Sustained a higher nadir pH than cows fed 10^6 or 10^7 and had a higher digestion rate of high moisture ear corn (HMEC) dry matter.	(Nocek et al., 2002)
Propionibacterium P15 *Enterococcus faecium* EF212	1×10^9 cfu/g	DMI and ruminal pH were not different. DFM resulted in numerically lower blood CO_2 concentrations and reduced risk of metabolic acidosis.	(Ghorbani et al., 2002)
Enterococcus faecium Yeast	5×10^9 cfu/g 5×10^9 cfu/g	Cows fed DFM consumed more DM, and produced 2.3 kg more milk/cow per day.	(Nocek and Kautz, 2006)
Propionibacterium P169	6×10^{10} cfu/cow 6×10^{11} cfu/cow	Cow fed high doses and low doses of P169 exhibited 7.1 and 8.5% increases above controls in daily 4% FCM, respectively.	(Stein et al., 2006)
Lactobacillus acidophilus LA747 *Propionibacteria freudenreichii* PF24 *Lactobacillus acidophilus* LA45	1×10^9 cfu/cow 2×10^9 cfu/cow 5×10^8 cfu/cow	No differences in average DMI, yield of 4% FCM, ruminal pH and total VFA concentration in the rumen were observed.	(Raeth-Knight et al., 2007)
Enterococcus faecium *Saccharomyces cerevisiae*	5×10^9 cfu/cow/d 5×10^9 cfu/cow/d	First lactation cows fed DFM produced more milk fat % and second lactation cows fed DFM received fewer antibiotic treatments.	(Oetzel et al., 2007)
Saccharomyces cerevisiae subspecies *boulardii* CNCM I-1079	0.5 g of yeast /steer/d	Treatments did not affect DMI, ADG, or feed efficiency during the experimental period.	(Keyser et al., 2007)
Prevotella bryantii	2×10^{11} cfu/dose	*Prevotella bryantii* treatment increased milk fat %, concentration of acetate, butyrate, and decreased lactate concentration 2 to 3 h after feeding.	(Chiquette et al., 2008)
Propionibacterium strain P169	6×10^{11} cfu/d	Cows fed P169 had lower concentrations of acetate, greater concentrations of propionate, and higher energetic efficiency.	(Weiss et al., 2008)
Propionibacterium strain P169 Yeast culture	6×10^{11} cfu/steer/d 56 g/steer/d	Feeding P169 tended increased molar proportions of propionate, however did not affect ruminal digestibility, microbial N synthesis, or particulate passage rates.	(Lehloenya et al., 2008)

faecium with yeast had higher ruminally available dry matter (DM), consumed more DM during both the pre- and postpartum periods, and produced more milk/cow per day. There were no differences in 3.5% fat-corrected milk between cows supplemented with DFM and controls. There were no differences in milk fat yield or milk protein percentage and yield. Cows consuming DFM had higher blood glucose postpartum, as well as lower beta-hydroxybutyrate levels both pre-partum and on day 1 postpartum. Oetzel et al. (2007) reported that *E. faecium* plus *S. cerevisiae* increased milk fat percentages when used as DFM for first lactation cows and increased milk protein percentages for second and greater lactation cows during the first 85 DIM. Second-lactation cows receiving DFM also received fewer antibiotic treatments before 85 DIM than cows receiving placebo. Raeth-Knight et al. (2007)

evaluated the effects of the combination of *L. acidophilus* LA747 and *P. freudenreichii* PF24 on 84 d dairy cattle performance and 28 d periods ruminal characterizations. DFM was top dressed on the TMR once daily. DFM did not affect performance including DM intake, 4% fat-corrected milk, percentage or yield of milk components, feed efficiency, apparent digestibility of DM, crude protein, neutral detergent fiber, starch, rumen pH or concentrations of ammonia or total volatile fatty acids.

DFM effects in the rumens of dairy cows have been studied in a feeding trial, in which mixtures of *E. faecium, L. plantarum*, and *S. cerevisiae* at a level of 10^5, 10^6, or 10^7 cfu/ml rumen fluid were directly administered via rumen cannula to cows in early lactation once daily for 21 d. Cows fed 10^5 cfu sustained a higher nadir pH than cows fed 10^6 or 10^7 cfu. Cows fed 10^5 cfu had a higher digestion rate of high moisture ear corn dry matter. Corn silage digestion was higher for cows fed 10^5 cfu and 10^6 cfu compared to those receiving 10^7 cfu (Nocek et al., 2002). Weiss et al. (2008) supplemented dairy cows from 2 wk before anticipated calving to 119 d in milk with *Propionibacterium* strain P169. Cows fed P169 had lower concentrations of acetate, greater concentrations of propionate and butyrate. Plasma and milk glucose or plasma beta-hydroxybutyrate levels were not affected by DFM. Cows fed P169 had greater concentrations of plasma NEFA on d 7 of lactation. Cows fed P169 during the first 17 wk of lactation produced similar amounts of milk with similar composition as cows fed a control diet. Calculated net energy use for milk production, maintenance, and body weight change were similar between treatments, but cows fed the P169 consumed less dry matter, which resulted in a 4.4% increase in energetic efficiency.

Ruminal anaerobic bacteria were also studied as DFM sources for dairy cows. *Prevotella bryantii* 25A was used as a DFM to dairy cows in early lactation (Chiquette et al., 2008). Six cows were given 2×10^{11} cells/dose of *P. bryantii* 25A, inoculated directly with a syringe through the rumen cannula. Administration of *P. bryantii* 25A did not change milk yield, but tended to increase milk fat in accordance with increased acetate and butyrate concentrations in the rumen. *P. bryantii* 25A also decreased lactate concentration after 2-3 h feeding compared with control treatments, thereby exhibiting the potential to prevent acidosis (Chiquette et al., 2008). Exogenous cellulolytic bacteria have been studied as DFM to improve ruminal fermentation (Chiquette et al., 2007). *Ruminococcus flavefaciens* NJ, isolated from the rumen of a wild moose, was supplemented into the rumens of non-lactating dairy cows fed either a high concentrate or a high forage diet daily. NJ modified the abundances of other cellulolytic bacterial populations, and improved *in sacco* digestibility of timothy hay in the rumen when fed as part of a high concentrate diet. The presence of *Aspergillus oryzae* or *S. cerevisiae*, or a change of concentrate to forage ratio in the diet did not succeed in establishing the new strain in the rumen. In an early study, genetically marked *Ruminococcus albus* was inoculated into the rumen of a goat and the extent of bacterial survival in the rumen was measured (Miyagi et al., 1995). *R. albus* persisted in the rumen for 14 d at 10^2 cells/ml of rumen contents.

STRATEGIES OF DFM APPLICATION FOR RUMINANT ANIMALS

Aero-tolerant microorganisms as DFM sources

As discussed above, microbials for DFM must be:

i) viable during preparation and delivery to animals
ii) able to survive in digestive environments

Cultivation and preparation of ready-to-use strict anaerobes may be cost-prohibitive. Any dosing method other than adding DFM to the diet is unlikely to be acceptable as a general on-farm practice (Nagaraja et al., 1997), especially for daily dosing. Individual administration may be labor and time-intensive and prohibitive for large feedlots. DFM studies of strict anaerobic bacterial species generally focus on establishment of exogenous or genetically modified strains after short-term administration (Jones and Megaritty, 1986; Robinson et al., 1992; Miyagi et al., 1995; Gregg et al., 1998; Chiquette et al., 2007), while studies of facultative or aero-tolerant anaerobic bacterial species include long-term daily supercharging in the rumen (Swinney-Floyd et al., 1999; Ohya et al., 2000; Elam et al., 2003; Krehbiel et al., 2003). *Synergesties jonesii* (Jones and Megaritty, 1986) and *B. fibrisolvens* (Gregg et al., 1998) established populations in the rumen, while *R. albus* strain A3 (Miyagi et al., 1995) and *R. flavefaciens* NJ (Chiquette et al., 2007) did not persist in the rumen at effective population sizes. However, repeated dosing increased cell numbers of *R. flavefaciens* NJ in the rumen. The chance to succeed as a DFM with one-time administration may be limited to only a few strains. Therefore, innate or acquired aero-tolerance may be an important criterion for DFM to be useful to supercharge populations daily or establish populations in the rumen.

An experiment was conducted to evaluate potentiality of aero-tolerant rumen LUB (Kim, 2007). Ruminal contents were collected from dairy cattle and enriched in lactic acid media anaerobically via two transfers (N2), and then used as inocula for further enrichments. A strict anaerobic preparation (N6) was enriched through four additional anaerobic subcultures. An aero-tolerant preparation (N2A2N2) was passed through two aerobic subculturing and then two anaerobic enrichments. An aerobic preparation (N2A4) passed 4 aerobic enrichments. When these enrichments were added to acidosis-inducing *in vitro*

Table 6. Effects of DFM containing bacilli on ruminant performance

Strains	Animals	Effects	References
Bacillus licheniformis *Bacillus subtilis*	Sheep and lambs	Control group tended to have higher mortality than the DFM treated group and produced significantly more milk.	(Kritas et al., 2006)
Bacillus licheniformis *Bacillus subtilis*	Holstein cows	Milk yield and protein were increased by supplementation of bacilli. *Bacillus licheniformis* increased ruminal digestibility and total VFA concentration.	(Qiao et al., 2009)
Bacillus subtilis	Holstein calves	Fecal shedding of presumptive *Clostridium perfringens* at day 7 was reduced in scouring calves treated with electrolytes plus DFM compared to scouring calves treated with electrolytes alone.	(Wehnes et al., 2009)
Bacillus licheniformis *Bacillus subtilis*	Holstein calves	Cows fed DFM had a higher ADG, final live weight.	(Kowalski et al., 2009)
Bacillus cereus var. Toyoi *Saccharomyces boulardii*	Sheep	Both probiotics enhanced humoral immunity.	(Roos et al., 2010)
Bacillus subtilis strain 166	Cattle	There were no dignificant differences observed between treatments for either hide or fecal prevalence of *E. coli* O157:H7.	(Arthur et al., 2010)

ruminal fermentation, N2A4 completely inhibited lactate accumulation, yielded greater total VFA and maintained higher pH than N6 or N2A2N2. Aerobic enrichment may increase the chances to isolate aero-tolerant lactic acid-utilizers by reducing strict anaerobes in the culture. The current study also supports the potential use of aero-tolerant rumen microorganisms as DFM for cattle. However, there are only a few species of aero-tolerant microorganisms. Aero-tolerance is required only during delivery to the rumen, and does not guarantee that a microorganism will be effective as DFM.

Spore forming bacteria as DFM sources

Tolerance of microorganisms to heat is also important for DFM since they have to survive processing during feed production. In general, most yeast and LAB are destroyed by heat during pelleting (Kung Jr, 2001). Spore forming bacteria have advantages as probiotics for humans and animals (Ripamonti et al., 2009). Ripamonti et al. (2009) suggested that the ability to form spores provides probiotics (DFM) with higher resistance to stresses during production and storage processes (Hyronimus et al., 2000) and also higher resistance to gastric and intestinal environmental conditions (Sanders et al., 2003; Hong et al., 2005).

Several recent studies demonstrated the probiotic (DFM) effects of bacilli, spore forming bacteria, on ruminant performance (Table 6). *Bacillus* species have specific mechanisms that inhibit gastrointestinal infection by pathogens or producing antimicrobials.

Kritas et al. (2006) examined the effects of DFM containing *Bacillus licheniformis* and *B. subtilis* on young lambs and milking ewes under field conditions. The addition of DFM tended to reduce the mortality of young lambs and increased the daily milk yield of ewes. Another experiment regarding bacilli DFM was conducted in China (Qiao et al., 2009), and yields of 4% fat corrected milk (FCM), FCM/dry matter intake, and milk protein percentages were increased after *B. licheniformis* supplementation. Total VFA and acetate concentrations were higher with *B. licheniformis* than in the other two groups, *B. subtilis* or animals that received no supplements.

In addition to the practical advantages of spore forming DFM, strong cellulolytic activity may support the potential of bacilli as DFM for ruminant or nonruminant animals by improving fiber digestion in the rumen and/or in the GIT by supplying oligosaccharides to beneficial microorganisms.

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REFERENCES

Abe, F., N. Ishibashi and S. Shimamura. 1995. Effect of administration of bifidobacteria and lactic acid bacteria to

newborn calves and piglets. J. Dairy Sci. 78:2838-2846.

Abu-Tarboush, H. M., M. Y. Al-Saiady and A. H. Keir El-Din. 1996. Evaluation of diet containing lactobacilli on performance, fecal coliform, and lactobacilli of young dairy calves. Anim. Feed Sci. Technol. 57:39-49.

Adams, M. C., J. Luo, D. Rayward, S. King, R. Gibson and G. H. Moghaddam. 2008. Selection of a novel direct-fed microbial to enhance weight gain in intensively reared calves. Anim. Feed Sci. Technol. 145:41-52.

Arthur, T. M., J. M. Bosilevac, N. Kalchayanand, J. E. Wells, S. D. Shackelfold, T. L. Wheeler and M. Koohmaraie. 2010. Evaluation of a direct-fed microbial product effect on the prevalence and load of escherichia coli o157:H7 in feedlot cattle. J. Food Prot. 73:366-371.

Axelsson, L. T., T. C. Chung, W. Dobrogosz and S. E. Lidgren. 1989. Production of a broad spectrum antimicrobial substance by *Lactobacillus reuteri*. Microb. Ecol. Health Dis. 2:131-136.

Beharka, A. A., T. G. Nagaraja and J. L. Morrill. 1991. Performance and ruminal function development of young calves fed diets with aspergillus oryzae fermentation extract. J. Dairy Sci. 74:4326-4336.

Carlsson, J., Y. Iwami and T. Yamada. 1983. Hydrogen peroxide excretion by oral streptococci and effect of lactoperoxidase thiocyanate-hydrogen peroxide. Inf. Immunol. 40:70-80.

Chaucheyras, F., G. Fonty, G. Bertin, J. M. Salmon and P. Gouet. 1995. Effects of a strain of *Saccharomyces cerevisiae* (Levucell SC), a microbial additive for ruminants, on lactate metabolism *in vitro*. Can. J. Microbiol. 42:927-933.

Chiquette, J., M. J. Allison and M. A. Rasmussen. 2008. Prevotella bryantii 25a used as a probiotic in early-lactation dairy cows: Effect on ruminal fermentation characteristics, milk production, and milk composition. J. Dairy Sci. 91:3536-3543.

Cotter, P. D., C. Hill and R. P. Ross. 2005. Bacteriocins: developing innate immunity for food. Nat. Rev. Microbiol. 3:777-788.

Cruywagen, C. W., I. Jordaan and L. Venter. 1996. Effect of lactobacillus acidophilus supplementation of milk replacer on preweaning performance of calves. J. Dairy Sci. 79:483-486.

Dicks, L. M. T. and M. Botes. 2010. Probiotic lactic acid bacteria in the gastro-intestinal tract: Health benefits, safety and mode of action. Benef. Microbes 1:11-29.

Dobrogosz, W. J., I. A. Casas, G. A. Pagano, T. L. Talarico, B. M.Sjöberg and M. Karlsson. 1989. *Lactobacillus reuteri* and the enteric microbiota. In: The Regulatory and protective role of the normal microflora (Ed. E. Norin). pp. 283-292. Stockton Press. New York.

Elam, N. A., J. F. Gleghorm, J. D. Rivera, M. L. Galyean, P. J. Defoor, M. M. Brashears and S. M. Younts-Dahl. 2003. Effects of live cultures of lactobacillus acidophilus (strains np45 and np51) and propionibacterium freudenreichii on performance, carcass, and intestinal characteristics, and escherichia coli strain o157 shedding of finishing beef steers. J. Anim. Sci. 81: 2686-2698.

Forestier, C., C. De Champs, C. Vatoux and B. Joly. 2001. Probiotic activities of *Lactobacillus casei rhamnosus*: *in vitro* adherence to intestinal cells and antimicrobial properties. Res. Microbiol. 152:167-173.

Frizzo, L. S., L. P. Sotto, M. V. Zbrun, E. Bertozzi, G. Sequeira, R. R. Armesto and M. R. Rosmini. 2010. Lactic acid bacteria to improve growth performance in young calves fed milk replacer and spray-dried whey powder. Anim. Feed Sci. Technol. 157:159-167.

Fuller, R. 1989. A review: Probiotics in man and animals. J. Appl. Bacteriol. 66:365-378.

Galyean, M. L., G. A. Nunnery, P. J. Defoor, G. B. Salyer and C. H. Parsons. 2000. Effects of live cultures of *Lactobacillus acidophilus* (Strains 45 and 51) and *Propionibacterium freudenreichii* PF-24 on performance and carcass characteristics of finishing beef steers. Available: http://www.asft.ttu.edu/burnettcenter/progressreports/bc8.pdf. Accessed June 27, 2002.

Ghorbani, G. R., D. P. Morgavi, K. A. Beauchemin and J. A. Z. Leedle. 2002. Effects of bacterial direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle. J. Anim. Sci. 80:1977-1985.

Gilliland, S. E. 1989. Acidophilus milk products: a review of potential benefits to consumers. J. Dairy Sci. 72:2483-2494.

Gregg, K., B. Hamdorf, K. Henderson, J. Kopecny and C. Wong. 1998. Genetically modified ruminal bacteria protect sheep from fluoroacetic acid poisoning. Appl. Environ. Microbiol. 64:3496-3498.

Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J. Anim. Sci. 73:2820-2833.

Holzapfel, W. H., R. Geisen and U. Schillinger. 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int. J. Food Microbiol. 24:343-362.

Hong, H. A., L. H. Duc and S. M. Cutting. 2005. The use of bacterial spore formers as probiotics. FEMS Microbiol. Rev. 29:813-835.

Hyronimus, B., C. Le Marrec, A. Hadj Sassi and A. Deschamps. 2000. Acid and bile tolerance of spore-forming lactic acid bacteria. Int. J. Food Microbiol. 61:193-197.

Jones, R. J. and R. G. Megaritty. 1986. Successful transfer of DHP-degrading bacteria from Hawaiian goats to Australian ruminants to overcome the toxicity of Leucaena. Aust. Vet. J. 63:259-262.

Jones, G. W. and J. M. Rutter. 1972. Role of K88 antigen in the pathogenesis of neonatal diarrhoea caused by *Escherichia coli* in piglets. Infect. Immun. 6:918-927.

Jouany, J. P., F. Mathieu, J. Senaud, J. Bohatier, G. Bertin and M. Mercier. 1999. Influence of protozoa and fungal additives on ruminal pH and redox potential. S. Afr. J. Anim. Sci. 29:65-66.

Keyser, S. A., J. P. McMeniman, D. R. Smith, J. C. MacDonald and M. L. Galyean. 2007. Effects of saccharomyces cerevisiae subspecies boulardii cncm i-1079 on feed intake by healthy beef cattle treated with florfenicol and on health and performance of newly received beef heifers. J. Anim. Sci. 85: 1264-1273.

Kim, S. W. 2006. Development of a direct-fed microbial for beef cattle. PhD Dissertation. Mich. Stat. Univ. East Lansing, MI.

Kowalski, Z. M., P. Gorka, A. Schlagheck, W. Jagusiak, P. Micek and J. Strzetelski. 2009. Performance of holstein calves fed milk-replacer and starter mixture supplemented with probiotic feed additive. J. Anim. Feed Sci. 18:399-411.

Krehbiel, C. R., S. R. Rust, G. Zhang and S. E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. J. Anim. Sci. 81:E120-132.

Kritas, S. K., A. Govaris, G. Christodoulopoulos and A. R. Burriel. 2006. Effect of *Bacillus licheniformis* and *Bacillus subtilis* supplementation of ewe's feed on sheep milk production and young lamb mortality. J. Vet. Med. Series A. 53:170-173.

Kung, L., Jr. and A. O. Hession. 1995. Preventing *in vitro* lactic acid accumulation in ruminal fermentations by inoculation with *Megasphaera elsdenii*. J. Anim. Sci. 73:250-256.

Kung Jr, L. 2001. Direct-fed microbials for dairy cows and enzymes for lactating dairy cows: New theories and applications. In: 2001 Pennsylvania State Dairy Cattle Nutrition Workshop, Grantville, PA. pp. 86-102.

Lee, Y. K., K. Y. Puong, A. C. Ouwehand and S. Salminen. 2003. Displacement of bacterial pathogens from mucus and Caco-2 cell surface by lactobacilli. J. Med. Microbiol. 52:925-930.

Lehloenya, K. V., C. R. Krehbiel, K. J. Mertz, T. G. Rehberger and L. J. Spicer. 2008. Effects of propionibacteria and yeast culture fed to steers on nutrient intake and site and extent of digestion. J. Dairy Sci. 91:653-662.

Lynch, H. A. and S. A. Martin. 2002. Effects of *Saccharomyces cerevisiae* culture and *Saccharomyces cerevisiae* live cells on *in vitro* mixed ruminal microorganism fermentation. J. Dairy Sci. 85:2603-2608.

Malik, R. and S. Bandla. 2010. Effect of source and dose of probiotics and exogenous fibrolytic enzymes (EFE) on intake, feed efficiency, and growth of male buffalo (*bubalus bubalis*) calves. Trop. Anim. Health Prod. 42:1263-1269.

Matsuguchi, T., A. Takagi, T. Matsuzaki, M. Nagaoka, K. Ishikawa and T. Yokokura. 2003. Lipoteichoic acids from *Lactobacillus* strains elicit strong tumor necrosis factor a-inducing activities in macrophage through Toll-like receptor 2. Clin. Diagn. Lab. Immunol. 10:259-266.

Miettinen, M., J. Vuopio-Varkila and K. Varkila. 1996. Production of human necrosis factor a, interleukin 6, and interleukin 10 is induced by lactic acid bacteria. Infect. Immun. 64:5403-5405.

Miyagi, T., K. Kaneichi, R. I. Aminov, Y. Kobayashi, K. Sakka, S. Hoshino and K. Ohmiya. 1995. Enumeration of transconjugated *Ruminococcus albus* and its survival in the goat rumen. Appl. Environ. Microbiol. 61:2030-2032.

Nagaraja, T. G., C. J. Newbold, C. J. Van Nevel and D. I. Demeyer. 1997. Manipulation of ruminal fermentation. pp. 523-632 in the Rumen Microbial Ecosystem (Ed. P. N. Hobson and C. S. Stewart). Blackie Academic & Professional, London, NY.

Nocek, J. E., W. P. Kautz, J. A. Z. Leedle and J. G. Allman. 2002. Ruminal supplementation of direct-fed microbials on diurnal ph variation and *in situ* digestion in dairy cattle. J. Dairy Sci. 85:429-433.

Nocek, J. E., W. P. Kautz, J. A. Z. Leedle and E. Block. 2003. Direct-fed microbial supplementation on the performance of dairy cattle during the transition period. J. Dairy Sci. 86:331-335.

Nocek, J. E. and W. P. Kautz. 2006. Direct-fed microbial supplementation on ruminal digestion, health, and performance of pre- and postpartum dairy cattle. J. Dairy Sci. 89:260-266.

Oetzel, G. R., K. M. Emery, W. P. Kautz and J. E. Nocek. 2007. Direct-fed microbial supplementation and health and performance of pre- and postpartum dairy cattle: A field trial. J. Dairy Sci. 90:2058-2068.

Ohya, T., T. Marubashi and H. Ito. 2000. Significance of fecal volatile fatty acids in shedding of *Escherichia coli* O157 from calves: experimental infection and preliminary use of a probiotic product. J. Vet. Med. Sci. 62:1151-1155.

Pratt, W. C. 2001. Methods for maintaining and administering live probiotic as feed additives for animals. US Patent 5401501. Available: http://www.patentstorm. us/patents/5401501-fulltext. html. Accessed Jun. 15, 2007.

Qiao, G. H., A. S. Shan, N. Ma, Q. Q. Ma and Z. W. Sun. 2009. Effect of supplemental bacillus cultures on rumen fermentation and milk yield in Chinese Holstein cows. J. Anim. Physiol. Anim. Nutr. 94:429-436.

Raeth-Knight, M. L., J. G. Linn and H. G. Jung. 2007. Effect of direct-fed microbials on performance, diet digestibility, and rumen characteristics of holstein dairy cows. J. Dairy Sci. 90: 1802-1809.

Reynolds, C. K., P. C. Aikman, B. Lupoli, D. J. Humphries and D. E. Beever. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. J. Dairy Sci. 86:1201-1217.

Ripamonti, B., A. Agazzi, A. Baldi, C. Balzaretti, C. Bersani, S. Pirani, R. Rebucci, G. Savoini, S. Stella, A. Stenico and C. Domeneghini. 2009. Administration of *Bacillus coagulans* in calves: Recovery from faecal samples and evaluation of functional aspects of spores. Vet. Res. Commun. 33:991-1001.

Robinson, J. A., W. J. Smolenski, R. C. Greening, M. L. Ogilvie, R. L. Bell, K. Barsuhn and J. P. Peters. 1992. Prevention of acute acidosis and enhancement of feed intake in the bovine by *Megasphaera elsdenii* 407A. J. Anim. Sci. 70 (Suppl. 1):310 (Abstr.).

Roger, V., G. Fonty, S. Komisarczuk-Bony and P. Gouet. 1990. Effects of physicochemical factors on the adhesion to cellulose Avicel of the ruminal bacteria *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* subsp. *succinogenes*. Appl. Environ. Microbiol. 56:3081-3087.

Rose, A. H. 1987. Responses to the chemical environment. In: *The Yeasts* (Ed. A. H. Rose and J. S. Harrisson) Vol. 2, Academic Press, London (1987), pp. 5-40.

Roos, T. B., V. C. Tabeleão, L. A. Dümmer, E. Schwegler, M. A. Goulart, S. V. Moura, M. N. Corrêa, F. P. L. Leite and C. Gil-Turnes. 2010. Effect of *Bacillus cereus* var. Toyoi and *Saccharomyces boulardii* on the immune response of sheep to vaccines. Food Agric. Immunol. 21:113-118.

Sanders, M. E., L. Morelli and T. A. Tompkins. 2003. Sporeformers as human probiotics: *Bacillus*, *Sporolactobacillus*, and *Brevibacillus*. Compr. Rev. Food Sci. Food Saf. 2:101-110.

Silva, M., N. V. Jacobus, C. Deneke and S. L. Gorbach. 1987. Antimicrobial substance from a human *Lactobacillus* strain. Antimicrob. Agents Chemother. 31:1231-1233.

Stein, D. R., D. T. Allen, E. B. Perry, J. C. Bruner, K. W. Gates, T. G. Rehberger, K. Mertz, D. Jones and L. J. Spicer. 2006. Effects of feeding propionibacteria to dairy cows on milk yield, milk components, and reproduction. J. Dairy Sci. 89:111-125.

Swinney-Floyd, D., B. A. Gardiner, F. N. Owens and T. Rehberger. 1999. Effects of inoculation with either *Propionibacterium* strain P-63 alone or in combination with *Lactobacillus acidophilus* strain LA53545 on performance of feedlot cattle. J.

Anim. Sci. 77 (Suppl.):77 (Abstr.).

Tamate, H., A. D. McGilliard, N. L. Jacobson and R. Getty. 1961. Effect of various dietaries on the anatomical development of the stomach in the calf. J. Dairy Sci. 45:408-420.

Yoon, I. K. and M. D. Stern. 1995. Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants: A review. Asian-Aust. J. Anim. Sci. 8:533-555.

Wehnes, C., K. Novak, V. Patskevich, D. Shields, J. Coalson, A. Smith, M. Davis and T. Rehberger. 2009. Benefits of supplementation of an electrolyte scour treatment with a bacillus-based direct-fed microbial for calves. Probiotics Antimicrob. Proteins 1:36-44.

Weiss, W. P., D. J. Wyatt and T. R. McKelvey. 2008. Effect of feeding propionibacteria on milk production by early lactation dairy cows. J. Dairy Sci. 91:646-652.