Journal of Integrative Agriculture 2015, 14(3): 526–533

Available online at www.sciencedirect.com

ScienceDirect

REVIEW

Direct-fed microbes: A tool for improving the utilization of low quality roughages in ruminants

Mona M Y Elghandour[1], Abdelfattah Z M Salem[1], Jose S Martínez Castañeda[1], Luis M Camacho[2], Ahmed E Kholif[3], Juan C Vázquez Chagoyán[1]

[1] *Faculty of Veterinary Medicine and Animal Science, Autonomous University of the State of Mexico, Toluca P.O. 50000, Mexico*
[2] *Faculty of Veterinary Medicine and Animal Science, Autonomous University of Guerrero, Altamirano P.O. 40660, Mexico*
[3] *Dairy Science Department, National Research Centre, 33 Bohouth st. Dokki, Giza P.O. 12311, Egypt*

Abstract

For many years, ruminant nutritionists and microbiologists have been interested in manipulating the microbial ecosystem of the rumen to improve production efficiency of different ruminant species. Removal and restriction of antibiotics sub-therapeutic uses from ruminant diets has amplified interest in improving nutrient utilization and animal performance and search for more safe alternatives. Some bacterial and fungal microorganisms as a direct-fed microbial (DFM) can be the most suitable solutions. Microorganisms that are commonly used in DFM for ruminants may be classified mainly as lactic acid producing bacteria (LAB), lactic acid utilizing bacteria (LUB), or other microorganism's species like *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, *Bacillus*, *Propionibacterium*, *Megasphaera elsdenii* and *Prevotellabryantii*, in addition to some fungal species of yeast such as *Saccharomyces* and *Aspergillus*. A definitive mode of action for bacterial or fungal DFM has not been established; although a variety of mechanisms have been suggested. Bacterial DFM potentially moderate rumen conditions, and improve weight gain and feed efficiency. Fungal DFM may reduce harmful oxygen from the rumen, prevent excess lactate production, increase feed digestibility, and alter rumen fermentation patterns. DFM may also compete with and inhibit the growth of pathogens, immune system modulation, and modulate microbial balance in the gastrointestinal tract. Improved dry matter intake, milk yield, fat corrected milk yield and milk fat content were obtained with DFM administration. However, the response to DFM is not constant; depending on dosages, feeding times and frequencies, and strains of DFM. Nonetheless, recent studies have supported the positive effects of DFM on ruminant performance.

Keywords: direct-fed microbial (DFM), mode of action, ruminants

1. Introduction

The main goals of rumen microbial studies are to improve feed utilization, animal production and health, and animal food safety, which may be achieved by facilitating desirable fermentation, minimizing ruminal disorders, and excluding pathogens. For the past few decades, a number of chemical feed additives such as antibiotics, ionophores, methane inhibitors and defaunating agents have been used in rumi-

Received 3 September, 2013 Accepted 6 May, 2014
Correspondence Abdelfattah Z M Salem, E-mail: asalem70@yahoo.com

doi: 10.1016/S2095-3119(14)60834-0

nant nutrition to manipulate the microbial ecosystem and fermentation characteristics in the rumen and intestinal tract of livestock (Seo *et al.* 2010). Due to probable toxicity problems to the host animals, these feed additives are not routinely used (Salem *et al*. 2014a, b). Recently, a great awareness from public health aspects such as residues of these chemicals in milk and meat, and bacterial resistance to antibiotics as a result of increased use in the food chains prohibits their use as feed additives (Barton 2000). These supplements have been criticized by the consumers' organizations on the ground of product safety and quality. The consumers' demands have stimulated to search for natural alternatives to chemical feed additives. Supplementation with probiotics that can survive in the rumen has become a suitable alternative (Fon and Nsahlai 2013).

Therefore, this review summarizes the effects of direct-fed microbial (DFM) on rumen fermentation, methane inhibition, microbial populations and ruminant performance as growth, milk production and the efficiency of feed utilization.

2. Direct-fed microbial

The term "probiotic" is composed from two parts of Greek words: "pro" which means in favor and "biotic" which means life. 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). However, as pointed out by Vanbelle *et al.* (1990), many researchers accept that probiotic refers to "selected and concentrated viable counts of lactic acid bacteria *Lactobacillus*, *Streptococcus*". Moreover, Kmet *et al.* (1993) defined the term probiotics as "live cultures of microorganisms that are deliberately introduced into the rumen with the aim of improving animal health or nutrition". The Food and Drug Administration of USA has required feed manufacturers to use the term "direct-fed microbial" instead of probiotic (Miles and Bootwalla 1991) and has narrowed the definition to "a source of live, naturally occurring microorganisms" (Yoon and Stern 1995). Krehbiel *et al.* (2003) and Yang *et al.* (2004) defined the DFM as "alive, naturally occurring microorganisms that have been used to improve digestive function of livestock". The definition of DFM is very broad and may include specific and nonspecific yeast, fungi, bacteria, cell fragments, and filtrates (Sullivan and Martin 1999; Oetzel *et al.* 2007; Elghandour *et al*. 2014b). DFM grow in the rumen and beneficially modify its microbial ecosystem and/or fermentation characteristics. The intestinal tract may also provide a suitable habitat for DFM (Seo *et al.* 2010).

There are many different types of DFM being used in livestock production. They can be classified into three main categories; bacterial, fungal, and a combination of both. The bacterial DFM is the most common. The bacterial DFM strains may be classified as lactic acid producing bacteria (LAB), lactic acid utilizing bacteria (LUB), or other microorganisms. *Lactobacillus*, *Propionibacterium*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, and *Bacillus*, all of which are common microorganisms used in bacterial DFM for ruminants, in addition to other distinctive bacterial species such as *Megasphaera elsdenii* and *Prevotella bryantii* (Kung 2006; Seo *et al.* 2010). Development of this organism for ruminant animals should be continued with emphasis on optimizing dose and timing of administration. Success with such organisms could allow feedlot producers to decrease the time it takes to adapt cattle to a high concentrate diet. It could also be useful by reducing chronic acidosis in lactating cows (Kung 2006). The response to DFM was inconstant in ruminants; however, it has been positive in many experiments.

3. DFM mode of action

3.1. Bacterial DFM

The mode of action of DFM depends on many factors, such as dosages, feeding times and frequencies, and strains of DFM. Some of DFM act within the rumen while others impact the gastrointestinal tract (Puniya *et al*. 2015).

(1) Within rumen: The mode of action of different DFM sources within the rumen depends mainly on LAB and LUB. LAB might affect the rumen positively through preventing 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 LUB. LUB have been proposed as DFM that can decrease concentrations of lactate and maintain ruminal pH. *Megasphaera elsdenii* is the major lactate-utilizing bacterium in the rumen that prevents the drastic pH drops caused by accumulation of lactate in the rumen when fed a highly fermentable diet (Yang *et al.* 2004; Kung 2006) or prevents lactic acidosis in steers (Robinson *et al.* 1992). This bacteria simultaneously uses lactate, glucose, and maltose (Russell and Baldwin 1978) and would compete with lactate-producing organisms for substrate. During the feeding of readily degradable soluble carbohydrates, *M. elsdenii* seems to be the major ruminal lactate utilizer because *Selenomonas ruminantium* undergoes catabolite repression (Russell and Baldwin 1978) and is relatively acid-intolerant (Mackie and Gilchrist 1979).

Another bacterial species is the *Propionibacteria* which is naturally found in high numbers in the rumen of animals fed forage and medium concentrate diets (Kung 2006). Propionate is quantitatively the most important single precursor of glucose synthesis among volatile fatty acids (VFA), and tissue distribution of nutrient (Nagaraja *et al.* 1997). Certain

species of *Propionibacteria* were reported to modify rumen fermentation and increase the molar portion of ruminal propionate (Stein *et al.* 2006). It can ferment lactate to propionate in early lactation dairy cows (Reynolds *et al.* 2003; Kung 2006) resulting in increased hepatic glucose production (Stein *et al.* 2006), providing more substrates for lactose synthesis, improving energetic efficiency and reducing ketosis (Weiss *et al.* 2008). For growing ruminants and lactating cows, propionate has been estimated to account for 61 to 67% of glucose release (Reynolds *et al.* 1994; Huntington 2000). Also, increased propionate has been accompanied with a decrease in methane (CH_4) production according to the stoichiometric laws of chemical balance and its equation (van Soest 1994). When the acetate:propionate ratio decreases, CH_4 production declines, and energy retention by cattle would theoretically increase (Wolin 1960).

Feeding *Propionibacterium* increased protozoa especially *Entodinium* with decreased amylolytic bacteria in the rumen of feedlot steers (Ghorbani *et al.* 2002). The mechanism by which bacterial DFM stimulate protozoa remains unclear (Ghorbani *et al.* 2002).

(2) Within the post-ruminal gastrointestinal tract. Many proposals were adjusted to elucidate the mode of action of DFM within post-ruminal gastrointestinal tract (GIT) (Seo *et al.* 2010). DFM can inhibit or prevent pathogen like *Escherichia coli* establishment attached to the intestinal mucosa *via* hydrophobic interactions and limit pathogens from attaching to the enterocytic receptor or producing enterotoxins that can induce diarrhea (Lee *et al.* 2003; Kung 2006). LAB was able to adhere to the intestinal tract and protect animals against *Salmonella* (Frizzo *et al.* 2010). In addition to the role of LAB of producing lactate and acetate as main metabolic end-products, it had critical roles in penetrating microbial cells and interfering with essential cell function (Holzapfel *et al.* 1995).

Another mechanism is that DFM like LAB can produce antibacterial compounds such as bacteriocin and hydrogen peroxide that have a competitive exclusion and probiotic characteristics. Hydrogen peroxide can oxidize the sulfhydryl groups in metabolic enzymes such as glucose transport enzymes, hexokinase, and glycerol aldehyde-3-phosphate dehydrogenase causing glycolysis blocking (Carlsson *et al.* 1983; Dicks and Botes 2010). In contrast, LAB bacteriocins can inhibit the binding of substrates to the subunit of ribonucleotide reductase so as to interfer with DNA-synthesis of target microorganisms (Cotter *et al.* 2005; Dicks and Botes 2010).

A newly discovered mechanism is that DFM have the ability to modulate host immune function. 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 (Krehbiel *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 *Lactobacillus casei* Shirota and *Lactobacillus rhamnosus* Lr23 stimulated macrophages to secrete TNF-α or promote development of regulatory dendritic cells (Seo *et al.* 2010).

3.2. Fungal DFM

Fungal DFM have been extensively used in ruminants for improving performance and normalizing rumen fermentation. *Saccharomyces cerevisiae* and *Aspergillus oryzae* are the most common used species (Elghandour *et al*. 2014a; Puniya *et al*. 2015).

A variety of mechanisms have been put to explain changes in ruminal fermentations and improvements in performance when ruminants are fed fungal-based DFM. The mode of action can be illustrated based on many facts. Yeast may have a buffering effect in the rumen by mediating the sharp drops in rumen pH (Elghandour *et al*. 2014a, b). Fungal cultures may improve the use of lactate by the ruminal organism, *Selenomonas ruminantium*, by providing a source of dicarboxcylic acids (e.g., malic acid) and other growth factors (Martin and Streeter 1995). Thus, yeast may help to buffer excess lactic acid production when ruminants are fed high concentrate diets (Kung 2006). Moreover, yeasts can remove oxygen on the surfaces of freshly ingested feed to maintain metabolic activity in the rumen (Newbold *et al.* 1996) and keep the rumen as anaerobic chamber. Another mechanism depends on the ability of yeast to decrease the redox potential in the rumen (Jouany *et al.* 1999) which provides a better condition for the growth of strict anaerobic cellulolytic bacteria, and 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), which preventing lactate accumulation in the rumen, providing growth factors, such as organic acids or vitamins in the rumen, and resulting in stimulated ruminal cellulolytic bacteria and LUB (Chaucheyras *et al.* 1995).

The effects on buffering are subtle, as added yeast cannot prevent lactic acidosis if the rumen is challenged with a diet rich in fermentable carbohydrates (Dawson and Hopkins 1991; Aslan *et al.* 1995). The effect of fungal cultures on ruminal VFA has been inconsistent. Newbold *et al.* (1991) reported that fungal extracts had no effect or

tended to increase the rumen acetate: propionate ratio, while active yeast either had no effect or decreased the acetate: propionate ratio. There is no direct evidence that yeast or fungal extracts affect digestion or metabolism in the lower gut. However, the potential for such effects should not be overlooked.

4. Effect of DFM on ruminant performance

4.1. Preruminant calves

The young calves differ from the adult ruminants that they can digest a significant amount of ration nutrients in their intestine with the risk of intestinal proliferation of detrimental organisms that increased the chance for diarrhea and weight loss. Here come the roles of DFM administration which may obtain positively modified GIT function (Abu-Tarboush *et al.* 1996; Kung 2001). For dairy calves, repaid 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 experiment, Nakanishi *et al.* (1993) found that Holstein calves supplemented with yogurt containing *Lactobacillus acidophilus* tended to ruminate more at 30 d than untreated calves, indicating that *L. acidophilus* may promote ruminal development.

Dicks and Botes (2010) suggested that *Bifidobacteria* produces 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 other experiments, LAB was also inoculated into young calves to improve growth performance (Adams *et al.* 2008; Frizzo *et al.* 2010). Adams *et al.* (2008) examined the effect of *Propionibacterium jensenii* 702 (PJ702) on growth performance of young calves. There were improvements in weight gains with the treated group during both the pre-weaning and the weaning period with heavier calves final weight. Frizzo *et al.* (2010) fed young calves on milk replacer and a large quantity of spray-dried whey powder to generate an intestinal imbalance. Under these conditions, calves fed LAB had higher daily gain, total feed intake, and starter diet intake as well as lower fecal consistency index, indicating that diarrhea incidence was reduced.

The most common DFM species to young calves are *Lactobacillus* and *Streptococcus* species. Many reports have been documented a decreased incidence of diarrhea (Abu-Tarboush *et al.* 1996). Abu-Tarboush *et al.* (1996) found that feeding *L. acidophilus* 27SC to calves significantly lowered the incidence of diarrhea in calves. 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*.

4.2. Dairy cows

Limited research has evaluated the efficiency of bacterial DFM for lactating dairy cows. High producing cows in early lactation would be the best candidates for such products because these cows are in negative energy balance and have diets that contain highly fermentable carbohydrates that sometimes lead to acidosis (Kung 2006). During the period of 3 wk prior to calving to 3 wk after calving (i.e., transition periods; Oetzel *et al.* 2007), cows may be subject to many metabolic disorders such as sub-acute acidosis as a result of calving stress, changing diets to rapidly fermented carbohydrate sources, and lactation (Oetzel *et al.* 2007; Chiquette *et al.* 2008). In this case, DFM should be used to improve performance of dairy cows through increasing dry matter intake, milk yield and milk protein content, higher blood glucose and insulin levels at the pre- and/or post-partum periods (Nocek *et al.* 2003; Nocek and Kautz 2006; Oetzel *et al.* 2007). In the study of Weiss *et al.* (2008), they supplemented dairy cows from 2 wk before anticipated calving to 119 d in milk with *Propionibacterium* P169. Cows fed P169 had lower concentrations of acetate with greater concentrations of propionate and butyrate. Plasma glucose and plasma β-hydroxybutyrate levels were not affected by DFM, with higher concentrations of plasma non-esterified fatty acids. Cows fed DFM produced similar amounts of milk with similar composition as cows fed the control diet. Calculated net energy used for milk production, maintenance, and body weight change were similar between treatments, but cows fed *Propionibacterium* P169 consumed less dry matter, which resulted in a 4.4% increase in energetic efficiency.

Chiquette *et al.* (2008) used *P. bryantii* 25A as a DFM to dairy cows in early lactation. They found that 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 of feeding compared with control treatments, thereby exhibiting the potential to prevent acidosis.

Exogenous cellulolytic bacteria have been studied as DFM to improve ruminal fermentation (Chiquette *et al.* 2008; Khattab *et al*. 2011). *Ruminococcus flavefaciens* NJ, was supplemented into the rumen of non-lactating dairy cows fed either a high concentrate or a high forage diet daily. *R. flavefaciens* NJ modified the abundance 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.

4.3. Beef production

In finishing beef cattle, it is very important to prevent ruminal acidosis caused by highly fermentable feeds that are commonly be used. Beef cattle fed DFM showed improved growth performance, meat production, and feed efficiency in many experiments (Ghorbani *et al.* 2002; Krehbiel *et al.* 2003). DFM can have an important role on lowering newly received beef calves under stress on both newly received stressed calves and adult feedlot cattle. Newly received calves entering the feedlot heard undergo a variety of stress conditions, such as recent weaning, traction, and dehorning. Such conditions can alter microorganisms in the rumen and lower gut (Williams and Mahoney 1984), resulting in decreased performance and increased mortality and death loss. Administration of bacterial DFM to repopulate the gut might reduce these changes in the microbial population. The response to bacterial DFM might be greater when newly weaned and/or received calves are more prone to health problems. Krehbiel *et al.* (2003) administered 5×10^9 CFU LAB (*Enterococcus faecium*, *L. acidophilus*, *Bifidobacterium thermophilum*, and *Bifidobacterium longum*) to 466 newly received calves, to study the effects of LAB administration on health and performance. Daily gain did not differ among calves received DFM *vs*. those received 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 received no DFM.

The effects of administrating DFM on stressed calves are limited. But in general, results suggest that the addition of DFM to the diet can improve health and performance of stressed stocker calves. These data suggested that DFM might improve recovery of morbid newly received feedlot calves.

Regarding to supplementing diets of feedlot with DFM, results showed that supplementing diets with LAB or LUB can improve feed efficiency and daily gain of feedlot cattle (Galyean *et al.* 2000). Huck *et al.* (1999) studied the effects of feeding *L. acidophilus* BG2FO4 and *Propionibacterium freudenreichii* P-63 as a DFM on growth performance and carcass characteristics of finishing heifers for 126 d. Feeding either *L. acidophilus* BG2FO4 or *P. freudenreichii* P-63 did not affect daily gain, dry matter intake (DMI), or feed efficiency. These authors suggested that growth performance of finishing cattle could be improved by targeting the appropriate DFM to a particular phase of production. Also, Krehbiel *et al.* (2003) summarized results of many reports and suggested 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 was inconsistent. In studies reviewed, carcass weight was generally increased by 6 to 7 kg.

Another role for DFM in case of feedlot cattle is reduction of *Escherichia coli* from GIT. The species of *E. coli* O157:H7 are commonly isolated from 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. Many studies suggested the possible application of bacterial DFM to reduce fecal shedding of *E. coli* O157:H7 from cattle. Based on those results, supplementing feed for cattle with certain DFM might decrease the incidence of *E. coli* O157:H7 in feedlot cattle. An increase in VFA, especially acetate, correlated with the reducing of *E. coli* O157:H7. For example, Ohya *et al.* (2000) used LAB of *Streptococcus bovis* LCB6 and *Lactobacillus gallinarum* LCB 12 to eliminate *E. coli* O157:H7 from experimentally infected Holstein calves.

5. Conclusion

It could be indicated that supplying DFM can contribute to the ability of the rumen ecosystem to manage lactic acid production and utilization can be beneficial, even for animals that do not have clinical acidosis.

Acknowledgements

Elghandour M M Y wishes to thank the National Council for Science and Technology (CONACyT, Mexico) for the scholarship for her Ph D at the Autonomous University of the State of Mexico. Kholif A E thanks the National Council for Science and Technology (CONACyT, Mexico) and the World Academy of Sciences (TWAS, Italy) for his Postdoctoral fellowship from the Faculty of Veterinary Medicine and Animal Science, Autonomous University of the State of Mexico.

References

Abu-Tarboush H M, Al-Saiady M Y, Keir El-Din A H. 1996. Evaluation of diet containing lactobacilli on performance, fecal coliform, and lactobacilli of young dairy calves. *Animal Feed Science and Technology*, **57**, 39–49.

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

Aslan V S, Thamsborg M, Jorgensen R J, Basse A. 1995. Induced acute ruminal acidosis in goats treated with yeast (*Saccharomyces cerevisiae*) and bicarbonate. *Acta Veterinaria Scandinavica*, **36**, 65–68.

Barton M D. 2000. Antibiotic use in animal feed and its impact on human health. *Nutrition Research Reviews*, **13**, 279–299.

Bruce B B, Gilliland S E, Bush L J, Staley T E. 1979. Influence of feeding cells of *Lactobacillus acidophilus* on the fecal flora of young dairy calves. *Oklahoma Agricultural Experiment Station*, **104**, 207–209.

Carlsson J, Iwami Y, Yamada T. 1983. Hydrogen peroxide excretion by oral streptococci and effect of lactoperoxidase thiocyanate-hydrogen peroxide. *Infection and Immunity*, **40**, 70–80.

Chaucheyras F, Fonty G, Bertin G, Salmon J M, Gouet P. 1995. Effects of a strain of *Saccharomyces cerevisiae* (Levucell® SC1), a microbial additive for ruminants, on lactate metabolism *in vitro*. *Canadian Journal of Microbiology*, **42**, 927–933.

Chiquette J, Allison M J, Rasmussen M A. 2008. *Prevotellabryantii* 25a used as a probiotic in early-lactation dairy cows: Effect on ruminal fermentation characteristics, milk production, and milk composition. *Journal of Dairy Science*, **91**, 3536–3543.

Cotter P D, Hill C, Ross R P. 2005. Bacteriocins: developing innate immunity for food. *Nature Reviews Microbiology*, **3**, 777–788.

Dawson K A, Hopkins D M. 1991. Differential effects of live yeast on the cellulolytic activities of anaerobic ruminal bacteria. *Journal of Animal Scienc*e, **69**(Suppl. 1), 531.

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

Elghandour M M Y, Vázquez Chagoyán J C, Salem A Z M, Kholif A E, Martínez Castañeda J S, Camacho L M, Buendía G, 2014a. *In vitro* fermentative capacity of equine fecal inocula of 9 fibrous forages in the presence of different doses of *Saccharomyces cerevisiae*. *Journal of Equine Veterinary Science*, **34**, 619–625.

Elghandour M M Y, Vázquez Chagoyán J C, Salem A Z M, Kholif A E, Martínez Castañeda J S, Camacho L M, Cerrillo-Soto M A. 2014b. Effects of *Saccharomyces cerevisiae* at direct addition or pre-incubation on *in vitro* gas production kinetics and degradability of four fibrous feeds. *Italian Journal of Animal Science*, **13**, 295–301.

Fon F N, Nsahlai I V. 2013. Effect of direct-fed microbial consortia on ruminal fermentation of maize stover in sheep. *Small Ruminant Research*, **111**, 71–75.

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

Fuller R. 1989. A review: Probiotics in man and animals. *Journal of Applied Bacteriology*, **66**, 365–378.

Galyean M L, Nunnery G A, Defoor P J, Salyer G B, Parsons C H. 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*. *Burnett Center Progress Report*. No. 8. [2013-12-1] . http://www.afs.ttu.edu/burnett_center/progress_reports/bc8.pdf

Ghorbani G R, Morgavi D P, Beauchemin K A, Leede J A Z. 2002. Effect of bacterial direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle. *Journal of Animal Science*, **80**, 1977–1986.

Gilliland S E, Bruce B B, Bush L J, Staley T E. 1980. Comparison of two strains of *Lactobacillus acidophilus* as dietary adjuncts for young calves. *Journal of Dairy Science*, **63**, 964–972.

Gilliland S E. 1989. Acidophilus milk products: A review of potential benefits to consumers. *Journal of Dairy Science*, **72**, 2483–2494.

Holzapfel W H, Geisen R, Schillinger U. 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International Journal of Food Microbiology*, **24**, 343–362.

Huck G L, Kriekemeier K K, Ducharme G A. 1999. Effect of feeding *Lactobacillus acidophilus* BG2F04 (Micro cell) and *Propionibacterium freudenrechii* P-63 (MicroCell PB) on growth performance of finishing heifers. *Journal of Animal Science*, **77**(Suppl. 1), 264.

Huntington G B. 2000. High-starch rations for ruminant production discussed. *Feedstuffs*, **12**, 13–23.

Jenny B F, Vandijk H J, Collins J A. 1991. Performance and fecal flora of calves fed a *Bacillus subtilis* concentrate. *Journal of Dairy Science*, **74**, 1968–1973.

Jouany J P, Mathieu F, Senaud J, Bohatier J, Bertin G, Mercier M. 1999. Influence of protozoa and fungal additives on ruminal pH and redox potential. *South African Journal of Animal Science*, **29**, 65–66.

Khattab H M, Gado H M, Kholif A E, Mansour A M, Kholif A M. 2011. The potential of feeding goats sun dried rumen contents with or without bacterial inoculums as replacement for berseem clover and the effects on milk production and animal health. *International Journal of Dairy Science*, **6**, 267–277.

Kmet V, Flint H J, Wallace R J. 1993. Probiotics and manipulation of rumen development and function. *Archives of Animal Nutrition*, **44**, 1–10.

Krehbiel C R, Rust S R, Zhang G, Gilliland S E. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. *Journal of Animal Science*, **81**, E120–E132.

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.

Kung Jr L. 2006. Direct-fed microbial and enzyme feed additives. In: *2006 Direct-Fed Microbial*, *Enzyme and Forage Additive Compendium*. Miller Publishing, Minnetonka, MN.

Lee Y K, Puong K Y, Ouwehand A C, Salminen S. 2003. Displacement of bacterial pathogens from mucus and Caco-2 cell surface by lactobacilli. *Journal of Medical Microbiology*, **52**, 925–930.

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

Mackie R I, Gilchrist F M C. 1979. Changes in lactate-producing and lactate-utilizing bacteria in relation to pH in the rumen of sheep during stepwise adaptation to a high-concentrate diet. *Applied and Environmental Microbiology*, **67**, 422–430.

Martin S A, Streeter M N. 1995. Effect of malate on *in vitro* mixed ruminal microorganism fermentation. *Journal of Animal Science*, **73**, 2141–2145.

Matsuguchi T, Takagi A, Matsuzaki T, Nagaoka M, Ishikawa K, Yokokura T. 2003. Lipoteichoic acids from *Lactobacillus* strains elicit strong tumor necrosis factor α-inducing activities in macrophage through Toll-like receptor 2. *Clinical and Diagnostic Laboratory Immunology*, **10**, 259–266.

Miles R D, Bootwalla S M. 1991. Direct-fed microbials in animal production. In: *Direct-Fed Microbials in Animal Production. A Review*. National Feed Ingredient Association, West Des Moines, Iowa, USA. pp. 117–132.

Nagaraja T G, Newbold C J, Van Nevel C J, Demeyer D I. 1997. Manipulation of ruminal fermentation. In: Hobson P N, Stewart C S, eds., *Rumen Microbial Ecosystem*. 2nd ed. Blackie Academic and Professional, London, UK. pp. 523–632.

Nakanishi Y, Arave C W, Stewart P H. 1993. Effects of feeding *Lactobacillus acidophilus* yogurt on performance and behavior of dairy calves. *Journal of Dairy Science*, **76**(Suppl. 1), 244.

Newbold C J, Brock R, Wallace R J. 1991. Influence of autoclaved or irradiated *Aspergillus oryzae* fermentation extract on fermentation in the rumen simulation technique (Rusitec). *Journal of Agricultural Science* (Cambridge), **116**, 159–162.

Newbold C J, Wallace R J, McIntosh F M. 1996. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. *British Journal of Nutrition*, **76**, 249–261.

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

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

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

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

Ohya T, Marubashi T, Ito H. 2000. Significance of fecal volatile fatty acids in shedding of *Escherichia coli* O157 from calves: Experimental infection and preliminary use of a probiotic product. *Journal of Veterinary Medical Science*, **62**, 1151–1155.

Puniya A K, Salem A Z M, Kumar S, Dagar S S, Griffith G W, Puniya M, Ravella S R, Kumar N, Dhewa T, Kumar R. 2015. Role of live microbial feed supplements with reference to anaerobic fungi in ruminant productivity: A review. *Journal of Integrative Agriculture*, **14**, 550–560.

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

Reynolds C K, Harmon D L, Cecava M J. 1994. Absorption and delivery of nutrients for milk protein synthesis by portal-drained viscera. *Journal of Dairy Science*, **77**, 2787- 2808.

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

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

Russell J B, Baldwin R L. 1978. Substrate preferences in rumen bacteria: Evidence of catabolite regulatory mechanisms. *Applied and Environmental Microbiology*, **36**, 319–329.

Salem A Z M, Kholif A E, Elghandour M M Y, Buendía G, Mariezcurrena M D, Hernandez S R, Camacho L M. 2014a. Influence of oral administration of *Salix babylonica* extract on milk production and composition in dairy cows. *Italian Journal of Animal Science*, **13**, 10–14.

Salem A Z M, Kholif A E, Olivares M, Elghandour M M Y, Mellado M, Arece J. 2014b. Influence of *S. babylonica* extract on feed intake, growth performance and diet *in vitro* gas production profile in young lambs. *Tropical Animal Health and Production*, **46**, 213–219.

Seo J K, Kim J S, Kim M H, Upadhaya S D, Kam D K, Ha J K. 2010. Direct-fed microbials for ruminant animals. *Asian-Australasian Journal of Animal Sciences*, **23**, 1657–1667.

van Soest P J. 1994. *Nutritional Ecology of the Ruminant*. 2nd ed. Comstock, Ithaca, NY.

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

Sullivan H M, Martin S A. 1999. Effects of a *Saccharomyces*

cerevisiae culture on *in vitro* mixed ruminal microorganism fermentation. *Journal of Dairy Science*, **82**, 2011–2016.

Vanbelle M, Teller E, Focant M. 1990. Probiotics in animal nutrition: A review. *Archive of Animal Nutrition*, **40**, 543–567.

Weiss W P, Wyatt D J, McKelvey T R. 2008. Effect of feeding propionibacteria on milk production by early lactation dairy cows. *Journal of Dairy Science*, **91**, 646–652.

Williams D L, Mahoney J H. 1984. Pre-weaning and post-weaning nutrition. In: *Annual Convention of the American Association of Bovine Practice*. Stillwater. p. 98.

Wolin M J. 1960. A theoretical rumen fermentation balance. *Journal of Dairy Science*, **43**, 1452–1459.

Yang W Z, Beauchemin K A, Vedres D D, Ghorbani G R, Colombatto D, Morgavi D P. 2004. Effects of direct-fed microbial supplementation on ruminal acidosis, digestibility, and bacterial protein synthesis in continuous culture. *Animal Feed Science and Technology*, **114**, 179–193.

Yoon I K, Stern M D. 1995. Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants: A review. *Asian-Australasian Journal of Animal Sciences*, **8**, 533–555.

(Managing editor ZHANG Juan)