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REVIEW

Role of live microbial feed supplements with reference to anaerobic fungi in ruminant productivity: A review

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Abstract

To keep the concept of a safe food supply to the consumers, animal feed industries world over are showing an increasing interest in the direct-fed microbials (DFM) for improved animal performance in terms of growth or productivity. This becomes all the more essential in a situation, where a number of the residues of antibiotics and/or other growth stimulants reach in milk and meat with a number of associated potential risks for the consumers. Hence, in the absence of growth stimulants, a positive manipulation of the rumen microbial ecosystem to enhance the feedstuff utilization for improved production efficiency by ruminants has become of much interest to the researchers and entrepreneurs. A few genera of live microbes (i.e., bacteria, fungi and yeasts in different types of formulations from paste to powder) are infrequently used as DFM for the domestic ruminants. These DFM products are live microbial feed supplements containing naturally occurring microbes in the rumen. Among different DFM possibilities, anaerobic rumen fungi (ARF) based additives have been found to improve ruminant productivity consistently during feeding trials. Administration of ARF during the few trials conducted, led to the increased weight gain, milk production, and total tract digestibility of feed components in ruminants. Anaerobic fungi in the rumen display very strong cell-wall degrading cellulolytic and xylanolytic activities through rhizoid development, resulting in the physical disruption of feed structure paving the way for bacterial action. Significant improvements in the fiber digestibility were found to coincide with increases in ARF in the rumen indicating their role. Most of the researches based on DFM have indicated a positive response in nutrient digestion and methane reducing potential during *in vivo* and/or *in vitro* supplementation of ARF as DFM. Therefore, DFM especially ARF will gain popularity but it is necessary that all the strains are thoroughly studied for their beneficial properties to have a confirmed 'generally regarded as safe' status for ruminants.

Keywords: anaerobic rumen fungi, bacterial DFM, direct-fed microbials, probiotics, rumen

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1. Introduction

Improved ruminant health and performance has always remained a primary objective of people associated with livestock production. Several compounds have been used to improve ruminant performance either by manipulation of the rumen environment (e.g., sodium bicarbonate) or by directly altering the composition and metabolic activities of the rumen microbes (e.g., ionophores). But, with the growing concerns towards the use of antibiotics and other growth stimulants in the ruminant feed industry, more emphasis has been given to increase public awareness, disease prevention and use of other natural growth promoters like direct-fed microbials (DFM). DFM are the mono or mixed cultures of live microbes which when fed to the host, exert beneficial health effects by improving its gastrointestinal tract microbial balance (Elghandour *et al*. 2014a, b, 2015). Aside from improving the digestibility and performance of the ruminants, DFM detoxify toxic compounds to modulate immune system and maintain gut peristalsis and intestinal mucosal integrity (Chaucheyras-Durand and Duran 2010; Sandri *et al.* 2014). The term DFM is different from "Probiotic" in a sense that it is only restricted to the use of "live, naturally occurring microbes" (Yoon and Stern 1995; Kenney 2013; Krehbiel *et al.* 2003). For domestic ruminants like cattle and buffaloes, yeasts and aerobic fungi have been successfully used to increase growth rate and production efficiency. But, nowadays use of anaerobic fungi is emphasized because of its ability to produce wide array of enzymes that can even degrade the lignified walls of plant cells. Many factors like infections, improper food, environmental conditions and ingestion of antibiotics have been described that result in imbalance of intestinal microflora of ruminants. For many years, studies related to supplementation of microbial feed additive in the diet for the improvement of health are under progress. Nowadays, there are growing evidences that DFM may be useful in managing conditions like irritable bowel syndrome, lactose intolerance, chronic liver disease, pancreatitis and even certain forms of cancers. The mechanisms suggested for the action for DFM include colonization of the lower intestine, thereby limiting the growth of any potential pathogens through 'competitive exclusion' or inhibit pathogens by lowering the pH of the intestinal lumen and by producing anti-microbial proteins (bacteriocins).

This paper will cover a number of aspects related to the type of DFM, their modes of action, environmental protection using DFM, their benefits when fed to the host, etc.

2. Bacterial DFM

There are many DFM based on bacteria that are commercially available for use in ruminant diets with more specific applications. Most of the DFM bacteria are lactic acid bacteria with lactobacilli being the most dominant microflora, followed by the bifidobacteria, enterococci and bacilli. Among lactobacilli, *Lactobacillus acidophilus* is the most commonly used in DFM (Abdel-Aziz *et al*. 2015; Elghandour *et al*. 2015b). Most bacterial-based DFM are probably beneficial because they have effects in the lower gut and not in the rumen. For example, *L. acidophilus* produces lactic acid, which may lower the pH in small intestines, and inhibit the growth of pathogenic microbes. Early research with DFM was focused on ruminants which are either stressed or have immature microbial ecosystems in their guts (Vandevoorde *et al.* 1991) like milk fed young calves, calves being weaned or cattle being shipped (Jenny *et al.* 1991).

2.1. Modes of action

In ruminants, mode of action of feeding bacterial DFM is variable, which 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 rodent models. Bacterial DFM have been reported to modify the balance of intestinal microbes, adhere to intestinal mucosa and prevent pathogen adherence or activation, influence gut permeability, and modulate immune function are discussed below.

Competitive attachment Early research (Jones and Rutter 1972) suggested that attachment to the intestinal wall was important for pathogenic strains of *Escherichia coli* to induce diarrhea. It is believed that the attachment supports proliferation and reduces peristaltic removal of organisms. Bacterial DFM could compete with pathogens for the sites of adherence on the intestinal surface and thus can facilitate their removal (Wisener *et al.* 2014). 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).

Antibacterial effect Many species of lactobacilli have demonstrated inhibitory activity against pathogens. *L. acidophilus* has been shown to be antagonistic toward entero-pathogenic *E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Clostridium perfringens* (Gilliland and Speck 1977). Mann *et al.* (1980) showed that the 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. Hydrogen peroxide produced by lactobacilli appears to be partially responsible for the antagonistic interaction (Gilliland and Speck 1977). Different reports suggest that antimicrobial proteins and/or bacteriocins either mediate or facilitate antagonism by *L. acidophilus* (Gilliland and Speck 1977; Barefoot and

Klaenhammer 1983). However, because of the presence of proteolytic enzymes, their importance might be limited. In addition, Walsh *et al.* (2012) suggested that DFM should not be considered as viable alternatives to in-feed antibiotics in a pathogen challenge situations.

Immune response Bacterial DFM have been shown to affect the innate, humoral and cellular arms of the immune system. Oral administration of lactobacilli generally result in an augmentation of innate immune responses (i.e., enhanced phagocytosis and natural killer cell activity), as well as an elevated production of immunoglobulin A (IgA) and a decrease immunoglobulin E (IgE) production in 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. Furthermore, some species of probiotics appear to be capable of altering the immunomodulatory effects exerted by other species. For instance, *L. reuteri* DSM12246 was shown to potentially suppress *Lactobacillus casei* induced production of IL-6, IL-12, and TNF-*α* in dendritic cells (Christensen *et al.* 2002), suggesting that the composition of bacterial DFM administered should be considered. Qiu *et al.* (2012) indicated that supplementation with the DFM also regulated in energy re-partitioning to the immune system and an increase in antibody production independent of changes in whole body metabolism or growth performance. Therefore, bacterial DFM also show promise as immune modulators, although, more research is needed to determine the underlying mechanisms.

2.2. Effect on ruminant performance

Pre-ruminant calves Generally, the importance of feeding DFM to neonatal livestock has been to establish and maintain normal intestinal microbes rather than as a production stimulant. In the neonate, the microbial population of the gastrointestinal tract (GIT) is in transition and extremely sensitive. Abrupt environmental or dietary changes may cause shifts in the microbial population of the GIT which often leads to an increased incidence of diarrhea in calves (Sadine 1979). 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. For dairy calves, rapid adaptation to solid feed by accelerating the establishment of rumen and intestinal microbes and avoiding the establishment of entero-pathogens, which often results in diarrhea, is the primary goal. Feeding calves with viable cultures of species of *Lactobacillus* and *Streptococcus* has been reported to decrease the incidence of diarrhea (Ewaschuk *et al.* 2004; Hossaini *et al.* 2010; Riddell *et al.* 2010). In addition, some studies have indicated that DFM in the diet improves weight gain, feed efficiency and feed intake (Timmerman *et al.* 2005; Adams *et al.* 2008). In an experiment by Hossaini *et al.* (2010), calves fed DFM containing *L. acidophilus*, *L. casei*, *Bacillus thermophilus*, *Enterococcus faecium* confirmed the beneficial effect of it. 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*.

Performance response is likely not important early in the pre-ruminant'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.

Lactating ruminants Modern day intensive production systems, especially with high producing dairy cows and buffaloes involve the feeding of high levels of concentrate in order to meet the metabolic demand for high milk yield. Feeding high levels of concentrate often lead to metabolic dysfunction and eventually rumen acidosis; especially under conditions of poor methods of feeding and/or composition of diets. The goal of the nutritionist, when implementing high concentrate feeding is to maximize performance and efficiency, while keeping digestive disturbances such as the rumen acidosis within acceptable limits through good nutritional management. Theoretically, a number of approaches can be followed to control the incidences of the rumen acidosis. One approach is to inhibit the growth of lactic acid producing bacteria such as *Streptococcus bovis* and *Lactobacillus* species through the use of feed supplements such as ionophores (Callaway and Martin 1997). Another approach is to use DFM such as *Megasphaera elsdenii*, a lactic acid utilizer, to regulate lactic acid levels in the rumen. Experimentally, there have been several bacteria that have potential as DFM for ruminants but have not been commercialized for different reasons. For example, *M. elsdenii* is the major lactate-utilizing organism in the rumen of adapted cattle fed high grain diets. However, when cattle are abruptly shifted from a high-forage to high concentrate diet, the numbers of *M. elsdenii* are often insufficient to prevent lactic acidosis. Similarly, *E. faecium* and yeast used were of limited value for feedlot cattle already adapted to high-grain diets (Beauchemin *et al.* 2003). Erasmus *et al.*

(1992) and Aikman *et al.* (2008) observed an increase in milk production for a high producing group of cows when *M. elsdenii* NCIMB 41125 was dosed compared to the control animals. Similar results were obtained in second lactating cows (Hagg and Henning 2007), where *M. elsdenii* NCIMB 41125 were dosed after calving.

Gomez-Basauri *et al.* (2001) reported 0.73 kg d^{-1} more milk with 0.42 kg less dry matter (DM) consumption, when cows were fed with lactic acid bacteria (*L. acidophilus*, *L. casei*, *E. faecium*; total lactic bacteria=10^9 CFU g^{-1}) and mannan-oligosaccharide, compared to the control. Furthermore, milk yields continued to increase over time for DFM- and mannan-oligosaccharide-fed cows, whereas control cows maintained constant milk yields. On similar lines, Boyd *et al.* (2011) reported that the addition of a DFM (*L. acidophilus* NP51 and *Proponibacterium freudenreichii* NP24) and dietary glycerol may improve yield and digestibility for cows subject to heat stress. However, strain difference (*L. acidophilus* LA747 and *P. freudenreichii* PF24) may not affect the performance, diet digestibility and rumen characteristics (Raeth-Knight *et al.* 2007).

Other experiments conducted with combinations of fungal cultures and lactic acid bacteria (Komari *et al.* 1999; Block *et al.* 2000) has shown higher milk yields when lactating cows were fed with *Saccharomyces cerevisae* in combination with *L. acidophilus* and/or *Lactobacillus plantarum*/*E. faecium*. Propionibacteria, which convert lactic acid and glucose to acetic and propionic acid, may also be beneficial if inoculated into the rumen, because higher concentrations of rumen propionate represents the energy status of the animal. These bacteria are naturally present in high numbers in the rumen of animals fed forage and medium concentrate diets. Their supplementations as DFM increased milk fat percentage and milk yield as well as improved health of prepartum and postpartum cows (Noeck and Kautz 2006; Oetzel *et al.* 2007).

3. Yeast and fungal DFM

In adult ruminants, fungal DFM have mostly been selected to target the rumen compartment, which is the main site for feed digestion. The fungal feed additives and supplements have been shown to affect the rumen fermentation patterns.

3.1. Mode of action

Several reasons for improvements in rumen fermentation from feeding fungal DFM have been suggested. First, DFM exerts beneficial changes in activity and numbers of the rumen microbes. For example, the total rumen anaerobes and cellulolytic bacteria increase with fungal extracts. Beharka *et al.* (1991) reported that young calves fed *Aspergillus oryzae* fermentation extract were weaned one week earlier than the untreated calves and that supplementation increased the rumen bacteria and volatile fatty acids (VFA) concentrations. *Aspergillus* fermentation extracts (Chang *et al.* 1999) and yeast cultures (Chaucheryas *et al.* 1995) have also been shown to stimulate the rumen fungi directly, which improved fiber digestion. Feeding *S. cerevisiae* increased the rumen protozoa and increased NDF digestion in steers fed straw-based diets (Plata *et al.* 1994). Yeasts have also been shown to stimulate acetogenic bacteria in the presence of methanogens (Chaucheryas *et al.* 1995), which might result in more efficient rumen fermentation.

Second, fungal DFM may also prevent the accumulation of excess lactic acid in the rumen when cattle are fed diets containing highly fermentable carbohydrates. Specifically, extracts of *A. oryzae* stimulated the uptake of lactic acid by the rumen lactate-utilizers *Selenomonas ruminantium* (Nisbet and Martin 1991) and *M. elsdenii* (Waldrip and Martin 1993) possibly by providing a source of malic acid. Increased metabolism of lactic acid should theoretically raise rumen pH and this may be one reason why DFM increased the rumen cellulolytic bacteria and improved fiber digestion (Arambel *et al.* 1987). Chaucheyras *et al.* (1995) reported that *S. cerevisiae* was able to prevent the accumulation of lactic acid production by competing with *S. bovis* for glucose and by stimulating the uptake of lactic acid by *M. elsdenii*, perhaps by supplying amino acids and vitamins. In contrast, added yeasts were unable to prevent acute episodes of lactic acidosis when fermentations were challenged with a diet rich in fermentable carbohydrates (Aslan *et al.* 1995). Yeast may improve rumen fermentation because they are able to scavenge excess oxygen (Newbold *et al.* 1996), creating a more optimal environment for the rumen anaerobic bacteria (Elghandour *et al*. 2014a, b). *Aspergillus* extracts may improve fiber digestion because they contain esterase enzymes (Varel *et al.* 1993).

Anaerobic rumen fungi (ARF) have also been supplemented as fungal DFM to ruminant for better utilization of fibrous feeds in terms of increased feed intake, body weight gain, enhanced milk production, and thus improved ruminant productivity (Dey *et al.* 2004; Thareja *et al.* 2006). ARF are the normal inhabitants of the rumen ecosystem. The fungi colonize the fibrous plant fragments in the rumen and penetrate plant tissues making more room for bacterial attack and thus increase the area susceptible to enzymatic attack (Dagar *et al.* 2011). The enzymes produced by ARF and their functions are shown in Table 1. These properties of ARF are suggestive of manipulation of fungal numbers for better utilization of fibrous feeds.

Table 1 Enzymes produced by anaerobic rumen fungi and their functions

Enzymes	Types	Function(s)	Reference(s)
Esterases	p-Coumaroyl esterase Feruloyl esterase	Cleave phenolic acid (p-coumaric and ferulic acid) residues from the lignin hemicellulose or lignin xylan complexes, loosening cell wall structures, thereby allowing access to previously protected polysaccharides	Atsushi *et al.* (1984); Yue *et al.* (2009)
	Acetyl esterase	Acetyl xylan esterases remove acetyl group more specifically from xylose moieties in the xylan main chain	Blum *et al.* (1999)
Cellulases	Endoglucanases Exoglucanase β-Glucosidase	These act in synergy to convert cellulose to glucose. Initial attack on the cellulose molecule is by the endo-glucanase, which cuts the linear cellulose chains internally. Exo-glucanase can then act at these nick sites, releasing cellobiose, which is in turn hydrolysed by β-glucosidase to glucose monomers	Teunissen and Op den Camp (1993); Gordon and Phillips (1998); Atanasova-Pancevska and Kungulovski (2008); Comlekcioglu *et al.* (2010)
Hemicellulases	Xylanase	Degrade xylan	Mountfort and Asher (1989); Teunissen and Op den Camp (1993); Breton *et al.* (1995); Blum *et al.* (1999); Novotna *et al.* (2010)
	Mannase	Degrade manose	Coughlan and Hazlewood (1993)
Pectinases	Endocellular pectin lyase Polygalacturonase		Kopecny and Hodrova (1995)
Proteases		The contribution made by protease of anaerobic fungi in degradation of dietary proteins remains unclear	Wallace and Joblin (1985)
Chitinases			Sakurada *et al.* (1995); Novotna *et al.* (2008)

3.2. Effect on ruminant performance

There have been numerous studies reporting positive effects of *S. cerevisiae* and *A. oryzae* on intake and milk production of lactating cows. Supplementing diets with *S. cerevisiae* was shown to increase total dry matter intake (DMI), total VFA and propionic acid production, besides higher propionate concentration and decreased acetate to propionate ratio were determined in some experiments (Schingoethe *et al.* 2004; Cakiroglu *et al.* 2010; Ondarza *et al.* 2010). Higher VFA, especially propionic acid are important in terms of enhanced lactose production, milk volume and overall energy balance (Miller-Webster *et al.* 2009). Erasmus *et al.* (1992) suggested that supplementation of *S. cerevisiae* tended to increase microbial protein synthesis in dairy cows and significantly altered the amino acid profile of the duodenal digesta. Wohlt *et al.* (1991) suggested that supplementing yeast culture before parturition and extending through peak lactation was necessary to evaluate the effect on lactating cows. Some field reports indicate increased DMI and milk production when yeast was fed during periods of heat stress, possibly reflecting the role in aiding appetite during time of stress (Huber 1998). In beef cattle, the addition of *S. cerevisiae* led to an increase of live weight by 7.5% depending on the type of diet tested. Improvement can reach 13% in feedlot conditions, with diets rich in starch and sugars. Wallace and Newbold (1993) reported that responses recorded in trials in beef cattle tended to be higher with corn silage rather than with grass silage. In dairy cows, an improvement by around 4% of the milk yield, often associated with increased feed intake was generally reported and response was greater in early as opposed to mid or late lactation (Ali-Haimoud-Lekhal *et al.* 1999). *A. oryzae* in diets of lactating cows increased milk production, feed efficiency and tolerance to heat stress in some (Gomez-Alarcon *et al.* 1990) but not all (Higginbotham *et al.* 1993; Yu *et al.* 1997) studies.

Among microbial additives, there are evidences of definite positive relationship between ARF in the rumen and the increased voluntary intake of low digestible fibrous feeds (Ha *et al.* 1994; McAllister *et al.* 1994; Dey *et al.* 2004; Saxena *et al.* 2010). The ARF have been isolated from animals of different parts of the world providing evidence to suggest that they may have an important role in the digestion of fibrous materials in the rumen (Trinci *et al.* 1994; Tripathi *et al.* 2007b; Dagar *et al.* 2011; Ishtiyak *et al.* 2013) through substantial colonization of plant material (Edwards *et al.* 2008). Different fungal species improved digestibility of dry matter and cell wall constituents of cereal straws (Manikumar *et al.* 2004; Khattab *et al.* 2013) as well as sugarcane bagasse (Shelke *et al.* 2009) in the *in vitro* system. Incorporation of fungus increased growth rate, rumen fermentation, nutrient

digestibility and nitrogen retention in sheep (Ha *et al.* 1994), crossbred calves (Dey *et al.* 2004), buffalo calves (Sehgal *et al.* 2008), and dairy goats (Kholif *et al*. 2014). Tripathi *et al.* (2007a, b) found that administration of *Piromyces* sp. increased the growth rate, feed efficiency and nutritive value of wheat straw based ration in buffalo calves.

Experiments, where ARF were either absent or eliminated, have provided a deep insight into the contribution of fungi to fibre digestion, feed intake, rumen fermentation and overall metabolism. Ford *et al.* (1987) showed a decrease in voluntary feed intake of sheep to 49% in groups where ARF were eliminated. Removal of ARF from the rumen of sheep reduced the voluntary intake of poor quality feed to about 70% (Gordon and Phillips 1993). The addition of fungal culture *Neocallimastix* sp. R1 increased the forage intake by 35% in early weaned calves (Theodorou *et al.* 1990). In fungi-free rumen of sheep, the dosing of *Neocallimastix* sp. SLI increased the intake of straw based diet to 40% (Gordon and Phillips 1993). The elimination of ARF significantly reduced the degradation of dry matter, neutral detergent fiber, acid detergent fiber, and the activity of carboxymethylcellulase (CMCase) in sheep rumen (Gao *et al.* 2013).

An increased feed digestibility was documented, when different strains of *Neocallimastix* were dosed into the rumen of fungi-free sheep (Elliott *et al.* 1987). Paul *et al.* (2004) studied the effect of *Piromyces* sp. FNG5 on *in vivo* rumen fermentation and digestion of nutrients in buffaloes. They found an increase in total tract DMD, organic matter, neutral detergent fibre and acid detergent fibre digestibility. An increase in VFAs and enzymatic activities (CMCase, xylanase, microcrystalline cellulase, acetyl esterase, feruloyl esterase and protease) was also noticed. In addition, *Piromyces* sp. FNG5 was also found to tolerate tannic acid concentration up to 20 g L^{-1} (Paul *et al.* 2006), suggesting its possible application in improving fibre digestion of tannin-containing feeds. The administration of ARF into the rumen of goat increased the DMD, concentrations of ammonia, total VFA and CMCase activity. On the other hand, their elimination from sheep and goat resulted in a decreased digestibility of straw based dry matter. In absence of ARF, the concentrations of acetate, butyrate and total VFA decreased significantly in the rumen of sheep (Gao *et al.* 2008). Sehgal *et al.* (2008) studied the influence of *Neocallimastix* sp. GR1 on growth, rumen fermentation and nutrient digestion in female buffalo calves and found a considerable increase in daily weight gain and better feed efficiency of total mixed ration compared to control calves. Tripathi *et al.* (2007b) found that the DMD was the highest in group fed with *Piromyces* sp. WNG-12 than *Orpinomyces* sp. C-14 fed group. A similar pattern of increased digestibility of crude protein, cell-wall contents and average body weight gain was also observed in treatment groups. The same cultures were used to study the digestibility of wheat straw: concentrate (50:50) based diet, effect on rumen fermentation and milk production in lactating buffalo (Saxena *et al.* 2010). An increase in milk production was recorded in the fungus fed groups. There was also an increase of 6% fat corrected milk yield per animal per day in treatment groups. A similar pattern of increase in DMD, crude protein, neutral detergent fibre, acid detergent fibre, cellulose and digestible energy were observed in fungus fed groups, extending the possibility of their use as DFM in lactating buffaloes for obtaining higher milk production, even on poor quality feed.

4. Environmental protection using DFM

Methane produced from enteric fermentation leads to loss of 6 to 15% of gross intake energy of ruminant's energy. Besides, methane, as the second most potent greenhouse gas, leads to the global warming and poses threats to the environment (Kumar *et al.* 2009, 2013a, b, 2014). Thus, the consequences of methanogenesis in the rumen are not only associated with low ruminant efficiency but also have a negative impact on the sustainability of their production. Since, the enteric fermentation emission is one of the major sources of methane; therefore, experiments were conducted using antibiotics and other chemicals for mitigating methane emissions. However, appearance of antibiotic-resistant bacteria restricts its convenient use. Moreover, the antibiotics excreted to manures without being absorbed have been scattered on the environment (Mwenya *et al.* 2006). The alternative to antibiotics is the use of DFM that include lactic acid bacteria and yeasts as they are also found to reduce methane emission (Kalmakoff *et al.* 1996; Teather and Forster 1998; Klieve and Hegarty 1999) and acetate:propionate ratio (Martin and Nisbet 1992; Gamo *et al.* 2002; Lila *et al.* 2004). Hydrogen, which is released in the rumen during fibre degradation by cellulolytic microbes like bacteria and ARF, is rapidly utilized by methanogens for its conversion to methane. On the other hand, acetogenic bacteria are also able to utilize hydrogen for acetate production; but their numbers are less in the rumen of adults. Therefore, the acetogenic bacteria could be potentially used to compete with methanogens for hydrogen utilization; thereby also preventing the energy loss occurring as a result of methane production. Chaucheyras *et al.* (1995) studied the effect of a live strain of *S. cerevisiae* on hydrogen utilization and acetate and methane production by an acetogen and a methanogen. They concluded that the addition of yeast cells enhanced the acetogenesis of the acetogenic strain by more than fivefold, while in absence of yeasts, hydrogen was principally used for methane synthesis. Therefore, the use of yeasts as ruminant feed additives could help reducing methane, increasing the rumen metabolism and

hence, promoting ruminant performance and health. Lopez *et al.* (1999) also found that acetogens depressed methane production when added to the rumen fluid *in vitro* and suggested that even if a stable population of acetogens could not be established in the rumen, it might be possible to achieve the same metabolic activity using the acetogens as a daily fed feed additive. In addition, methane oxidisers can also be used as DFM. The oxidation reaction competes with the production of methane, which is a strictly anaerobic process. Methane oxidisers from gut and non-gut sources could be screened for their activity in the rumen fluid *in vitro* and then selected methane oxidisers could be introduced into the rumen on a daily basis.

5. Practical applications of DFM

There are varieties of DFMs such as powder, paste, gel, and capsules available commercially. These different forms may be mixed in feed, top-dressed, given as a paste, or mixed into the drinking water or milk replacer. However, their use must be managed effectively as viability of organism can be largely affected on interactions with chlorine, water, temperature, minerals, flow rate, and antibiotics. Bacterial DFM pastes are formulated with vegetable oil and inert gelling ingredients. Non-hydroscopic whey is generally used as a carrier for bacteria-based DFM. Fungal DFM products are formulated with grain by-products as carriers. Some DFM are developed for one-time dosing while others are developed for feeding on a daily basis. Most DFMs contain live bacteria; however, some contain only bacterial or fungal extracts or fermentation by-products. The best response can be observed during the following situations: (i) when a newborn animal acquire beneficial bacteria from environment, (ii) during weaning or dietary changes, (iii) periods of stress, i.e., shipping, vaccination, and other situations , and (iv) antibiotic therapy. The stability of DFMs is crucial because the microbes must be delivered live to the animal to be effective. For this, most DFMs require storage in a cool and dry area, away from heat, direct sunlight, and high levels of humidity. They must not only survive during processing and storage but also in the gut environment. The metabolites present in culture extracts have been suggested to be the “active” ingredients.

6. Conclusion and futuristic approaches

In light of international regulations and consumer demands to withdraw the growth-enhancing antibiotics and limiting the use of treatment related antibiotics, the DFM offer an option. For ruminants, ARF as DFM have been used successfully for improving the rumen and gastro-intestinal health, enhancing milk production, feed efficiency and daily gain in animals. On the other hand, methanogenesis, which accounts for significant loss of ruminant’s energy and increased greenhouse gases in environment, is also a major concern in the present scenario. Therefore, the use of DFM for improving production efficiency without compromising animal health and environmental sustainability is most advocated.

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