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

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1

2 **Role of Live Microbial Feed Supplements with Reference to Anaerobic Fungi in Ruminant**

3 **Productivity**

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17

18 **ABSTRACT**

19 To keep the concept of a safe food supply to the consumers, animal feed industries world over are  
20 showing an increasing interest in the direct fed microbials (DFM) for improved animal performance  
21 in terms of growth or productivity. This becomes all the more essential in a situation, where a  
22 number of the residues of antibiotics and/or other growth stimulants reach in milk and meat with a  
23 number of associated potential risks for the consumers. Hence, in the absence of growth stimulants,  
24 a positive manipulation of the rumen microbial ecosystem to enhance the feedstuff utilization for  
25 improved production efficiency by ruminants has become of much interest to the researchers and  
26 entrepreneurs. A few genera of live microbes (i.e., bacteria, fungi and yeasts in different types of  
27 formulations from paste to powder) are infrequently used as DFM for the domestic ruminants.  
28 These DFM products are live microbial feed supplements containing naturally occurring microbes  
29 in the rumen. Among different DFM possibilities, anaerobic rumen fungi (ARF) based additives

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30 have been found to improve ruminant productivity consistently during feeding trials. Administration  
31 of ARF during the few trials conducted, led to the increased weight gain, milk production, and total  
32 tract digestibility of feed components in ruminants. Anaerobic fungi in the rumen display very  
33 strong cell-wall degrading cellulolytic and xylanolytic activities through rhizoid development,  
34 resulting in the physical disruption of feed structure paving the way for bacterial action. Significant  
35 improvements in the fiber digestibility were found to coincide with increases in ARF in the rumen  
36 indicating their role. Most of the researches based on DFM have indicated a positive response in  
37 nutrient digestion and methane reducing potential during *in vivo* and/ or *in vitro* supplementation of  
38 ARF as DFM. Therefore, DFM especially ARF will gain popularity but it is necessary that all the  
39 strains are thoroughly studied for their beneficial properties to have a confirmed 'generally regarded  
40 as safe' status for ruminants.

41

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

43

#### 44 **INTRODUCTION**

45 Improved ruminant health and performance has always remained a primary objective of people  
46 associated with livestock production. Several compounds have been used to improve ruminant  
47 performance either by manipulation of the rumen environment (e.g., sodium bicarbonate) or by  
48 directly altering the composition and metabolic activities of the rumen microbes (e.g., ionophores).  
49 But, with the growing concerns towards the use of antibiotics and other growth stimulants in the  
50 ruminant feed industry, more emphasis has been given to increasing public awareness, disease  
51 prevention and use of other natural growth promoters like direct-fed microbials (DFM). DFM are  
52 the mono or mixed cultures of live microbes which when fed to the host, exert beneficial health  
53 effects by improving its gastrointestinal tract microbial balance. Aside from improving the  
54 digestibility and performance of the ruminants, DFM detoxify toxic compounds to modulate  
55 immune system and maintain gut peristalsis and intestinal mucosal integrity (Chaucheyras-Durand  
56 and Duran 2010, Sandri *et al.* 2014). The term DFM is different from "Probiotic" in a sense that it is  
57 only restricted to the use of "live, naturally occurring microbes" (Yoon and Stern 1995; Krehbiel *et*  
58 *al.* 2003; Kenney 2013). For domestic ruminants like cattle and buffaloes, yeasts and aerobic fungi  
59 have been successfully used to increase growth rate and production efficiency. But, now a day's use  
60 of anaerobic fungi is emphasized because of its ability to produce wide array of enzymes that can  
61 even degrade the lignified walls of plant-cells. Many factors like infections, improper food,  
62 environmental conditions and ingestion of antibiotics have been described that result in imbalance

63 of intestinal microflora of ruminants. For many years, studies related to supplementation of  
64 microbial feed additive in the diet for the improvement of health are under progress. Now days,  
65 there are growing evidences that DFM may be useful in managing conditions like irritable bowel  
66 syndrome, lactose intolerance, chronic liver disease, pancreatitis and even certain forms of cancers.  
67 The mechanisms suggested for the action for DFM include colonization of the lower intestine,  
68 thereby limiting the growth of any potential pathogens through ‘competitive exclusion’ or inhibit  
69 pathogens by lowering the pH of the intestinal lumen and by producing anti-microbial proteins  
70 (bacteriocins).

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

73

#### 74 **BACTERIAL DFM**

75 There are many DFM based on bacteria that are commercially available for use in ruminant diets  
76 with more specific applications. Most of the DFM bacteria are lactic acid bacteria with lactobacilli  
77 being the most dominant microflora, followed by the bifidobacteria, enterococci and bacilli. Among  
78 lactobacilli, *Lactobacillus acidophilus* is the most commonly used in DFM. Most bacterial-based  
79 DFM are probably beneficial because they have effects in the lower gut and not in the rumen. For  
80 example, *L. acidophilus* produces lactic acid, which may lower the pH in small intestines, and  
81 inhibit the growth of pathogenic microbes. Early research with DFM was focused on ruminants  
82 which are either stressed or having immature microbial ecosystems in their guts (Vandevoorde *et al.*  
83 1991) like milk fed young calves, calves being weaned or cattle being shipped (Jenny *et al.* 1991).

84

#### 85 **Modes of action**

86 In ruminants, mode of action of feeding bacterial DFM is variable, which emphasizes the need for  
87 greater understanding of underlying mechanisms. Research conducted to determine the potential  
88 mode of action of bacterial DFM has most often used the rodent models. Bacterial DFM have been  
89 reported to modify the balance of intestinal microbes, adhere to intestinal mucosa and prevent  
90 pathogen adherence or activation, influence gut permeability, and modulate immune function are  
91 discussed below.

92 *Competitive attachment* Early research (Jones and Rutter 1972) suggested that attachment to the  
93 intestinal wall was important for pathogenic strains of *E. coli* to induce diarrhea. It is believed that  
94 the attachment support proliferation and reduce peristaltic removal of organisms. Bacterial DFM  
95 could compete with pathogens for the sites of adherence on the intestinal surface and thus can

96 facilitate their removal (Wisener *et al.* 2014). Adhesion is thought to be mediated either  
97 nonspecifically by physicochemical factors, or specifically by adhesive bacterial surface molecules  
98 and epithelial receptor molecules (Holzapfel *et al.* 1998).

99 *Antibacterial effect* Many species of lactobacilli have demonstrated inhibitory activity against  
100 pathogens. *L. acidophilus* has been shown to be antagonistic toward entero-pathogenic *E. coli*,  
101 *Salmonella typhimurium*, *Staphylococcus aureus* and *Clostridium perfringens* (Gilliland and Speck  
102 1977). Mann *et al.* (1980) showed that the strain of *E. coli*, which causes illness and death when it is  
103 the sole microbial species in young lambs, could be tolerated in the presence of lactobacilli.  
104 Hydrogen peroxide produced by lactobacilli appears to be partially responsible for the antagonistic  
105 interaction (Gilliland and Speck 1977). Different reports suggest that antimicrobial proteins and/or  
106 bacteriocins either mediate or facilitate antagonism by *L. acidophilus* (Gilliland and Speck 1977;  
107 Barefoot and Klaenhammer 1983). However, because of the presence of proteolytic enzymes, their  
108 importance might be limited. In addition, Walsh *et al.* (2012) suggested that DFM should not be  
109 considered as viable alternatives to in-feed antibiotics in a pathogen challenge situations.

110 *Immune Response* Bacterial DFM have been shown to affect the innate, humoral and cellular arms  
111 of the immune system. Oral administration of lactobacilli generally result in an augmentation of  
112 innate immune responses (i.e., enhanced phagocytosis and natural killer cell activity), as well as an  
113 elevate production of immunoglobulin (IgA) and a decrease IgE production in animals (Erickson  
114 and Hubbard 2000; Isolauri *et al.* 2001). However, influence of DFM on cytokine production and T  
115 and B cell responses show mixed results depending on the strain, dose and duration of feeding DFM,  
116 as well as the type of tissues and cells analyzed. Furthermore, some species of probiotics appear to  
117 be capable of altering the immunomodulatory effects exerted by other species. For instance,  
118 *L.reuteri* DSM12246 was shown to potentially suppress *Lactobacillus. casei* induced production of  
119 IL-6, IL- 12, and TNF- $\alpha$  in dendritic cells (Christensen *et al.* 2002), suggesting that the composition  
120 of bacterial DFM administered should be considered. Qiu *et al.* (2012) indicated that  
121 supplementation with the DFM also regulate in energy re-partitioning to the immune system and an  
122 increase in antibody production independent of changes in whole body metabolism or growth  
123 performance. Therefore, bacterial DFM also show promise as immune modulators, although, more  
124 research is needed to determine the underlying mechanisms.

125

## 126 **Effect on ruminant performance**

127 *Pre-ruminant calves* Generally, the importance of feeding DFM to neonatal livestock has been to  
128 establish and maintain normal intestinal microbes rather than as a production stimulant. In the

129 neonate, the microbial population of the gastrointestinal tract (GIT) is in transition and extremely  
130 sensitive. Abrupt environmental or dietary changes may cause shifts in the microbial population of  
131 the GIT which often leads to an increased incidence of diarrhea in calves (Sadine 1979). In terms of  
132 ruminant production systems, the efficacy of bacterial DFM has been studied most extensively in  
133 the neonatal dairy calf. Bacterial DFM, such as species of *Lactobacillus*, *Enterococcus*,  
134 *Streptococcus*, and *Bifidobacterium* have been studied in young calves and the data have been  
135 reviewed. For dairy calves, rapid adaptation to solid feed by accelerating the establishment of  
136 rumen and intestinal microbes and avoiding the establishment of entero-pathogens, which often  
137 results in diarrhea, is the primary goal. Feeding calves with viable cultures of species of  
138 *Lactobacillus* and *Streptococcus* has been reported to decrease the incidence of diarrhea (Ewaschuk  
139 *et al.* 2004; Hossaini *et al.* 2010; Riddell *et al.* 2010). In addition, some studies have indicated that  
140 DFM in the diet improves weight gain, feed efficiency and feed intake (Timmerman *et al.* 2005;  
141 Adams *et al.* 2008). In an experiment by Hossaini *et al.* (2010), calves fed DFM containing *L.*  
142 *acidophilus*, *L. casei*, *Bacillus. thermophilus*, *Enterococcus. faecium* confirmed the beneficial effect  
143 of it. The decreased incidence of diarrhea might be associated with a consistently increased  
144 shedding of *Lactobacillus* (Gilliland *et al.* 1980; Jenny *et al.* 1991; Abu-Tarboush *et al.* 1996) and  
145 an inconsistent decreased shedding of coliforms (Bruce *et al.* 1979) in feces in response to  
146 supplements of *Lactobacillus*.

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

153 *Lactating Ruminants* Modern day intensive production systems, especially with high producing  
154 dairy cows and buffaloes involve the feeding of high levels of concentrate in order to meet the  
155 metabolic demand for high milk yield. Feeding high levels of concentrate often lead to metabolic  
156 dysfunction and eventually rumen acidosis; especially under conditions of poor methods of feeding  
157 and/or composition of diets. The goal of the nutritionist, when implementing high concentrate  
158 feeding is to maximize performance and efficiency, while keeping digestive disturbances such as  
159 the rumen acidosis within acceptable limits through good nutritional management. Theoretically, a  
160 number of approaches can be followed to control the incidences of the rumen acidosis. One  
161 approach is to inhibit the growth of lactic acid producing bacteria such as *Streptococcus bovis* and

162 *Lactobacillus* species through the use of feed supplements such as ionophores (Callaway and  
163 Martin 1997). Another approach is to use DFM such as *Megasphaera elsdenii*, a lactic acid utilizer,  
164 to regulate lactic acid levels in the rumen. Experimentally, there have been several bacteria that  
165 have potential as DFM for ruminants but have not been commercialized for different reasons. For  
166 example, *M. elsdenii* is the major lactate-utilizing organism in the rumen of adapted cattle fed high  
167 grain diets. However, when cattle are abruptly shifted from a high-forage to high concentrate diet,  
168 the numbers of *M. elsdenii* are often insufficient to prevent lactic acidosis. Similarly, *E. faecium* and  
169 yeast used were of limited value for feedlot cattle already adapted to high-grain diets (Beauchemin  
170 *et al.* 2003). Erasmus (1992) and Aikman *et al.* (2008) observed an increase in milk production for a  
171 high producing group of cows when *M. elsdenii* NCIMB 41125 was dosed compared to the control  
172 animals. Similar results were obtained in second lactating cows (Hagg and Henning 2007), where *M.*  
173 *elsdenii* NCIMB 41125 were dosed after calving.

174 Gomez-Basauri *et al.* (2001) reported 0.73 kg/d more milk with 0.42 kg less DM consumption,  
175 when cows were fed with lactic acid bacteria (*L. acidophilus*, *L. casei*, *E. faecium*; total lactic  
176 bacteria= $10^9$  cfu g<sup>-1</sup>) and mannan-oligosaccharide, compared to control. Furthermore, milk yields  
177 continued to increase over time for DFM- and mannan-oligosaccharide-fed cows, whereas control  
178 cows maintained constant milk yields. On similar lines, Boyd *et al.* (2011) reported that the addition  
179 of a direct-fed microbial (*L. acidophilus* NP51 and *Propionibacterium freudenreichii* NP24) and  
180 dietary glycerol may improve yield and digestibility for cows subject to heat stress. However, strain  
181 difference (*L. acidophilus* LA747 and *Propionibacterium freudenreichii* PF24) may not affect the  
182 performance, diet digestibility and rumen characteristics (Raeth-Knight *et al.* 2007).

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

192

193 **YEAST AND FUNGAL DFM**

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

197

### 198 **Mode of action**

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

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

224 Anaerobic rumen fungi (ARF) have also been supplemented as fungal DFM to ruminant for better  
225 utilization of fibrous feeds in terms of increased feed intake, body weight gain, enhanced milk  
226 production, and thus improved ruminant productivity (Dey *et al.* 2004; Thareja *et al.* 2006). ARF



227 are the normal inhabitants of the rumen ecosystem. The fungi colonize the fibrous plant fragments  
228 in the rumen and penetrate plant tissues making more room for bacterial attack and thus increase the  
229 area susceptible to enzymatic attack (Dagar *et al.* 2011). The enzymes produced by ARF and their  
230 functions are shown in table 1. These properties of ARF are suggestive of manipulation of fungal  
231 numbers for better utilization of fibrous feeds.

232

### 233 **Effect on ruminant performance**

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

255 Among microbial additives, there are evidences of definite positive relationship between ARF in the  
256 rumen and the increased voluntary intake of low digestible fibrous feeds (McAllister *et al.* 1994; Ha  
257 *et al.* 1994; Dey *et al.* 2004; Saxena *et al.* 2010). The ARF have been isolated from animals of  
258 different parts of the world providing evidence to suggest that they may have an important role in  
259 the digestion of fibrous materials in the rumen (Trinci *et al.* 1994; Tripathi *et al.* 2007b; Dagar *et al.*

260 2011; Ishtiyak *et al.* 2013) through substantial colonization of plant material (Edwards *et al.* 2008).  
261 Different fungal species improved digestibility of dry matter and cell wall constituents of cereal  
262 straws (Manikumar *et al.* 2004) as well as sugarcane bagasse (Shelke *et al.* 2009) in the *in vitro*  
263 system. Incorporation of fungus increased growth rate, rumen fermentation, nutrient digestibility  
264 and nitrogen retention in sheep (Ha *et al.* 1994), crossbred calves (Dey *et al.* 2004), and buffalo  
265 calves (Sehgal *et al.* 2008). Tripathi *et al.* (2007a, b) found that administration of *Piromyces* sp.  
266 increased the growth rate, feed efficiency and nutritive value of wheat straw based ration in buffalo  
267 calves.

268 Experiments, where ARF were either absent or eliminated have provided a deep insight into the  
269 contribution of fungi to fibre digestion, feed intake, rumen fermentation and overall metabolism.  
270 Ford *et al.* (1987) showed a decrease in voluntary feed intake of sheep to 49% in groups where ARF  
271 were eliminated. Removal of ARF from the rumen of sheep reduced the voluntary intake of poor  
272 quality feed to about 70% (Gordon and Phillips 1993). The addition of fungal culture  
273 *Neocallimastix* sp. R1 increased the forage intake by 35% in early weaned calves (Theodorou *et al.*  
274 1990). In fungi free rumen of sheep, the dosing of *Neocallimastix* sp. SL1 increased the intake of  
275 straw based diet to 40% (Gordon and Phillips 1993). The elimination of ARF significantly reduced  
276 the degradation of dry matter, neutral detergent fiber, acid detergent fiber, and the activity of  
277 CMCase in sheep rumen (Gao *et al.*, 2013).

278 An increased feed digestibility was documented, when different strains of *Neocallimastix* were  
279 dosed into the rumen of fungus free sheep (Elliott *et al.* 1987). Paul *et al.* (2004) studied the effect  
280 of *Piromyces* sp. FNG5 on *in vivo* rumen fermentation and digestion of nutrients in buffaloes. They  
281 found an increase in total tract DMD, organic matter, neutral detergent fibre and acid detergent fibre  
282 digestibility. An increase in VFAs and enzymatic activities (carboxymethylcellulase (CMCase),  
283 xylanase, microcrystalline cellulase, acetyl esterase, feruloyl esterase and protease) was also noticed.  
284 In addition, *Piromyces* sp. FNG5 was also found to tolerate tannic acid concentration up to 20 g/L  
285 (Paul *et al.* 2006), suggesting its possible application in improving fibre digestion of tannin-  
286 containing feeds. The administration of ARF into the rumen of goat increased the DMD,  
287 concentrations of ammonia, total VFA and CMCase activity. On the other hand, their elimination  
288 from sheep and goat resulted in a decreased digestibility of straw based dry matter. In absence of  
289 ARF, the concentrations of acetate, butyrate and total VFA decreased significantly in the rumen of  
290 sheep (Gao *et al.* 2008). Sehgal *et al.* (2008) studied the influence of *Neocallimastix* sp. GR1 on  
291 growth, rumen fermentation and nutrient digestion in female buffalo calves and found a  
292 considerable increase in daily weight gain and better feed efficiency of total mixed ration compared

293 to control calves. Tripathi *et al.* (2007b) found that the DMD was highest in group fed with  
294 *Piromyces* sp. WNG-12 than *Orpinomyces* sp. C-14 fed group. A similar pattern of increased  
295 digestibility of crude protein, cell-wall contents and average body weight gain was also observed in  
296 treatment groups. The same cultures were used to study the digestibility of wheat straw: concentrate  
297 (50:50) based diet, effect on rumen fermentation and milk production in lactating buffalo (Saxena *et*  
298 *al.* 2010). An increase in milk production was recorded in the fungus fed groups. There was also an  
299 increase of 6% fat corrected milk yield/ animal/ day in treatment groups. A similar pattern of  
300 increase in DMD, crude protein, neutral detergent fibre, acid detergent fibre, cellulose and  
301 digestible energy were observed in fungus fed groups, extending the possibility of their use as DFM  
302 in lactating buffaloes for obtaining higher milk production, even on poor quality feed.

303

#### 304 **ENVIRONMENTAL PROTECTION USING DFM**

305 Methane produced from enteric fermentation leads to loss of 6 to 15% of gross intake energy of  
306 ruminant's energy. Besides, methane is the second most potent green house gas, lead to the global  
307 warming and poses threats to the environment (Kumar *et al.* 2009, 2013a, b, 2014). Thus, the  
308 consequences of methanogenesis in the rumen is not only associated with low ruminant efficiency  
309 but also have a negative impact on the sustainability of their production. Since, the enteric  
310 fermentation emission is one of the major sources of methane; therefore, experiments were  
311 conducted using antibiotics and other chemicals for mitigating methane emissions. However,  
312 appearance of antibiotic-resistant bacteria restricts its convenient use. Moreover, the antibiotics  
313 excreted to manures without being absorbed have been scattered on the environment (Mwenya *et al.*  
314 2006). The alternative to antibiotics is the use of DFM that include lactic acid bacteria and yeasts as  
315 they are also found to reduce methane emission (Kalmakoff *et al.* 1996; Teather and Forster 1998;  
316 Klieve and Hegarty 1999) and acetate: propionate ratio (Martin and Nisbet 1992; Gamo *et al.* 2002;  
317 Lila *et al.* 2004). Hydrogen, which is released in the rumen during fibre degradation by cellulolytic  
318 microbes like bacteria and ARF, is rapidly utilized by methanogens for its conversion to methane.  
319 On the other hand, acetogenic bacteria are also able to utilize hydrogen for acetate production; but  
320 their number is less in the rumen of adults. Therefore, the acetogenic bacteria could be potentially  
321 used to compete with methanogens for hydrogen utilization; thereby also preventing the energy loss  
322 occurring as a result of methane production. Chaucheyras *et al.* (1995) studied the effect of a live  
323 strain of *S. cerevisiae* on hydrogen utilization and acetate and methane production by an acetogen  
324 and a methanogen. They concluded that the addition of yeast cells enhanced the acetogenesis of the  
325 acetogenic strain by more than fivefold, while in absence of yeasts, hydrogen was principally used

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

335

### 336 **PRACTICAL APPLICATIONS OF DFM**

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

353

### 354 **CONCLUSION AND FUTURISTIC APPROACHES**

355 In light of international regulations and consumer demands to withdraw the growth-enhancing  
356 antibiotics and limiting the use of treatment related antibiotics, the DFM offer an option. For  
357 ruminants, ARF as DFM have been used successfully for improving the rumen and gastro-intestinal  
358 health, enhancing milk production, feed efficiency and daily gain in animals. On the other hand,

359 methanogenesis, which accounts for significant loss of ruminant's energy and increased green  
360 house gases in environment, is also a major concern in present scenario. Therefore, the use of DFM  
361 for improving production efficiency without compromising animal health and environmental  
362 sustainability is most advocated.

363

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369

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630 **Table 1:** Enzymes produced by anaerobic rumen fungi and their functions  
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Enzymes	Types	Function(s)	Reference(s)
Esterases	p-Coumaroyl 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 <i>et al.</i> (1984); Yue <i>et al.</i> (2009)
	Feruloyl esterase		
	Acetyl esterase	acetyl xylan esterases remove acetyl group more specifically from xylose moieties in the xylan main chain	Blum <i>et al.</i> (1999)
Cellulases	Endoglucanases	These act in synergy to convert cellulose to glucose. Initial attack on the cellulose molecule is by the endoglucanase, which cuts the linear cellulose chains internally. Exo-glucanase can then act at these nick sites, releasing cellobiose, which is in turn hydrolysed by $\beta$ -glucosidase to glucose monomers	Teunissen and Op den Camp (1993); Gordon and Phillips (1998); Atanasova-Pancevska and Kungulovski(2008); Comlekcioglu <i>et al.</i> (2010)
	Exoglucanase		
	$\beta$ -glucosidase		
Hemicellulases	Xylanase	Degrade Xylan	Mountfort and Asher (1989); Teunissen and Op den Camp (1993); Breton <i>et al.</i> (1995); Blum <i>et al.</i> (1999); Novotna <i>et al.</i> (2010)
	Mannase	Degrade manose	Coughlan and Hazlewood (1993)
Pectinases	Endocellular pectin lyase		Kopecny and Hodrova (1995)
	Polygalacturonase		
Proteases		The contribution made by protease of anaerobic fungi in degradation of dietary proteins remains unclear	Wallace and Joblin (1985)
Chitinases			Sakurada <i>et al.</i> (1995); Novotna <i>et al.</i> (2008)

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