ABSTRACT

Nonbacterial, direct-fed microbials added to ruminant diets generally consist of Aspergillus oryzae fermentation extract, or Saccharomyces cerevisiae cultures, or both. Results from in vivo research have been variable regarding effects of direct-fed microbials on ruminant feedstuff utilization and performance. Some research has shown increased weight gains, milk production, and total tract digestibility of feed components, but others have shown little influence of direct-fed microbials on these parameters. In vitro research with mixed ruminal microorganisms likewise has been inconsistent regarding the effects of direct-fed microbials. Several researchers observed that direct-fed microbials increased cellulolytic bacterial numbers in the rumen and stimulated the production of some fermentation end products. This suggests that direct-fed microbials may be providing growth factors for the ruminal microbes. However, other researchers have reported no effect of direct-fed microbials on in vitro fiber digestion. Recent research demonstrated that growth of the predominant ruminal bacterium Selenomonas ruminantium in lactate medium as well as lactate uptake by whole cells of Sel. ruminantium were markedly increased by an A. oryzae fermentation extract and an S. cerevisiae culture. In addition, both products increased the production of acetate, propionate, succinate, total VFA, and cell yield (grams of cells per mole of lactate). Therefore, it appears that these direct-fed microbials provide soluble factors that stimulate lactate utilization by Sel. ruminantium. Evidence is presented indicating that the malate content of the A. oryzae fermentation extract and S. cerevisiae culture may be involved in this stimulation.

(Key words: direct-fed microbials, rumen, fermentation, lactate utilization)

INTRODUCTION

For many years, ruminant nutritionists and microbiologists have been interested in manipulating the microbial ecosystem of the rumen to improve production efficiency by domestic ruminants. Several studies in the 1960s evaluated, with mixed results, the effects of dietary enzyme supplements on ruminant feedstuff utilization and performance (5, 20, 34, 38). Burroughs et al. (5) reported that weight gains were increased 7% in beef cattle under feedlot conditions, but other researchers observed no changes in weight gain or feed digestibility (20, 38).

As much as 12% of the energy in feed may be converted to methane and lost via eructation in ruminant animals; thus, much research has been conducted over the past 20 yr aimed at reducing feed energy losses associated with methanogenisis in the rumen (32). Perhaps the most studied and widely used group of feed additives that have been shown to reduce methane production is the ionophores (32). Generally, ionophores are thought to improve feed utilization by increasing the amount of metabolizable energy available to the animal as propionate, resulting in a decreased feed: gain ratio (4, 32). This theory is supported by the observation that propionate-producing ruminal bacteria (i.e., Selenomonas ruminantium)
Growing concern over the use of antibiotics and other growth stimulants in the animal feed industry increased interest in evaluating the effects of direct-fed microbials (DFM) on animal performance. However, compared with the ionophores, little research has been done to evaluate the effects of DFM on ruminal microbial fermentation and ruminant performance. In addition, limited information is available documenting the effects of DFM on the physiology of predominant ruminal microorganisms. Therefore, the objective of this paper was to provide an overview of recent research on the effects of DFM on ruminal fermentation.

**DFM VERSUS PROBIOTICS**

Terminology associated with the incorporation of fungal and yeast cultures into ruminant diets has been inconsistent and at times confusing. Probiotic has been defined as "a live microbial feed supplement [that] beneficially affects the host animal by improving its intestinal microbial balance" (13). However, as pointed out by Vanbelle et al. (39), many researchers accept that probiotic refers to "selected and concentrated viable counts of lactic acid bacteria" (i.e., *Lactobacillus, Streptococcus*). In 1989, the US FDA required manufacturers to use the term DFM rather than probiotic (26). The FDA defines DFM as "a source of live (viable) naturally-occurring microorganisms", and this includes bacteria, fungi, and yeast (26). This paper will focus on the use of fungi and yeast as DFM in domestic ruminants.

**IN VIVO STUDIES WITH DFM**

Only a brief review of the effects of DFM on ruminant performance will be given here because Williams and Newbold (43) recently published a detailed summary of ruminant production responses to DFM. Nonbacterial DFM added to ruminant diets generally consist of *Aspergillus oryzae* fermentation extract, or *Saccharomyces cerevisiae* cultures, or both. It should be emphasized that these products contain viable cells plus the growth medium (43). Current recommended usage for DFM in ruminants ranges between 3 and 110 g/d per animal.

**Aspergillus oryzae**

Incorporation of *A. oryzae* into dairy cattle or sheep diets has produced variable results. Van Horn et al. (40) reported that cows receiving *A. oryzae* supplementation exhibited significant improvements in the digestibility of DM and ADF, but no differences in milk production or feed intake were observed. Studies with nonlactating Holstein cows showed that *A. oryzae* treatment increased the total tract digestibility of DM, CP, and hemicellulose (41). However, *A. oryzae* had little effect on site or extent of digestion of orchardgrass hay by Holstein heifers (10). Judkins and Stobart (17) observed that *A. oryzae* treatment did not influence ruminal fermentation or ingesta passage in wether lambs, but some increase in plant cell-wall digestion was noted. Furthermore, Fondevila et al. (11) reported that *A. oryzae* addition increased the initial rate of barley straw degradation by sheep but did not alter the extent of degradation.

Recent research showed that *A. oryzae* supplementation increased ruminal and total tract digestibility of fiber fractions, but ruminal VFA and NH₃ production was not affected (14). In addition, Gomez-Alarcon et al. (15) found that *A. oryzae* treatment increased milk production in early lactation cows, and this finding was consistent with previous research (19). Another interesting observation was the tendency for *A. oryzae*-treated, lactating Holstein cows to have lower rectal temperatures during hot summer months (15).

**Saccharomyces cerevisiae**

Most of the limited in vivo research with *S. cerevisiae* has involved studies with dairy cattle. Total tract digestibility of CP and hemicellulose was increased by *S. cerevisiae* supplementation in nonlactating Holstein cows, but digestibilities of DM and ADF remained unchanged (41). No changes in rumen fermentation products or ruminal digesta flow kinetics were observed with *S. cerevisiae* treatment (41). A combination of *S. cerevisiae* plus *A. oryzae* increased total tract digestibility of DM,
CP, and hemicellulose (41). Harrison et al. (16) reported that S. cerevisiae decreased ruminal pH, acetate, and the acetate:propionate ratio and increased molar proportions of propionate and valerate. However, ruminal liquid dilution rate and total tract apparent nutrient digestibilities were not altered by S. cerevisiae treatment (16). When S. cerevisiae was added to the diets of beef steers and wether lambs, few changes in ruminal fermentation products, liquid dilution rate, and digestibility were detected, but some increase in daily feed intake was noted (1).

Published data describing the effects of S. cerevisiae on milk production is minimal. Saccharomyces cerevisiae culture had no effect on DMI, milk production, or milk composition in midlactation Holstein cows (9). Some increase in milk production was noted upon addition of S. cerevisiae to diets that contained high levels of concentrates, and the increase in milk production was associated with increased feed intake (43, 44). Wohlt et al. (45) likewise found that S. cerevisiae supplementation stimulated DMI and milk production in Holstein cows fed a combination of corn silage, grain, and hay. The greatest stimulation in milk production was observed during early lactation with S. cerevisiae (45), which is similar to the response reported for A. oryzae (14, 19).

IN VITRO STUDIES WITH DFM

Mixed Ruminal Microorganisms

Early research with DFM primarily focused on in vivo experiments, and the limited in vitro research involved ruminal bacterial enumeration and in vitro DM disappearance studies. These types of experiments provide some information regarding changes in bacterial populations within the rumen, but they do not adequately address microbial metabolism. Therefore, over the past few years, several studies have been conducted to evaluate the effects of DFM on the in vitro mixed ruminal microorganism fermentation (Table 1).

As pointed out by Dawson et al. (8), a common feature associated with DFM treatment appears to be an increase in the numbers of cellulolytic bacteria in the rumen (Table 1). Frumholtz et al. (12) found that rumen protozoal numbers were reduced 45% by A. oryzae treatment, but Oellermann et al. (31) reported that A. oryzae supplementation had little effect on the numbers of ruminal protozoa, total viable bacteria, amylolytic bacteria, cellulolytic bacteria, and anaerobic fungi. Even though cellulolytic bacterial numbers are increased (8, 12, 16, 41), the extent of fiber digestion does not always increase correspondingly. Only two in vitro studies, both using A. oryzae, reported an increase in fiber or DM digestion (14, 27), and Newbold et al. (27) suggested that A. oryzae stimulated the rate rather than extent of digestion. Evidence supporting this hypothesis came from the observation that A. oryzae stimulated DM digestion after 24 h, but not after 48 h, of incubation using the rumen simulation technique (Rusitec) (27). Harrison et al. (16) found that cellulolytic bacterial numbers were greater in Holstein cows fed S. cerevisiae, but the rate of cellulose disappearance in vitro was decreased in samples of ruminal fluid from cows receiving S. cerevisiae.

In addition to fiber or DM digestion, the effects of DFM on culture pH and production of various fermentation end products by mixed ruminal microorganisms in vitro have been evaluated (Table 1). Results were variable regarding pH and production of gas, VFA, and NH3. This variation between studies likely is due to differences in the type and concentration of DFM, substrates, and culture conditions used. When mixed ruminal microorganisms were cultured using Rusitec, A. oryzae treatment did not affect L-lactate production (12, 27). Several studies demonstrated that DFM treatment stimulated NH3 production by the mixed ruminal population (2, 12, 22, 23), suggesting that DFM enhance proteolysis in vitro. Arambel et al. (2) proposed that this increase in NH3 production could be due to the DFM providing additional nutrients to the ruminal microorganisms or possibly by endogenous proteolytic activity of the DFM. However, Oellermann et al. (31) reported that numbers of proteolytic bacteria in the rumen were lower in Holstein cows fed different concentrations of A. oryzae. Preliminary experiments in our laboratory have detected low proteolytic activity with either A. oryzae or S. cerevisiae (Martin, unpublished data).
TABLE 1. Effects of direct-fed microbials (DFM), Aspergillus oryzae fermentation extract, or Saccharomyces cerevisiae culture on numbers of ruminal microorganisms and on the in vitro mixed ruminal microorganism fermentation. 1,2

<table>
<thead>
<tr>
<th>Added DFM</th>
<th>Microbial numbers</th>
<th>pH</th>
<th>Gas</th>
<th>VFA</th>
<th>NH3</th>
<th>L-Lactate</th>
<th>Fiber or DM digestion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cerevisiae or A. oryzae</td>
<td>↑ cellulolytic bacteria</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>(41)</td>
<td></td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>↑ Va, Iv</td>
<td>↑</td>
<td>...</td>
<td>NE</td>
<td>(2)</td>
</tr>
<tr>
<td>A. oryzae</td>
<td>↑ total VFA, Ac:Pr ↓ Pr</td>
<td>...</td>
<td>...</td>
<td>↑</td>
<td>...</td>
<td>NE</td>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>↑ cellulolytic bacteria ...</td>
<td>NE</td>
<td>↓ total VFA</td>
<td>NE</td>
<td>...</td>
<td>↓ cellulose disappearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. oryzae</td>
<td>↑ cellulolytic bacteria ↓ protozoa</td>
<td>↑ CH₄</td>
<td>NE</td>
<td>↑</td>
<td>NE</td>
<td>NE</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>...</td>
<td>↓ CH₄</td>
<td>↑ Ac, Pr, Bu, Va, Iv, Tb, total VFA ↓ Ac:Pr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. oryzae</td>
<td>...</td>
<td>↓ H₂, CH₄</td>
<td>↑ Ac, Pr, Bu, Va, Iv, total VFA ↓ Ac:Pr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. oryzae</td>
<td>...</td>
<td>...</td>
<td>↑ Pr</td>
<td>NE</td>
<td>...</td>
<td>...</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>↑ proteolytic bacteria</td>
<td>...</td>
<td>...</td>
<td>↑</td>
<td>...</td>
<td>...</td>
<td>(31)</td>
<td></td>
</tr>
<tr>
<td>A. oryzae</td>
<td>↓ proteolytic bacteria</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>(27)</td>
<td></td>
</tr>
</tbody>
</table>

1↑ = increase, ... = not measured, ↓ = decrease, NE = no effect.
2Ac = Acetate, Pr = propionate, Bu = butyrate, Tb = isobutyrate, Iv = isovalerate, Va = valerate, Ac:Pr = acetate: propionate ratio.

Pure Culture Studies

Compared with data available that describe the effects of DFM on the in vitro fermentation by mixed ruminal microorganisms, there is minimal information in the literature detailing the effects of DFM on the metabolism of predominant ruminal bacteria. Russell et al. (33) emphasized that ruminal microbiology is not just an enumeration and analysis of microbial diversity. If progress is to be made in ruminant nutrition, a detailed understanding of basic physiological functions (i.e., nutrient transport mechanisms) as well as factors (i.e., ionsophores, DFM) that affect these functions in predominant ruminal bacteria is needed. Therefore, our research has focused on studying nutrient transport mechanisms in several ruminal bacteria, including Sel. ruminantium (24, 33, 42).

The common Gram-negative ruminal bacterium, Sel. ruminantium, can account for up to 51% of the total viable bacterial counts in the rumen (6). Previous research showed that Sel. ruminantium HD4 requires L-aspartate, carbon dioxide, p-aminobenzoic acid, and biotin for
Figure 1. Effects of aspartate (Asp), fumarate (Fum), and malate (Mal) on lactate uptake by whole cells of Selenomonas ruminantium (29).

TABLE 2. Effects of Aspergillus oryzae fermentation extract and Saccharomyces cerevisiae culture on lactate uptake by whole cells of Selenomonas ruminantium.

<table>
<thead>
<tr>
<th>Concentration (g/L)</th>
<th>Specific activity (nmol/mg of protein per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. oryzae</td>
</tr>
<tr>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>0.5</td>
<td>6.3</td>
</tr>
<tr>
<td>1.0</td>
<td>7.2</td>
</tr>
<tr>
<td>2.5</td>
<td>5.0</td>
</tr>
<tr>
<td>5.0</td>
<td>4.9</td>
</tr>
<tr>
<td>10.0</td>
<td>12.9</td>
</tr>
<tr>
<td>50.0</td>
<td>4.7</td>
</tr>
</tbody>
</table>

1Data from Nisbet and Martin (29, 30).
2DFM = Direct-fed microbial.
strate addition to mixed ruminal microorganisms cultured by using Rusitec (12). These results indicated that both DFM were affecting lactate metabolism by ruminal microorganisms. One possibility was that A. oryzae and S. cerevisiae stimulated lactate utilization by ruminal bacteria (i.e., Sel. ruminantium) capable of fermenting lactate. Therefore, experiments were conducted to examine the effects of A. oryzae and S. cerevisiae on lactate utilization by Sel. ruminantium HD4 (29, 30).

Aspergillus oryzae fermentation extract stimulated L-lactate uptake at all concentrations tested, and S. cerevisiae increased uptake at concentrations between 2.5 and 10 g/L (Table 2). When 50 g/L of each DFM were added, uptake was reduced, but, with A. oryzae, activity was still 4-fold higher than that of the control. Incubation of both DFM in the absence of Sel. ruminantium resulted in uptake rates that were less than controls (29, 30).

To evaluate whether some soluble component was involved in the stimulation of lactate uptake, a filter-sterilized filtrate of each DFM was used (29, 30). Uptake was enhanced over 4-fold by all concentrations of each filtrate, and the 25 μl/ml level of the S. cerevisiae filtrate increased uptake approximately 9-fold (Table 3). Both filtrates also stimulated growth of Sel. ruminantium on lactate (Figure 2), cell yield (grams of cells per mole of lactate), and VFA production (29, 30). Based on these results, A. oryzae and S. cerevisiae apparently provide soluble factors involved in enhancing lactate utilization by Sel. ruminantium.

Fumarate and malate are produced by A. oryzae, and malate is present in large amounts in all strains tested (3, 35). Malate is also an intermediary metabolite found in fairly high concentrations in S. cerevisiae (36). Therefore, the concentration of L-malate in the filter-sterilized filtrates of both A. oryzae and S. cerevisiae was determined. The concentrations of L-malate in A. oryzae and S. cerevisiae filtrates were 1.45 and 4.9 mM, respectively (29, 30). The presence of malate in both filtrates supports involvement of this dicarboxylic acid in the stimulation of lactate utilization by Sel. ruminantium treated with A. oryzae or S. cerevisiae. However, it is likely that both DFM are providing other growth factors such

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### Table 3. Effects of filter-sterilized filtrates of *Aspergillus oryzae* fermentation extract and *Saccharomyces cerevisiae* culture on lactate uptake by whole cells of *Selenomonas ruminantium*.

<table>
<thead>
<tr>
<th>Filtrate concentration (μl/ml)</th>
<th>Specific activity (nmol/mg of protein per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>A. oryzae</strong></td>
</tr>
<tr>
<td></td>
<td>X</td>
</tr>
<tr>
<td>0</td>
<td>.3</td>
</tr>
<tr>
<td>10</td>
<td>4.3</td>
</tr>
<tr>
<td>25</td>
<td>4.0</td>
</tr>
<tr>
<td>50</td>
<td>4.2</td>
</tr>
<tr>
<td>100</td>
<td>3.7</td>
</tr>
</tbody>
</table>

1 Data from Nisbet and Martin (29, 30).

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**Figure 2. Effect of *Aspergillus oryzae* fermentation extract (○) on *Saccharomyces cerevisiae* culture (△) on growth of *Selenomonas ruminantium* on lactate. Control incubations (●, ▲) were performed in the absence of direct-fed microbial (29, 30).**
as fumarate, B vitamins, including biotin and p-aminobenzoic acid, as well as AA, which are required by *Sel. ruminantium* for growth on lactate (18, 21). It should be noted that low concentrations of succinate (2.5 mM) were detected when *Sel. ruminantium* HD4 was grown on lactate plus 2% *S. cerevisiae* filtrate (30). These results provide additional evidence that dicarboxylic acids (malate, fumarate) in *S. cerevisiae* may be involved in stimulating lactate utilization by *Sel. ruminantium* HD4. Malate and *A. oryzae* filtrate also enhanced D-lactate uptake and growth on D-lactate by *Sel. ruminantium* HD4 (28), whereas the *S. cerevisiae* filtrate had little effect (Nisbet and Martin, unpublished data).

**Future Considerations**

Much interest has been generated over the past few years in manipulating the ruminal microflora to enhance feedstuffs utilization and alleviate problems associated with current feeding practices. Stabilization of ruminal fluid pH by enhancing the lactate-utilizing capability of ruminal bacteria without using antibiotics or ionophores has the potential to improve performance by overcoming the economic losses associated with acidosis of the rumen. The growing concern by consumers about the use of antibiotics in the animal feed industry as well as the need for a safe food supply should provide motivation to investigate and develop new nonantibiotic, or "natural", feed additives. Controversy associated with the proposed use of bST in the dairy industry is a good example of how public perception can be used against the animal industry.

When compared with other microorganisms, little is known about the physiology and genetics of ruminal microorganisms, especially if one takes into account the complexity and diversity of the rumen microbial ecosystem. Because bacterial growth cannot occur unless soluble nutrients are transported into the cell, a strong emphasis should be placed on a detailed understanding of the fundamental aspects of nutrient transport in ruminal bacteria. In addition, factors that may affect the transport process need to be evaluated.

Another area that deserves attention is the improvement of currently marketed feed additives or the development of new feed additives. Based on our research, there seems to be potential for the use of DFM and malate as feed additives, but additional research is needed. Given recent advances that have been made using recombinant DNA technology, particularly with fungi and yeast, there seems to be great potential for developing feed supplements for the future. However, much research still is needed to understand fully the physiological mechanisms and the specific requirements of predominant ruminal microorganisms under different feeding conditions. Once the physiology of these microbes is understood fully, products could be developed to take advantage of this microbial potential to improve feed digestion.

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