ABSTRACT: The ability of ruminants to convert plant biomass unsuitable for human consumption into meat and milk is of great societal and agricultural importance. However, the efficiency of this process is largely dependent on the digestibility of plant cell walls. Supplementing ruminant diets with exogenous enzymes has the potential to improve plant cell wall digestibility and thus the efficiency of feed utilization. Understanding the complexity of the rumen microbial ecosystem and the nature of its interactions with plant cell walls is the key to using exogenous enzymes to improve feed utilization in ruminants. The variability currently observed in production responses can be attributed to the array of enzyme formulations available, their variable activities, the level of supplementation, mode of delivery, and the diet to which they are applied as well as the productivity level of the host. Although progress on enzyme technologies for ruminants has been made, considerable research is still required if successful formulations are to be developed. Advances in DNA and RNA sequencing and bioinformatic analysis have provided novel insight into the structure and function of rumen microbial populations. Knowledge of the rumen microbial ecosystem and its associated carbohydrases could enhance the likelihood of achieving positive responses to enzyme supplementation. The ability to sequence microbial genomes represents a valuable source of information in terms of the physiology and function of both culturable and unculturable rumen microbial species. The advent of metagenomic, metatranscriptomic, and proteomic techniques will further enhance our understanding of the enzymatic machinery involved in cell wall degradation and provide a holistic view of the microbial community and the complexities of plant cell wall digestion. These technologies should provide new insight into the identification of exogenous enzymes that act synergistically with the rumen microbial populations that ultimately dictate the efficiency of feed digestion.

Key words: carbohydrases, cattle, exogenous enzymes, rumen, plant cell wall

INTRODUCTION

The efficiency by which ruminants obtain energy from structural plant polysaccharides and in turn produce high quality meat and milk protein is increasingly important if the demands of an expanding human population are to be met. Due to both economic considerations and maintenance of rumen health, forage will almost always be a component of the diet of ruminants (Krause et al., 2003). However, digestibility of forage cell walls ultimately limits nutrient availability as conditions for fiber digestion are often suboptimal in the rumen.

Exogenous enzymes are increasingly considered as a cost-effective means of improving feed efficiency (Krause et al., 2003), yet production responses to exogenous enzymes are still highly variable. Several reviews (Krause et al., 2003; Beauchemin et al., 2004; Beauchemin and Holtshauser, 2011; Tricarico et al., 2008) have been published on the use of exogenous enzymes in ruminants; however, this paper aims to provide an updated perspective on current research and new techniques being used to use these products as a means of improving feed efficiency. Most research has centered on the use of fibrolytic enzymes to increase fiber digestion and thus digestible energy intake, but responses have been vari-
able. Increases in milk (Gado et al., 2009; Klinger-merge et al., 2009), ADG (Beauchemin et al., 1999; McAllister et al., 1999), and DM and fiber digestion in situ, in vitro (Yang et al., 1999; Hristov et al., 2008), and in vivo (Rode et al., 1999; Beauchemin et al., 2000) have been reported. However, in many cases, the efficiency of growth or milk production in ruminants has not been improved (ZoBell et al., 2000; Eun et al., 2009; Arriola et al., 2011).

Characterization of rumen microbial populations using “-omics” technologies provides insight into the functionality of rumen microbiota (Morgavi et al., 2012) and the construction of gene catalogues through metagenomics and genomic sequencing expands our understanding of the interactions between community members and the carbohydrates they produce. These knowledge advancements are providing new insight into the formulation of exogenous enzymes that act synergistically with the carbohydrates of rumen microbial communities in a manner that enhances the efficiency of plant cell wall digestion.

**SOURCES OF ENZYMES**

Commercial enzyme products used in the livestock industry are of fungal (*Aspergillus oryzae* and *Trichoderma reesei*) and bacterial (*Bacillus subtilis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Enterococcus faecium* spp.) origin (Muirhead, 1996; McAllister et al., 2001). Beginning with a seed culture and growth media, enzymes for the feed industry are produced through microbial fermentation and although the source organisms only constitute a very limited group, the types and activity of enzymes produced can be diverse depending on the strain selected, the substrate they are grown on, and the culture conditions used (Considine and Coughlan, 1989; Gashe, 1992; Lee et al., 1998).

A vast array of enzymes is required to degrade the complex arrangements of structural carbohydrates in plant cell walls (Morgavi et al., 2012). Although commercial enzyme preparations are commonly referred to as cellulases or xylanases, secondary enzyme activities such as amylases, proteases, esterases, or pectinases are invariably present as these preparations seldom consist of a single pure enzyme (McAllister et al., 2001). This diversity is advantageous, as it facilitates targeting of a range of substrates using a single product, yet it complicates the identification of the specific enzymes responsible for any positive responses observed in feed digestion. Degradation of cellulose and hemicellulose alone requires a number of glycosidic hydrolases (Chesson and Forsberg, 1997; Krause et al., 2003) and differences in the relative proportions and activity of individual enzymes affects the overall efficacy of cell wall degradation (McAllister et al., 2001). A common approach is to use an enzyme that may not be suited to a specific feed but instead to formulate enzyme mixtures that are suitable for a range of feed types (Beauchemin et al., 2003). However, this approach of adding enzymes to diets without consideration for specific substrates has contributed to the highly variable results observed when enzymes are used in ruminants, an outcome that has undoubtedly discouraged and delayed the adoption of the technology.

**MEASUREMENT OF ENZYME ACTIVITY**

The activity of enzymes is assayed by measuring the generation of the product from the biochemical reaction that the enzyme catalyzes over time and is expressed as the amount of product produced per unit of time (McAllister et al., 2001; Beauchemin et al., 2003). In the case of carbohydrases, the production of free sugars is the most common product measured. Measurement of enzyme activities must be conducted under closely regulated conditions as variations in temperature, pH, ionic strength, substrate concentration, and substrate type influence enzyme activity (McAllister et al., 2001). Synthetic substrates can also be used to assay enzyme activity by measuring the release of a dye or chromophore (Higginbotham et al., 1996). However, these conditions fail to replicate those of the digestive tract, nor are the substrates representative of intact feeds that are fed to ruminants (McAllister et al., 2001). Consequently, these assays may have limited relevance to the potential worth of an enzyme as a feed additive for ruminants.

Biological assays using mixed ruminal microorganisms incubated with complex substrates has been one approach to identify enzyme preparations that are more suitable for use in ruminants. After the addition of enzymes, these in vitro incubations measure the digestion of ingredients commonly included in ruminant diets (i.e., grains, hay, silage, or straw) by recording the production of gas that arises from the fermentation process and the digestibility of the feed DM and fiber at a given incubation time. Using this system, several enzyme preparations can be simultaneously screened for their effectiveness with different application methods and rates (Muirhead, 1996; Iwaasa et al., 1998; Morgavi et al., 2000b). However, these systems are not very representative of in vivo conditions (Hristov et al., 2012) and do not account for animal-to-animal variation in the microbial community, making extrapolation of these results to whole animal scenarios challenging. Additionally, these systems do not account for the possible impact of exogenous enzymes on biological parameters such as feed intake, rate of passage, or postruminal digestion of nutrients (McAllister et al., 2001). Even in vitro systems may require more enzyme than what is typically produced by most laboratory-based expression systems. To overcome this problem we have developed a micro-in vitro
system that enables the synergistic interactions of milligram quantities of exogenous enzymes with rumen microbial enzymes to be assessed in a reaction mixture as small as 250 μL (Badhan, A., Wang, Y., and McAllister, T.A. [Agriculture and Agri food Canada, AB]; Patton, D., Powlowski, J., and Tsang A. [Centre for Structural and Functional Genomics, Concordia University, QC]). We used the system to identify carbohydrates from thermophilic and anaerobic fungi that could enhance the liberation of sugars from alfalfa hay and barley straw by mixed rumen enzymes. Such mini-assays could prove invaluable for identifying those enzyme activities that merit production scale up for animal experiments and the formulation of efficacious enzyme cocktails.

In vitro screening of exogenous enzymes provides a cost effective, less time consuming means of screening large numbers of products with specific substrates and can be used to predict possible in vivo responses. However, the final assessment of the true value of exogenous enzymes for ruminants in terms of improving feed utilization can only be assessed through the use of animal production trials.

**PRODUCTION RESPONSES TO EXOGENOUS ENZYMES**

**Dairy Cattle**

A search of the Commonwealth Agricultural Bureaux International (CABI) database (www.cabdirect.org) and the Journal of Dairy Science (www.journalofdairyscience.org) for “enzyme” and “dairy” in the title returned 328 and 39 publications, respectively. From these, 28 were selected and are summarized in Table 1. Criteria for this selection were that the study had to be published in English in a refereed journal, be with lactating dairy cows, and have a valid experimental design. “Field” trials where control over cow grouping was not clearly described or if there was potential for confounding factors were excluded from the analysis. Studies judged as having major flaws in experimental design or statistical analysis of the data were also excluded from Table 1. A large portion of the studies were not included as they did not involve the use of lactating cows.

Naturally, the main objective of using exogenous enzymes in dairy cow nutrition is to improve milk production and yield of milk components and as previous reviews (McAllister et al., 2001; Beauchemin et al., 2004; Tricarico et al., 2008) have focused on the dairy-specific effects of exogenous enzymes on ruminal fermentation and intestinal digestion, this review will focus on production effects. Most products tested in dairy cows are described as cellulases and/or xylanases, with proteases and amylases being investigated in fewer instances. It is practically impossible to compare exogenous enzyme preparations on an equal activity basis, as there is a distinct lack of standardization in the methodology used to assess enzyme activities among labs. Even when the same methods are used, it is difficult to standardize enzyme products because they contain multiple activities and can only be standardized for 1 or 2 activities at a time. For example, when 2 products are used to supply the same level of endoglucanase activity, they may supply vastly different levels of xylanase activity. Another common limitation of the enzyme literature is the lack of repeatability of the effects and repeated investigations of a common exogenous enzyme preparation as most are only examined in a single experiment. Experimental cost is perhaps a major reason for this lack of repetition, but the possibility that unfavorable results were not published cannot be excluded. Interestingly, the commercial α-amylase product Amaize (Alltech Inc., Nicholasville, KY) has been examined in 3 studies discussed in this review (DeFrain et al., 2005; Tricarico et al., 2005; Klingerman et al., 2009). All of these studies fed a diet containing either alfalfa hay, alfalfa haylage and maize silage, or a mixed legume hay and maize silage diet and observed no effects on milk yield or composition of milk components. The dose of enzyme and the portion of the diet to which it was applied varied across studies raising questions about the enzymes applicability for use in lactating dairy cows fed current diets.

Few studies have reported positive effects of exogenous enzymes on milk components; for example, Beauchemin et al. (2000) reported a 2% increase in milk true protein with a β-glucanase/xylanase/endoglucanase product. Similarly, Bowman et al. (2002), Sutton et al. (2003), and Eun and Beauchemin (2005) reported increased milk fat or protein. A few instances of increasing milk yield have also been observed. For example, Yang et al. (1999) found milk yield was increased by 1.9 kg/d when an exogenous enzyme (Pro-Mote; Biovance Technologies Inc., Omaha, NE) composed predominantly of cellulasse and xylanase activities was applied to hay at 2 g of enzyme mixture/kg. This effect was attributed to a 12% increase in nutrient digestibility. Despite these results, the application of exogenous enzymes to dairy cow diets has shown extremely variable results and largely failed to improve production efficiency. Of the studies compared in this paper, the majority showed no effect on milk yield (DeFrain et al., 2005; Hristov et al., 2008; Bernard et al., 2010; Peters et al., 2010; Ferraretto et al., 2011) or the production of milk components (Yang et al., 1999; Holtshausen et al., 2009; Bernard et al., 2010; Arriola et al., 2011).

A recent study by Holtshausen et al. (2011) screened 5 doses of a fibrolytic enzyme additive (AB Vista, Marlowborough, UK) and further assessed its efficacy in situ before the enzyme additive was fed to lactating Holstein
<table>
<thead>
<tr>
<th>Source, Year</th>
<th>Experimental design</th>
<th>Product/manufacturer</th>
<th>Declared primary activities</th>
<th>Application level</th>
<th>Forage level in basal diet</th>
<th>Milk production, kg/d</th>
<th>Effects</th>
<th>Milk components</th>
<th>Total tract digestibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen et al., 1995</td>
<td>Completely randomized (36)</td>
<td>Digest M, Loveland Industries Inc., Greeley, CO</td>
<td>Amylase and protease</td>
<td>209 g/t</td>
<td>34%</td>
<td>34 to 37</td>
<td>-</td>
<td>-</td>
<td>↑CP</td>
</tr>
<tr>
<td>Rode et al., 1999</td>
<td>Completely randomized (20)</td>
<td>Pro-Mote</td>
<td>Xylamase and cellulase</td>
<td>1.3 kg/TMR DM</td>
<td>39%</td>
<td>36 to 40</td>
<td>-</td>
<td>-</td>
<td>↓MF</td>
</tr>
<tr>
<td>Schingoethe et al., 1999</td>
<td>Completely randomized block (50)</td>
<td>FinnFeeds Int.</td>
<td>Cellulase and xylanase</td>
<td>0.7 to 1.5 L/t forage DM</td>
<td>55%</td>
<td>25 to 28</td>
<td>-</td>
<td>-</td>
<td>↑</td>
</tr>
<tr>
<td>Yang et al., 1999</td>
<td>Latin square (4)</td>
<td>Pro-Mote</td>
<td>Cellulase and xylanase</td>
<td>0.5 to 1 g/kg TMR DM</td>
<td>55%</td>
<td>24 to 26</td>
<td>-</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Beauchemin et al., 2000</td>
<td>Latin square (6)</td>
<td>Natra grain 33-L</td>
<td>β-glucanase, xylanase, and endocellulase</td>
<td>1.22 to 3.67 LA TMR</td>
<td>45%</td>
<td>30 to 31</td>
<td>↑</td>
<td>-</td>
<td>↑MMP</td>
</tr>
<tr>
<td>Kung et al., 2000</td>
<td>Completely randomized (30)</td>
<td>FinnFeeds Int.</td>
<td>Cellulase, hemicellulase, 2 to 10 L/t fresh forage and xylanase</td>
<td>50%</td>
<td>33 to 35 and 36 to 39</td>
<td>-</td>
<td>↑ (both experiments)</td>
<td>- or ↓MF and MMP</td>
<td>↑</td>
</tr>
<tr>
<td>Yang et al., 2000</td>
<td>Completely randomized block (43)</td>
<td>Biovance Technol. Inc.</td>
<td>Xylanase</td>
<td>50 mg/kg TMR DM</td>
<td>38%</td>
<td>35 to 37</td>
<td>-</td>
<td>-</td>
<td>↑DM</td>
</tr>
<tr>
<td>Zheng et al., 2000</td>
<td>Completely randomized block (48)</td>
<td>Bovizyme 4017</td>
<td>Cellulase and xylanase</td>
<td>1.25 LA forage DM</td>
<td>50 to 65%</td>
<td>33 to 37</td>
<td>-</td>
<td>-</td>
<td>↑</td>
</tr>
<tr>
<td>Bowman et al., 2002</td>
<td>Latin square (8)</td>
<td>Promote N.E.T.</td>
<td>Xylanase and cellulose</td>
<td>1 g/cow per d</td>
<td>55%</td>
<td>29 to 30</td>
<td>-</td>
<td>-</td>
<td>↑MF and MMP</td>
</tr>
<tr>
<td>Knowlton et al., 2002</td>
<td>Switchover (12)</td>
<td>Loveland Industries Inc., Greeley, CO</td>
<td>Cellulase</td>
<td>204 g/TMR</td>
<td>45 to 61%</td>
<td>31 to 43</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kung et al., 2002</td>
<td>Completely randomized (30)</td>
<td>FinnFeeds Int.</td>
<td>Cellulase and xylanase</td>
<td>10 L/t fresh forage</td>
<td>45%</td>
<td>36 to 39</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sutton et al., 2003</td>
<td>Latin square (4)</td>
<td>Biotech, Technologies, Inc.</td>
<td>Xylanase and endoglucanase</td>
<td>2 kg/TMR DM</td>
<td>57%</td>
<td>34 to 36</td>
<td>-</td>
<td>-</td>
<td>↑MMP</td>
</tr>
<tr>
<td>Vicini et al., 2003</td>
<td>Completely randomized (257 and 122)</td>
<td>FinnFeeds Int. and Biotech, Technologies Inc.</td>
<td>Xylanase and endoglucanase</td>
<td>1.25 to 2 LA TMR DM</td>
<td>43 to 57%</td>
<td>32 to 33 and 28 to 29</td>
<td>-</td>
<td>-</td>
<td>↑</td>
</tr>
<tr>
<td>Defran et al., 2005</td>
<td>Completely randomized block (24)</td>
<td>Amaize</td>
<td>Amylase</td>
<td>0.1% TMR DM</td>
<td>47 to 69%</td>
<td>38</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eun and Beauchemin, 2005</td>
<td>Latin square (8)</td>
<td>Protex 6L</td>
<td>Protease</td>
<td>1.25 mg/kg TMR DM</td>
<td>34 to 60%</td>
<td>41 to 48</td>
<td>↓</td>
<td>↓</td>
<td>↑MF; and ↓MMP</td>
</tr>
<tr>
<td>Tricano et al., 2005</td>
<td>Latin square (20)</td>
<td>Amaize</td>
<td>Amylase</td>
<td>240 to 720 dextrinizing units/kg TMR DM</td>
<td>55%</td>
<td>29 to 30</td>
<td>NR</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Elwakeel et al., 2007</td>
<td>Split plot (24)</td>
<td>Saf Agri, Milwaukee, WI</td>
<td>β-glucanase and xylanase</td>
<td>15 g/cow per d</td>
<td>37%</td>
<td>43 to 44</td>
<td>-</td>
<td>-</td>
<td>↑</td>
</tr>
<tr>
<td>Knowlton et al., 2007</td>
<td>Completely randomized (24)</td>
<td>Cattle-Ase-P</td>
<td>Cellulase and phytase</td>
<td>297 g/TMR DM</td>
<td>37%</td>
<td>37 to 39</td>
<td>-</td>
<td>-</td>
<td>↑</td>
</tr>
<tr>
<td>Reddish and Kung, 2007</td>
<td>Switchover (24)</td>
<td>Alltech Inc.</td>
<td>Cellulase and xylanase</td>
<td>10 g/cow per d</td>
<td>50%</td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hristov et al., 2008</td>
<td>Latin square (4)</td>
<td>Alltech, Inc.</td>
<td>Amylase and xylanase</td>
<td>10 g/cow per d</td>
<td>40%</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>↑DM, OM, and CP</td>
</tr>
</tbody>
</table>

*Table 1. Summary of exogenous polysaccharide-degrading enzyme effects on production traits and total tract apparent digestibility of nutrients in lactating dairy cows.*
Table 1. (continued)

<table>
<thead>
<tr>
<th>Source1</th>
<th>Experimental design (number of cows)</th>
<th>Product/manufacturer</th>
<th>Declared primary activities</th>
<th>Application level</th>
<th>Forage level in basal diet</th>
<th>Milk production, kg/d</th>
<th>Milk yield</th>
<th>Milk components</th>
<th>Effects2</th>
<th>Total tract digestibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miller et al., 2008</td>
<td>Completely randomized block (72)</td>
<td>Roxazyme G2 Liquid23</td>
<td>Xylanase and endoglucanase</td>
<td>2.15 and 4.30 mL/kg concentrate</td>
<td>Pasture and 6.7 kg/d grain supplement</td>
<td>28 to 29</td>
<td>-</td>
<td>-</td>
<td>NR</td>
<td>-</td>
</tr>
<tr>
<td>Gado et al., 200924</td>
<td>Completely randomized (20)</td>
<td>ZADO24</td>
<td>Protease, amylase, and cellulase</td>
<td>40 g/cow per d</td>
<td>70%</td>
<td>13 to 16</td>
<td>↑</td>
<td>↑</td>
<td>-</td>
<td>↑DM, OM, NDF, and ADF</td>
</tr>
<tr>
<td>Klingerman et al., 2009</td>
<td>Latin square (28)</td>
<td>Amai17 and an experimental preparation25</td>
<td>Amylase</td>
<td>0.4 g/kg TMR DM and 0.88 to 4.4 mL/kg</td>
<td>50%</td>
<td>44 to 47</td>
<td>↑</td>
<td>↑25</td>
<td>-</td>
<td>↑DM, OM, CP, and NDF25</td>
</tr>
<tr>
<td>Bernard et al., 2010</td>
<td>Completely randomized (44)</td>
<td>Promote N.E.T.-L26</td>
<td>Cellulase</td>
<td>4 g/cow per d</td>
<td>50 to 54%</td>
<td>40 to 42</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Peters et al., 2010</td>
<td>Switchover (6)</td>
<td>Roxazyme G223</td>
<td>Cellulase and xylanase</td>
<td>6.2 mL/kg TMR DM</td>
<td>50%</td>
<td>26 to 27</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Holshausen et al., 2011</td>
<td>Completely randomized (60)</td>
<td>AB Vista, Marlborough, UK</td>
<td>Xylanase and endoglucanase</td>
<td>0.5 to 1.0 mL/kg DM</td>
<td>52%</td>
<td>38</td>
<td>↓</td>
<td>-</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>Arriola et al., 2011</td>
<td>Completely randomized block (66)</td>
<td>Dynacid International Inc., Jupiter, FL</td>
<td>Xylanase, exoglucanase, and endoglucanase</td>
<td>3.4 mg/g TMR DM</td>
<td>52 to 67%</td>
<td>32 to 36</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>↑All</td>
</tr>
<tr>
<td>Ferrareto et al., 2011</td>
<td>Completely randomized (45)</td>
<td>Ronozyme RumiStar CT23</td>
<td>Amylase</td>
<td>300 kilo novo units/kg TMR DM</td>
<td>50%</td>
<td>49.5 to 52.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>↑MMP</td>
</tr>
</tbody>
</table>

1 In chronological order.
2 ↑ = increase; ↓ = decrease; - = no statistically significant effect; NR = not reported; MF = milk fat percentage; MMP = milk protein percentage; All = all nutrients studied.
3 Applied to the grain portion of the diet.
4 Interaction with grain processing.
5 Biovance Technologies Inc., Omaha, NE.
6 TMR = total mixed ration.
7 FinnFeeds International, Marlborough, Wiltshire, UK.
8 Reported increase of 3.5% fat corrected milk.
9 These studies investigated enzyme application method.
10 For one of the application methods.
11 BASF Corporation, Ludwigshafen, Germany.
12 Only the low enzyme application level.
13 Only the high enzyme application level.
14 Two experiments.
15 Agribrands International, St. Louis, MO.
16 Experimental diets were fed from 21 d before expected calving through 21 d in milk.
17 Alltech Inc., Nicholasville, KY.
18 Genencor International, Rochester, NY.
19 Only low-forage (34%) diet.
20 Animal Feed Technologies, Greeley, CO.
21 Trends for increased NDF and ADF digestibility.
22 Digestion experiment with sheep.
23 DSM Nutritional Products Ltd., Basel, Switzerland.
24 Molecular Biology Laboratory of the Ain Shams University, Cairo, Egypt. Questionable data; see discussion.
25 Milk yield and digestibilities increased only by low level of an experimental amylase enzyme.
26 Cargill Animal Nutrition, Minneapolis, MN.
dairy cows. The enzyme product improved fat corrected milk (FCM) production efficiency in a dose dependent manner up to 11.3%. Similarly, Arriola et al. (2011) screened varying amounts of a fibrolytic enzyme product in situ before conducting a feeding trial. Milk production efficiency was increased in cows fed this enzyme product with a low-concentrate diet as compared with those fed either an untreated low-concentrate diet or a high-concentrate diet (treated or untreated). Therefore, it is evident that careful attention needs to be paid to the type and dose of enzymes being applied to dairy cattle diets. The use of in vitro screening can help to elucidate appropriate doses and predict production responses when combined with specific substrates. However, it remains difficult to assess the consistency of animal responses to individual enzyme products, as most products are experimental and can vary in activity over time. In many instances the same product formulation is often used in only a limited number of studies of widely differing experimental conditions.

**Beef Cattle**

Although the first reports of exogenous enzymes improving beef cattle BW gains were over 50 yr ago (Burroughs et al., 1960), adoption of enzyme technology has been slow as the cost of enzymes outweighs that of other additives, such as ionophores, antibiotics, and implants (Beauchemin et al., 2006). We used a similar criterion as described above for dairy production studies to identify beef production studies (CABI and the *Journal of Animal Science* [www.journalofanimalscience.org]) with a search for “enzyme” and “beef cattle” in the title returning 383 and 70 titles, respectively. From these, 11 studies were selected and summarized in Table 2. Experimental criteria for selection were the same as for dairy cattle, with studies having to be conducted with growing, backgrounding, or finishing beef cattle. Although responses to exogenous enzymes are expected to be greater in beef cattle fed roughage-based diets as compared with high-grain diets, many exogenous enzyme formulations have shown promising effects in cattle fed high-grain finishing diets, at least when included in barley-based diets (Beauchemin and Holtshausen, 2011). However, these responses are still variable depending on the dosage of the enzyme applied, the time of application in relation to feeding, and the portion of the diet to which they are applied. Application of a mixture of xylanase and cellulase products (Xylanase B [Biovance Technologies Inc., Omaha, NE] and Spezyme CP [Genencor, Rochester, NY]) increased ADG of steers fed alfalfa hay or timothy hay by 30 and 36%, respectively, but had no effect when applied to barley silage (Beauchemin et al., 1995). These positive responses were attributed to an increase in digestible DM intake; however, it was noted that forage type influenced the optimal dose required to elicit these responses (0.25 to 1.0 L/t DM for alfalfa hay versus 4 L/t DM for timothy hay), demonstrating the importance of interactions between dosage, enzyme, and substrate. A subsequent study with steers assessed the same enzyme formulation in high-concentrate diets (95% DM) containing either barley or corn grain (Beauchemin et al., 1997). Application to a barley grain diet improved feed efficiency by 11%, yet performance was unaffected when added to corn. Supplementing a similar exogenous enzyme mixture (FinnFeeds Int. Ltd., Marlborough, UK) increased ADG of steers by 10% when applied to both the grain and forage portions of the diet (McAllister et al., 1999) and resulted in a 28% increase in ADF digestibility (Krause et al., 1998). These studies indicate the application of a xylanase and cellulase enzyme formulation is promising in terms of increasing ADG when applied to either barley grain or forage diets; however, the use of this enzyme formulation is not recommended in diets based on corn grain or barley silage due to its apparent lack of effectiveness with these feeds. Conversely, a study by Tricarico et al. (2007) reported an *A. oryzae* extract containing α-amylase activity quadratically increased ADG when included in either a cracked corn or high-moisture corn and corn silage diet but had no effect when included with alfalfa hay, cotton seed hulls, or steam-flaked corn. Similarly, DiLorenzo et al. (2011) observed no effects of supplementing an amylase enzyme formulation (600 kilo novo units/kg of dietary DM; Rumist; DSM Nutritional Products, Inc., Kaiseraugst, Switzerland) to either a dried-rolled corn or steam-flaked corn diet, indicating the need for further investigation of the various enzyme types and their applicability for feeds commonly supplied to beef cattle. A lack of response to amylases may reflect the fact that starch digestion is generally not limited in the rumen, provided that the grains are adequately processed (McAllister and Cheng, 1996).

Recently, the addition of an experimental exogenous proteolytic enzyme (Danisco-Agtech, Waukesha, WI) during the growing phase increased DMI of steers by 14.8%, but an increase in ruminal passage rate reduced NDF digestibility (4.1%) and as a result this increase in DMI was not reflected in improvements in BW gain or feed efficiency nor were any effects observed when this same enzyme was added to a finishing diet (Vera et al., 2012). ZoBell et al. (2000) observed no effects on ADG or feed efficiency when applying the same enzyme product to either a barley-based growing (65:35 forage to concentrate ratio; DM basis) or finishing diet (20:80 forage to concentrate ratio; DM basis) as McAllister et al. (1999), who observed an increase in DMI when this enzyme was applied (0.5 L/t DM) to barley silage as well as increase an in ADG when it was applied at (3.5 L/t total mixed ration [TMR]) to a finishing TMR. Comparatively, Balci et
al. (2007) applied Promote N.E.T. (60 g/d; Agribands Int., St. Louis, MO) with cellulase and xylanase activities to a corn and barley diet and observed increases in ADG and feed conversion efficiency. Eun et al. (2009) supplemented both growing and finishing diets with a commercial enzyme product (Fibrozome; Alltech Inc.) and observed no effect on growth performance, despite minor improvements in carcass characteristics. Lewis et al. (1996) applied Grasszyme (FinnFeeds Int. Ltd., Marlborough, UK) to a grass hay and barley diet (70:30) and measured the impact of application time before feeding and the portion of the diet to which the enzyme was applied. No effects on DMI were observed; however, digestibility of DM, NDF, and ADF increased when the enzyme was added to the forage either 24 h before or at the time of feeding. Similarly, Krueger et al. (2008) applied an enzyme mixture (Biocellulase A20; Loders Croklaan, Channahon, IL) to Bermudagrass hay at 3 different stages, immediately after cutting, at bailing, or at feeding and although enzyme treatment at cutting increased DMI, no effect was observed on final live weight, ADG, or G:F, regardless of the time of application. Evidently, most studies have observed inconsistent responses after enzyme supplementation in beef cattle despite the commonality of a barley-based diet, hampering the adoption of this technology on an industry-wide scale. Similarly, the studies that exhibited increases in ADG used different application methods whereby the enzyme was applied to the TMR in the study by McAllister et al. (1999) in the study of Balci et al. (2007), it was only applied to the concentrate portion of the diet, and as these studies were the only reports of positive effects on production performance, it is difficult to discern at this point which method, if either, would best ensure the efficacy of exogenous enzymes in beef cattle.

Small Ruminants

Generally, the application of exogenous enzymes to the diets of small ruminants has had little impact on production performance. Miller et al. (2008) fed a barley-based diet treated with a commercial exogenous enzyme (Roxazyme G2 Liquid; DSM Nutritional Products Pty Ltd, Basel, Switzerland) to Dorset-cross ewe lambs and observed no effects on DMI, ADG, feed conversion, or wool growth. Similarly, no effects were observed on milk yield, milk composition, or DMI when Promote (Agribands Int., St. Louis, MO) was applied to the diets of lactating Manchega and Lacaune ewes (Flores et al., 2008). Additionally, Rojo et al. (2005) fed exogenous amylases from *Bacillus licheniformis* and *Aspergillus niger* (up 2.90 g enzyme/kg DM sorghum; ENMAX, Mexico City, Mexico) and observed no effects on production performance in Suffolk lambs. As such, the majority of exogenous enzyme studies found in sheep and goats have focused on investigating the impact of exogenous enzymes on diet digestibility. In a study by Reddish and Kung (2007) lambs were fed a commercial diet supplemented with an enzyme mixture (4 g/lamb daily; Alltech Inc.); however, no effect on apparent digestibility of DM, ADF, NDF, or N was observed. A study by Avellaneda et al. (2009) fed Suffolk lambs Guinea grass in conjunction with fibrolytic enzymes (3 g/lamb daily; Fibrozyme; Alltech Inc.) and also reported no effects on DMI, ruminal fermentation, or ruminal or total tract digestion. Giraldo et al. (2008) delivered exogenous fibrolytic enzymes (12 g/lamb daily; Fibrozyme; Alltech Inc.) directly into the rumen of fistulated Merino sheep fed a grass–hay concentrate diet (70:30; DM basis) without affecting diet digestibility. By supplementing the enzyme directly into the rumen the prefeeding feed–enzyme interaction was negated, yet the enzymes were able to stimulate fibrolytic activity and the growth of cellulolytic bacteria. Conversely, Bala et al. (2009) applied 2 levels of exogenous enzymes, described as a cellulase and a xylanase (4,000 and 12,500 or 8,000 and 18,750 IU/kg, respectively), to the diets of lactating Beetle-sannen crossbred goats. The greatest amounts of supplementation decreased DMI (g/kg FCM yield) and increased the digestibility of DM, OM, CP, NDF, ADF, and total carbohydrates and improved FCM yield (kg/d) in the last quarter of lactation. These studies suggest that the application of existing exogenous enzymes to lamb diets has limited impact on diet digestibility or growth performance. Additional studies are required to determine if the response to exogenous enzymes in lactating goats is a reflection of their high metabolic demand for milk production.

**MODES OF ACTION**

**Preconsumption Effects**

Application of exogenous enzymes before consumption appears to be the most effective when they are applied in a liquid form to dry as opposed to wet forage as there appears to be components in silage that can inhibit exogenous enzymes (Morgavi et al., 2000a; Nsereko et al., 2000; Wallace et al., 2001). Even the low moisture content in dry feeds, such as hay and grain, appears sufficient to enable hydrolysis of carbohydrates from complex polymers (Morgavi et al., 2000b). This release of sugars arises, at least partially, from the solubilization of NDF and ADF (Morgavi et al., 2000b; Morrison and Miron, 2000; Devillard et al., 2004) encouraging rapid microbial growth and reducing the lag time required for microbial colonization (Beauchemin et al., 2004). The type of exogenous enzyme and substrate determines the degree of sugar release, yet this represents only a minute portion of the
Table 2. Summary of exogenous polysaccharide-degrading enzyme effects on production traits and total tract apparent digestibility of nutrients in beef cattle

<table>
<thead>
<tr>
<th>Source</th>
<th>Experimental design (number of cows)</th>
<th>Product/manufacturer</th>
<th>Declared primary activities</th>
<th>Application level</th>
<th>Forage level in basal diet</th>
<th>Effects 2</th>
<th>FCR 3</th>
<th>Total tract digestibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beauchemin et al., 1995</td>
<td>Completely randomized (72)</td>
<td>Xylanase B⁴ and Spezyme CP⁵</td>
<td>Xylanase and cellulase</td>
<td>40 to 316 FPU⁶/kg DM</td>
<td>91 to 96.7%</td>
<td>↑⁷</td>
<td>↑⁸</td>
<td>-</td>
</tr>
<tr>
<td>Lewis et al., 1996</td>
<td>Latin square (5)</td>
<td>Grasszyme, FinnFeeds Int.⁹</td>
<td>Xylanase and cellulase</td>
<td>1.65 mL/kg forage DM</td>
<td>70%</td>
<td>-</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Beauchemin et al., 1997</td>
<td>Completely randomized block (56)</td>
<td>Xylanase B⁴ and Spezyme CP⁵</td>
<td>Xylanase and cellulase</td>
<td>4.0 L/t concentrate DM</td>
<td>4.90%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Krause et al., 1998</td>
<td>Latin square</td>
<td>Pro-Mote⁴</td>
<td>Xylanase and cellulase</td>
<td>1.5 g/kg DM</td>
<td>5%</td>
<td>-</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Beauchemin et al., 1999</td>
<td>Completely randomized block (1,000)</td>
<td>Pro-Mote⁴</td>
<td>Xylanase and cellulase</td>
<td>1.4 L/t DM</td>
<td>7.8%</td>
<td>-</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>McAllister et al., 1999</td>
<td>Completely randomized (98 and 66)</td>
<td>FinnFeeds Int.⁹</td>
<td>Xylanase and cellulase</td>
<td>1.25 to 5.0 L/t DM</td>
<td>70 to 82.5%</td>
<td>↑¹⁰</td>
<td>↑¹¹</td>
<td>-</td>
</tr>
<tr>
<td>ZoBell et al., 2000</td>
<td>Completely randomized (32)</td>
<td>FinnFeeds Int.⁹</td>
<td>Xylanase and endoglucanase</td>
<td>15,880 and 5,580 IU/kg TMR¹³ DM</td>
<td>20 to 65%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Balci et al., 2007</td>
<td>Completely randomized (16)</td>
<td>Promote N.E.T.¹⁴</td>
<td>Xylanase and cellulase</td>
<td>60 g/d Ad libitum wheat straw</td>
<td>NR</td>
<td>↑</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Tricarico et al., 2007</td>
<td>Completely randomized block (120, 96, and 56)</td>
<td>Amaize¹⁵</td>
<td>Amylase</td>
<td>580 to 1,160 DU¹⁶/kg DM</td>
<td>-</td>
<td>↑¹⁷</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>Krueger et al., 2008</td>
<td>Completely randomized (50)</td>
<td>Biocellulase A20, Loders Croklaan, Channahon, IL</td>
<td>Xylanase and cellulase</td>
<td>16.5 g/t Ad libitum access to hay</td>
<td>↑¹⁸</td>
<td>-</td>
<td>-</td>
<td>↑DM, NDF, and CP¹⁸</td>
</tr>
<tr>
<td>Eun et al., 2009</td>
<td>Completely randomized (60)</td>
<td>Fibrozyme¹⁵</td>
<td>Endoglucanase, exoglucanase, xylanase, and amylase</td>
<td>1 to 2 g/kg TMR DM</td>
<td>20 to 58%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DiLorenzo et al., 2011</td>
<td>Completely randomized block (32)</td>
<td>RumiStar¹⁹</td>
<td>Amylase</td>
<td>400 kilo novo units/kg DM</td>
<td>5.1%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vern et al., 2012</td>
<td>Completely randomized (48)</td>
<td>Danisco-Agtech, Waukesha, WI</td>
<td>Protease</td>
<td>0.52 g/kg DM TMR</td>
<td>25 to 63.4%</td>
<td>↑²⁰</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

¹ In chronological order.
² ↑ = increase; ↓ = decrease; - = no statistically significant effect; NR = not reported.
³ FCR = feed conversion rate.
⁴ Biovance Technologies Inc., Omaha, NE.
⁵ Genencor, Rochester, NY.
⁶ FPU = filter paper units of cellulase.
⁷ Dependant on forage and application rate (increases seen at alfalfa level 3).
⁸ Dependant on forage and application rate (increases seen at alfalfa level 1, 2, and 3 and at timothy hay level 5).
⁹ Finnfeeds International, Marlborough, Wiltshire, UK.
¹⁰ Only the greatest amount of enzyme application (5.0 L/t) in the backgrounding study.
¹¹ Only in the finishing stage.
¹² Digestion experiment with sheep.
¹³ TMR = total mixed ration.
¹⁴ Cargill Animal Nutrition, Minneapolis, MN.

continued
total carbohydrate present in the diet. As such, it is difficult to attribute production responses solely to the generation of soluble carbohydrates before consumption.

Fibrolytic enzymes that bind to the feed appear to be more active, possibly because of increased resistance to proteolytic inactivation in the rumen (Fontes et al., 1995). Similarly, maximizing the proportion of the diet to which the enzyme is added is considered to increase the chances that the enzymes will remain active in the rumen. Bowman et al. (2002) reported that enzymes were more effective when added to rolled grain, which comprised 45% of the diet, compared with if they were added to a finely ground premix, which comprised 0.2% of the diet (DM basis).

**Ruminal Effects**

Exogenous enzymes have been shown to be more stable in the rumen environment than originally proposed (Hristov et al., 1998b; Morgavi et al., 2000b). For example, Morgavi et al. (2001) found 4 commercial enzymes remained stable when incubated in ruminal fluid, pepsin, or pancreatin. Enzyme stability in the rumen is considered to be a result of glycosylation and is usually enhanced by adding exogenous enzymes to feed before consumption (Fontes et al., 1995). However, non-glycosylated enzymes may also resist ruminal proteolysis, but their persistence in the rumen may depend on the microbial source from which they were derived (Fontes et al., 1995). Variation in enzyme stability may contribute to the inconsistent production responses observed when enzymes are included in ruminant diets.

Supplementing ruminant diets with exogenous enzymes increases the rate but seldom the extent of feed digestion. This suggests that positive responses to present exogenous enzymes are not a result of these preparations solubilizing substrates that would not be normally digested if retained in the rumen for a sufficient period of time. However, an increase in total enzymatic activity in the rumen can increase ruminal hydrolytic capacity, which can enhance the digestibility of the complete diet rather than just being limited to the specific components targeted by the enzyme (Beauchemin et al., 2004). As such, digestibility of both nonfibrous and fibrous fractions can increase, explaining why fibrolytic enzymes can also be effective in increasing the digestibility of nonfiber fractions in high grain diets (Beauchemin et al., 1999).

Given that exogenous enzymes represent only a fraction of enzyme activity in the rumen combined with the inherent capacity of the ruminal microbiota to digest fiber, it is difficult to attribute an increase in fiber degradation by exogenous enzymes to direct hydrolysis alone (McAllister et al., 2001). A synergistic relationship between exogenous enzymes and rumen microbiota and an increase in bacterial attachment are other likely modes of action of exogenous enzymes in the rumen. Synergism acts to increase the effects of both indigenous ruminal microbes and exogenous enzymes so that the combined response exceeds the additive effects of each individual component (Morgavi et al., 2000a). Identification and supplementation with exogenous enzymes not produced by ruminal microbes could be theorized to further heighten this synergistic response. Current enzyme preparations do not appear to introduce novel enzyme activity into the rumen as they typically increase only the rate and not the extent of cell wall digestion (Wallace et al., 2001; Krause et al., 2003; Jalilvand et al., 2008). Exogenous enzymes can also stimulate the attachment of ruminal microbes to plant fiber (Morgavi et al., 2000a), yet the mechanism by which this occurs is unknown. Reduced amounts of exogenous enzymes have been shown to enhance attachment of ruminal bacteria to fiber resulting in a disruption of the hydrogen bonds within the cellulose matrix (White et al., 1993). However, increased amounts of exogenous enzymes can also compete with the ruminal microbial population for cellulose binding sites on feed (Morgavi et al., 2000b), potentially explaining the lack of or even negative responses observed with the increased amounts of exogenous enzyme supplementation in vivo. For exogenous enzymes to be effective, it is important that they complement and not replace the
existing natural enzyme activities produced by ruminal microbes.

Enzymes have also been associated with a reduction in digesta viscosity in poultry (Chocht, 2006); if a similar reduction was observed in ruminants, an increase in passage rate through the rumen would be expected, reducing gut fill and subsequently increasing intake. From a production perspective, increasing intake is beneficial. However, if the enzymes are in the fluid phase and there is a rapid flow of digesta through the rumen, they may not be afforded sufficient time to degrade the fibrous portion of the diet before they are largely inactivated by exposure to the low pH and pepsin in the abomasum (Hristov et al., 1998b).

**Postruminal Effects**

Hristov et al. (1996, 1998a) were the first to report that approximately 30% of xylanases can escape ruminal fermentation and are active in intestinal digesta of ruminants. These findings confirmed previous reports in vitro (Fontes et al., 1995) and studies with pigs (Chesson, 1993; Inbarr et al., 1994). Depending on application level, other enzymes may also bypass the rumen and increase polysaccharide-degrading activities in intestinal digesta (Chesson, 1994). Glycosylation confers proteolytic stability to exogenous enzymes (Gorbacheva and Rodionova, 1977), but as demonstrated by Fontes et al. (1995), nonglycosylated enzymes may also resist proteolysis. Hristov et al. (1998a) identified the abomasum as a major barrier to active exogenous enzymes entering the intestine. A follow-up study by Morgavi et al. (2001) confirmed that some exogenous enzymes survive ruminal fermentation and the abomasal environment and may exert activity for a period of time in the small intestine. In general, xylanases are more stable in the rumen and abomasum than cellulases and consequently xylanase activity in the small intestine that is attributable to exogenous enzymes is usually greater than cellulase activity.

The few studies designed to investigate ruminal stability and bypass of exogenous enzymes have clearly shown that some exogenous enzymes are remarkably resistant to microbial proteases, bypass the abomasums, and remain active in the small intestine and have even been shown to linearly increase polysaccharide-degrading activities in feces (Hristov et al., 2000). However, the practical implication of these effects remains unclear. In theory, exogenous enzymes could improve animal performance by not only enhancing ruminal carbohydrate degradability but also by reducing the viscosity of digesta and improving postruminal nutrient absorption. Presently, although postruminal effects can be documented, they are thought to account for a minor component of any positive responses observed with existing enzyme preparations with improvements primarily arising from positive alterations in rumen function.

**NEW APPROACHES TO FORMULATING EXOGENOUS ENZYMES**

**Role of Metagenomics in Enzyme Discovery**

Before the development and application of the “-omics” disciplines, culturing isolates in a laboratory was the only method of identifying rumen microbial species. Such cultivation-based techniques applied to the rumen identified approximately 60 to 70 genera and 300 to 400 species of bacteria, protozoa, and fungi (Krause et al., 2007). However, this represents less than 10% of the total microbial species that inhabit this environment (Edwards et al., 2004; Krause et al., 2007), raising the question as to whether the numerically dominant or functionally significant members of the rumen remained unidentified. The diversity and complexity of the rumen ecosystem possess a major challenge in terms of determining the functionality and prevalence of the various carbohydrate-degrading enzymes present in ruminal microorganisms over a range of dietary conditions (Morgavi et al., 2012). Although the genomes of 3 of the most highly fibrolytic culturable bacterial species (Fibrobacter succinogenes, Ruminococcus albus, and Ruminococcus flavefaciens) have been sequenced (Krause et al., 2003), the enzymatic strategies used by each of these species to hydrolyze cellulose are still not well understood. Consequently, culture-independent approaches such as metagenomics, metatranscriptomics, and proteomics have been used to further characterize the carbohydrolases produced in the rumen without necessarily attributing their presence to any 1 specific microorganism. With the rapid advancements that have occurred in these sequence- or function-based technologies, an integrated and holistic picture of the metabolic potential and activity of this complex microbial ecosystem is being generated.

Although sequenced-based metagenomics may use various techniques and approaches, the main aim remains to develop a catalogue of all genes present in the rumen (Brule et al., 2009). A recent study by Hess et al. (2011) assembled 15 microbial genomes that were previously unculturable and identified more than 27,000 putative genes coding for carbohydrolases. Functional metagenomics, in this instance, aims to use these catalogues or libraries to identify and isolate specific hydrolytic enzymes involved in structural plant digestion in the rumen (Zhao et al., 2010).
Exogenous enzymes in ruminant production

al. (2005) identified 9 endogulcanases, 12 esterases, and 1 cyclodextrinases within a metagenomic library derived from the rumen of a dairy cow. The success of this approach in identifying novel enzymes and metabolic pathways, however, is still dependent on the availability of appropriate bioassays and the development of innovative strategies to screen for enzyme activities of interest. Current interest is focusing on the degradation of cellulose and hemicellulose by enzyme members of the glycoside hydrolase family 5 (GH5; Morgavi et al., 2012). This family of glycosyl hydrolases represents the most abundant cellulases from both cultured (Krause et al., 2003) and uncultured ruminal bacteria (Ferrer et al., 2005). As such, it is not surprising that even though Duan et al. (2009) identified and characterized novel cellulases from the rumen, they were identified as members of the GH5. Recently a metatranscriptomic approach was used by our laboratory (Qi et al., 2011) to examine carbohydrases associated with fungi and protozoa in the rumen of musk oxen, identifying a greater percentage of cellulases per gigabase of sequence than Hess et al. (2011). The application of this technology can be broadened to study carbohydrase complexes in the rumen of a variety of ruminant species over a range of ecological niches. In fact, New Zealand researchers are presently leading an international research group with the objective of collecting samples from ruminants across the globe. This global survey could provide new insight into the carbohydrases that are key to plant cell wall digestion in a variety of species. Identification of carbohydrases that are lacking within ruminal environment could provide the knowledge needed to specifically formulate exogenous enzymes that can fill these deficiencies.

A Focus on Rate-Limiting Enzymes

Lignin is the most recalcitrant of the 3 main heterogeneous polymers in lignocellulose (Himmel et al., 2007; Sanchez, 2009). The formation of ferulate-polysaccharide-lignin complexes that cross-link cell wall polymers is a major factor limiting the rate and extent of enzymatic dissolution of cell walls as it interferes with hydrolysis by preventing the binding of xylanases to their target substrate (Hatfield et al., 1999; Buanafina et al., 2008). However, the cellulolytic capacity of the rumen is not considered to limit cellulose digestion; rather, it is the surface area available for enzyme attachment that limits lignocellulose digestion in the rumen (Weimer et al., 1990). True degradation of lignin is an oxidative process that is primarily performed by aerobic fungi. As the rumen is anaerobic, lignin is not truly degraded, but rather its solubilization is a key step in increasing the amount of cellulose and hemicellulose available for microbial fermentation. Chemical pretreatment with either acidic (e.g., sulfuric acid; Himmel et al., 2007) or alkali solutions (e.g., ammonium hydroxide, sodium hydroxide) aims to increase the susceptibility of cellulose to enzymatic hydrolysis by breaking the ester bonds that link lignin with the plant cell wall (Buanafina et al., 2008). In biorefineries, cellulosic feedstocks are subjected to pretreatment strategies including thermochemical pretreatment, where dilute sulfuric acid is applied at 140 to 200°C, rendering the cellulose in cell walls more accessible to saccharifying enzymes. Alternatively, the application of alkalines through processes such as ammonia fiber expansion renders cell walls considerably more susceptible to enzyme hydrolysis (Himmel et al., 2007). Such pretreatments can reduce the recalcitrance of cellulose to enzymatic hydrolysis; however, they are costly, require heat or pressurization, and can reduce the availability of fermentable carbohydrate (Wyman, 2007; Weimer et al., 2009).

Carbohydrases have advantages over the use of alkali treatment as chemical treatments can be detrimental to the digestibility of other plant components and denature plant-associated enzymes (Mathew and Abraham, 2004). Ferulic acid esterases, produced by A. oryzae, perform a function similar to that of alkali de-esterification of lignin–hemicellulose linkages in plant cell walls (Tenkanen et al., 1991). Ferulic acid esterases act synergistically with xylanases and pectinases to facilitate access of hydrolytases to the backbone of cell wall polymers. However, the source and type of ferulic acid esterase can influence the amount of ferulic acid released and thus the accessibility of cellulose (Faulds et al., 2003, 2006).

The success of this technology is reliant on its ability to encompass a vast array of dietary and production scenarios. Therefore, it becomes essential to examine variations in ruminal microbial communities across vastly different production environments. The use of the metagenomic and metatranscriptomic procedures described above to identify high activity esterases that could be used to complement existing enzyme products could be one approach to improving the efficacy of these preparations (Fig. 1.). Similarly, the development and refinement of biomass pretreatment strategies that can be used in conjunction with exogenous enzymes to enhance hydrolysis of cellulose in the rumen could further improve the efficacy of enzyme preparations. Effective use of these technologies is considered the most promising way to identify novel enzyme cocktails and thus advance enzyme technology by minimizing the variability currently seen in production responses of ruminants.
Conclusion

The use of exogenous enzymes in ruminant diets is still an emerging technology. Although progress has been made, previous strategies have been largely unsuccessful at improving ruminant production or produced highly variable results due to our lack of understanding of the carbohydrase complex and microbial interactions within the rumen ecosystem. Enzymes have been added to diets with the aim of increasing cell wall digestibility and have shown some improvements in milk yield and feed conversion efficiency, yet the true mechanistic effects responsible for these positive outcomes remain elusive. New approaches such as metagenomics, meta-
transcriptomics, and proteomics are providing novel insights into the structure, interactions, and function of the rumen microbial community to a degree that was previously impossible with lab-based culture techniques. Such approaches have the potential to allow for formulation of exogenous enzymes based on optimal properties for specific ruminal conditions. Ideally, responses to these additives will need to be broad based across a range of diet types as development of a specific exogenous enzyme formulation and dose rate for each diet type and feeding level would introduce a degree of complexity in on-farm application that would likely discourage adoption by producers.

**LITERATURE CITED**


