Technologies for Improving Fiber Utilization

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Introduction

Forages typically account for 40 to 100 % of the ration of dairy cows and are vital for maintaining animal productivity and health. The high fiber content of forages is the main nutritional factor that differentiates them from concentrates and results in a relatively lower energy value. Nevertheless, fiber plays a fundamentally important role in ruminant livestock production, health, and welfare. In addition to being an important energy source, it stimulates chewing and salivation, rumination, gut motility, and health, buffers ruminal acidosis, regulates feed intake, produces milk fat precursors and is the structural basis of the scaffolding of the ruminal raft, which is vital for digestion of solid feed particles in the rumen. Cellulose and hemicellulose – the main components of fiber – are intrinsically ruminally digestible. However, their close association with lignin and hydroxycinnamic acids like ferulic acid in the plant cell wall is the greatest hindrance to complete digestion of feeds, particularly forages and byproducts, and to utilization of the nutrients and energy they contain. The degree of association with lignin and hydroxycinnamic acids and various plant anatomical features differentiate digestible from indigestible fiber. This paper describes the strategic importance of increasing forage fiber utilization and then discusses the efficacy and mode of action and benefits and disadvantages of different technologies for improving fiber digestion.

Importance of Increasing Fiber Digestion

It is critically important to increase fiber digestion for productivity, profitability and environmental reasons. Incomplete fiber digestion reduces the profitability of dairy production by limiting intake and hence, animal productivity, and increasing manure production. A 1-unit increase in forage NDF digestibility (**NDFD**) is associated with 0.17 and 0.25 kg/d increases in DMI and milk production, respectively (Oba and Allen, 1999). In addition, in perennial ryegrass (*L. perenne*), a 5–6 % increase in digestibility was estimated to increase milk production by up to 27% (Smith et al., 1998). Consequently, each percentage unit increase in lignin concentration in forage cell walls severely constrains DMI and milk production.

A second reason to increase fiber digestion is to increase energy supply from fibrous feeds that are not consumed by humans. Grains are high in energy and various

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processes have been developed for increasing the efficiency of energy extraction from such feeds for ruminants and non-ruminants. However, the growing demand for grains due to competition with the non-ruminant feed, biofuel and human food sectors, results in considerable price hikes and volatility. Fibrous feeds for ruminants are less subject to such competing demands but their recalcitrant lignocellulose matrix reduces the availability of the energy they contain, necessitating effective strategies for increasing the rate and efficiency of utilization of forage fiber and the energy therein.

It is equally necessary to increase fiber digestion for environmental reasons. Compared to that from starch, ruminal fermentation of fiber-derived hexoses generates more hydrogen ions that reduce carbon dioxide to methane. Consequently, fibrous feed fermentation results in greater production of methane and less energy supply than concentrate feeds. In addition to being a significant drain on energy supply to the cow, enteric methane production is a significant contributor to greenhouse gas emissions. In addition, enteric methane is the main source of agriculture-related methane emissions also, which has resulted in advocation of vegan diets for environmental reasons (Poore and Nemecek, 2018) and cessation of livestock production, despite the critical role livestock and livestock products play in human nutrition, income generation and livelihoods (Adesogan et al., 2018) or the fact that because ruminants convert forages that humans cannot consume into high quality food protein, only 14% of feed dry matter ingested by livestock is edible to humans (Mottet et al., 2017). Consequently, it is of paramount importance to increase forage fiber digestion to enhance animal productivity and environmental stewardship of livestock farming.

Various animal, plant, and environmental factors that modulate the intake and digestibility of forage fiber have been described in excellent reviews (Galyean and Goetsch, 1993; Fales and Fritz, 2007). The main plant-based factors affecting the digestibility and intake of forage fiber are: 1) chemical composition of tissues in organs, 2) tissue type and proportion within organs, and 3) organ type and proportion in plants. The interplay between these factors has a major effect on the intake and digestion of nutrients by ruminants (Jung, 2012), with chemical composition becoming more predominant than the other factors with increasing levels of mechanical processing.

Strategies to Increase Forage Fiber Digestion

Mechanical Processing. Mechanical processing is a crucial complementary step in forage production due to its impact on forage physical properties that cause gut fill and limit feed intake. Consequently, numerous studies have examined the influence of mechanical processing on particle size measures, typically the chop length of hay, straw, or silage or the particle size distribution of diets for lactating cows. Forage particle size is critically important in dairy cattle diets, which must contain sufficient physically effective NDF (**peNDF**; Hall and Mertens, 2017) – a combination of both physical (i.e. particle size) and chemical (i.e. NDF concentration) fiber characteristics (Mertens, 1997) – to stimulate chewing and salivation and reduce gut fill, without reducing digestibility. In this context, grinding of forages or byproducts will not be discussed because it removes the physical effectiveness of fiber.

Chopping. Despite the undeniable benefits of coarser forage particles on ruminal mat formation, chewing activity, digestion, and milk fat content (Allen, 1997; Mertens, 1997), long forage particles may limit intake through reduced ruminal passage rate and increased fill (Mertens, 1987). Furthermore, they promote dietary sorting (Leonardi and Armentano, 2003), and enhance the time spent consuming a meal (Grant and Ferraretto, 2018). Although particle size can be manipulated to enhance fiber digestibility, research findings have been inconsistent, and the outcome is not related to alterations in the chemical composition of forage fiber. A meta-analysis of published studies (Ferraretto and Shaver, 2012b) reported that digestibility of dietary NDF, DMI, and milk production were not altered by chop length of corn silage. This should not be surprising as fiber digestion is influenced by many factors and the combination of the benefits of long or short forage particles may be countered by the disadvantages. For instance, short forage particles have greater surface area for bacterial attachment, which may enhance forage digestibility despite their faster passage rate (Johnson et al., 1999). In contrast, coarse particles are retained for longer periods in the rumen and require more chewing leading to greater ruminal pH (Allen, 1997), which is conducive for cellulolytic bacteria and forage digestion in general.

Shredding. Shredding ensiled whole-plant corn at harvest is an effective method to alter the physical characteristics of the silage. A recently developed form of silage, called corn shredlage, is produced when corn forage is harvested with a self-propelled forage harvester fitted with cross-grooved crop-processing rolls set at approximately 20% greater roll speed differential and chopped at a greater theoretical length of cut (22 to 26 mm) than the norm. Despite the longer chop length used, the shredding process causes greater damage to coarse stover particles and kernels than conventional harvesting. Compared to conventionally processed silage, yields of 3.5% FCM and actual milk were increased by 1.0 and 1.5 kg/cow per day when whole-plant corn was harvested as corn shredlage using either conventional (Ferraretto and Shaver, 2012a) or brown midrib (**BMR;** Vanderwerff et al., 2015) hybrids, respectively. These results were attributed to the greater kernel breakage obtained by shredding whole-plant corn and the corresponding improvements in ruminal in situ and total tract in vivo starch digestibility (Ferraretto and Shaver, 2012a; Vanderwerff et al., 2015). Surprisingly, despite what appeared to be more thorough damage to the fibrous portion of the forage, Vanderwerff et al. (2015) reported that total tract NDFD was 2 percentage units lower when cows were fed corn shredlage instead of conventionally processed corn silage. These authors associated this response with the negative effects of the greater digestibility of shredlage starch on total tract NDFD. This premise was supported by the fact that ruminal in situ NDF digestibility of undried and unground corn silage samples did not differ among treatments. Finally, near-infrared reflectance spectroscopy-predicted 30-h NDFD was lower in corn shredlage (55.0 vs. 53.4% of NDF) compared to conventionally processed corn silage in an assessment of 3,900 commercial samples (Ferraretto et al., 2018). Although the benefits of harvesting corn silage with a shredlage processor are undeniable, some factors must be considered when evaluating the cost effectiveness. In addition to the costs associated with acquiring the processor (or a new self-propelled forage harvester), other factors such as changes in fuel usage and roll wear must be considered as they may differ from those involved in conventional processing. To our knowledge, this information is unavailable in the literature and should be the focus of future research.

Pelleting. In addition to the aforementioned effects of particle size, pelleting may enhance handling, storage and transportation (Bonfante et al., 2016), and it enhances the use of certain bulky forage or crop residues as livestock feeds (Mani et al., 2006). The use of forage pellets, however, is not a new concept. For example, Clifton et al. (1967) evaluated coastal Bermudagrass (*Cynodon dactylon (*L.*) Pers.*) fed as either silage or pellets. Cows fed forage pellets had greater intake but did not have greater animal performance. Caution is needed when forages are fed as pellets to due to the risk of acidosis from the reduced saliva production and resulting reduction in ruminal acid buffering caused by pelleting, but the effect may depend on the production stage of the cows. Bonfante et al. (2016) fed a pelleted TMR to growing heifers and did not observe adverse effects on ruminal health, though the authors advocated examining the pellets for longer feeding periods. Total tract digestibility of the potentially digestible NDF fraction was reduced, presumably due to reduced ruminal retention time. This suggests that using pelleted TMR for growing heifers may not adversely affect rumen health, but caution is needed to ensure digestibility is not reduced. In contrast, when alfalfa (*Medicago sativa* L.) pellets were substituted for alfalfa hay to induce subacute ruminal acidosis in dairy cows (Khafipour et al., 2009; 8% - unit increments from 50 to 10%, DM basis) over 6 weeks without altering forage to concentrate ratio and starch concentration, a gradual increase in consumption of pellets instead of hay was evident but yields of milk and milk fat and ruminal pH decreased in a linear manner. The latter results emphasize that pelleting TMR for lactating cows can be detrimental and reinforces the need to account for peNDF during diet formulation.

Genetic Improvement

Brown-midrib mutants. Improvements to fiber digestibility of forages are often accomplished by reducing lignin or indigestible NDF concentrations (Grant and Ferraretto, 2018). Brown midrib mutant forages (e.g., corn and sorghum) consistently have lower lignin concentrations compared to conventional forages (Sattler et al., 2010) resulting in greater milk production when the BMR forages are fed. In this context, several studies have reported greater DMI, passage rate, and rate of NDF digestion in cows fed BMR compared to conventional corn silage (Oba and Allen, 2000; Ebling and Kung, 2004). In a meta-analysis of published studies, Ferraretto and Shaver (2015) reported increases in total tract NDFD (44.8 vs. 42.3% of intake), DMI (24.9 vs. 24.0 kg/d), yields of milk (38.7 vs. 37.2 kg/d) and protein (1.18 vs. 1.13) for cows fed BMR diets instead of conventional corn silage diets. These benefits are associated with lower rumen gut fill as conventional forage-based diets may have lower rates of passage and digestion, causing physical constraints in the rumen (Allen, 1996) that limit intake.

As for corn, BMR sorghum (*Sorghum bicolor)* has lower lignin concentration and greater fiber digestibility compared to conventional sorghum (Sattler et al., 2010). A meta-analysis by Sánchez-Duarte et al. (2019) compared conventional to BMR sorghum silage

(**BMRSS**) in diets for dairy cows and revealed that cows fed BMRSS had greater intake (+0.8 kg/d), milk production (+1.6 kg/d) and milk fat concentration (+0.09%-units) than cows fed conventional sorghum. In addition, when compared with conventional corn silage, cows fed BMRSS had greater milk fat (+0.10%-units) but lower milk protein (-0.06%-units) concentrations. No differences in intake and milk yield were observed. Such BMR hybrids would be particularly desirable in areas or situations unsuitable for corn production. Some studies have shown that lodging can be a problem for some BMR sorghum hybrids particularly when sown at high seeding rates (Pedersen et al., 2005), but the incidence may be reduced by increasing plant spacing or planting brachytic dwarf hybrids that are less prone to lodging (Bernard and Tao, 2015).

Genetic improvement is resulting in BMR hybrids that are higher yielding than earlier hybrids. Nevertheless, it is important to account for lower yields of certain BMR hybrids than conventional hybrids when deciding on which hybrid to grow. Such lower yields may be outweighed by the improved animal performance from BMR hybrids but the magnitude of the improvements may vary from farm to farm based on the prevailing conditions. Producers should consider establishing guidelines for using BMR hybrids such as feeding them to high-producing cows in early lactation while feeding less digestible conventional hybrids to cows in mid-to-late lactation. Such guidelines should be based on recommendations of animal nutritionists and agronomists who are familiar with the prevailing conditions on the dairy farm.

Reduced-lignin alfalfa. Feeding reduced-lignin alfalfa to dairy cows has been studied for over a decade. Guo et al. (2001) examined the lignin concentration and IVNDFD of 6 independent transgenic alfalfa lines with reduced lignin concentration compared to control lines (non-transgenic) and reported a range from 13 to 29% in lignin concentration. Furthermore, they observed an increase of 8% in IVNDFD for one of these transgenic lines compared to its isogenic counterpart. Mertens and McCaslin (2008) fed transgenic alfalfa hay with reduced lignin concentration (5.3 vs. 5.8% of DM) to young lambs and observed greater NDF intake (1.6 vs. 1.42% of BW/d) and digestibility (57.5 vs. 49.1% of NDF intake) compared to a non-transgenic line. When this same transgenic alfalfa variety was fed to dairy cows by Weakley et al. (2008), total tract NDFD was greater for cows fed transgenic compared to non-transgenic alfalfa but no differences in DMI, milk yield or milk fat concentration were observed. Li et al. (2015) tested effects of 2 transgenic alfalfa cultivars (Roundup-ready vs. Roundup-ready low-lignin) and reported greater in vitro total tract NDFD for the low-lignin alfalfa. This response was primarily driven by alterations in the NDF to lignin ratio as lower NDF (30.1 vs. 31.6% of DM) but similar lignin (5.6 vs. 5.5% of DM) concentrations were reported for the Roundup-ready conventional versus Roundup-ready low-lignin cultivar. However, no peer-reviewed dairy cow feeding studies on low-lignin alfalfa was found, so caution is required when interpreting these results; further studies evaluating responses of dairy cows are warranted. As for BMR hybrids, it is important to account for potential variations in yields and prices when choosing between reduced-lignin alfalfa and conventional varieties.

Chemical Treatment

Alkali treatment. Alkali treatments break hemicellulose-lignin and lignocellulose bonds, hydrolyzing uronic and acetic acid esters, and disrupting cellulose crystallinity by inducing cellulose swelling (Jung and Deetz, 1993). These processes increase cell wall degradability and enable ruminal microorganisms to attack the structural carbohydrates and increase degradation of hemicellulose and cellulose (Jung and Deetz, 1993; Sun et al., 1995). Additionally, alkali treatment has potential to degrade lignin, thereby increasing its water solubility and allowing it to be removed from the cell wall (Chesson, 1988). Various alkalis including ammonia, sodium hydroxide (NaOH), calcium oxide (CaO) and calcium hydroxide ($Ca(OH)_2$) have been used to increase fiber digestion and hence nutritive value of low quality forages, particularly crop residues (Singh and Klopfenstein, 1998). However, their widespread adoption to improve forage quality has been hindered by factors like the cost of application, the hazardous nature, or their corrosiveness.

Ammoniation. Improves forage digestibility by hydrolyzing linkages between lignin and structural polysaccharides (Dean et al., 2008). Ammoniation of low quality forages like bermudagrass hay (Dean et al., 2008; Krueger et al., 2008), Bahiagrass hay (Krueger et al., 2008), cereal straws including barley, wheat, and oat (Horton and Steacy, 1979) has resulted in improved intake, increased DM and NDF digestibility, and N concentration, and improved milk production (Kendall et al., 2009). However, the effects of feeding ammoniated forages on lactation performance by cattle are not consistent (Brown et al., 1992). Also, ammoniation has not gained widespread commercial acceptance due to its high cost, and caustic effect of the alkali when inhaled or ingested excessively by humans and animals (Krueger, 2006). Urea treatment is a safer and easier method of ammoniation that poses far less handling and safety risks (Sundstol and Coxworth, 1984). In addition, it is easy to transport and store. However, the amount of urease activity and moisture content of forages determine the efficacy of urea treatment, as both are required for the formation of ammonia from urea.

Ammonia-fiber expansion. The ammonia-fiber expansion (**AFEX**) is an alternative to direct ammoniation that combines chemical and physical treatments. The method involves ammoniating low quality forages at high temperature and pressure, with subsequent pressure release and ammonia removal (Campbell et al., 2013; Griffith et al., 2016) or recycling. Recently, Griffith et al. (2016) reported 35 and 27% greater in vitro dry matter digestibility (**IVDMD**) and IVNDFD due to AFEX treatment of barley straw. Mor et al. (2018) reported improved nutrient digestibility (DM, OM, CP, NDF, and ADF) of AFEX-treated wheat straw. However, acetamide, a co-product of the AFEX treatment (Weimer et al., 1986) may remain with the treated biomass. Early research indicates that ruminal accumulation of acetamide from AFEX treatment is transient in the rumen because certain ruminal bacteria can grow on the amide (Mor and Mok, 2018). More research on the effects and fate of residual acetamide in cattle fed AFEX-treated forages is needed to ensure that meat and milk are not contaminated.

Sodium hydroxide. Sodium hydroxide treatment originally entailed soaking forage with the dilute alkali for several days followed by washing to remove unreacted residues (Jackson, 1977). This was effective at improving in vitro OM digestibility of a low-quality forages like rye straw from 46 to 71% (Sundstol, 1988), but it contributes to environmental pollution via the effluent. The dry method of NaOH treatment involves spraying dilute NaOH onto the forage without rinsing prior to feeding. Treating rice straw with 4% NaOH via the dry method improved net energy value and increased DMI, and growth performance in steers and feeder lambs (Garrett et al., 1974). Similarly, NaOH treatment (4% DM basis) of corn stalks wetted with 50% moisture from added water increased OM digestibility by 20% compared to untreated stalks (Klopfenstein et al., 1972). The main advantage of the dry method is that it is less labor intensive and issues with wastewater pollution are avoided. However, because excess NaOH is not rinsed off, there are greater chances of toxicity if forage samples are not uniformly treated. Like ammonia, NaOH is caustic and hazardous.

Calcium oxide or calcium hydroxide. Alternatives to ammonia and NaOH are less hazardous alkalis such as CaO and $Ca(OH)_2$. Wanapat et al. (2009) observed greater DMD and 3.5% FCM production by dairy cows fed rice straw treated with a combination of urea (2.2%) and $Ca(OH)_2$ (2.2%). Chaudhry (1998) treated wheat straw with CaO and reported greater OM, NDF and ADF digestibility. Similarly, Shreck et al. (2015) reported greater DMI, feed efficiency, and average daily gain in beef steers with diets containing 5% CaO treated-corn stover or wheat straw compared to diets using the untreated forages.

Acid treatment. Acid hydrolysis for pretreatment of lignocellulosic materials is hydrolyzes hemicellulose, decreases cellulose crystallinity, and increases the porosity of treated biomass (Sun and Cheng, 2005). To foster ease of handling and cost-effectiveness, dilute acid treatment is preferred. Torget et al. (1990) treated switchgrass with dilute sulfuric acid (H_2SO_4; 0.45-0.50%, v/v) and reported 95% xylan hydrolysis and concomitant improvement in cellulose digestibility. Similar results were observed with dilute H_2SO_4 pretreatment of corn cobs and corn stover (Torget et al., 1991). Acid hydrolysis can also improve subsequent enzyme-mediated increases in cell wall digestibility by increasing the pore size of the treated material as reported for corn stover (Ishizawa et al., 2007). However, acid pretreatment is not widely used of the cost, health hazards and corrosive nature of the acids.

Studies have also reported use of inorganic (H_2SO_4) and organic acids (formic acid) as silage preservatives (O'Kiely et al., 1989; Henderson, 1993). Sulfuric acid reduces the pH of forage thereby inhibiting the activity of undesirable bacteria such as enterobacteria and clostridia, and stimulating lactic acid bacteria; however, the effects on animal performance are not promising (O'Kiely et al., 1989). Organic acids, in particular formic acid, induce antibacterial activity and restrict the activity of lactic acid-producing bacteria thereby conserving water soluble carbohydrates for animals (Bosch et al., 1988). Studies have reported decreased acetic acid, lactic acid, and ammonia-N concentrations along with greater sugar concentrations with formic acid-treated alfalfa (Nagel and Broderick, 1992) or ryegrass (Mayne, 1993), compared to the control silage. The effects of feeding formic acid treated silage on animal performance are inconsistent. The use of acids as silage preservatives has declined due to their corrosive effects on machinery and potential health hazards for humans (Lorenzo and Kiely, 2008). Ammonium tetraformate is a buffered form of formic acid, which is less corrosive in nature and easier to handle.

Broderick et al. (2007) fed ammonium tetraformate-treated alfalfa silage to lactating dairy cows and reported greater DMI, and yields of milk, milk protein, FCM and greater N-efficiency compared to untreated alfalfa silage.

Exogenous Fibrolytic Enzymes

Cellulase-xylanase enzymes. Limited understanding of the composition and mode of action of exogenous fibrolytic enzymes (**EFE**) has restricted the development of effective EFE preparations that consistently improve fiber digestion and the performance of cattle (Beauchemin and Holtshausen, 2010; Adesogan et al., 2014). The effects of EFE on forage nutritive value are influenced by various factors including the dose, activity and composition (Eun and Beauchemin, 2007), proteomic profile (Romero et al., 2015a), prevailing pH and temperature (Arriola et al., 2011), presence of metal ion cofactors (Ca^{2+}, Co^{2+}, Fe^{2+}, Mg^{2+}, and Mn^{2+}) (Romero et al., 2015b), animal performance level (Schingoethe et al., 1999), and the dietary fraction to which the EFE is applied (Dean et al., 2013).

The effects of EFE in ruminant diets can be classified as pre-ingestive, ruminal, and post-ruminal (McAllister et al, 2001). When EFE are applied to fibrous substrates before feeding, fiber hydrolysis can be observed as partial solubilization of NDF and ADF and release of sugars and free or monomeric hydroxycinnamic acids (Krueger et al., 2008; Romero et al., 2015c). These factors may contribute to improvements in in vitro fiber digestibility (Romero et al., 2015a) and microbial growth (Forsberg et al., 2000). Adding EFE to the diet increases the hydrolytic capacity of the rumen mainly due to increased bacterial attachment (Wang et al., 2001) and stimulation of ruminal microbial populations (Nsereko et al., 2002). Furthermore, Morgavi et al. (2000) showed that synergism between EFE and ruminal microbes enhanced ruminal cellulose, xylan and corn silage digestion. Adding EFE may also increase the hydrolytic capacity of the rumen by adding complementary enzyme activities that are absent. For instance, rumen metagenomic and metatranscriptomic studies have shown that the glycoside hydrolase family GH7 is absent in the rumen but is present in certain aerobic microorganisms (Dai et al., 2015).

Application of EFE often increases fiber hydrolysis and NDFD of forages, which partially explains their ability to improve animal performance. A recent meta-analysis of published studies reported that EFE application to dairy cow diets resulted in an increase in milk yield (0.83 kg/d) and this was attributed to a tendency for EFE to improve NDF and DM digestibility (Arriola et al., 2017). This meta-analysis also reported that application to the TMR instead of the concentrate or forage tended to improve milk protein concentration. Another recent meta-analysis reported an increase in milk yield (1.9 kg/d) when cows were fed enzyme-treated diets containing high forage to concentrate ratios (≥ 50%), but no increase occurred when diets with low forage to concentrate ratios (< 50%) were fed (Tirado-Gonzalez et al., 2017). The latter study reported also that cellulose-xylanase enzyme treatment of high-forage, legume-based diets increased milk production by cows (2.3 kg/d) as did xylanase treatment of high-forage grass-based diets (3.1 kg/d).

Though the meta-analyses cited above indicate that overall effects of EFE on fiber digestion and milk yield by dairy cows are positive, the results of individual studies have been variable. This is partly because of inadequate understanding of enzyme nomenclature and activity, which results in some ineffective preparations (Adesogan et al., 2014), degradation of enzymes and loss of their activities in the rumen (Colombatto and Beauchemin, 2003; Arriola et al., 2017), adaptation of enzymes developed for paper, textile and other applications for ruminant nutrition (Beauchemin et al., 2003; Adesogan et al., 2014) and formulation of enzyme products that do not complement ruminal enzyme activities (Ribeiro et al., 2018). Recent approaches like proteomics, metagenomics and metatranscriptomics, are providing a better understanding of the structure, interaction, and functions of the ruminal microbial community (Meale et al., 2014). The knowledge generated by these new techniques should be exploited in formulating enzyme preparations that will persist in the rumen and effectively and consistently improve fiber digestion.

Esterase and etherase enzymes. When ferulic acid cross-links arabinoxylans and lignin via ester and ether linkages, in plant cell wall, the extent of digestion is reduced dramatically (Jung and Deetz, 1993). Jung and Allen (1995) hypothesized that the ester portion of the ferulic acid bridge is not available to enzymes because the lignin polymer is in such close proximity, impeding substrate attachment. Ferulic and p-coumaric acid esterases have been used recently to increase the potency of EFE in ruminant diets (Beauchemin et al., 2003; Krueger et al., 2008).

Etherase enzymes are required to hydrolyze ether linkages and release ether-linked ferulic acid from cell walls but they are produced rarely by fungi and are not present in the rumen environment. Mathieu et al. (2013) reported no β-etherase activity from 26 fungal strains (including *Humicola grisea*, *Aspergillus* sp. and *Trichoderma viride*) within 3 ecological groups (white, brown, and soft - rot fungi) cultured with Tien and Kirk medium and supplemented with or without sawdust. The authors concluded that cleavage of β-aryl ether linkage by extracellular β-etherase is a rare and nonessential activity among wood-decaying fungi.

Bacterial inoculants. Applying EFE with microbial inoculants to forages at ensiling is beneficial as they may hydrolyze plant cell walls into sugars that serve as fermentation substrates, thus improving silage fermentation, nutrient preservation and utilization of the silage by animals (Muck and Bolsen, 1991). Consequently, some silage inoculant preparations contain fibrolytic enzymes, mainly cellulases or xylanases, and some studies have reported increased silage NDFD due to application of such products (Filya and Sucu, 2010; Queiroz et al., 2012).

Certain inoculants contain bacteria that secrete fibrolytic enzymes including cellulases, xylanases, and ferulic acid esterase (FAE) that may contribute to increased fiber digestion. Addah et al. (2011) reported that using a mixed bacterial culture containing *L. buchneri* LN4017 that produces FAE, and contains *L. plantarum* and *L. casei,* increased in situ NDF disappearance after 24 and 48 h of incubation by 40.5 and 14.5 %, respectively. Unfortunately, the enzyme activities or enzyme-secreting ability of inoculant bacteria are rarely declared on inoculant labels. Nevertheless, in recent meta-analyses, although no effects on NDFD were observed when bacterial homofermentative and facultative heterofermentative inoculants were applied to forages, milk yield was improved (Oliveira et al., 2017). More information is needed on the enzyme activities produced by inoculant bacteria, as this may lead to development of inoculants that are more potent at increasing fiber digestion.

Expansins. Expansins and expansin-like proteins are a recently discovered group of non-hydrolytic proteins with the unique ability to induce cell-wall relaxation or loosening (Cosgrove, 2000). They are relatively small proteins (between ~26 to 28 kDa) with disruptive activity that weakens cellulose fibers thereby enhancing accessibility and hydrolysis by cellulases and hemicellulases (Kim et al., 2009). Perhaps the most remarkable characteristic of expansins and expansin-like proteins is their ability to synergize with EFE to increases hydrolysis of cellulose and hemicellulose (Kim et al., 2009; Bunterngsook et al., 2015; Liu et al., 2015). Previous studies have demonstrated that synergistic effects between BsEXLX1 and exogenous fibrolytic enzymes (EFE) increased hydrolysis of cellulose and hemicellulose more than 5-fold compared to EFE alone (Kim et al., 2009). Recently, it was demonstrated that BsEXLX1 has greater affinity towards substrates with high concentrations of lignin (Kim et al., 2013), which further confirms that they may be particularly effective at increasing the efficacy of EFE at digesting the forage lignocellulose complex, particularly in C_4 grasses and legumes, which tend to be more lignified than C_3 grasses.

The recombinant expression of expansin and expansin-like protein is currently the only viable method to study these proteins due to lack of commercially available products. Bacterial expansin-like proteins (**BsEXLX1**) from *Bacillus subtilis* have been used as the gold standard to study the disruptive effects of expansins and expansin-like proteins on hydrolysis of cellulose. Preliminary studies have shown greater IVDMD and IVNDFD of bermudagrass silage by approximately 4% and 16%, respectively by applying both additives instead of the EFE alone (Pech-Cervantes et al., 2017). In contrast, no synergistic increases in IVDMD or IVNDFD were observed with corn silage (Pech-Cervantes et al., 2017).

Live yeast, yeast culture and yeast fermentation products. Yeast products include live yeast, yeast culture and yeast fermentation products that can be produced from different strains of *Saccharomyces cerevisiae.* Various studies have shown that yeast products improved fiber utilization and animal performance (Ferraretto et al., 2012; Jiang et al., 2017a). However, some studies have shown that they did not improve nutrient digestibility (Ouellet and Chiquette, 2016), dairy cow performance (Ferraretto et al., 2012), or ruminal fermentation and microbiome composition (Bayat et al., 2015). A meta-analysis by Desnoyers et al. (2009) showed that adding live yeast to dairy cow diets increased OM digestibility by 0.8 percentage-units, DMI by 0.44 kg/d, and milk yield by 1.2 kg/d by dairy cows. Similarly, in another meta-analysis by Poppy et al. (2012), yeast culture addition to the diet increased milk and milk fat and protein yields by 1.18, 0.06, and 0.03 kg/d. Increases in milk production by yeast supplementation may be due to improvement in fiber utilization.

Yeast supplementation has decreased lactate production and enhanced lactate utilization (Lynch and Martin, 2002); thereby stabilizing ruminal pH and increasing NDFD (Marden et al., 2008). In addition, live yeasts have the ability to scavenge O_2 and reduce the redox potential of ruminal fluid (Newbold et al., 1996). These changes supposedly make the ruminal environment more conducive for the growth of anaerobic microorganisms including cellulolytic bacteria (Newbold et al., 1996; Marden et al., 2008). Additionally, yeast product supplementation may provide soluble growth factors such as vitamin B, amino acids, and organic acids that are beneficial for the growth of major cellulolytic bacteria (Callaway and Martin, 1997; Jiang et al., 2017 a, b).

Overall, supplementation with live yeast or yeast culture increased milk yield by dairy cows but the magnitude of the response varies with the lactation stage of cows, the yeast product, diet composition and stressors on the cow. Therefore, future studies should aim to optimize yeast products to achieve consistent improvements in fiber digestion and animal performance over a wide range of conditions. In addition, research should identify the relative importance of the main active ingredients of yeast products, particularly yeast cultures or fermentation products to determine their mode of action.

White-rot fungi The white-rot fungi achieve lignin depolymerization through the activity of their ligninolytic enzymes, which include lignin peroxidase, manganese peroxidase, versatile peroxidase, laccase, and H_2O_2-forming enzymes (such as (methyl) glyoxal oxidase and aryl alcohol oxidase) (Wong, 2009; Sindhu et al., 2016). In addition, white-rot fungi also use extracellular reactive oxygen species, which may initiate lignocellulose decay, as lignocellulose-degrading enzymes are too large to penetrate an intact cell wall (Srebotnik et al., 1988, Blanchette et al., 1997). In addition to ligninolytic enzymes, certain white-rot fungi also produce cellulose-degrading enzymes (β-glucosidase, cellobiohydrolase, and β-xylosidase) (Vrsanska et al., 2016), resulting in simultaneous degradation of lignin and cellulose components by several strains (*Trametes versicolor, Heterobasidium annosum*, and *Irpex lacteus*). Consequently, white-rot fungi can improve digestibility and nutritive value of low-quality forages such as wheat straw and bermudagrass (Akin et al., 1993, Nayan et al., 2018). Tuyen et al. (2012) reported that 9 of 11 species of white-rot fungi increased in vitro NDF and ADF digestibilities of wheat straw. However, excessive carbohydrate degradation is one of the main drawbacks to using some strains of white-rot fungi to improve utilization of fiber by ruminants (Wong, 2009, Sarnklong et al., 2010). Nonetheless, considerable variability in fiber degradation potential exists among different strains of the same species.

Few studies have involved feeding white rot fungi to animals. A notable exception is the study of Fazaeli et al. (2004) in which treatment of wheat straw with a lignin-selective strain, *Pleurotus ostreatus* (P-41) increased DMI (12.2 vs. 10.6 kg/d), DM digestibility (58.8 vs 52.3 %), NDFD (42.3 vs. 34.3 %), milk yield (9 vs. 7.5 kg/d), and BW gain (743 vs. 272 g/d) of dairy cattle in late lactation. Similarly, Shrivastava et al. (2014) reported greater DMI (per kg metabolic BW), DM digestibility (57.82 vs. 52.07 %), NDFD

(53.3 vs. 45.8 %) and 50 g/d higher average BW gain, when buffalo calves were fed wheat straw treated with *Crinipellis sp.* RCK-1 instead of untreated wheat straw.

Despite these positive responses, white-rot fungi are not widely used for increasing ruminant fiber digestion due to the long pretreatment time required (van Kuijk et al., 2015) and more importantly, the risk of degradation of digestible carbohydrates, thus reducing the nutrient content and digestibility of the residual forage. While careful strain selection can help minimize such problems, it should be noted that laccase, is a potential inhibitor of cellulase activity (Moreno et al., 2012; Yingjie et al., 2018) and fungal delignification is an aerobic process (van Kuijk et al., 2015) that does not occur in the anerobic rumen.

Brown-rot fungi. Brown-rot fungi can degrade lignocellulose polysaccharides by supposedly modifying rather than removing lignin (Highley, 1991) and producing enzymes that selectively depolymerize cellulose and hemicellulose, leaving a brown-colored rot (Gao et al., 2012). The modifications to lignin include demethylation, hydroxylation, and side chain oxidation (Arantes et al., 2012; Martinez et al. 2011).

Several studies have used brown-rot fungi to pretreat biomass for biofuel production, but few animal nutrition studies have used them to increase fiber utilization in ruminant diets. Gao et al. (2012), pretreated corn stover with different strains of white- and brown- rot fungi and reported that the greatest conversion of cellulose to glucose occurred with a strain of brown-rot fungi, *G. trabeum* (KU-41), after 20 days of pretreatment. These authors reported 32.0 and 31.4% conversion of xylan to xylose with 2 strains of *G. trabeum*, KU-41 and NBRC6430, respectively, compared to 11.2% for the control treatment, after 48 h of enzymatic hydrolysis. El-Banna et al. (2010) reported that in vivo digestibility of CP, NDF, ADF, hemicellulose and cellulose of sugarcane bagasse treated with the brown rot fungi, *Trichoderma reesei* (F-418), were increased compared to the untreated control, when fed to sheep. The authors reported that incubation of crop residues (bean straw, rice straw, corn stalk and sugarcane bagasse) with *T. reesei* for 14 days decreased concentrations of NDF and ADF by 14.4 and 10.0%, respectively. Furthermore, NDFD was increased when brown-rot fungi-treated bean straw was fed to sheep (El-Banna et al., 2010). However, Nurjana et al. (2016) reported that a different *T. reesei* strain, QM6a, decreased NDF and ADF concentrations of Napier grass but did not affect NDFD.

Lack of *in vitro* and *in vivo* studies to examine digestibility and animal performance-enhancing effects of brown-rot fungi are attributable to the long pretreatment time, the need for aerobic conditions for the treatment, and the fact that certain strains of brown-rot fungi degrade desirable polysaccharides, which could reduce the residual nutrient content of treated forages. More research is needed to identify strains that remove or modify lignin in ways that increase accessibility to cellulose and hemicellulose, without degrading these beneficial polysaccharides.

Summary

Using brown midrib hybrids has been among the most consistent, cost effective and adopted strategies to increase forage fiber digestion and milk production by dairy cows. In this context, more research is needed to examine and validate the efficacy and cost effectiveness of other genetic technologies like low-lignin alfalfa or grasses, seedling-ferulate ester mutants, and transgenic fibrolytic-enzyme secreting forages. Mechanical treatment methods that reduce forage particle size vary in effects on fiber digestibility depending on the particle size achieved. A balance between maintaining physical effectiveness of the fiber and reducing the particle size is critical for such approaches even when they increase intake and facilitate handling and transport of feeds. Chemical treatment methods of improving fiber digestibility are consistently effective, but their widespread adoption has been limited by their caustic nature and high costs. Among the biological treatment techniques, some (yeast products, enzymes and inoculants) have increased fiber digestion and milk production by dairy cows in recent meta-analyses though responses in individual studies have varied. Omic technologies should be exploited to make such products more potent and consistently effective. Other biological treatments (brown and white rot fungi) have considerable potential to improve fiber utilization provided strains used avoid or minimize carbohydrate degradation. Combination treatments like Ammonia-Fiber Expansion or steam-pressure-thermal treatment can reduce the integrity of fiber and increase the digestibility but they are not feasible on farms, as they occur in reactors. More studies on the cost effectiveness of feeding the products are needed as well as studies on adapting the technology for on-farm use.

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SESSION NOTES