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Evaluation of Enzyme Effectiveness at Enhancing Fiber Digestion in Small Ruminants

Rachel Ranells

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Abstract

Feed costs account for up to 70% of total production costs making it vital that livestock producers maximize feed efficiency in a cost-effective manner (Bach, 2012). Producers often utilize feed additives to optimize nutrient absorption and increase productivity (McGrath et al., 2018). This study was conducted to determine the effects of an enzyme supplement containing *Aspergillus niger* and *Aspergillus oryzae* on DM, OM, ADF, and NDF intake and digestibility by lambs fed ad libitum diets of either alfalfa or bermudagrass hay. The study was set up in a 2×2 factorial treatment arrangement in which 20 lambs were weighted, stratified by weight, then randomly assigned to one of the four treatments: 1) alfalfa hay plus enzyme, 2) alfalfa hay with no enzyme, 3) bermudagrass hay plus enzyme, and 4) bermudagrass hay with no enzyme. Intake or digestibility of DM, OM, ADF, or NDF were not affected ($P \geq 0.42$) by enzyme supplementation. Intake of DM and OM and digestibility of DM were greater ($P < 0.05$) from lambs offered alfalfa than from those offered bermudagrass, resulting in greater ($P < 0.05$) digestible DM and OM intake by lambs offered alfalfa hay. Intake of NDF or ADF was not different ($P \geq 0.21$) between forages, but NDF and ADF digestibility was greater in lambs fed alfalfa diets ($P < 0.01$). Ruminal pH was greater in lambs offered enzyme ($P < 0.05$). The enzyme had no effect on total or individual ruminal VFA concentrations ($P \geq 0.10$), but total ruminal VFA concentrations were higher, and acetate concentrations were lower in lambs offered alfalfa ($P < 0.05$). Forage by time interactions were observed for all individual ruminal VFA concentrations, with the exception of acetate ($P < 0.05$). Therefore, the *Aspergillus* enzyme blend increased ruminal pH but had no effects on intake or digestibility of alfalfa or bermudagrass hay.

Abbreviations: DM = dry matter; OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber.

Introduction

For economic purposes, animal producers often seek to maximize efficiency. For ruminant animals, this is typically achieved by enhancing feed intake and digestibility, especially of fiber, to maximize nutrient utilization, leading to a boost in overall animal health, and often, an increased level of production (Yousef et al., 2017). This is traditionally achieved by feeding high quality, nutrient dense feeds, such as legume hays. However, such feeds are often significantly more costly than less nutrient dense forages, such as grass hays. Therefore, producers often rely on feed additive enzymes as a more cost-effective means of enhancing intake and digestion. Such enzymes have been widely tested to determine their efficacy, but mixed results are often reported. These inconsistencies are thought to be due in part to differences in experimental protocols, such as methods of enzyme administration and diet fed to animals (Jung and Ralph, 1990).

Increased DM (Humphry et al., 2002) and OM (Caton et al., 1993) intake and in vitro DM digestibility (Caton et al., 1993) as well as ADF and NDF degradation and increasing VFA and rumen bacterial counts (Beharka and Nagaraja, 1993) were observed with the addition of *Aspergillus oryzae* fermentation extract to either the diet or in vitro cultures. Likewise, *A. niger* addition has also resulted in positive effects on digestion, increasing DM degradability (Yousef et al., 2017) and NDF disappearance (Yousef et.al., 2017; Regalado et al., 2011). However, *A. niger* had no effect on intake or digestibility in other studies (Rojo et al., 2005). Therefore, further research is necessary to understand inconsistencies and determine how to optimize the effects of these enzyme extracts. Furthermore, little research has been conducted in which both enzyme extracts were administered together. The objective of this study was to evaluate the

efficacy of a direct-fed enzyme blend consisting of *A. niger* and *A. oryzae* on intake, digestibility and ruminal VFA concentrations from lambs offered alfalfa and bermudagrass hay.

Literature Review

Alfalfa and bermudagrass

Alfalfa is a perennial legume, typically harvested from spring to late fall. Alfalfa is an excellent source of quality protein and fiber, as well as vitamins and minerals. In fact, crude protein in early-bloom alfalfa is typically greater than 19%, crude fiber around 28%, and calcium at 1.41% (NRC, 2007). These values do vary considerably however, depending on the maturity at which the alfalfa is harvested; the most nutrient dense alfalfa is cut pre-bloom, as the nutrients are contained in the leaf instead of migrating into the flower and stem (Palmonari et al., 2014). Early-bloom alfalfa neutral detergent fiber (NDF) content is around 45%, and its acid detergent fiber (ADF) concentration is approximately 35%, whereas full bloom alfalfa has approximately 52 and 40% NDF and ADF, respectively (NRC, 2007). Additionally, alfalfa is a highly palatable, easily digested forage.

Bermudagrass is a warm season perennial grass. Bermudagrass is typically quite resilient, heat and drought tolerant, and fairly easy to grow (Ye et al., 2016). However, it is significantly lower in overall nutrient content. While there are several varieties and hybrids of bermudagrass, its average crude protein content is 10%, with crude fiber at 30%, and calcium at 0.46%. Average concentrations of NDF and ADF are 78 and 39%, respectively (NRC, 2007). Bermudagrass hay is also lower in palatability and digestibility than alfalfa hay.

Forage intake by ruminants

Determining ruminant forage intake can be a very complex process, as there are several interdependent factors involved. According to Tarazona et al. (2012) these factors can be sectioned into 3 categories: those directly relating to the animal, the animal's environment, and social influences. Of those factors relating to the individual animal, ruminal fill is the most obvious. An animal can only consume the amount of feed that its digestive tract can hold. Therefore, gut capacity greatly dictates individual intake.

A second factor directly pertaining to the individual animal is nutrient requirements. There have been several studies supporting the long-standing concept that how much ruminants consume is largely based on their individual nutrient requirements. This theory is supported by studies reporting positive relationships between animal body weight and intake, and production level of dairy cows and intake (Peyraud et al., 1996; Faverdin et al., 2007). Furthermore, a meta-analysis investigating the nutrient requirements of dairy cows in relation to factors including body weight, changes in body weight, and milk yield determined that 71% of the total variation observed in dry matter intake were due to these factors (Vazquez and Smith, 2000). However, Baumgardt (1970) examined results from 15 feed intake experiments involving cattle and sheep and found a general increase in intake of digestible energy for diets with increasing digestible energy content of up to about 12MJ/kg and then tendencies to decline with higher digestible energy concentrations. Therefore, if ruminants are provided with high quality, high energy forages, their intake decreases, as they eat to fulfill their requirements. However, as forage quality and energy content decrease, intake is regulated by gut fill rather than the need to fulfill energy requirements.

Next, environmental factors also influence ruminant animal intake. Ruminants in warmer climates (above 25 C) are often forced to deviate from their natural feeding schedule in order to

avoid feeding during the warmest parts of the day. Therefore, they refrain from mid-day meals and try to compensate by early morning or night grazing. However, this is rarely enough, and often results in decreased grazing time, and ultimately, decreased intake (Baumont et al., 2000).

Social factors also contribute to intake by animals. Hierarchies naturally form in ruminant populations, creating a divide between dominant and submissive individuals. Dominant individuals are generally granted access to superior feeding areas, as well as the ability to feed first. Therefore, dominant animals often have a greater intake than subordinate animals (Haskell et al., 2019).

Feed enzymes

Many studies have reported positive results with *A. niger* as an active enzyme ingredient. When fed to lambs, *A. niger* enhanced DM digestibility from 62.0 to 88.5% and 82.1 to 87.1% from guinea grass and rice husks, respectively. Additionally, NDF degradation also improved, increasing from 43.8 to 63.7% for guinea grass and 39.8 to 62.5% for rice husks (Yousef et al, 2017).

Similarly, *A. niger* improved fiber digestion of highly fibrous corn stover in a solid state fermentation system. Corn stover pre-treated with an alkaline solution containing the enzymatic extract was exposed to ruminal fluid for 48 or 72 h of digestion, resulting in a 2.5% and 5.3% increase in in vitro ruminal digestibility, respectively. In vitro true digestibility was also positively affected, with increases of 9% and 10% exhibited in pre-treated corn stover in contact with the enzymatic extract after 48 or 72 h exposure to ruminal fluid for digestion, respectively. Additionally, pre-treated corn stover showed significantly lower NDF, ADF, hemicellulose, cellulose, and lignin values, (4.3, 2.1, 11.9, 9.6, and 14.6%, respectively) after 8 to 12 h of enzyme contact time. While no significant difference was found in in vitro ruminal digestibility,

in vitro true digestibility, or hemicellulose content between corn stover with or without the pre-treatment, it is important to note that the study cited that their results confirm that *A. niger* requires the pre-degradation provided by the alkaline pre-treatment in order for it to gain access to its substrate. Therefore, although the study offers strong support for the use of *A. niger* as a feed enzyme, it is evident that *A. niger* did not achieve these improvements in digestibility without outside assistance. This indicates the need for additional studies to better understand the potential benefits of *A. niger* as a feed additive (Regalado et al, 2011).

Other studies reported no benefit of supplementation with *A. niger*. The enzyme decreased starch digestibility and had no effect on intake when fed to lambs on diets high in concentrate (Rojo et al., 2005). However, the enzyme did increase ruminal pH, protozoa, and lactate levels. These authors concluded that there were too many inconsistencies when feeding *A. niger,* and further research was needed. In light of this, researchers sought to determine if the effect of *A. niger* could be optimized by developing enzyme blends.

A blend of A. *niger* and *Trichhoderma reesei* enhanced cellulose, amylase, and xylanase activity in a solid state fermentation mixture of bermudagrass and corn cobs. Addition of the enzyme mixture enhanced ADF, NDF, lignin, and cellulose degradability by 24.8, 35.9, 2.9, and 21.9% respectively. Furthermore, 2.4-fold and 1.4-fold improvements in in vitro and true digestibility of DM were reported, respectively (Amaro-Reyes et al., 2016). In contrast, an enzyme blend of *A. niger* and *Trichoderma longibachiatum* did not alter DM intake by lambs fed varying forage to concentrate ratios (400:600, 500:500, or 600:400 kg/kg) or in vitro DM digestibility; the only significant factor was the forage to concentrate ratio (Pinos-Rodriguez et al., 2008).

One of the most widely-tested fungal feed additives of the 1990s, *Aspergillus oryzae*, has also been plagued with inconsistent results. These irregularities are evident when analyzing the effect of the enzyme on intake. Dry matter intake was increased by 4% in heifers fed ad libitum diets of tall fescue hay (Humphry et al., 2002) and organic matter intake was increased by 9.7% in steers grazing cool-season smooth bromegrass pasture (Caton et al., 1993). In contrast, beef and dairy cows on diets of 60% concentrate and 40% timothy hay (Chiquette, 1995), and beef cows on ad libitum diets of alfalfa or bromegrass hay (Varel and Kreikemeir, 1994) experienced no effect on forage intake.

Likewise, *A. oryzae* had varying effects on digestion measurements. In a study analyzing the effect of the enzyme at various concentrations on in vitro fiber digestion of various feedstuffs, alfalfa, bromegrass, and high-endophyte fescue hays all demonstrated an increase in fiber digestibility, while the enzyme had no effect on digestibility of pure cellulose, low-endophyte fescue, wheat straw, corn silage, or prairie hay. The only substrate demonstrating an increase in fiber degradation at the 0.4g/L of fermentation mixture dose of enzyme was high-endophyte fescue hay, which increased NDF and ADF degradation by 5.9 and 3.7%, respectively. At the 0.8g/L dose, alfalfa displayed a 5.5% increase in NDF degradation, but no significant effect on ADF degradation. At this dose, bromegrass hay exhibited a 4.9% and 3.7% increase in NDF and ADF degradation, respectively. At the 1.2g/L dose of enzyme, there was no effect on NDF or ADF degradation of high-endophyte fescue hay. However, at this same dose, alfalfa hay experienced an increase in NDF and ADF degradation of 6.4% and 3.0%, respectively, and bromegrass NDF and ADF degradation increased 5.6% and 3.9%, respectively. When whole rumen fluid (WRF) was added to the enzyme-substrate mixtures, NDF and ADF degradation increased further, ranging from a 7 to 12% increase in NDF degradation and a 12 to

15% increase in ADF degradation for both alfalfa and bromegrass hays. Interestingly, high-endophyte fescue hay did not experience a significant increase in NDF or ADF degradation at the 1.2% dose but did demonstrate a 5.2 to 6.4% increase in NDF and a 3.8 to 4.0% increase in ADF degradation at the 0.4 and 0.8 dosages (Beharka and Nagaraja, 1993). Similarly, in vitro DM digestibility was improved by 5.9% with the addition of A. *oryzae* for steers on smooth bromegrass (Caton et al., 1993). In contrast to the previous two studies which either reported enhanced fiber digestion for bromegrass or bromegrass and alfalfa hays, Varel and Kreikemeier (1994) found no enzymatic effect on fiber or organic matter degradation for either forage. Other studies also reported no effect of *A. oryzae* fermentation extract on DM, ADF, and NDF digestibility of tall fescue hay (Humphry et al., 2002), as well as ruminal and total tract DM digestibility from a 60% concentrate, 40% timothy diet (Chiquette, 1995).

Additional discrepancies have also occurred in the effects of *A. oryzae* fermentation extract on ruminal fermentation. Increased VFA concentrations and increased bacterial counts were reported in conjunction with the positive effects on digestion in an in vitro fermentation study reported by Beharka and Nagaraja (1993). In other studies, increased concentrations of acetate, propionate, and total VFAs (Chiquette, 1995) and increased total number of ruminal anaerobes (Varel and Kreikemeier, 1994). were reported although no enzymatic effects on digestion were observed. Conversely, *A. oryzae* fermentation extract did not affect VFA concentrations, ruminal pH, or ammonia concentrations in spite of enhanced DM digestion in another study (Caton et al., 1993).

Although numerous studies have been performed to evaluate the efficacy of various feed additive enzymes at improving feed intake and digestibility, several enzymes have failed to produce consistent results. Consequently, the efficacy of many highly-tested enzymes are still in

question. Therefore, in spite of extensive previous testing, additional studies are necessary to determine the optimal situations in which to use *A. oryzae* and *A. niger* fermentation extracts to improve intake and digestion in ruminant animals. Furthermore, information pertaining to the effects of a combination of the two fermentation extracts on digestive characteristics is limited. Therefore, the objective of this research was to evaluate the effects of a combination of *A. oryzae* and *A. niger* on digestion of alfalfa and bermudagrass hay diets by lambs.

Methods and Materials

All procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee protocol no. 18118. Twenty crossbred lambs (29.7 ± 5.80 kg average. initial BW) were stratified by weight and randomly assigned to treatments in an experiment with a 2 × 2 factorial arrangement of a completely randomized design to provide 5 observations per treatment. Main effect treatments consisted of alfalfa hay or bermudagrass hay offered for ad libitum consumption either with no supplemental enzyme or an enzyme mixture of *A. niger* and *A. oyzae* offered at 4.6 g/d of actual enzyme in a calcium carbonate carrier. Animals were fed their respective diets for ad libitum consumption split into three daily feedings at 1800, 2200, and 0700h. Orts were gathered and all lambs were offered soybean meal (2.5 g/kg BW) and a commercial mineral supplement[1] (~10 g/d) 20 min. prior to the 1800 h feeding. Enzyme was added with the soybean meal at this time. All lambs had ad libitum access to water.

Lambs were housed in 1 × 1.5m individual pens fitted with expanded metal grate flooring in a room set at an ambient temperature between 10 and 16°C with 14h of lighting. Lambs were

[1] Preferred Mineral for Sheep and Goats (Ragland Mills Inc., Neosho, MO, USA) The mineral contained 350-400 g/kg salt, 90-100 g/kg Ca, and not less than 80 g/kg P, 10 g/kg Mg, 10 g/kg K, 125 ppm Co, 150 ppm I, 5,000 ppm Fe, 10 ppm Se, 140 ppm Zn, 352,000 IU/kg of Vitamin A, 88,000 IU/kg of Vitamin D3, and 330 IU/kg of Vitamin E.

removed from their pens, comingled, and allowed a minimum of 2 h outside each week for exercise and socialization. Water, but no forage was provided during this time.

Lambs were given an adaptation period of 14 d followed by a 7–d total fecal collection period. The total fecal output was collected as it was excreted from each sheep by placing trays directly underneath each pen and collecting feces as it passed through the expanded metal flooring. Total feces were weighed, then dried to a constant weight at 50°C. Samples from both forages, soybean meal, and enzyme were gathered as each feed component was being weighed prior to feeding beginning 2 d prior to the start of fecal collections and ending 2 d prior to the end of fecal collections. Total ort collections from each sheep began 1 d prior to fecal collections and ended 1 d prior to the termination of fecal collections. A sub-sample from the total orts were taken and weighed. Ort and feed samples were also dried to a constant weight at 50°C. All samples will be allowed to acclimate to atmospheric moisture, then ground to pass through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA). All samples were analyzed for total ash content (AOAC, 2000), and NDF and ADF (Vogel et al., 1999). On the final day of the study, rumen samples were also collected via stomach tube immediately prior to feeding, and 3 and 6 h after feeding. Rumen samples were analyzed for pH using a portable pH meter and volatile fatty acids by gas chromotography.

Intake and digestibility data were analyzed statistically using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA) as a 2 × 2 factorial treatment arrangement, using the individual animal (n = 5) as the experimental unit. Forage type, enzyme and their interaction were considered as fixed effects. Ruminal pH and VFA data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc.) as a 2 × 2 factorial treatment arrangement with repeated measurements. Effects of forage type, enzyme, sampling time and their 2- and 3-way interactions

were considered fixed effects. Time was considered a repeated measurement and the individual animal was the subject. When an interaction of a main effect with time occurred ($P < 0.05$), linear and quadratic orthogonal polynomial effects were determined across sampling times within each main effect treatment.

Results

Chemical composition of the feedstuffs offered to lambs is presented in Table 1. The fiber concentrations of the alfalfa were 17 and 7% higher for ADF and NDF, respectively, than those reported by the National Research Council. That of bermudagrass also differed from the National Research Council's report, with ADF concentrations 6% higher and NDF 5% lower than reported (NRC, 2007).

None of the intake and digestibility measurements were affected ($P \geq 0.12$) by the hay type by enzyme interaction (Table 2). Likewise, enzyme treatment had no effect ($P \geq 0.042$) on intake or digestibility measurements. Dry matter and OM intake ($P < 0.05$) and DM digestibility were greater ($P < 0.05$) for alfalfa vs. bermudagrass hay but OM digestibility did not differ ($P = 0.20$) between hays. Digestibilities of NDF and ADF were also greater ($P < 0.05$) for alfalfa than bermudagrass hay.

Ruminal pH, VFA, and molar percentage of acetate were not impacted by sampling time ($P \geq 0.05$) or interactions involving sampling time ($P \geq 0.15$; Table 3). Ruminal pH was greater ($P < 0.05$) in lambs offered enzyme and tended ($P = 0.06$) to be greater from lambs offered alfalfa. Lambs fed alfalfa had greater ($P < 0.05$) total ruminal VFA concentrations than lambs fed bermudagrass. Ruminal acetate concentrations were greater ($P < 0.05$) from lambs offered

bermudagrass compared with those offered alfalfa, but supplementation with enzyme did not affect ($P \geq 0.75$) total VFA or acetate concentrations.

No enzyme effects or interactions with sampling time were observed ($P \geq 0.10$) for any of the individual VFA concentrations (Table 4). Forage type affected ($P < 0.05$) the percentage of individual VFA present in the rumen. However, for each acid with the exception of acetate, a forage × time interaction occurred ($P < 0.05$; Figures 1 through 6). Ruminal propionate concentrations responded linearly and quadratically ($P < 0.05$) within both alfalfa and bermudagrass treatments (Figure 1). Immediately prior to feeding, ruminal propionate concentrations were 9.4% greater for lambs fed alfalfa than lambs fed bermudagrass hay. Within lambs offered alfalfa, ruminal propionate concentrations increased at 3 h post-feeding then declined by 6 h after feeding. Propionate concentrations also increased in the 3-h sample from lambs offered bermudagrass but did not decrease at the 6-h sample within those lambs. Propionate concentrations were 17.6 and 10.8% greater from lambs offered alfalfa vs. bermudagrass at 3 and 6 h post-feeding, respectively. The ratio of acetate to propionate (Figure 2) in lambs fed alfalfa responded linearly and quadratically ($P < 0.05$), being highest immediately prior to feeding, then decreasing at 3 h post-feeding, then increasing in samples taken 6 h post-feeding. The acetate to propionate ratio decreased linearly ($P < 0.05$) across sampling times in lambs offered bermudagrass.

Ruminal concentrations of isobutyrate (Figure 3) declined between samplings taken immediately prior to feeding and those taken 3 h post-feeding, then increased by 6 h post-feeding in lambs offered alfalfa (quadratic response; $P < 0.05$). However, within lambs offered bermudagrass diet, isobutyrate levels remained constant between hour zero and 3 h post-feeding, but then increased between the second and third sampling (linear; $P < 0.05$). Butyrate

concentrations (Figure 4) responded quadratically ($P < 0.05$) for both diets, but the decline in butyrate concentrations between the initial sampling and samples take 3 h post-feeding was more pronounced in lambs offered the alfalfa diet.

Isovalerate concentrations (Figure 5) followed a similar pattern as was observed for isobutyrate for both forages, with concentrations from alfalfa responding quadratically ($P < 0.05$) and those from bermudagrass increasing ($P < 0.05$) linearly. Isovalerate concentrations from lambs offered alfalfa declined at 3 h post-feeding but increased by 6 h post-feeding (quadratic; $P < 0.05$), whereas isovalerate concentrations from lambs offered bermudagrass increased linearly ($P < 0.05$) across sampling times. Concentrations of valerate responded linearly and quadratically ($P < 0.05$) for both forages (Figure 6), but valerate concentrations from lambs offered alfalfa increased sharply by 3 h post-feeding but then declined by 6 h post-feeding, but those from lambs offered bermudagrass increased by 3 h post-feeding and then remained at the same concentration at 6 h post feeding.

Discussion

Numerous studies have been conducted to determine if there is a relationship between intake and digestibility, however many contrasting hypotheses have been developed. For example, the nutrient requirement theory stating that animals eat until they fulfill their nutritional needs suggests that intake increases with decreasing diet quality, whereas intake is lower for higher quality diets, as nutrient needs will be reached more quickly. This theory was somewhat supported by an analysis of 15 feed intake studies involving sheep and cattle in which an overall trend of increased intake was observed for diet of up to 12MJ/kg of digestible energy, whereas intake declined with diets of greater than 12MJ/kg of digestible energy (Baumgardt, 1970). Our results did not concur with the nutrient requirement theory, as DM and OM intake were higher

for alfalfa hay which had much lower NDF and ADF concentrations than the bermudagrass hay used in this study. Also in direct contrast to this theory, Meyer et al. (2010), summarizes many studies that report decreased intake with decreasing forage quality. In fact, this idea of decreased intake with decreased forage quality is considered common knowledge in applied agricultural science (Van Soest, 1994). Additional studies support this theory and even led to its expansion. Cordova et al. (1978) and Meissner and Paulsmeier (1995) hypothesized that forage intake decreases with forages of increasing fiber content, as fibrous forages require an increased ruminal retention time for digestion. Interestingly, in support of this theory, a significant negative correlation was reported between apparent digestibility and rate of rumen outflow by cows fed 1:1 forage to concentrate diets (r=-0.80; Ørskov et al., 1988). The study also reported a positive correlation between voluntary intake and rate of rumen outflow. In contrast to the theory, Ørskov et al. (1988) reported no significant correlation between intake and digestibility in diets of 1:1 straw to concentrate. Although ruminal pH was greater from lambs offed the enzyme mixture, no other measurements of intake or digestibility were affected by enzyme supplementation. These findings were comparable to those of Varel and Kriekemeier (1994), who also had no enzyme effect on OM, ADF, or NDF intake or digestibility when feeding cows *A. oryzae* with alfalfa or bromegrass, or those of Humphry et al (2002), in which *A. oryzae* did not affect DM, ADF, or NDF digestibility when fed to heifers on a full forage diet. However, Humphry et. al. (2002) reported increased DM intake when cows were offered *A. oryzae* fermentation extract. Furthermore, *A. oryzae* fermentation extract did not impact intake or digestion by steers offered bermudagrass hay with supplemental ground corn (Galloway et al., 1991). In contrast, other studies reported beneficial effects on intake or digestibility when *A. oryzae* fermentation extract was added to diets or continuous cultures. Degradation of NDF and ADF from alfalfa,

bromegrass, and high-endophyte tall fescue hays were improved when *A. oryzae* was added to their individual fermentation mixtures (Berharka and Nagaraja, 1993). Similarly, both DM and NDF degradability of guinea grass and rice husk increased when exposed to A. *niger* (Yousef et al, 2017), and increased OM intake and DM digestibility resulted when *A. oryzae* was given to steers on bromegrass pasture (Caton et al., 1993).

While there may be some benefits associated with feeding the enzyme blend, results are largely inconsistent. In the present study, the enzyme was shown to have no effect on intake, digestion, or ruminal VFA concentrations on diets of alfalfa or bermudagrass. Therefore, we conclude that the enzyme blend of *A. niger* and *A. oryzae* is ineffective in improving intake or digestion in poor or high-quality forage diets.

References

Amaro-Reyes, A., Gracida, J., Huizache-Peña, N., Elizondo-García, N., Salazar-Martínez, J., Garcia Almendárez, B. E., Regalado, C., 2016. On-site hydrolytic enzymes production from fungal co-cultivation of bermuda grass and corn cob. *Bioresource Technol., 212,* 334-337.

AOAC. 2000. *Official Methods of Analysis of the Association of Official Analytical Chemists International (17th Ed.)* Gaithersburg, MD.

Bach, A., 2012. Key indicators for measuring dairy cow performance. *Proceedings of the FAO Symposium: Optimization of Feed Use Efficiency in Ruminant Production Systems, Food and Agriculture Organization of the United Nations and Asian-Australasian Association of Animal Production Societies*, pp. 33-34.

Baumgardt, B. R., 1970. Regulation of feed intake and energy balance. *Physiology of Digestion and Metabolism in the Ruminant*, 235-253.

Baumont, R., Prache, S., Meuret, M., Morhand, F. P., 2000. How forage characteristics influence behavior and intake in small ruminants: A review. *Livestock Prod. Sci., 61*, 15-28.

Beharka, A. A., Nagaraja, T. G., 1993. Effect of Aspergillus oryzae extract alone or in combination with antimicrobial compounds on ruminal bacteria. *J. Dairy Sci., 81,* 1591-1598.

Caton, J. S., Erickson, D. O., Carey, D. A., Ulmer, D. L., 1993. Influence of Aspergillus oryzae fermentation extract on forage intake, site of digestion, in situ degradability, and duodenal amino acid flow in steers grazing cool-season pasture. *J. Anim. Sci., 71,* 779-787.

Chiquette, J. 1995., Saccharomyces cerevisiae and *Aspergillus oryzae*, used alone or in combination, as a feed supplement for beef and dairy cattle. *Can. J. Anim. Sci., 75*, 405-415.

Cordova, F. J., Wallace, J. D., Pieper, A. R. D., 1978. Forage intake by grazing livestock: A review. *J. Range Manage., 31*, 430-438.

Faverdin, P., Delaby, L., Delagarde, R., 2007. L'ingestion d'aliments par les vaches laitières et sa prévision au cours de la lactation. *INRA Prod. Anim., 20*, 151-162.

Galloway, D. L. Sr., Goetsch, A. L., Sun, W., Forster, L. A. Jr., 1991. Effects of additions of sodium bicarbonate, salt, *Aspergillus oryzae* culture extract, niacin, lysine, or phenylalanine to ground corn-based supplements on feed intake and digestion by Holstein steers consuming bermudagrass (*Cynodon dactylon*) hay. *Anim. Feed Sci. Tech.*, *32*:261-273.

Haskell, M. J., Rooke, J. A., Roehe, R., Turner, S. P., Hyslop, J. J., Waterhouse, A., Duthie, C., 2019. Relationships between feeding behaviour activity, dominance and feed efficiency in finishing beef steer. *Applied Anim. Behavior Sci., 210,* 9-15.

Humphry, J. B., Coffey, K. P., Moyer, J. L., Brazle, F. K., Lomas, L. W., 2002. Intake, digestion, and digestive characteristics of *Neophodium coenophialum*-infected and unifected fescue by heifers

offered hay diets supplemented with *Aspergillus oryzae* fermentation extract or laidlomycin propionate. *J. Anim. Sci., 80*, 225-234.

Jung, H. G., Ralph, J., 1990. Phenolic-carbohydrate complexes in plant cell walls and their effect on lignocellulose utilization. *Microbial and Plant Opportunities to Improve Lignocellulose Utilization by Ruminants*. 173-182.

McGrath J., Duval S. M., Tamassia L. F. M., Kindermann M., Stemmler R. T., de Gouvea, V. N., Celi, P., 2018. Nutritional strategies in ruminants: A lifetime approach. *Res. Vet. Sci., 116*, 28-39.

Meissner, H. H., Paulsmeier, D. V., 1995. Plant compositional constituents affecting between-plant and animal species prediction of forage intake. *J. Anim. Sci., 73*, 2447-2457.

Meyer, K., Hummel, J., Clauss, M., 2010. The relationship between forage cell wall content and voluntary food intake in mammalian herbivores. *Mammal Rev., 40*, 221-245.

National Research Council, 2007, *Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids*. 2007. National Academic Press, Washington, D.C.

Ørskov, E. R., Ojwang, I., Reid, G. W., 1988. A study on consistency of differences between cows in rumen outflow rate of fibrous particles and other substrates and consequences for digestibility and intake of roughages. *Anim. Prod., 47*, 45-51.

Palmonari, A., Fustini, M., Canestrari, G., Grilli, E., Formigoni, A., 2014. Influence of maturity on alfalfa hay nutritional fractions and indigestible fiber content. *J. Dairy Sci., 97*, 7729-7734.

Peyraud, J. L., Comeron, E. A., Wade, M. H., Lemaire, G., 1996. The effect of daily herbage allowance, herbage mass and animal factors upon herbage intake by grazing dairy cows. *Ann. Zootech., 45*, 201-207.

Pinos-Rodríguez, J. M., Moreno, R., González, S. S., Robinson, P. H., Mendoza, G., Álvarez, G., 2008. Effects of exogenous fibrolytic enzymes on ruminal fermentation and digestibility of total mixed rations fed to lambs. *Anim. Feed Sci. Technol., 142*, 210-219.

Regalado, C., Vázquez-Obregón, I., García-Almendárez, B. E., Domínguez-Domínguez, J., Aguilera-Baeyro, A., Amaro-Reyes, A., 2011. Xylanolytic enzymes production by *Aspergillus niger* GS1 from solid-state fermentation on corn stover and their effect on ruminal digestibility. *Electronic J. of Biotechnol., 14.*

Rojo, R., Mendoza, G. D., González, S. S., Landois, L., Bárcena, R., Crosby, M. M., 2005. Effects of exogenous amylases from *Bacillus licheniformis* and *Aspergillus niger* on ruminal starch digestion and lamb performance. *Anim. Feed Sci. Technol., 123*, 655-665.

Tarazona, A. M., Zoot. MSc., Ceballos, M.C., Zoot., Naranjo, J. F., Zoot, Cuartas, C., 2012. Factors affecting forage intake and selectivity in ruminants, *Colombian J. Anim. Sci.*, 25, 473-487.

Van Soest, P. J., 1994. *Nutritional Ecology of the Ruminant*. Cornell University Press, Ithaca, New York, USA.

Varel, V. H., Kreikemeier, K. K., 1994. Response to various amounts of *Aspergillus oryzae* fermentation extract on ruminal metabolism in cattle. *J. Dairy Sci., 77,* 3081-3086.

Vazquez, O. P., Smith, T. R., 2000. Factors affecting pasture intake and total dry matter intake in grazing dairy cows. *J. Dairy Sci., 83*, 2301-2309.

Vogel, K. P., Pedersen, J. F., Masterson, S. D., Toy, J. J., 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. *Crop Sci*., 276-279.

Ye, T., Shi, H., Wang, Y., Yang, F., Chan, Z., 2016. Contrasting proteomic and metabolomic responses of bermudagrass to drought and salt stresses. *Front. Plant Sci., 7*, 1694.

Yousef, M. B., Kolade, I. O., Adetitun, D. O., Arekemase, M. O., 2017. Effect of laboratory produced xylanase from *Aspergillus niger* on fibre digestibility of rice husk and guinea grass. *Ethiopian J. Environ. Stud. Manage.*, 10, 668-674.

Table 1. Chemical composition of feedstuffs offered to lambs in a digestion study

Item	Bermudagrass	Alfalfa	Soybean meal
	---------- g/kg DM ----------		
Organic matter	917	864	901
Neutral detergent fiber	730	516	164
Acid detergent fiber	521	449	96

Table 2. Intake and digestibility by lambs offered alfalfa or bermudagrass hay with or without supplementation with a mixture of *A. oryzae* and *A. niger* fermentation extracts.

Item	Alfalfa Without Enzyme	Alfalfa With Enzyme	Bermudagrass Without Enzyme	Bermudagrass With Enzyme	Standard Error	Effect[a]
DM intake, g/kg BW	38	35	26	31	2.7	F
DM digest, g/kg DMI	628	603	583	584	14.2	F
Digest. DMI, g/kg BW	24	21	15	18	1.9	F
OM intake, g/kg BW	33	30	24	28	2.4	F
OM Digest, g/kg OMI	629	604	596	598	14.4	ns
Digest. OMI, g/kg BW	21	18	14	17	1.7	F
NDF intake, g/kg BW	18	16	18	21	1.8	ns
NDF digest, g/kg NDF	555	509	603	621	18.6	F
ADF intake, g/kg BW	16	14	13	15	1.3	ns
ADF digest, g/kg ADF	532	490	571	597	21.2	F

[a] F = alfalfa differed from bermudagrass ($P < 0.05$); ns = no forage or enzyme effects ($P < 0.05$).

Table 3. Ruminal pH and total volatile fatty acid and acetate concentrations from lambs offered alfalfa or bermudagrass hay with or without supplementation with a mixture of *A. oryzae* and *A. niger* fermentation extracts.

	Alfalfa Without Enzyme	Alfalfa with Enzyme	Bermudagrass Without Enzyme	Bermudagrass With Enzyme	Standard Error	Effects[a]
pH	6.7	6.7	6.5	6.7	0.05	f, E, f*e, t
Total VFA mmol/mL	82.0	88.2	77.8	74.3	4.34	F
Acetate, (% of total VFA	67.7	67.7	73.9	73.9	0.74	

[a] F, f = alfalfa differed from bermudagrass ($P < 0.05$ and 0.10, respectively; E = enzyme differed from no enzyme ($P < 0.05$); f*e = tendency for a forage × enzyme interaction ($P < 0.10$). t = tendency for an effect of sampling time ($P < 0.10$).

Table 4. Concentration of Individual VFAs by lambs with and without enzyme supplementation and averaged across those offered alfalfa and bermudagrass hay[a]

Item	Without enzyme	With enzyme	SE
Acetate	70.8	70.9	0.55
Propionate	17.4	17.7	0.36
Ace:Pro ratio	4.1	4.1	0.12
Isobutyrate	1.3	1.2	0.09
Butyrate	8.2	7.5	0.29
Isovalerate	1.6	1.6	0.13
Valerate	1.1	1.1	0.05

[a] The enzyme is a mixture of *Aspergillus oryzae* and *Aspergillus niger* fermentation extracts.

Figure 1. Ruminal propionate concentrations (% of total VFA) at multiple times from lambs offered either alfalfa or bermudagrass hay

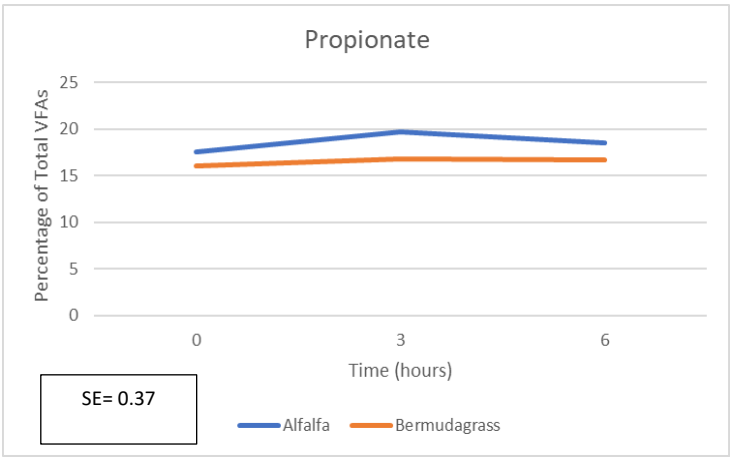

Figure 2. Ruminal acetate:propionate ratios at multiple times from lambs offered either alfalfa or bermudagrass hay

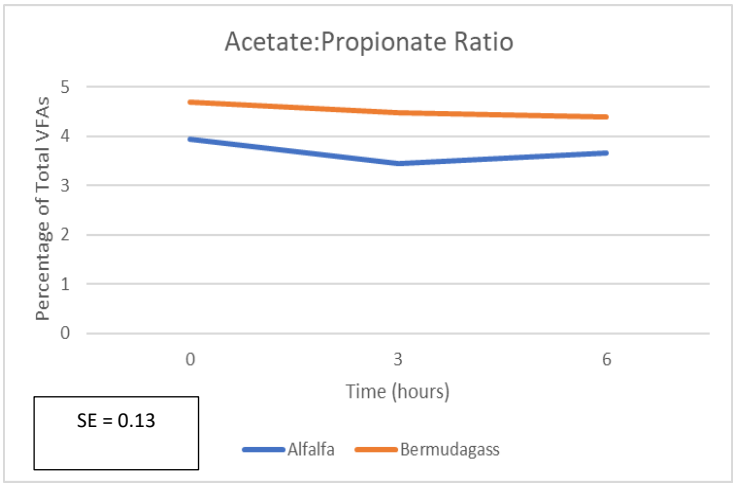

Figure 3. Ruminal isobutyrate concentrations (% of total VFA) at mutiple times from lambs offered either alfalfa or bermudagrass hay

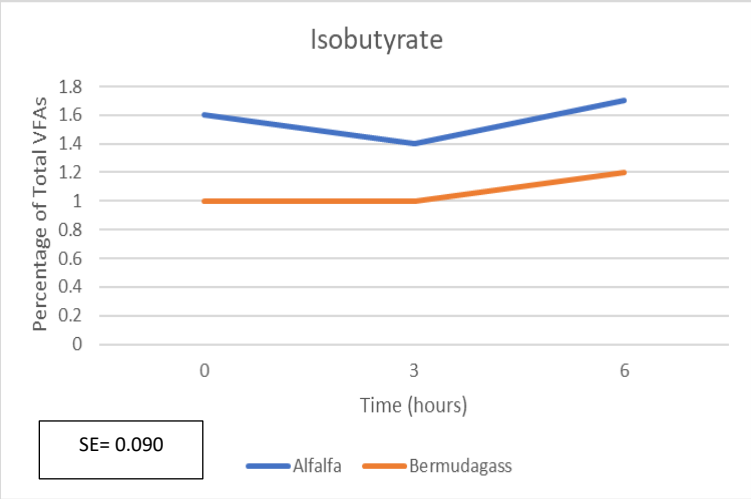

Figure 4. Ruminal butyrate concentrations (% of total VFA) at multiple times from lambs offered either alfalfa or bermudagrass hay

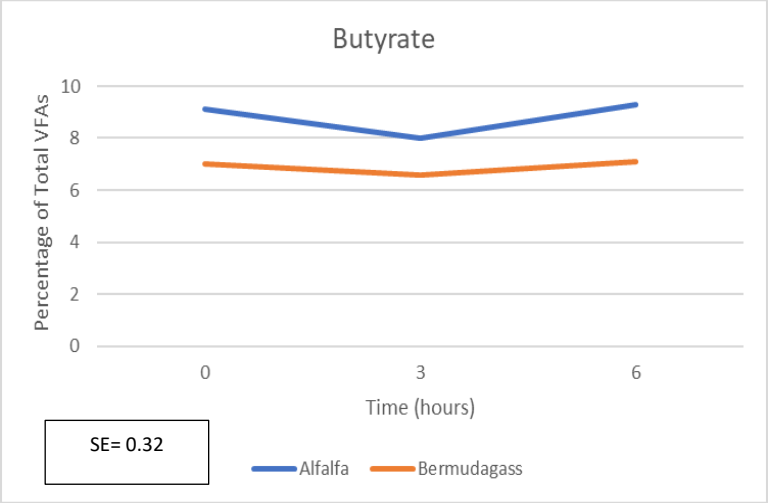

Figure 5. Ruminal isovalerate concentrations (% of total VFA) at multiple times from lambs offered either alfalfa or bermudagrass hay

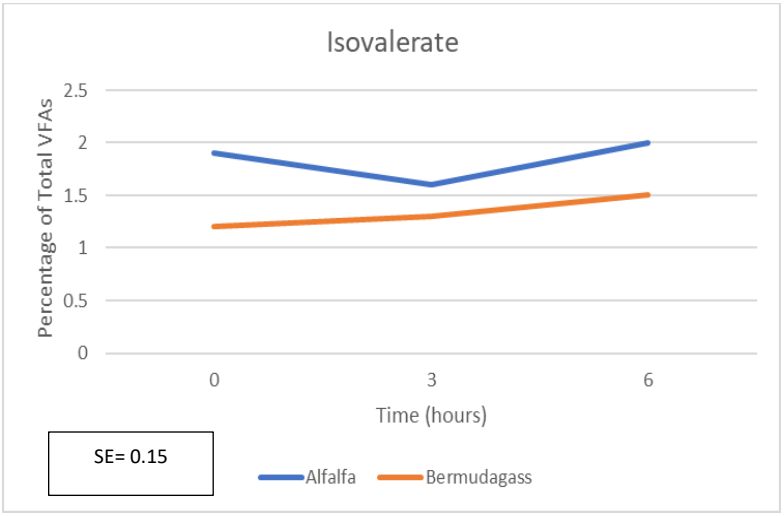

Figure 6. Ruminal valerate concentrations (% of total VFA) at multiple times from lambs offered either alfalfa or bermudagrass hay