IMPROVING FIBER BY-PRODUCT UTILIZATION IN HIGH CONCENTRATE DIETS

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled:

IMPROVING FIBER BY-PRODUCT UTILIZATION IN HIGH CONCENTRATE DIETS

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A candidate for the degree of Master of Science,

And hereby certify that in their opinion it is worthy of acceptance.

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IMPROVING FIBER BY-PRODUCT UTILIZATION IN HIGH CONCENTRATE DIETS

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ABSTRACT

The effects of starch and fiber combinations in feedlot diets were examined to determine optimum combinations for diet digestibility and animal performance as well as identify opportunities for fibrolytic enzyme inclusion improvement. Previous work was not conclusive on the optimum corn inclusion rate in fiber-based diets to optimize diet digestibility and animal performance. Fibrolytic enzyme addition also generated inconsistent results due to variations in activity, inclusion rates, strains and target substrates. Experiments in this thesis were performed to pinpoint the maximum corn inclusion rate in high fiber diets and determine the effects of fibrolytic enzyme addition to whole shell corn (WSC) diets with increasing fiber inclusion. Soybean hull (SH) diets with increasing WSC inclusion rates were compared in continuous culture fermenters and feedlot cattle. The fermenter study evaluated digestibilities, VFA production, lactic acid production and pH. Fiber digestibility was reduced as starch inclusion increased though an optimum balance between DM and fiber digestibility was between 80 and 90% SH inclusion. The subsequent feedlot study determined the maximum starch inclusion for optimum digestibility was 80:20, SH:WSC. Despite the optimum digestibility values, ADG and G:F decreased as SH inclusion increased, reinforcing the need to improve performance in animals fed diets containing fiber byproducts. Enzymes were evaluated as an option for improving fiber byproduct digestibility in high corn diets. An *in vitro* fermentation study identified 0.045% as the minimum dietary inclusion rate of a commercial proprietary mix of *Bacillus subtilis, Aspergillus oryzae* and *Trichoderma viride* (ENZ; Cattlemace, R & D Life Sciences LLC, Menomonie, WI). In continuous culture, increasing SH inclusion in corn diets improved NDF digestibility (NDFd) but reduced DM and OM digestibility. Adding 0.045% ENZ generated greater acetate, reduced propionate and increased acetate:propionate, indicating a favorable shift in fiber fermentation. Steers were fed the same WSC diets with increasing SH inclusion and ENZ addition. Despite a linear NDFd improvement in continuous culture, there was a cubic response in NDFd due to SH inclusion. Although ADF digestibility decreased in ENZ diets, there was a DIETxENZ interaction in G:F. There was no difference in ADG or G:F for steers consuming 100% WSC diets versus 14-28% SH diets with ENZ included. There was no G:F difference between ENZ and non-ENZ diets containing 7% or less SH or hay thus adequate substrate was not present to generate an ENZ response. An 80:20 SH:WSC diet optimizes fiber digestibility and animals can consume WSC diets containing 14-28% SH and 0.045% ENZ without negative effects on growth performance as compared to all WSC diets.

CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

The fractionation of grains generates a myriad of byproducts often high in fiber content as well as other nutrients. The ability of ruminant animals to facilitate high fiber feed digestion gives producers an advantage when these feeds are priced competitively compared to source grains. The primary byproducts from soybean oil production include a high CP meal as well as a low protein hull fraction that is high in digestible fiber (Archer Daniels Midland, 2008). Similarly, the gluten feed from corn starch production and distillers grains generated by ethanol production are also higher in fiber but contain medium levels of protein as compared with other grain processing by-products. The high fiber byproducts available to cattle producers are not limited to corn and soybean sources. Cottonseed oil production generates a high protein meal and a high fiber, low protein hull fraction. Wheat flour production leaves a high fiber middling fraction. The byproducts available near grain processing facilities are usually produced throughout the year and a high fiber product typically results. Along with forages, these byproducts can provide a source of fiber and potentially a cost-effective dietary energy source though not typically a complete primary energy source substitute. As a substitute for forage fiber in mixed dairy diets, 35% of the required NDF can come from byproducts (Sarwar et al., 1991). Even though forages and fibrous byproducts contain similar levels of total energy, the forage feeding value is typically lower and is associated with the cell wall component (Jung 1987) as plant cell walls make up the fiber component (Beauchemin et al., 2004). Fiber composition varies by plant species as well as growth stage and therefore so does digestibility though it is also dependent on diet inclusion and animal type. Genetic and agronomic advances have improved cell wall degradation but digestibility is still typically limited (Beauchemin et al., 2003). In beef feedlot situations where the diet is largely grain-based, fiber digestibility has been estimated at less than 50% (Beauchemin et al., 2004). Despite limited digestibility, fiber byproduct use in ruminants is still an advantage over other species. Pigs can only consume diets containing up to 15% soybean hulls without adverse effects (Kornegay, 1981) thereby limiting the ration cost advantages in monogastrics as compared to ruminants. Understanding the composition of fiber and the explanations for limited digestibility are key to finding means of improvement in ruminant diets.

FIBER COMPOSITION

Fiber is composed primarily of cellulose, hemicellulose and lignin. The proportions of cellulose and hemicellulose vary by species as soybean hulls have a greater proportion of cellulose while corn fiber contains a greater degree of hemicellulose (Garleb et al., 1988; Ipharraguerre and Clark, 2003). Cellulose is a homopolymer composed entirely of glucose molecules bound via β-1, 4-glycosidic bonds (Demeyer, 1981). The polysaccharide chains are tightly bound together in parallel fashion by hydrogen bonds to form microfibrils. The length of the glucose chains in cellulose can vary greatly depending on the number of glucose molecules therein, but length appears to have no effect on digestibility (Baker et al., 1959). Digestibility is affected by cellulose crystallinity, as greater crystallinity reduces digestibility whereas amorphous structures are more digestible. Although amorphous cellulose is typically only a small percentage of the total cellulose in many plants (Pérez et al., 2002), forages are largely composed of amorphous cellulose structures. Due to the increased amorphous cellulose proportion in forages, Baker et al. (1959) postulated that forage cellulose is digested as long as contact is made with a cellulosic organism or enzyme, implying that forage degradation is heavily influenced by surface area. The cellulose surface area and subsequent degradation are particle size and accessibility dependent, as cellulose microfibrils are embedded in the macromolecule of lignin and hemicellulose (Demeyer, 1981).

While cellulose is homogenously composed of glucose alone, hemicellulose is made of several sugars, namely xylose and arabinose (Garleb et al., 1988). In addition to xylose and arabinose, galactose, mannose, glucuronic acid and other hemicellulosic sugars are present in varying concentrations (Van Soest and McQueen, 1973). The respective hemicellulosic sugar concentrations vary by plant species as corn is composed primarily of xylose and arabinose along with lesser amounts of galactose and glucuronic acid while wheat contains no glucuronic acid but instead contains small amounts of mannose and glucose (Saha, 2003). Similarly, rice bran contains xylose, arabinose, galactose, glucose and anhydrouronic acid. Interestingly, cottonseed hull hemicellulose appears to contain xylose alone (Garleb et al., 1988). The various hemicellulosic sugars are bound together with β-1,4-glycosidic bonds in addition to a smaller proportion of β-1,3-glycosidic bonds that further facilitate lateral side chains of different sugars (Pérez et al., 2002). With some exceptions, hemicellulose is generally less digestible than cellulose in ruminants (Keys et al., 1969). Compared to cellulose, a greater proportion of the hemicellulose digestion takes place in the lower gastrointestinal tract than the rumen. As a result, the fiber digestion advantage in ruminants versus monogastrics is less pronounced in plants with greater concentrations of hemicellulose than in heavily cellulosic plants (Keys et al., 1969). Hydrogen bonds bind the cellulose microfibrils to the surrounding hemicellulose (Jung, 1987) and as bond frequency increases digestibility decreases (Garleb et al., 1988).

Interlaced with hemicellulose, is the third of the primary fiber components, lignin. While both cellulose and hemicellulose are carbohydrate polymers, lignin is composed of phenyl-propane units (Demeyer, 1981) and is insoluble in water (Pérez et al., 2002). The purpose of lignin is to provide structural support as well as resistance to microbial and oxidative breakdown. Lignin is categorized as either core or non-core lignin (Jung, 1987). Core lignin is composed of highly condensed polymeric matrices while non-core lignin is composed of low molecular weight phenolic monomers. Core lignin typically contains units that are covalently-bound with two or more linkages, creating the polymeric matrix. Non-core lignin typically has only one covalent bond that is associated with either hemicellulose or core lignin. Of the acids in non-core lignin, ferulic acid is generally linked to core lignin while para-coumaric acid is generally associated with hemicellulose. Non-core lignin contains both ester-linked and ether-linked phenolic groups however the esterified groups are most prevalent. There are some non-core lignin units that possess a second covalent bond and thereby serve as the crosslink between hemicellulose and core lignin. Crosslinks most likely contain both an ester and ether linkage rather than two ester linkages (Jung, 1987). As frequency of crosslinks between core lignin and hemicellulose increases, the digestibility of the cell walls is reduced as is seen in oat hulls, wherein the cell walls are heavily crosslinked and digestibility is low (Garleb et al., 1988). In addition to the non-core lignin crosslinking, ester bonds between core lignin and the arabinose of hemicellulose are a significant crosslinkage in forage cell walls as well (Jung, 1987). The core lignin concentration has a negative correlation with digestibility in C4 grasses however there is no correlation in C3 grasses. The lignin and hemicellulose association is responsible for the reduced hemicellulose digestibility.

The cellulose, hemicellulose and lignin proportions vary based on species as well as age and maturity of the plant (Pérez et al., 2002). During early plant development the cell walls are interspersed with small amounts of pectin, however these proportions are reduced during plant maturation as lignification increases (Jung, 1997). Like hemicellulose, pectins are heterogeneous mixtures of sugars, mainly galacturonic acid, galactose, and arabinose (Van Soest and McQueen, 1973). Pectin is found in greater concentrations in legumes than grasses (Jung, 1997). The cell walls are composed primarily of primary cell walls during early development while the plant tissue is actively growing and elongating. Once elongation has subsided, secondary cell wall development begins. Lignification progresses in the secondary wall as the plant develops. The maturation of the plant and development of secondary cell walls reduces digestibility due to lignification and an increase in crystalline cellulose development in the thicker, secondary walls (Leschine, 1995). As maturation occurs, the proportions of p-coumaric acid and ratio of p-coumaric:ferulic acid increase in grasses however little change occurs in legumes (Jung, 1987). A combination of factors including total lignin content, cross linkage of core lignin with hemicellulose, cellulose crystallinity, and hydrogen bonding of cellulose fibrils with hemicellulose are responsible for reducing forage and fibrous byproduct digestibility in ruminants.

FIBER DEGRADATION

Ruminants and rumen microbiota degrade and utilize high fiber feedstuffs despite the compositional challenges presented by cellulose, hemicellulose and lignin. The primary roles of the animal are to increase feed particle surface area by chewing and provide an anaerobic environment in the rumen that is conducive to microbial growth and proliferation. In turn, the microbial population generates sources of energy and protein for the animal in the form of short chain fatty acids and microbial cells (Russell and Hespell, 1981). Besides mechanically breaking down feedstuffs and providing anaerobic conditions, the rumen serves as an optimum environment due to a relatively constant temperature, a regular supply of nutrients, an ability to prevent major changes in pH via saliva buffer capacity, and a system to absorb end-products toxic to the microbial population across the rumen wall. These conditions provide an environment for approximately 10^{11} bacteria and 10^{6} protozoa per milliliter of rumen contents along with a smaller number of fungi. Both fungi and protozoa participate in feedstuff digestion however fermentation is predominantly driven by bacteria (Huntington, 1997). The degradation of fiber in particular can be accomplished without the involvement of protozoa (Windham and Akin, 1984) however Leschine (1995) suggests ciliate protozoa may be responsible for a third or more of the microbial fiber degradation in the rumen when present. Some protozoa species generate hydrolytic enzymes extracellularly while other protozoa engulf polysaccharides for further degradation. Chewing by the animal is the primary source of mechanical digestion and surface area improvement but the hyphae of fungi present in the rumen may help compromise the surface of fiber particles and facilitate increased bacterial colonization (Huntington, 1997). In addition to improving surface area, anaerobic fungi appear to contribute to fiber degradation by penetrating cell walls and solubilizing polysaccharides that are inaccessible to bacteria (Leschine, 1995). Fungal analysis shows the ability to degrade up to 34% of lignin in plant tissue however this ability is compromised by the slow growth rate of fungi and the dilution rate of the rumen (Krause et al., 2003). Bacterial digestion of fiber starts with attachment (Huntington, 1997) and therefore increased surface area generates more opportunities for attachment. Reducing forage particle size via mechanical means prior to chewing by the animal helps increase surface area but has been shown to reduce chewing time by the animal (Grant, 1997). Consequently, salivary buffering is reduced, leaving animals subject to lower ruminal pH. This is important for fiber degradation as cellulolytic bacteria are typically less tolerant of low pH than the prevalent amylolytic species (Russell and Hespell, 1981; Russell and Wilson, 1996). Fiber digestibility is typically inhibited below pH 6.2 (Trater et al., 2001) and some cellulolytic species, such as *Ruminococcus flavefaciens* have shown complete washout by pH 6.15 (Russell and Dombrowski, 1980). Similarly, *Bacteriodes succinogenes* washed out at pH 6.0, *Ruminococcus albus* by pH 5.9 and *Butyrivibrio fibrisolvens* by pH 5.70. *R. albus*, *F. succinogenes* and *R. flavefaciens* are the primary cellulolytic species in the rumen along with *B. fibrisolvens* to a lesser extent (Leschine, 1995). Like bacteria, protozoa cannot survive acidic conditions for long, especially at or below pH 5.5 (Demeyer, 1981). Although fiber particle size is important for optimum rumination in high fiber diets (Grant, 1997), particle length has no effect on animal performance or ruminal fermentation in low-fiber feedlot diets (Shain et al., 1999).

Once bacterial attachment to the fiber has occurred, enzymatic activity takes over. There are three cellulolytic enzyme classes that break cellulose microfibrils down to glucose molecules. Endoglucanases hydrolyze the β-1-4 glycosidic bonds of cellulose at random locations and therefore generate both reducing and non-reducing ends on the resultant molecules (Leschine, 1995). Exoglucanases work at the non-reducing ends of the cellulose chains, cleaving off cellobiose units. Cellobiose, a disaccharide, is composed of 2 glucose molecules still bound by a β-1-4 glycosidic linkage. The third class of cellulolytic enzymes, β-glucosidases hydrolyze the bond in cellobiose, generating two glucose monomers. Glucose is converted via fermentative and cellulolytic bacteria to volatile fatty acids for animal use.

The primary VFAs are acetate, propionate, and butyrate. Carbon dioxide and methane also result from feedstuff breakdown but are released via eructation. Hemicellulose is degraded in similar fashion and several cellulolytic species also degrade hemicellulose, though these species do not necessarily utilize the resultant sugars (Dehority, 1973). Hemicellulose is typically lower in digestibility than cellulose, Keys et al. (1969) reported less than 47% of hemicellulose was digested in sheep fed alfalfa hay and this was attributed to lignification. As previously discussed, the lignification of hemicellulose is the primary reason for indigestibility of the polysaccharide however lignin concentrations have been shown to have negative effect on cellulose degradation as well (Jung, 1987). Rumen delignification releases free phenolic compounds that can be toxic to some microbe species. This causes a shift in the microbial population towards more acetate-producing species, a contributing factor in the increased acetate:propionate ratio observed in high-fiber diets. Substantial hemicellulose delignification requires an alkaline environment not found in ruminants (Demeyer, 1981).

Not all degradation of fiber and the resulting substituents takes place in the rumen as hemicellulose hydrolysis largely occurs post-ruminally (Keys et al., 1969). The degradation of hemicellulose generally requires a lower pH than cellulose as is evidenced by the removal of hemicellulose but not cellulose during the ADF procedure (Van Soest and McQueen, 1973) and *in vitro* work confirms the yield of hemicellulosic sugars from acid hydrolysis (Myhre and Smith, 1960). Before particles can flow post-ruminally, size reduction must generally occur. Fiber optic observation has shown the passage of alfalfa hay up to ten millimeters in length flowing through the reticulo-omasal orifice with no restriction though the possibility of omasal expulsion of large particles back into the reticulo-rumen for further degradation was not ruled out (McBride et al., 1984). Martz and Belyea (1986) measured post-ruminal particle size and determined there is a strong resistance to the passage of particles exceeding 1.2 millimeters though the use of polypropylene ribbon has shown significant amounts of 5-10 millimeter particles in the feces. It may be that pliable particles pass with greater ease despite size as compared with the rigid structures of many fiber sources. Though not typically relied upon for most of the fiber digestion that occurs in a functioning ruminant, the large intestine and caecum perform the majority of the limited fiber digestion that takes place in an animal whose rumen has been defaunated (Demeyer, 1981). Caecal and large intestinal fiber digestion is likely limited to remaining cellulose and delignified hemicellulose as Bailey and Macrae (1970) reported caecum and large intestine microbial populations were unable to hydrolyze hemicellulose that had not been delignified in sheep fed red clover hay. Though the lower digestive tract plays a role, fiber digestion is accomplished primarily in the rumen by a diverse and pH-sensitive microbiota.

FIBER DIETS

In order to optimize fiber digestibility, a ruminal environment must be maintained to prevent fibrolytic microbe inhibition. Supplementing forages with fibrous byproducts may be an option to increase energy density while utilizing low-cost forages. Grigsby et al. (1992) fed bromegrass hay to cannulated steers with increasing soybean hull replacement (0, 15, 30, 45, and 60% soybean hulls) and reported an acetate concentration increase, propionate decrease and acetate:propionate increase as soybean hull inclusion increased. Ruminal pH also increased as soybean hull inclusion increased, as did DM digestibility and OM digestibility. The determination was that soybean hulls can replace at least 60% of low quality forage without negatively affecting digestibility. In younger stock, Soto-Navarro et al. (2004) supplemented nursing calves with a 49% soybean hull, 44% wheat middling creep feed at a rate of 0.57% BW and found no negative effect on rumen fermentation characteristics, microbial efficiency, OM intake or OM total tract digestibility.

Taking a different approach, Trater et al. (2001) evaluated the results of holstein steers fed soybean hull diets with increasing alfalfa hay inclusion (10.4, 20.7, or 30.9%)

and reported DM and OM digestibilities decreasing linearly as alfalfa hay inclusion increased. There were quadratic effects of hay inclusion on digestibility and the authors determined there were positive associative effects of the soybean hull and hay combination on DM, OM and NDF digestibilities (Trater et al., 2001). Additionally, Trater et al. (2001) postulated the improved digestibilities due to hay inclusion were a result of a pH increase to levels less inhibitory to fibrolytic microbes rather than slower soybean hull passage since liquid passage rates increased with increasing hay inclusion. Soybean hull supplementation does not negatively affect forage digestibility. Furthermore, positive associative effects can result from a dietary combination of forage and byproducts, raising the question of non-fiber supplementation effects on fiber digestibility.

CONCENTRATE IN FIBER DIETS

Fibrous byproducts improve digestibility when fed alongside forages. Supplementing fiber diets with concentrate may be necessary to further increase energy concentration and potentially greater animal performance. Over the course of five grazing trials, Anderson et al. (1988) supplemented cattle with varying levels of soybean hulls or corn and concluded supplementation improved gain as compared to no supplement when fed at rates ranging from 0.5-0.7% BW. At 0.5 and 0.55% BW supplementation levels, no differences between supplements were observed however at 0.7% BW, cattle fed soybean hulls had greater ADG than cattle fed corn. In the final trial, cattle were fed 0.43% BW soybeans hulls continually, 0.86% soybean hulls continually or had supplement rate increased throughout the grazing season from 0.43 to 0.86% soybean hulls. The low supplement level did not improve gain but the greater level and increasing level did improve gain. Anderson et al. (1988) concluded soybean hull supplementation had reduced acidosis risk as compared to corn and that digestible fiber in the form of soybean hulls did not affect ruminal fermentation in the same manner as starch from corn. Similar conclusions were reached in a two year study supplementing steers and sheep on bermudagrass hay with a 92.5% corn supplement, a soybean hulls supplement or a 93.6% wheat middlings supplement (Garcés-Yépez et al., 1997). Steers were supplemented at two rates, less than 0.5% or greater than 0.8% of BW. Between supplements there was no difference in G:F, and at supplement levels below 0.5% BW there was no difference in ADG. Furthermore, at DMI less than 0.5% BW, all supplements improved ADG as compared to unsupplemented control. At 0.83% BW the corn supplement reduced gain as compared to both the middlings and soybean hulls consumed at rates exceeding 0.9% BW. The sheep trials demonstrated reduced NDF digestibility in animals supplemented with corn or wheat middlings at a rate exceeding 0.8% BW. In a similar comparison providing 0.5% BW corn or 0.7% BW soybean hulls to cattle consuming bermudagrass or orchardgrass hay, Galloway et al. (1993) reported reduced NDF digestibility in steers fed 0.5% BW corn as compared to the steers fed soybean hulls.

Corn inclusion exceeding 0.5% BW appears to have a negative effect on fiber digestibility and animal performance as compared to soybean hulls in forage-based diets (Anderson et al., 1988). Corn supplementation less than 0.5% BW has been postulated as the maximum feeding rate as well. Cannulated steers were fed bromegrass hay and a supplement at a rate of 0.63% BW (Grigsby et al., 1993). Supplements were 0.63% soybean hulls, 0.21% corn with 0.42% soybean hulls, 0.42% corn with 0.21% soybean hulls, 0.63% corn and a control with no supplement. There was a quadratic response to increasing corn in the rumen as DM, OM and NDF digestibilities were greatest for 0.21% corn though 0.42% corn appeared advantageous over 0.63% corn as well. Also, microbial efficiency was greatest for 0.42% corn. Finally, acetate and butyrate concentrations decreased linearly as corn increased but there was no effect on acetate:propionate. There was a trend for decreased pH as corn increased and the conclusion was that corn can be fed at up to 0.21% BW with no negative associative effects on digestibility and ruminal fermentation (Grigsby et al., 1993). Based on the relative performance of the supplement rates, an argument may be made for a corn supplementation rate of 0.42% BW however growth performance would strengthen this conclusion.

Even when corn was included in forage diets at less than 0.5% BW to avoid negative associative effects, there was no energy supplementation benefit if forage quality was high. Buttrey et al. (2012) reported no ADG improvement from 0.44% BW corn supplementation versus no supplement in Hereford steers grazing high quality winter wheat pasture containing 23.7% CP and 34.7% NDF. Though energy was not limiting and the wheat pasture CP was high, providing a different CP source to fulfill AA needs was beneficial, as 0.44% BW dried distillers grains improved ADG in the same study (Buttrey et al., 2012). Distillers grains and corn gluten feed contain more CP than soybean hulls and other low protein fiber byproducts, an advantage when supplementing low protein diets.

FIBER IN CONCENTRATE DIETS

Corn included in a fiber-based diet causes negative associative effects on digestibility and performance if fed at rates exceeding 0.2 to 0.5% BW however soybean hulls contain less digestible energy, causing performance differences in animals on fiber-based diets versus animals on concentrate diets. Löest et al. (2001) reported reduced ADG and G:F in heifers fed soybean hull-based diets as compared with heifers fed corn-based diets. In addition, DM digestibility was reduced in the soybean hull diets. Despite the reduced growth performance possible with fiber feedstuffs, they are included to reduce ration cost or reduce metabolic issues associated with high concentrate diets. Ferreira et al. (2011a) investigated increasing soybean hull inclusion rates in corn-based diets with 10% hay fed to rams. Dry matter intake linearly increased and G:F linearly decreased as soybean hull inclusion increased. There were no effects on ADG or carcass characteristics. The conclusion was up to 45% of corn in a sheep diet could be replaced with soybean hulls with no effect on ADG or carcass values, despite an 11.6% reduction in G:F from 0% soybean hulls to 45%. In a subsequent study, Ferreira et al. (2011b) replaced corn at the same rates (0, 15, 30, 45% soybean hulls) in fistulated ram diets and found that DM digestibility decreased and NDF digestibility tended to increase as soybean hull inclusion increased while ADF and CP digestibilities were unaffected. Additionally, the acetate concentration increased linearly with soybean hull inclusion. Propionate concentration and acetate:propionate were both linear and quadratic with propionate greatest for 15% soybean hull inclusion and acetate:propionate greatest for

45% soybean hulls. Likely causing the numerical trend for increased NDF digestibility, pH increased linearly with soybean hull inclusion.

Utilizing an additional fibrous grain byproduct, Ludden et al. (1995) fed 5% ground corn cobs in dry-rolled corn diets along with increasing soybean hulls (0, 20, 40, 60% inclusion) to steers and wethers. In steers, ADG and G:F decreased linearly as soybean hulls increased whereas DMI increased linearly. In the wether digestibility experiment, DMI also increased linearly with soybean hull inclusion. There was no difference in DM digestibility however NDF digestibility was linear and was greatest for 60% soybean hulls. Animals consuming the 0% soybean hull diet gained 0.01 kilogram more per day than the 20% soybean hull diet. Diets including soybean hulls at levels greater than 20% should be based on economics however the magnitude of difference in growth performance between 0 and 20% soybean hulls was minor. Summarizing several studies, Ipharraguerre and Clark (2003) determined soybean hulls included in diets can replace up to 30% of the corn in high grain diets without negatively affecting digestibility, fermentation or performance in mid to late lactation females.

An advantage over the lower protein fibrous byproducts like soybean hulls, the protein content of corn gluten feed can increase its value in diets by reducing necessary protein supplementation. Bowman and Paterson (1988) fed wet, dry, and ensiled corn gluten feed to wether lambs in high concentrate diets and reported corn gluten feed could be substituted in a corn diet at up to 50% DM inclusion and compare favorably to corn and urea diets as well as corn and soybean meal diets. Similar results were found in a group of steers receiving increasing inclusion rates of wet corn gluten feed (0, 10, 20, 25, 30, 35% DM basis) in a steam-flaked corn diet containing 10% corn silage (Macken et al., 2004). There was no effect of corn gluten feed inclusion on DMI, ADG, G:F or any measured carcass trait indicating that corn gluten feed could be included in diets at a rate of at least 35% with no negative effect on performance, well within the 50% inclusion rate proposed by Bowman and Paterson (1988). Likewise, Block et al. (2005) assigned steers to steam-flaked corn diets containing 0, 20, 30, or 40% wet corn gluten feed with 10% corn silage and reported ADG, G:F and HCW responded quadratically to increasing corn gluten feed and were greatest for the 20% inclusion rate. Dry matter intake responded quadratically and was greatest for 30% corn gluten feed. There were no other effects of corn gluten feed on growth or carcass characteristics. In this experiment, 20% corn gluten feed was the optimum inclusion rate in a steam-flaked corn diet with 10% hay to maximize ADG and G:F.

In addition to corn, distillers grains are produced from sorghum, wheat and other grains depending on regional ethanol production, however there appears to be little difference in performance at moderate distillers grains inclusion levels despite different source grains. Depenbusch et al. (2009) compared wet and dry forms of corn and sorghum distillers grains included as 15% of a finishing diet fed to steers with or without 6% alfalfa hay inclusion and reported no difference in growth performance, digestibility or carcass characteristics of steers fed corn or sorghum distillers grains. Dry matter and OM digestibilities were reduced in steers fed 15% versus 0% distillers grains though there was no difference in growth or carcass traits. Similarly, 6% hay inclusion reduced diet digestibility but led to an improvement in growth and carcass characteristics. Farran et al. (2006) fed steers dry-rolled corn diets containing 0, 3.75, or 7% alfalfa hay and 0 or 35% wet corn gluten feed. Average daily gain, DMI and HCW increased as hay inclusion increased. The corn gluten feed diets had greater DMI and tended to have greater ADG and HCW. Gain:feed did not differ across hay inclusion rates or corn gluten feed inclusion. Hay was less important in diets containing 35% wet corn gluten feed and could be reduced to reduce fecal excretion. Evaluating soybean hulls, distillers grains, and corn gluten feed use in high grain diets with and without the addition of forages or fibrous feedstuffs, there was no difference in growth performance of animals fed a high grain diet with 20-30% of the corn substituted for a highly digestible fibrous byproduct and minimal forage. Although there was no difference in growth performance, digestibility was reduced in high grain diets with fiber inclusion. Improving fiber digestibility in high grain diets containing 20-30% fibrous byproducts may provide additional metabolizable energy to meet or exceed cattle performance on all concentrate diets.

FIBER DIGESTION IMPROVEMENT

Improving fiber digestibility will improve feed efficiency by providing more metabolizable energy per kilogram feed consumed. A wide variety of techniques have been utilized to improve digestion. Ammoniation is the application of anhydrous ammonia on forages stored anaerobically for 6 weeks prior to feeding (Krueger et al., 2008). Ammoniated forage is inherently higher in nitrogen content and therefore CP concentration. Additionally, ammoniation increased DMI due to the increase in DM, NDF and CP digestibilities. Ammonia application poses a safety concern, as exposure can cause serious injuries to the skin, eyes and respiratory system (Millea et al., 1989).

Alkali solutions such as hydrogen peroxide (Kerley et al., 1986), sodium hydroxide (Petersen et al., 1981; Coombe et al., 1979) or calcium hydroxide (Petersen et al., 1981) can be applied to forage. Hydrogen peroxide treated wheat straw was included in corn diets and fed to cannulated wethers, the hydrogen peroxide increased DM, NDF and cellulose digestibilities (Kerley et al., 1986). Coombe et al. (1979) fed fistulated steers wheat straw treated with sodium hydroxide and reported sodium hydroxide increased DM, OM and ADF digestibilities as compared to untreated straw or straw treated with water. The straw treated with water prior to ensiling was not different in digestibility than the untreated straw. Petersen et al. (1981) examined the effects of sodium hydroxide, calcium hydroxide and a 50:50 combination of sodium hydroxide and calcium hydroxide on Kentucky bluegrass straw fed to lambs. Compared to the untreated control, sodium hydroxide increased OM, NDF, ADF and ADL digestibilities. Both calcium hydroxide and the 50:50 alkaline mix improved NDF, ADF and ADL digestibilities. Sodium hydroxide treatment resulted in greater digestibilities than calcium hydroxide for NDF and ADF. Evaluating the various alkaline treatments, DM and fiber digestibilities are improved. Like anhydrous ammonia, alkaline exposure is also a safety concern when treating forages as eye exposure to alkali solutions can cause irritation and even tissue necrosis (Hughes, 1946) while brief skin exposure to calcium hydroxide is associated with severe burns (Narayanan et al., 2000). Additionally, both ammoniation and alkaline treatments require forages to remain enclosed or ensiled for several weeks prior to feeding. Additional time and labor are required for either treatment.

Though the time and labor investments are similar to ammoniation and alkali treatments, treating forages with a fibrolytic enzyme prior to storage is safer for handlers and has shown digestibility improvements as well. Bermudagrass hay was treated at cutting, baling or just prior to feeding with 16.5 grams of biocellulase per ton of air-dried hay and fed to steers along with a 1% BW supplement of soybean hulls and cottonseed meal (Krueger et al., 2008). All three enzyme treatments increased DM digestibility. Enzyme applied at cutting or baling improved NDF and CP digestibilities while enzyme applied just prior to feeding tended to increase NDF digestibility. Krueger et al. (2008) also noted the handler safety advantage of enzyme treatments versus treatments like ammoniation. In a similar fashion, Krause et al. (1998) fed cannulated steers a diet of rolled barley and 5% forage with cellulase and xylanase enzyme applied to the treatment diet’s barley prior to storage periods. Treating the rolled barley with enzyme increased total tract ADF digestibility of the diet and tended to decrease acetate:propionate suggesting starch digestion was greater due to greater availability by enhancing digestion of the hull surrounding the starch portion of the grain. In a dairy setting, Yang et al. (1999) fed fistulated Holstein cows a diet consisting of alfalfa hay cubes and corn with one gram of a cellulase/xylanase enzyme mixture applied per kg hay, two grams per kg hay, or one gram applied per kg of both hay and corn. The hay was treated and cubed two weeks prior to the start of the study. Compared with no enzyme, total tract OM digestibility and fat-corrected milk production were greater for the diets containing the high-dosage treated hay or both feedstuffs treated. Neutral detergent fiber and CP digestibilities were greater for the diet containing hay with the higher enzyme dosage. There was a dosage response to the total enzyme in the diet. Reducing exposure time of feedstuffs to enzyme treatments, Lewis et al. (1996) fed a 70:30 grass hay:barley diet to cannulated steers with a cellulase, xylanase, cellobiose and glucose oxidase enzyme treatment applied to hay at 24 hours pre-feeding, to hay immediately before feeding, to barley immediately before feeding, infused ruminally two hours post-feeding or not treated. Dry matter, NDF and ADF digestibilities tended to be greater for hay treated immediately prior to feeding or 24 hours prior to feeding as compared to the untreated diet. Total VFA production was greater for treated hay diets however no differences existed in individual VFAs. Differences in digestibility can be elicited from feedstuffs treated with enzymes for shorter periods of time than the two weeks or greater shown in other enzyme investigations.

Further evaluating enzyme efficacy when administered at feeding, cannulated lambs were fed alfalfa or ryegrass with or without the addition of an enzyme placed directly into the rumen at feeding (Pinos-Rodríguez et al., 2002). Dry matter intake by lambs and total VFA concentrations were greater in enzyme treated diets. Additionally, the enzyme increased apparent digestibility of CP, hemicellulose and NDF in the alfalfa diets. In a similar study, Giraldo et al. (2008a) fed an endoglucanase/xylanase enzyme to lambs consuming a 70:30 grass hay:concentrate diet. Of the 30% of the diet composed of concentrate, 9.45% was starch grain, 12.12% was fibrous byproduct, and the remainder was protein and mineral sources. The enzyme treatment resulted in an increased propionate proportion, decreased acetate:propionate and tended to increase cellulolytic bacteria at four hours post-feeding. There was no difference in digestibilities in spite of an increased rate of disappearance for DM and NDF in enzyme-treated lambs. Despite no difference in digestibility, the conclusion was enzyme supplementation increased fibrolytic activity and stimulated cellulolytic bacterial growth without a prefeeding feed-enzyme exposure period. High concentrate diets provide the most challenge to fibrolytic activity. Giraldo et al. (2008b) examined the effects of various enzymes and dosages on a 30:70 grass hay:corn substrate *in vitro*. Xylanase from *Trichoderma viride*, cellulase from *Aspergillus niger* and cellulase from *T. longibrachiatum* all decreased NDF content compared to untreated controls. The *T. longibrachiatum* cellulase also decreased ADF as compared to the other treatments and control. Hemicellulose content was decreased by all enzymes while acetate, propionate and total VFA proportions were all increased. Administering exogenous fibrolytic enzymes immediately prior to feeding appears to improve fiber degradation but more work is needed, especially in high concentrate diets to determine efficacy in feedlot diets.

CONCLUSION

Fibrous feedstuffs can improve profitability when priced competitively compared to grains if performance is unaffected or cost of gain is reduced. Ruminant animals provide an anaerobic environment that supports a microbial population capable of digesting a wide variety of feedstuffs including forages and fibrous byproducts poorly utilized by other species. The varying combinations of lignin content, degree of lignin-hemicellulose crosslinkage, cellulose crystallinity, and cellulose-hemicellulose linkage reduce fibrous feedstuff digestibility in ruminants. Understanding fiber composition and degradation gives insight into the reduced performance associated with high fiber diets and high concentrate diets containing fibrous feedstuffs. In order to optimize fiber digestibility, a ruminal environment must be maintained to prevent fibrolytic microbe inhibition as is often caused by low pH due to starch supplementation. Additionally, determining an acceptable level of fiber inclusion in high concentrate diets that won’t detract from animal performance provides more flexibility in feedlot diet formulation..

Fibrous byproduct supplementation has no negative effect on forage diet digestibility and often generates positive associative effects. Similarly, limiting concentrate supplementation to 0.2 to 0.5% of BW in fibrous diets can produce positive associative effects while fibrous byproduct inclusion in a high concentrate diet appears to have negligible effects on performance when inclusion does not exceed 20-30%. Despite the negligible effect of 20-30% fibrous byproduct inclusion on performance, fiber digestibility is often reduced. Improving fiber digestibility in high concentrate diets has the potential to increase metabolizable energy value, improving feed efficiency and profitability. A variety of techniques like ammoniation and alkaline treatment exist for improving fiber degradation though exogenous enzyme addition tends to be safer for handlers, require less storage time and require less labor. Administering exogenous fibrolytic enzymes at feeding holds promise but work is limited in high concentrate diets. The hypothesis is that the maximum corn supplementation in fibrous diets is 0.4% BW and fibrolytic enzyme inclusion in high concentrate diets containing a fibrous feedstuff will improve feed efficiency by improving digestibility.

CHAPTER 2

Effect of corn inclusion on soybean hull diet digestibility by rumen microbiota.

ABSTRACT

Soybean hull (SH) diets with increasing corn proportions were fed to continuous culture fermenters to determine increasing starch inclusion effects on digestibility and microbial efficiency (MoEff). The hypothesis was fiber digestibility would respond quadratically to starch level in the diet with a breakpoint where starch inclusion improves fiber digestion. Proportionately, the diets contained 100 (SH100), 90 (SH90), 80 (SH80), 60 (SH60) or 20 (SH20) % SH with the remaining proportion corn. Diets were randomly distributed over 24 continuous culture fermenters. The NDF digestibility decreased linearly (P=0.04) and ADF digestibility tended to decrease linearly (P=0.09) as corn increased. The DM and OM digestibilities were greatest for SH20 followed by SH90 and were cubic (P<0.02). Bacterial nitrogen and MoEff responded linearly (P<0.01) and were greatest for SH20. Lactic acid (LAC), VFA, and pH were measured at 0, 4 and 8 h post-feeding. The only diet x time interaction was for LAC. Acetate was linear (P<0.01) and greatest for SH100. Propionate was quadratic (P<0.01) and greatest for SH100 followed by SH20. Butyrate and total VFA were quadratic (P<0.01) and greatest for SH20. Acetate:propionate was quadratic (P<0.01) and greatest for SH80. Lactic acid was cubic (P<0.01) with the greatest value for SH20. The pH was quadratic (P<0.01) and greatest for SH80. Hours 4 and 8 were not different (P>0.05) for pH but both were less (P<0.02) than hour 0.

Fiber digestion was reduced across the range of starch inclusion rates and an optimum balance of diet and fiber digestibilities was between 10 and 20% corn inclusion.

Keywords: digestibility, fiber, soybean hulls

INTRODUCTION

Alternative dietary energy sources are important as conventional energy sources like corn increase in cost. Previous studies utilized soybean hulls (SH) as the primary energy source in growing beef cattle diets (Garrigus et al., 1967; Löest et al., 2001). Compared to corn, SH and other fibrous byproducts are cost-competitive but typically have lower feeding value (Ludden et al., 1995) and may reduce feedlot performance (Löest et al., 2001). Providing corn along with SH could increase dietary energy concentration without markedly increasing diet cost. Providing corn in a fiber-based diet can cause negative associative effects by reducing fiber digestibility (Chase and Hibberd, 1987; Garcés-Yépez et al., 1997). Anderson et al. (1988) reported 0.5% BW was the maximum corn inclusion rate in a fibrous diet to avoid negative associative effects whereas Grigsby et al. (1993) and Pordomingo et al. (1991) reported 0.2% as the optimum rate. The experiment objective was to determine the corn inclusion rates in a fibrous diet in which fiber digestion was compromised in order to help identify opportunities for avoiding negative associative effects. The hypothesis was fiber digestibility would respond quadratically to starch level in the diet with a breakpoint where starch inclusion improves fiber digestion.

MATERIALS AND METHODS

Experimental animal use was approved by the University of Missouri Animal Care and Use Committee. Twenty-four single-flow fermenters were used in a continuous culture system. Each fermenter consisted of a two liter polycarbonate jar (Nalgene, Rochester, NY) with a continuous volume of approximately 1460 mL. Each fermenter received buffer (McDougall, 1948) at a rate of 6% per hour^{-1} via a peristaltic pump (Masterflex model 7520-10, Cole Parmer Instrument Co., Chicago, IL). Fermenters received a steady supply of carbon dioxide to maintain anaerobic conditions, were continuously stirred with magnetic stir plates and were kept in a water bath heated to 39°C by a thermostatically-controlled water heater (model 730, Fisher Scientific, Pittsburgh, PA).

Five treatment diets (TRT) were randomly distributed across 24 fermenters. Proportionately, the diets contained 100 (SH100), 90 (SH90), 80 (SH80), 60 (SH60) or 20 (SH20) % SH with the remaining proportion corn. Four fermenters were allocated to SH100 while five fermenters were allocated to SH20, SH60, SH80, and SH90. Treatment diets were formulated (Tables 2.1, 2.2) to meet RDP and RDN requirements (Russell et al., 1992) based on estimated MoEff and nutrient dilution rate calculations (Meng et al., 1999). Mineral inclusion rates were determined using a 2.3% BW intake value for a 273 kg calf and were formulated to meet NRC recommended levels (NRC, 2000). Ingredients were ground through a 2-mm screen in a Wiley Mill (Arthur H. Thomas Company, Philadelphia, PA) prior to mixing. Rumen fluid was collected from two lactating, rumen-fistulated Holstein cows housed at the University of Missouri-Columbia Foremost Dairy Research Center. Fluid was strained through two layers of cheesecloth, combined, and mixed with buffer (McDougall, 1948) prior to fermenter introduction at a 3:1 ratio (buffer:rumen fluid). Fermenters were fed 50g per day divided into two feedings at 0800 and 2000 hours. Fermenters were allowed four adaptation days followed by three sampling days.

Fermenter pH was recorded and 10mL samples were collected at 0800, 1200, and 1600 hours during the three sampling days. Fermenter pH was measured using a glass bulb pH meter recalibrated prior to each timepoint. The 10mL fermenter samples were stored at 4°C until analyses for ammonia, VFA and lactic acid (LAC) concentration. Effluent outflow was individually collected for each fermenter in plastic graduated cylinders surrounded with ice. During sampling, a 1L subsample was collected daily from each effluent outflow at 0800 and shaken thoroughly to ensure a representative sample once the total volume was recorded. The subsamples from all three days were combined for each fermenter and stored at 4°C until analysis. At the conclusion of the third sample day, fermenter contents were blended (model 34BL22, Waring, New Hartford, CT) for 30 seconds to encourage separation of microbes from feed particles, strained through two layers of cheesecloth to remove large feed particles, and stored at 4°C. Strained fermenter contents were centrifuged at 1,000 x *g* for five minutes at 4°C to further remove feed residue. The resulting supernatant was centrifuged at 27,000 x *g* for 30 minutes, supernatant was removed and the pellets from each fermenter were transferred to a plastic cup using distilled water to transfer any pellet residue not transferred by spatula. The cups were then lyophilized at 10°C (Genesis, Virtis, Gardiner, NY) and the dry bacteria were ground with a mortar and pestle. A 500mL subsample of each 3-day effluent composite was lyophilized at 10°C (Genesis, Virtis, Gardiner, NY) and then ground using a mortar and pestle. Samples of feed, effluent residue, and fermenter bacteria were analyzed for DM, OM and total N. The DM was determined by drying 1g replicates of each sample at 105° C for 24 hours. Organic matter was determined by incinerating the DM samples at 500° C for 12 hours and total N was determined by combustion (LECO FB-428, LECO Corporation, St. Joseph, MI). Effluent residue and feed samples were analyzed for NDF and ADF (ANKOM NDF and ADF methods, ANKOM Technology, Macedon, NY). Bacterial N (BactN) was determined by analyzing effluent and fermenter pellet samples for purine content (effluent purine x fermenter N / fermenter purine; Zinn and Owens, 1986). Bacterial N and OM values were used to calculate MoEff (g microbial N / kg OM digested). Volatile fatty acid and LAC concentrations were analyzed with gas chromatography (Salanitro and Muirhead, 1975; Model 3400, Varian, Palo Alto, CA). Ammonia was determined colorimetrically (DU-65 spectrometer, Beckman, Palo Alto, CA) using a phenol-hypochlorite assay (Broderick and Kang, 1980).

PROC MIXED procedure of SAS was used to separate TRT effects at $P < 0.05$ for the VFA, LAC, pH and ammonia values which had multiple time points (SAS 9.2, SAS Institute, Cary, NC). PROC GLM procedure of SAS was used to separate TRT effects at $P < 0.05$ for measures with a single value per fermenter (SAS 9.2, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Increasing dietary corn inclusion linearly decreased (*P*=0.04) NDF digestibility (NDFd) and tended to linearly decrease (*P*=0.09) ADF digestibility (ADFd; Table 2.3). Increasing corn had a cubic effect (*P*<0.02) on DM digestibility (DMd) with the greatest value for SH20 and the lowest value for SH80. Similarly, starch inclusion had a cubic effect (*P*=0.02) on OM digestibility (OMd) wherein the greatest digestibility was in SH20 while SH60 was lowest. These results agreed with Aikman et al. (2006) where increasing starch decreased NDFd and ADFd while increasing DMd and OMd in Jersey cows fed a forage-based diet with increasing wheat inclusion and decreasing SH inclusion. In a 273 kg calf consuming 2.3% BW, the SH80 calculated to approximately 0.45% bodyweight corn intake and the SH90 was approximately 0.22%. Comparing the fiber digestibility TRT values and quadratic responses, there was a greater difference between SH80 and SH90 than other adjacent TRT indicating a breakpoint in fiber digestibility between those values. This was similar to the 0.5% bodyweight corn intake limit reported by Anderson et al. (1988) and the 0.21% reported by Grigsby et al. (1993). In treatments containing at least 60% SH, OMd was greatest for 10-20% dietary corn inclusion. Nitrogen digestibility was not affected by TRT (*P*>0.2). Bacterial N and MoEff increased linearly (*P*<0.009) as starch increased. In isonitrogenous diets, Grigsby et al. (1993) reported a quadratic effect of BactN to increasing starch in cannulated steers with a peak at 0.2% bodyweight, approximately equivalent to SH90. The RDP and RDN inclusion was increased in the diet as corn inclusion increased and this caused the response of BactN and MoEff because dilution rates were constant across treatments. The only treatment x post-feeding time interaction occurred for total VFA concentration (TVFA; *P*=0.04) thus all VFA, LAC and pH analysis focuses on treatment or time main effects. In table 2.4, acetic acid (ACE) decreased linearly (*P*=0.001) as corn inclusion increased. Propionic acid (PRO) responded quadratically (*P*=0.008) with the greatest values for SH100 and SH20. Butyric acid (BUT) had linear and quadratic responses (*P*=0.001) and was greatest for SH20. Total VFA (ACE, PRO, BUT, isobutyrate, valerate and isovalerate) was quadratic (*P*=0.002) and greatest for SH100. The ACE:PRO ratio (A:P) was quadratic (*P*<0.04). Treatment SH80 had the greatest A:P. Lactic acid was cubic (*P*<0.005) and greatest for SH100. Grigsby et al. (1993) reported linear decreases in ACE and BUT with increasing corn but no effect on PRO or A:P. The quadratic A:P response agreed with the digestibility results as SH80 and SH90 produced greater A:P than SH60 and SH20, demonstrating a shift from amylolytic, propionate-producing bacteria to fibrolytic, acetate-producing bacteria. The A:P for both SH80 and SH90 were also greater than SH100, a positive associative effect pattern because A:P was expected to be greatest in an all fiber diet.

The concentrations of ACE, PRO, TVFA and LAC did not differ (*P*>0.05) between hours 4 and 8 but were greater (*P*<0.05) than hour 0 (Table 2.4). Hour 0 was greater than hour 8 for A:P, but hour 4 did not differ (*P*>0.05) from either timepoint. Treatment had linear and quadratic effects on pH with the greatest value for SH80. The pH was greatest (*P*<0.02) prior to feeding at hour 0. Hours 4 and 8 did not differ (*P*>0.05) for pH. Trater et al. (2001) reported pH 6.2 as the minimum to prevent fiber digestion inhibition, a greater pH than the SH80 which still exceeded the washout pH values of many cellulolytic species (Russell and Dombrowski, 1980). The SH20 and SH60 treatments each had a mean below pH 5.7, an explanation for the reduced NDFd and ADFd compared to diets containing less than 20% corn. The greater pH values generated by SH80 and SH90 strengthen the argument that optimum fiber digestibility was produced by diets that were proportionately 10-20% corn as greater pH values increased fibrolytic microbe survivability and activity. Ammonia measurements were zero for all TRT, indicating N was entirely utilized and RDN may have been inadequate. The RDN was originally balanced to account for N recycling found *in vivo*, however with no *in vitro* recycling additional RDN may be necessary. Additional RDN may have further increased fibrolytic activity because ammonia was the sole source of N for cellulolytic bacteria (Griswold et al, 2003). Analysis of corn inclusion effects on MoEff, BactN and ammonia in high fiber diets would be better explained with diets balanced for AA, RDP and RDN based on equivalent performance expectations and adequate RDN to account for no *in vitro* N recycling.

Fiber digestibility decreased as corn inclusion increased due to negative effects on pH. Decreasing pH reduces fibrolytic activity but had no negative effect on overall diet digestibility in diets containing 20% or greater SH. The optimum corn inclusion rate in a highly digestible fiber diet lies between 10 and 20% of diet DM. Efforts to improve fiber digestibility may be most beneficial in fibrous diets exceeding 20% corn.

TABLES

Table 2.1 - Ingredient and analytical composition of diets (%, DM basis)

	Diets[1]				
Ingredient	SH20	SH60	SH80	SH90	SH100
Corn	69.8	37.7	19.6	9.9	-
Soybean hulls	17.5	56.9	77.8	88.7	98.5
Soybean meal	7	-	-	-	-
Fish meal	2.1	2.1	-	-	-
Blood meal	2	2	1	-	-
Limestone	1.1	0.7	0.5	0.3	0.2
Supplement[2]	0.4	0.4	0.4	0.4	0.4
Magnesium oxide	-	0.1	0.2	0.2	0.2
Dicalcium phosphate	-	-	0.5	0.5	0.6
Analysis					
DM, as-fed basis	87.7	87.8	87.2	87.3	87.3
OM	4.3	5.1	5.8	5.8	6.4
CP	12.4	11.2	10.3	8.7	9.2
NDF	19.7	38.4	46	53	57.4
ADF	10.1	24.9	32.2	37.9	41.6

[1] Diets: SH20 = 20% soybean hulls with 80% corn; SH60 = 60% soybean hulls with 40% corn; SH80 = 80% soybean hulls with 20% corn; SH90 = 90% soybean hulls with 10% corn; SH100 = 100% soybean hulls with no corn

[2] Composition: 50% Sodium chloride, 25% Beef trace mineral premix (10,000 ppm Copper; 25,000 ppm Iron; 20,000 ppm Manganese; 30,000 Zinc; 100 ppm Cobalt; 100 ppm Selenium; 500 ppm Iodine), 25% Beef vitamin premix (3,006,302 IU/kg Vitamin A; 751,575 IU/kg Vitamin D; 75,157 IU/kg Vitamin E)

Table 2.2 - Digestibility, microbial efficiency and bacterial nitrogen production due to increasing soybean hull to corn proportions in continuous culture fermenter diets

	Diets[1]						*P*-values			
Item[2]	SH20	SH60	SH80	SH90	SH100	SEM	Model	Linear	Quadratic	Cubic
DMd, %	45.77	37.14	36.40	38.59	37.64	1.6	<0.01	<0.01	<0.01	0.02
OMd, %	47.12	40.15	42.97	43.73	42.24	1.6	0.04	0.2	0.1	0.02
NDFd, %	65.28	66.39	65.86	71.42	74.89	3.7	0.3	0.04	0.4	1.0
ADFd, %	64.48	66.32	64.98	71.41	73.75	4.5	0.5	0.09	0.6	0.9
Nd, %	66.55	59.33	62.12	62.47	56.92	3.3	0.3	0.1	0.9	0.1
BactN, g	48.64	36.61	33.14	32.9	31.05	0.04	<0.01	<0.01	0.1	0.3
MoEff[3]	24.79	21.82	18.61	18.54	17.93	2.0	0.07	<0.01	0.3	1.0

[1] Diets: SH20 = 20% soybean hulls with 80% corn; SH60 = 60% soybean hulls with 40% corn; SH80 = 80% soybean hulls with 20% corn; SH90 = 90% soybean hulls with 10% corn; SH100 = 100% soybean hulls with no corn

[2] DMd = DM digestibility; OMd = OM digestibility; NDFd = NDF digestibility; ADFd = ADF digestibility; Nd = nitrogen digestibility; BactN = Bacterial nitrogen; MoEff = Microbial efficiency

[3] Grams of bacterial nitrogen per kilogram OM digested

Table 2.3 – VFA concentrations, lactic acid concentrations and pH due to increasing soybean hull to corn proportions in continuous culture fermenter diets

	Diets[1]						*P*-values			
Item[2]	SH20	SH60	SH80	SH90	SH100	SEM	Model	Linear	Quad	Cubic
ACE, m*M*	46.3	49.6	53	55.5	60.2	2.1	<0.01	<0.01	0.8	0.7
PRO, m*M*	24.7	24.4	20.9	22.4	27.2	1.8	0.04	0.5	<0.01	0.2
BUT, m*M*	17.8	13.5	9.3	9.2	10.1	1.4	<0.01	<0.01	<0.01	0.8
TVFA[3], m*M*	99.5	94.0	89.9	93.6	102.8	3.5	0.03	0.5	<0.01	0.7
LAC, m*M*	3.24	0.35	0.38	0.26	0.65	0.3	<0.01	<0.01	<0.01	<0.01
A:P	2.06	2.1	2.54	2.49	2.23	0.1	<0.01	0.04	<0.01	0.07
pH	5.32	5.62	5.87	5.83	5.78	0.03	<0.01	<0.01	<0.01	0.7

[1] Diets: SH20 = 20% soybean hulls with 80% corn; SH60 = 60% soybean hulls with 40% corn; SH80 = 80% soybean hulls with 20% corn; SH90 = 90% soybean hulls with 10% corn; SH100 = 100% soybean hulls with no corn

[2] ACE = acetate; PRO = propionate; BUT = butyrate; TVFA = total VFA; LAC = lactic acid; A:P = acetate:propionate

[3] Total VFA includes acetate, propionate, isobutyrate, butyrate, isovalerate and valerate

Table 2.4 – VFA concentrations, lactic acid concentrations and pH at 0, 4 and 8 hours post-feeding in continuous culture fermenters fed soybean hull and corn diets

	Hours[1]				
Item[2]	0	4	8	SEM	*P*-value
ACE, m*M*	50.6^{b}	54^{a}	54.1^{a}	1.0	<0.01
PRO, m*M*	21.6^{b}	24.6^{a}	25.6^{a}	0.8	<0.01
BUT, m*M*	11.7	12.1	12.1	0.6	0.75
TVFA[3], m*M*	90.9^{b}	97.9^{a}	99.1^{a}	1.8	<0.01
LAC, m*M*	0.72^{b}	1.14^{a}	1.07^{a}	0.14	<0.01
A:P	2.38^{a}	2.28^{ab}	2.19^{b}	0.06	<0.01
pH	5.74^{a}	5.65^{b}	5.67^{b}	0.02	<0.01

[1] Hours post-feeding

[2] ACE = acetate; PRO = propionate; BUT = butyrate; TVFA = total VFA; LAC = lactic acid; A:P = acetate:propionate

[3] Total VFA includes acetate, propionate, isobutyrate, butyrate, isovalerate and valerate

[a,b] Within row, least squares means without common superscript differ ($P<0.05$)

CHAPTER 3

Effect of corn inclusion on soybean hull diet digestibility and growth performance of beef calves.

ABSTRACT

Soybean hull (SH) diets with increasing corn proportions were fed to crossbred beef cattle to determine increasing corn inclusion effects on digestibility and performance. The hypothesis was fiber digestibility would be greatest at a corn intake of 0.4% BW and otherwise decrease as starch inclusion increased. Proportionately, the diets contained 100 (SH100), 90 (SH90), 80 (SH80), 60 (SH60) and 20 (SH20) % SH with the remaining proportion corn. Corn intake of 0.4% BW was represented by SH80. Forty steers (347 ± 29 kg BW) and 50 heifers (374 ± 24 kg BW) were blocked by gender, stratified by BW and distributed across diet. Diets were fed for 70 d with titanium dioxide included in the diet for the final 14 d. Fecal samples were collected on day 70 to measure digestibility. Diets were balanced for absorbable AA requirements and RDP based on available ME. Individual DMI was measured using Growsafe Feed Intake System. Average daily gain and G:F increased linearly ($P<0.01$) as corn inclusion increased. Using SH100 ADG as a base value SH had 69% energy equivalency of corn in SH20 but 76% energy equivalency in SH80. There was no DMI difference ($P>0.46$) due to diet. Dry matter digestibility (DMd) and OM digestibility (OMd) were both linear ($P<0.05$), increasing as corn inclusion increased. Both NDF and ADF digestibilities responded quadratically (*P*=0.04) and were greatest for SH80. Fiber digestibility was optimum at 0.4% bodyweight corn intake.

Keywords: digestibility, fiber, soybean hulls

INTRODUCTION

As conventional dietary energy sources increase in cost, soybean hulls (SH) and other fibrous byproducts become advantageous on a cost per ton basis. Cost of gain must be considered as cattle fed primarily SH diets gain less than corn-fed cattle (Löest et al., 2001; Garrigus et al., 1967). Supplementing SH diets with corn can increase dietary energy concentration but corn inclusion in fiber-based diets negatively affects fiber digestion (Chase and Hibberd, 1987; Faulkner et al., 1994). Pordomingo et al. (1991) reported 0.2% BW intake as the maximum corn feeding rate to avoid negative associative effects in fiber-based diets. Grigsby et al. (1993) determined 0.21% BW as the maximum though 0.42% BW was also advantageous over greater corn supplementation rates. Anderson et al. (1988) reported 0.5% BW as the maximum corn supplementation. Determining maximum corn inclusion rates may help avoid negative associative effects while improving dietary energy concentration, diet digestibility and feedlot performance in SH-based diets. The hypothesis was fiber digestibility would be greatest at a corn intake of 0.4% BW and would otherwise decrease as starch inclusion increased.

MATERIALS AND METHODS

Experimental animal use was approved by the University of Missouri Animal Care and Use Committee. Fifty crossbred heifers (374 ± 24 kg BW) and 40 crossbred steers (347 ± 29 kg BW) were fed experimental diets for 70 d in confinement. Steers and heifers were separated throughout the study duration. All calves received an electronic ID tag (Allflex US Inc., Dallas-Fort Worth Airport, TX) in the left ear to facilitate intake measurement using a GrowSafe FI system (GrowSafe Systems Ltd., Airdrie, AB, Canada). A pour-on (Cydectin, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) was administered to control internal and external parasites. All calves were placed on a receiving diet for 7 days prior to the onset of the five treatment diets. Steers were stratified by initial BW (IBW) to pen with 1 pen per diet (8 steers/diet). Heifers were blocked by weight into a heavy and light pen per diet (5 heifers/pen, 2 pens/diet). Steers were housed in concrete pens (7.3m x 16.5m) with access to shelter and two GrowSafe bunks that allowed two animals to eat at a time. Heifers were housed in concrete pens (5m x 9.1m) with access to shelter and one GrowSafe bunk. *Ad libitum* water was provided at all times via automatic waterers (Ritchie Industries Inc., Conrad, IA). Feed ingredients were blended in a truck-mounted mixer (Reel Auggie 3120, KUHN North America Inc., Brodhead, WI) and fed on a daily basis. Diets were mixed in a random order each day. Initial BW was measured for two consecutive days and calves were assigned to treatment pens following the second days weight (day 0). All steers were implanted (Revalor IS, Intervet Inc., Millsboro, DE) on day 0 but heifers were not implanted to allow for replacement heifer selection following the study. Intermediate weights were measured on day 29 and day 56. Starting on day 57, diets contained titanium dioxide (.098%, Table 3.1) to facilitate digestibility calculations. Final BW (FBW) was measured on day 69 and 70 of the study and fecal grab samples were collected. All weights were measured prior to the daily feeding.

The five diets were formulated (Table 3.1, 3.2) to meet RDP and RDN requirements (Russell, 1992) based on estimated MoEff as well as nutrient dilution rate calculations (Meng, 1999). Amino acid requirements were based on available metabolizable energy, average IBW and predicted ADG. Amino acid requirements were met using Amino Plus (Ag Processing Inc., Omaha, NE) and Alimet (88% Methionine; Novus International, St. Charles, MO). Mineral inclusion rates were formulated to meet or exceed NRC recommended levels for growing cattle (NRC, 2000). Rumensin 90 (200g/kg Monensin; Elanco Animal Health, Indianapolis, IN) was included in the diet to provide 28 grams monensin per 909kg DM. Choice white grease was included in the pelleted supplement at a rate of 2% of pelleted supplement to improve pellet quality. With the exception of the whole shell corn (WSC), SH pellets and fescue hay, all ingredients were pelleted. The WSC, hay, SH and pelleted supplement were combined on farm in a truck-mounted mixer.

Feed samples were gathered on a weekly basis, ground through a 2mm screen in a Wiley Mill (Arthur H. Thomas Company, Philadelphia, PA) and analyzed for DM, OM, total N, NDF and ADF. The DM was determined by drying 1g replicates of each sample at 105°C for 24 hours. Organic matter was determined by incinerating the DM samples at 500°C for 12 hours and total N was determined by combustion (LECO FB-428, LECO Corporation, St. Joseph, MI). NDF and ADF were determined using the ANKOM method of fiber analysis (ANKOM Technology, Macedon, NY). The fecal samples collected on day 70 were weighed and dried for 2 weeks at 55°C. The dried samples were reweighed and ground through a 2mm screen. Fecal samples were analyzed for DM, OM, total N, NDF, ADF and titanium dioxide content. Samples were prepared using methods outlined by Myers et al. (2004) and titanium content was determined colorimetrically (DU-65 spectrometer, Beckman, Palo Alto, CA). Individual intake and dietary titanium inclusion were used to determine individual titanium dioxide intake.

PROC GLM procedure of SAS was used to separate TRT effects at $P<0.05$ (SAS 9.2, SAS Institute, Cary, NC). Animal gender was included in the model.

RESULTS AND DISCUSSION

Initial BW on day 0 was not different ($P=1$; Table 3.3). Average daily gain was linear ($P<0.0001$), increasing as corn inclusion increased. Using SH100 average daily gain as the base, SH in SH20 had 69% energy equivalency of corn. The SH in SH80 had 76% energy equivalency of corn, a positive associative effect. Final bodyweight increased linearly ($P=0.004$) as corn inclusion increased. The linear response in FBW was expected due to the ADG linear response and lack of difference in IW. The G:F response was also linear ($P<0.001$), increasing as corn increased. There was no curvilinear DMI response ($P>0.46$) across diets. The growth results agreed with Ludden et al. (1995) wherein steers fed corn and SH diets had decreased ADG and G:F with increasing SH as well as linearly increasing DMI. Despite the poorest ADG among treatments, the SH100 cattle had the same G:F as limit-fed heifers in a previous SH study

(Löest et al., 2001). Feed conversion was consistent between SH diets thus the difference in ADG between studies was likely due to differences in DMI, BW and dietary CP. Additionally, SH100 contained 10% hay which has been shown to improve digestibility in SH diets (Trater et al., 2001) and ADG in concentrate diets (Shain et al., 1999).

Dry matter digestibility (DMd) increased linearly (*P*=0.05) as corn inclusion increased and agreed with previous work (Ludden et al., 1995; Löest et al., 2001; Table 3.4). Organic matter digestibility (OMd) responded like DMd, increasing linearly (*P*=0.04) as corn increased. The greater starch digestibility versus SH explains the increased DMd and OMd. Despite numerical increases in DMd and OMd for SH80, there was no quadratic response (*P*=0.86). Both NDF and ADF digestibilities (NDFd, ADFd) responded quadratically (*P*=0.04) and were greatest for SH80. Nitrogen digestibility (Nd) had no curvilinear response to diet (*P*>0.13) though SH20 was numerically greatest, followed by SH80. The NDFd increase from SH20 to SH80 agreed with previous work (Ludden et al., 1995) in which NDFd increased linearly as diet SH inclusion increased from 0 to 60%. Grigsby et al. (1993) reported quadratic responses for ruminal DMd, OMd and NDFd that were greatest for steers fed 0.21% BW corn but no curvilinear response in total tract digestibilities. The SH90 diet was equivalent to approximately 0.2% BW corn and SH80 was equivalent to 0.4% BW. Though the NDFd was greatest for 0.4% versus the 0.21% reported by Grigsby et al. (1993), it was within the 0.5% BW corn maximum reported by Anderson et al. (1988). The decrease in fiber digestibility at levels greater than 0.4% was likely due to pH based on an accompanying continuous culture study using the same SH-corn proportions (Russell et al., 2013b) wherein pH responded quadratically to corn inclusion and was greatest for SH80. Evaluating the effects of forage inclusion in SH diets, Trater et al. (2001) also postulated that SH digestibility improvement was due to a pH increase. The numerical increase in Nd for SH80 was congruent with the quadratic NDFd and ADFd responses, an indication ammonia-consuming fibrolytic microbes were less suppressed by low pH than in greater starch inclusion diets.

Feeding 0.4% BW corn resulted in positive associative effects on NDF digestibility and improved ADG versus diets containing less corn. Despite the positive associative effects of 0.4% BW corn supplementation, greater corn inclusion rates generated improved DMd, OMd, ADG and G:F. Fiber digestibility was optimum at 0.4% BW corn.

TABLES

Table 3.1 - Ingredient composition of diets (%, DM basis)

	Diets[1]				
Ingredient	SH20	SH60	SH80	SH90	SH100
Whole shell Corn	59	31.4	16.1	8.2	-
Soybean Hulls Pellets	14.9	47.1	64.7	73.8	82.3
Amino Plus[2]	14.1	9.9	7.8	6.7	6.5
Fescue hay	10	10	10	10	10
Limestone	1.14	0.67	0.38	0.23	0.1
Choice White Grease[3]	0.32	0.23	0.18	0.16	0.15
Dry Supplement[4]	0.27	0.27	0.27	0.27	0.27
Sodium Chloride	0.18	0.15	0.15	0.15	0.14
AliMet[5]	0.06	0.08	0.09	0.1	0.1
Dicalcium Phosphate	-	0.16	0.31	0.39	0.45

[1] Diets: SH20 = 20% soybean hulls with 80% corn; SH60 = 60% soybean hulls with 40% corn; SH80 = 80% soybean hulls with 20% corn; SH90 = 90% soybean hulls with 10% corn; SH100 = 100% soybean hulls with no corn

[2] Amino Plus: Ag Processing Inc., Omaha, NE

[3] Added 18.2 kg choice white grease per 910 kg of pelleted supplement to improve pellet quality

[4] Composition: 40.7% Beef Vitamin Premix (3,006,302 IU/kg Vitamin A; 751,575 IU/kg Vitamin D; 75,157 IU/kg Vitamin E), 27.1% Beef Trace Mineral Premix (10,000 ppm Copper; 25,000 ppm Iron; 20,000 ppm Manganese; 30,000 Zinc; 100 ppm Cobalt; 100 ppm Selenium; 500 ppm Iodine), 26.7% Titanium dioxide (digestibility marker included during final 14d) and 5.4% Rumensin 90 (200g/kg Monensin; Elanco Animal Health, Indianapolis, IN)

[5] Alimet: 88% Methionine; Novus International, St. Charles, MO

Table 3.2 - Analytical composition of diets (%, DM basis)

	Diets[1]				
Item	SH20	SH60	SH80	SH90	SH100
DM, as-fed basis	87.7	88.3	88.1	88.4	88.5
CP	15.7	15	14.6	14.8	15
ME, Mcal/kg	2.95	2.86	2.82	2.79	2.77
NDF	27.1	45.2	54.9	60.6	62.8
ADF	15.2	30.5	37.7	42.4	45

[1] Diets: SH20 = 20% soybean hulls with 80% corn; SH60 = 60% soybean hulls with 40% corn; SH80 = 80% soybean hulls with 20% corn; SH90 = 90% soybean hulls with 10% corn; SH100 = 100% soybean hulls with no corn

Table 3.3 – Bodyweight, growth and digestibility in cattle consuming diets with increasing soybean hull to corn proportions

	Diets[1]						*P*-Values			
Item[2]	SH20	SH60	SH80	SH90	SH100	SEM	Model	Linear	Quad	Cubic
IBW, kg	362	358	359	360	361	6.5	1	1	0.7	0.8
FBW, kg	477	451	458	445	445	7.4	<0.01	<0.01	0.3	0.4
ADG, kg/d	1.63	1.32	1.41	1.2	1.2	0.07	<0.01	<0.01	0.2	0.4
DMI, kg	10.6	10.1	11.3	10.2	10.4	0.3	0.04	0.8	0.5	0.7
G:F	0.15	0.13	0.13	0.12	0.12	0.006	<0.01	<0.01	0.1	0.4
Digestibility										
DM, %	70.5	62.5	67.5	60.5	59.8	4.2	0.2	0.05	0.9	0.6
OM, %	73.2	65.6	69.8	63.8	62.5	4	0.2	0.04	0.9	0.5
NDF, %	55	60.9	68.3	65.2	59.5	4.7	0.2	0.3	0.04	0.8
ADF, %	52.8	60.3	65.9	62	56.0	5.1	0.3	0.6	0.04	1
Nitrogen, %	68.2	59.2	63.7	58.8	59.7	4	0.3	0.1	0.5	0.5

[1] Diets: SH20 = 20% soybean hulls with 80% corn; SH60 = 60% soybean hulls with 40% corn; SH80 = 80% soybean hulls with 20% corn; SH90 = 90% soybean hulls with 10% corn; SH100 = 100% soybean hulls with no corn

[2] IBW = initial BW; FBW = final BW

CHAPTER 4

Effect of enzyme inclusion rate on in vitro digestibility of soybean hulls and fescue hay.

ABSTRACT

Soybean hulls (SH) and fescue hay were used to evaluate the effect of a commercial dried fermentation product (ENZ) on *in vitro* NDF and ADF digestibility (NDFd; ADFd). The hypothesis was NDFd and ADFd would increase due to ENZ inclusion. The ENZ was a proprietary *B. subtilis, A. oryzae* and *T. viride* mix included with the feedstuff at 4 inclusion rates (0, 0.025, 0.045, 0.09%). Four digestion jars were used in each block with 1 ENZ rate per jar. Twelve hay replicates and 12 SH replicates were evaluated for each ENZ inclusion rate and three blocks were performed, resulting in 36 replicate samples per feedstuff per ENZ rate. Samples were incubated for 48 hours in a mixture of buffer and strained rumen fluid. Following incubation, samples were analyzed for indigestible NDF and ADF. The ENZ rate had no effect (P>0.4) on NDFd and ADFd. Feed and feed x rate had effects (P<0.01) on NDFd and ADFd. Hay NDFd and ADFd were greater (P<0.02) for 0.09 compared to 0.025 and 0.045% ENZ inclusion. The hay with 0 and 0.09% ENZ did not differ (P>0.5) for NDFd and ADFd. Hay NDFd tended to be greater (P=0.06) for 0 versus 0.025% ENZ. There were no differences (P<0.05) in SH NDFd and ADFd though 0.045% ENZ tended to be greater than 0.025% ENZ for NDFd (P=0.14) and ADFd (P=0.09). There was no difference in SH or hay fiber digestibility due to ENZ inclusion compared to untreated feedstuffs.

Keywords: digestibility, enzyme, fiber

INTRODUCTION

Incorporating fibrous byproducts like soybean hulls (SH) in corn-based cattle diets can reduce feed cost. Growth performance in cattle fed corn-based diets decreased as SH inclusion increases (Ludden et al., 1995) and fiber digestibility was compromised in animals consuming corn at rates exceeding 0.2 to 0.4% BW (Pordomingo et al., 1991; Grigsby et al., 1993; Russell et al., 2013a). Adding exogenous enzymes has shown promise in fiber-based diets by increasing NDF and ADF digestibility (NDFd; ADFd) in steers (Lewis et al., 1996). Similarly, Feng et al. (1996) reported greater *in situ* NDF disappearance rate as well as improved total tract DM digestibility (DMd) and NDFd for enzyme-treated grass hay compared to untreated hay despite no change in ruminal pH in cannulated steers. The current study utilized dried fibrolytic fermentation products derived from *Aspergillus oryzae*, *Bacillus subtilis* and *Trichoderma viride* combined in a commercial proprietary blend (Cattlemace, R&D Life Sciences LLC, Menomonie, WI). Enzyme activity differs between fibrolytic species and strains (Beauchemin et al., 2003) thus *in vitro* evaluation helps compare inclusion rates and digestibility effects on specific substrates. Soybean hulls and fescue hay were used to evaluate the effect of a commercial dried fermentation product (ENZ) on *in vitro* NDFd and ADFd. The hypothesis was NDFd and ADFd would increase due to ENZ inclusion.

MATERIALS AND METHODS

Experimental animal use was approved by the University of Missouri Animal Care and Use Committee. Soybean hulls (SH) and fescue hay (HAY) were ground through a 2mm screen in a Wiley Mill (Arthur H. Thomas Company, Philadelphia, PA). The feedstuffs were combined (Table 4.1) with a commercial dried fermentation product (ENZ; Cattlemace, R&D Life Sciences LLC, Menomonie, WI) at four ENZ inclusion rates (0, 0.025, 0.045 and 0.09 % as-fed). The ENZ was a proprietary mix of *Aspergillus oryzae*, *Bacillus subtilis* and *Trichoderma viride* fermentation products. Prior to substrate addition, ENZ was ground through a 1mm screen in a Cyclotec Sample Mill (FOSS, Hillerod, Denmark) to increase surface area and improve distribution in the feedstuffs. The 8 feedstuff/ENZ combinations (TRT; 2 feedstuffs x 4 ENZ rates) were weighed into ANKOM F57 filter bags (0.25 grams/bag; ANKOM Technology, Macedon, NY) that had been rinsed in acetone for 5 minutes and dried prior to weighing. Twelve bags were weighed and heat sealed for each TRT. Four digestion jars were utilized and each was randomly assigned to one of the ENZ rates. The 12 SH bags and 12 hay bags for each ENZ inclusion rate were combined and placed in a digestion jar along with an empty, heat-sealed filter bag as a control. Rumen fluid was collected from a lactating, rumen-fistulated Holstein cow housed at the University of Missouri-Columbia Foremost Dairy Research Center and was strained through 2 layers of cheesecloth. Two buffers were prepared: Buffer A (10g KH_2PO_4, 0.5g $MgSO_47H_2O$, 0.5g NaCl, 0.1 $CaCl_22H_2O$, 0.5g urea / L distilled water) and Buffer B (15g Na_2CO_3, 1g Na_2S9H_2O / L distiller water). Both buffers were heated to 39°C. While receiving a continuous carbon dioxide flow to maintain an anaerobic environment, 400mL of strained rumen fluid, 1320mL of Buffer A and 280mL of Buffer B were combined for each digestion jar. The sealed jars were placed in an incubator (DAISY II Incubator, ANKOM Technology, Macedon, NY) set to 39°C. During the 48 hour incubation, the jars were continuously rotated horizontally. After the incubation period, the bags were removed and rinsed under cold tap water until the water ran clear. The bags were then analyzed for NDF and ADF (ANKOM NDF and ADF methods, ANKOM Technology, Macedon, NY). An undigested sample of each TRT was analyzed for NDF, ADF, DM and Ash. The DM was calculated by drying two 1g sample replicates at 105°C for 24 hours. Organic matter was calculated by incinerating the DM samples at 500°C for 12 hours and determining ash content. The entire experiment was performed three times to generate 36 replicates per TRT.

Data were analyzed as a split plot design using PROC MIX procedure of SAS to separate TRT effects at $P<0.05$ (SAS 9.2, SAS Institute, Cary, NC). Block and block x ENZ were entered as random effects.

RESULTS AND DISCUSSION

Feedstuff and feedstuff x ENZ rate had effects ($P<0.01$) on NDFd (Table 4.2). The ENZ inclusion rate had no effect ($P>0.4$) on NDFd and ADFd. Hay NDFd and ADFd were greater ($P<0.02$) for 0.09 compared to 0.045% ENZ inclusion and tended to be greater ($P=0.12$) for 0.09 versus 0.025% ENZ. The hay with 0 and 0.09% ENZ did not differ ($P>0.5$) for NDFd and ADFd. Hay NDFd tended to be greater ($P=0.06$) for 0 versus 0.025% ENZ. Feng et al. (1996) reported improved DM and NDF disappearance with high enzyme inclusion versus untreated grass however low enzyme inclusion had no effect. Feng et al. (1996) also reported greater *in situ* NDF disappearance rate, total tract DMd and total tract NDFd for enzyme-treated grass hay than the untreated control. Insufficient ENZ inclusion or enzyme activity variation may have caused differences in the current study compared to previous grass and enzyme combinations. Pinos-Rodríguez et al. (2002) compared alfalfa and ryegrass hay digestibilities with fibrolytic enzyme application in cannulated lambs and reported an increase in enzyme-treated alfalfa NDFd but not in ryegrass, indicating enzyme preparations were more effective on higher quality forages.

There were no differences ($P<0.05$) in SH NDFd and ADFd. The 0.045% ENZ tended to be greater than 0.025% ENZ for NDFd ($P=0.14$) and ADFd ($P=0.09$). The 0.045% ENZ was numerically greater than 0% ENZ by 1.1% NDFd and 1.6% ADFd. The 0.045% ENZ was numerically greater than 0.09% ENZ by 0.9% NDFd and 1.2% ADFd. An advantage for lower exogenous enzyme inclusion over higher inclusion has been observed *in vivo* as Beauchemin et al. (1995) reported greater ADFd in steers fed alfalfa hay with lower enzyme inclusion rates versus higher rates. Milk production records in lactating Holstein cows consuming diets with enzyme-treated forage followed a quadratic pattern and were greater for a medium enzyme application rate than a high application rate (Lewis et al., 1999).

There was no improvement in SH or hay fiber digestibility due to ENZ inclusion compared to untreated feedstuffs. Current results agreed with previous work as enzyme activity was limited in low quality forage. Due to enzymatic activity variation between fibrolytic species and strains (Beauchemin et al., 2003), further ENZ analysis would be beneficial to determine effects on fermentation characteristics and animal performance.

TABLES

Table 4.1 - Enzyme inclusion with soybean hulls or grass hay

	Enzyme with soybean hulls				Enzyme with grass hay			
Item	0	0.025	0.045	0.09	0	0.025	0.045	0.09
Enzyme[1], % as-fed	-	0.025	0.045	0.09	-	0.025	0.045	0.09
SH, % as-fed	100	99.975	99.955	99.91	-	-	-	-
Grass hay, % as-fed	-	-	-	-	100	99.975	99.955	99.91
Enzyme, g	-	0.01	0.018	0.036	-	0.01	0.018	0.036
Soybean hulls, g	40	39.99	39.982	39.964	-	-	-	-
Grass hay, g	-	-	-	-	40	39.99	39.982	39.964

[1] Enzyme: dry fermentation product, *B. subtilis, A. oryzae* and *T. viride*, Cattlemace, R&D Life Sciences, Menomonie, WI

Table 4.2 – Digestibility of soybean hulls or grass hay with different enzyme inclusion rates (%, DM basis)

	Enzyme[1] with soybean hulls (%, as-fed)				Enzyme with hay (%, as-fed)					*P*-values		
Digestibility	0	0.025	0.045	0.09	0	0.025	0.045	0.09	SEM	Feed	Rate[2]	FxR[3]
NDF	88.8^{a}	88.2^{a}	89.9^{a}	89^{a}	44.8^{bc}	43.9^{bc}	43.4^{c}	45.7^{b}	1	<0.01	0.7	<0.01
ADF	88.6^{a}	88.1^{a}	90.2^{a}	89^{a}	45.9^{bc}	43.5^{cd}	43.4^{d}	46.6^{b}	1.4	<0.01	0.4	<0.01

[1] Enzyme: dry fermentation product, *B. subtilis, A. oryzae* and *T. viride*, Cattlemace, R&D Life Sciences, Menomonie, WI

[2] Enzyme inclusion rate

[3] Feed x Rate interaction

[a,b,c,d] Within row, least squares means without common superscript differ ($P<0.05$)

CHAPTER 5

Effect of soybean hull and enzyme inclusion on corn-based diet digestibility

ABSTRACT

A continuous culture study was conducted to determine the effects of increasing fiber inclusion and enzyme addition on digestibility, microbial efficiency (MoEff), VFA and lactic acid (LAC) production. The hypothesis was fiber digestibility, acetate (ACE) and acetate:propionate (A:P) would increase while propionate (PRO) would decrease with the inclusion of soybean hulls (SH) and a commercial dried fibrolytic fermentation product. Eight treatments (TRT) were arranged in a 4x2 factorial in a complete randomized design comprised of 4 diets (DIET) with increasing SH to corn proportions (0, 7, 14 and 28% SH) and 2 inclusion rates (ENZ) of a proprietary mix of *B. subtilis, A. oryzae* and *T. viride*; 0.045% (E45) or 0% (E0). The TRT were randomly distributed over 24 fermenters and two replications were performed (n=48). Three fermenter and effluent sample collection days followed 4 acclimation days. VFA samples were collected at 0, 4 and 8 hours post-feeding on sample days and compiled by timepoint. There were no interactions (*P*>0.05) with the exception of a DIETxENZ interaction (*P*<0.05) for LAC. The NDF digestibility (NDFd), ACE and A:P increased linearly (*P*<0.05) as SH inclusion increased but PRO decreased linearly (*P*<0.05). Nitrogen digestibility was quadratic (*P*<0.05) and greatest for 7% SH. MoEff and LAC responded quadratically (*P*<0.05) with the greatest values generated by 0% SH and the lowest by 14% SH. The butyrate (BUT) was also linear and quadratic (*P*<0.05) though BUT was greatest for 14% SH and lowest for 0%. The ACE and A:P were greater ($P<0.05$) for E45 but PRO was greater ($P<0.05$) for E0. The ACE, LAC and PRO were all quadratic ($P<0.05$). Acetate and LAC were greatest at hour 4 and PRO was greatest at hour 8. Both BUT and A:P were linear ($P<0.05$) as BUT increased over time while A:P decreased. The conclusion was fiber digestibility increased as SH inclusion increased in corn-based diets. Based on the increased A:P, enzyme addition improved fiber fermentation.

Keywords: digestibility, fermentation, soybean hulls

INTRODUCTION

Although soybean hull (SH) inclusion in concentrate diets can reduce feed cost, growth performance was reduced as inclusion increased (Ludden et al., 1995). Ferreira et al. (2011a) reported ground corn in high concentrate lamb diets could be replaced by up to 45% SH with no negative effect on ADG whereas Ludden et al. (1995) reported a maximum 20% SH inclusion rate in corn-based cattle diets to avoid negative effects on ADG. Both studies (Ferreira et al., 2011a; Ludden et al., 1995) agreed G:F was negatively affected by increasing SH inclusion. The decreasing performance as SH inclusion increased may be attributed to decreasing DM digestibility (DMd) and OM digestibility (OMd). The same trend was reported by Ferreira et al. (2011b) in a lamb study with increasing acetate (ACE), decreasing propionate (PRO), increasing ACE:PRO (A:P) and increasing NDF digestibility (NDFd) as SH inclusion increased. Methods for improving digestibility in corn-based diets containing SH could increase digestible energy and improve G:F. Beauchemin et al. (1997) supplemented high concentrate diets with fibrolytic enzymes and reported no growth performance effects in corn-fed steers but reported a G:F improvement in barley-fed steers. When fiber substrate was abundant, fibrolytic enzymes had improved digestibility in steers (Feng et al., 1996). The objective of the current study was to determine the effects of increasing fiber inclusion and enzyme addition on digestibility, microbial efficiency (MoEff), VFA and lactic acid (LAC) production. The hypothesis was fiber digestibility, ACE and A:P would increase while PRO would decrease with the inclusion of SH and a commercial dried fibrolytic fermentation product.

MATERIALS AND METHODS

Experimental animal use was approved by the University of Missouri Animal Care and Use Committee. Twenty-four single-flow fermenters were used in this continuous culture system and two replicates were performed for a total of 48 experimental units. The fermenters consisted of a 2L polycarbonate jar (Nalgene, Rochester, NY) with a continuous volume of approximately 1460mL. Buffer (McDougall, 1948) was delivered to fermenters at a rate of 6% per hour^{-1} via a peristaltic pump (Masterflex model 7520-10, Cole Parmer Instrument Co., Chicago, IL). Anaerobic conditions were maintained with a continuous influx of carbon dioxide. The fermenter contents were stirred continuously with magnetic stir plates and kept at 39°C by a thermostatically-controlled water heater (model 730, Fisher Scientific, Pittsburgh, PA) in a water bath.

Four corn-based diets were compared. Proportionately, the diets contained 0 (SH0), 7 (SH7), 14 (SH14) and 28 (SH28) % SH with the remaining proportion corn (Tables 6.1, 6.2). Each diet was replicated with the addition of a commercial dried fermentation product (ENZ; Cattlemace, R&D Life Sciences LLC, Menomonie, WI) for a total of eight treatments. The ENZ was a mix of *Bacillus subtilis, Aspergillus oryzae* and *Trichoderma viride* included at 0.045% of the diet (E45) or 0% (E0). The eight treatments were randomly distributed across the 24 fermenters for six experimental units per treatment during each rep. Diets were formulated (Table 5.1) to meet RDP and RDN requirements (Russell et al., 1992) based on estimated MoEff and nutrient dilution rate calculations (Meng et al., 1999). A 273kg animal model was used to calculate amino acid requirements and determine mineral inclusion rates based on NRC recommended levels (NRC, 2000). Ingredients were ground through a 2mm screen in a Wiley Mill (Arthur H. Thomas Company, Philadelphia, PA) prior to mixing. Rumen fluid was collected from two lactating, rumen-fistulated Holstein cows housed at the University of Missouri-Columbia Foremost Dairy Research Center. The rumen fluid was strained through two layers of cheesecloth at which point the fluid from both cows were combined and mixed with buffer (McDougall, 1948) at 3:1 (buffer:rumen fluid) before being introduced to the fermenters. Each fermenter was fed 50g per day divided into two feedings at 0900 and 2100 hours. Following four days for adaptation, the fermenters were sampled for three days.

Ten-mL samples were collected from each fermenter at 0800, 1200, and 1600 hours during the three sampling days and fermenter pH was measured at these times. Fermenter pH was measured using a glass bulb pH meter that was recalibrated prior to each measurement period. The 10-mL fermenter samples were combined by sampling time (30mL per fermenter per timepoint) and stored at 4°C until sampling days were completed. Each fermenter's effluent outflow was individually collected in a plastic graduated cylinder. The graduated cylinders were surrounded with ice to inhibit further microbial activity. During the three sample days, individual daily effluent outflows were recorded, shaken to encourage even distribution of contents, and 1L subsamples were collected at 0800 hours. The subsamples from three days were combined for each fermenter and stored at 4°C until analysis. At the conclusion of the third sample day, the contents of each fermenter were blended (model 34BL22, Waring, New Hartford, CT) for 30 seconds to improve microbe separation from feed particles. The blender-agitated contents were strained through 2 layers of cheesecloth to remove large feed particles, and stored at 4°C. Strained fermenter contents were centrifuged at 1,000 x *g* for 5 minutes at 4°C to further remove remaining feed residue. The resulting supernatant was centrifuged at 27,000 x *g* for 30 minutes. Following supernatant removal, the final pellets from each fermenter were transferred to a plastic cup using distilled water to capture the entire pellet. The cups were then lyophilized at 10°C (Genesis, Virtis, Gardiner, NY) and the dry bacteria were ground with a mortar and pestle. The 3-day effluent composite from each fermenter was agitated to ensure even distribution and a 500mL subsample was removed for lyophilization at 10°C (Genesis, Virtis, Gardiner, NY). The dry effluent residue was ground using a mortar and pestle. Fermenter bacteria, effluent residue, and treatment feed samples were analyzed for DM, OM and total N. The DM was calculated by drying two 1g sample replicates at 105°C for 24 hours. Organic matter was calculated by incinerating the DM samples at 500°C for 12 hours and determining ash content. Total

N was determined by combustion (LECO FB-428, LECO Corporation, St. Joseph, MI). Effluent residue and feed samples were analyzed for NDF and ADF (ANKOM method, ANKOM Technology, Macedon, NY). Fermenter and effluent residue samples were analyzed for purine content (Zinn, 1986) and the values were used to calculate microbial N (effluent purine x fermenter N / fermenter purine). Microbial efficiency was calculated using microbial N and OM values (g microbial N / kg OM digested). VFA and lactic acid concentrations were analyzed using gas chromatography (Salanitro, 1975; Model 3400, Varian, Palo Alto, CA). A phenol-hypochlorite assay was performed (Broderick, 1980) to determine ammonia (DU-65 spectrometer, Beckman, Palo Alto, CA).

PROC MIXED procedure of SAS was used to separate TRT effects at $P<0.05$ (SAS 9.2, SAS Institute, Cary, NC) for the VFA, lactic acid, and ammonia values due to the multiple time points. PROC GLM procedure of SAS was used to separate TRT effects at $P<0.05$ (SAS 9.2, SAS Institute, Cary, NC) for measures with a single value per fermenter (experimental unit). Rep was included in the model. Main effects were analyzed due to limited DIET x ENZ interactions.

RESULTS AND DISCUSSION

There were no DIETxENZ interactions ($P>0.05$) with the exception of LAC ($P=0.01$) and pH ($P=0.02$). There was no DMd or OMd effect ($P>0.17$) due to increasing SH inclusion despite numerical decreases as SH inclusion increased (Table 5.3). This agreed with results reported by Ferreira et al. (2011b) in which DMd and OMd did not differ but tended to decrease as SH inclusion increased in corn-based diets. Similarly, Ludden et al. (1995) reported a linear decrease in DMd as SH increased in corn-based diets. Neutral detergent fiber digestibility was quadratic (*P*=0.003) and greatest for SH28. The NDFd increased quadratically as SH inclusion increased thus NDFd may reach a maximum digestibility at a SH inclusion above 28%. Unlike NDFd, there was no ADFd effect (*P*>0.181) due to increasing SH inclusion. Both Ferreira et al. (2011b) and Ludden et al. (1995) reported tendencies for linearly increasing NDFd as SH increased and Ferreira et al. (2011b) reported no response in ADFd.

Nitrogen digestibility responded quadratically (*P*=0.04) to SH inclusion and was greatest for SH7 (Table 5.3). Microbial efficiency responded cubically (*P*<0.04) and was lowest for SH14. Accordingly, the ammonia concentrations were quadratic (*P*<0.0001) and lowest for SH14. Ammonia and MoEff responded inversely, reaffirming that increased N consumption for microbial growth decreases ammonia concentrations in the rumen. As SH inclusion increased, ACE increased linearly (*P*<0.0001), PRO decreased linearly (*P*=0.0007) and A:P increased quadratically (*P*<0.032; Table 5.4). The ACE, PRO and A:P responses were congruent with expectations based on NDFd response because greater dietary NDF composition and NDFd were associated with greater ACE production (Ferreira et al., 2011b) whereas greater PRO was associated with greater starch levels such as SH0. Similar results were reported by Ferreira et al. (2011b) in which ACE and A:P increased linearly due to increasing SH inclusion. Butyrate was quadratic (*P*=0.04) and greatest for SH14. The A:P was also quadratic (*P*=0.03) though numerically greatest for SH28 and similar results have been observed in previous studies (Ferreira et al., 2011b). Fermenter pH responded quadratically (*P*=0.02) and was greatest for SH14 though all means exceeded the pH 6.2 considered a minimum for avoiding fiber digestion inhibition (Trater et al., 2001). Lactic acid responded linearly and quadratically (*P*<0.0002) and was lowest for SH14. Understandably, the lowest pH values align with the greatest LAC as increased LAC was commonly associated with decreased rumen pH (Wilson et al., 1975).

There were no effects (*P*>0.2) of ENZ on calculated digestibility measures (Table 5.5) nor was there an effect (*P*>0.3) of ENZ on fermenter pH though there were differences in VFA production (Table 5.6). Acetate was greater (*P*=0.02) for E45 than E0 whereas PRO was lower (*P*=0.025) for E45 than E0. The increase in ACE and decrease in PRO produced a greater (*P*<0.0001) A:P for E45 than E0. Though there was no NDFd difference between E45 and E0, the increased ACE and decreased PRO was characteristic of a shift toward increased fiber fermentation (Demeyer, 1981). Unlike the current study, Giraldo et al. (2008a) reported decreased ACE and increased PRO due to enzyme addition in a predominantly fibrous diet yet reported a similarity in increased A:P but added that variation between enzyme investigations was often due to substrate and enzyme activity variation. There was no ENZ effect (*P*>0.26) on ammonia, LAC or BUT concentrations. Giraldo et al. (2008a) was not different, reporting no enzyme effect on ammonia or BUT.

Fermenter samples were collected and fermenter pH was measured prior to the 0800 feeding (hour 0) and again 4 and 8 hours following the morning feeding. Acetate, PRO, BUT and LAC concentrations were not different (*P*>0.05) for hours 4 and 8, and were lowest (*P*<0.01) for hour 0 (Table 5.7). Acetate:propionate was greatest (*P*=0.02) for hour 0 and hours 4 and 8 did not differ (*P*>0.05). Reports from *in vivo* VFA determination in lambs agreed as ACE was lowest prior to feeding, however PRO and

A:P were quadratic reaching the lowest ratio at hour 4 in that study (Ferreira et al., 2011b). The explanation for the difference in responses was unclear and was likely due to differences in enzyme activity and the inherent differences between *in vivo* and *in vitro* work. Ammonia was greatest (P<0.01) for hour 4 and lowest (P<0.01) for hour 8. Due to the greater VFA and LAC concentrations in hours 4 and 8, the pH was lowest (P<0.01) for hours 4 and 8. Hour 0 was greatest (P<0.01) for pH.

Including SH in diets containing more than 66% corn tends to decrease DMd and OMd despite increases in NDFd. The ENZ inclusion shifted VFA concentrations indicating greater fiber fermentation despite no measured effect on fiber digestibility. Due to the challenges associated with comparing *in vivo* and *in vitro* investigations, *in vivo* comparisons utilizing the current diets and ENZ may clarify effects. The conclusion was SH and 0.045% ENZ inclusion increased fiber digestibility in corn-based diets.

TABLES

Table 5.1 - Ingredient composition of diets (%, DM basis)

	E0 diets[1]				E45 diets[2]			
Ingredient	SH0	SH7	SH14	SH28	SH0e	SH7e	SH14e	SH28e
Corn	89.7	84.1	78.5	66.6	89.655	84.055	78.455	66.555
Soybean Hulls	-	6.3	12.8	25.9	-	6.3	12.8	25.9
Amino Plus[3]	7.33	6.81	6.2	5.43	7.33	6.81	6.2	5.43
Limestone	1.4	1.4	1.3	1.05	1.4	1.4	1.3	1.05
Urea	0.94	0.86	0.77	0.59	0.94	0.86	0.77	0.59
Supplement[4]	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Potassium chloride	0.3	0.2	0.1	-	0.3	0.2	0.1	-
Dicalcium phosphate	-	-	-	0.1	-	-	-	0.1
Enzyme	-	-	-	-	0.045	0.045	0.045	0.045

[1] Diets: S0 = no soybean hulls; S7 = 7% of corn replaced with soybean hulls; S14 = 14% of corn replaced with soybean hulls; S28 = 28% of corn replaced with soybean hulls

[2] E45 diets: 0.045% dry fermentation product included, *B. subtilis, A. oryzae* and *T. viride*, Cattlemace, R&D Life Sciences, Menomonie, WI

[3] Amino Plus: Ag Processing Inc., Omaha, NE

[4] Composition: 60.6% Sodium chloride, 24.2% Beef vitamin premix (3,006,302 IU/kg Vitamin A; 751,575 IU/kg Vitamin D; 75,157 IU/kg Vitamin E), 15.2% Beef trace mineral premix (10,000 ppm Copper; 25,000 ppm Iron; 20,000 ppm Manganese; 30,000 Zinc; 100 ppm Cobalt; 100 ppm Selenium; 500 ppm Iodine)

Table 5.2 - Analytical composition of diets (%, DM basis)

	E0 diets[1]				E45 diets[2]			
Item	SH0	SH7	SH14	SH28	SH0e	SH7e	SH14e	SH28e
DM, as-fed basis	90.1	89.9	90.3	90.8	90.2	89.9	90.0	90.2
OM	95.9	95.5	95.9	95.3	96.2	96.0	95.6	95.4
CP	14.5	15.4	14.5	14.4	14.7	14.9	15.8	14.9
NDF	13.3	17.0	19.8	26.7	13.7	17.2	18.5	27.7
ADF	3.1	6.2	8.6	14.1	3.4	5.8	8.3	15.2

[1] Diets: S0 = no soybean hulls; S7 = 7% of corn replaced with soybean hulls; S14 = 14% of corn replaced with soybean hulls; S28 = 28% of corn replaced with soybean hulls

[2] E45 diets: 0.045% dry fermentation product included, *B. subtilis, A. oryzae* and *T. viride*, Cattlemace, R&D Life Sciences, Menomonie, WI

Table 5.3 – Digestibility and microbial efficiency due to increasing soybean hull to corn proportions in continuous culture fermenter diets

	Diets[1]					*P*-Values			
Item[2]	SH0	SH7	SH14	SH28	SEM	Model	Linear	Quadratic	Cubic
DMd, %	22.4	22.6	21	20.8	1	0.5	0.17	0.8	0.5
OMd, %	67.2	67.3	66.9	65.8	0.8	0.6	0.2	0.5	1
NDFd, %	30.5	48.8	58.4	60.3	2.7	<0.01	<0.01	<0.01	0.9
ADFd, %	79.7	84.6	81	81. 8	1.6	0.2	0.7	0.2	0.06
Nd, %	87.8	96.2	93.5	93	2.1	0.6	0.2	0.04	0.2
MoEff[3]	32.9	31.6	28.4	31	0.8	<0.01	0.02	0.02	0.04

[1] Diets: S0 = no soybean hulls; S7 = 7% of corn replaced with soybean hulls; S14 = 14% of corn replaced with soybean hulls; S28 = 28% of corn replaced with soybean hulls

[2] DMd = DM digestibility; OMd = OM digestibility; NDFd = NDF digestibility; ADFd = ADF digestibility; Nd = nitrogen digestibility; MoEff = Microbial efficiency

[3] Grams of bacterial nitrogen per kilogram OM digested

Table 5.4 – VFA concentrations, lactic acid concentration, ammonia concentration and pH due to increasing soybean hull to corn proportions in continuous culture fermenter diets

	Diets[1]					*P*-Values			
Item[2]	SH0	SH7	SH14	SH28	SEM	Model	Linear	Quadratic	Cubic
ACE, m*M*	51.5	57.2	59	61.9	1.1	<0.01	<0.01	0.2	0.3
PRO, m*M*	26.1	24.5	23.2	23	0.7	<0.01	<0.01	0.3	0.8
BUT, m*M*	13.9	15	18.7	17	0.7	<0.01	<0.01	0.04	0.01
LAC, m*M*	0.81	0.57	0.4	0.44	0.08	<0.01	<0.01	<0.01	0.4
A:P	2	2.4	2.6	2.7	0.06	<0.01	<0.01	0.03	0.3
NH_3, mg/dl	4.87	7.42	7.9	6.55	0.2	<0.01	<0.01	<0.01	0.8
pH	6.64	6.66	6.67	6.65	0.007	0.08	0.6	0.02	0.3

[1] Diets: S0 = no soybean hulls; S7 = 7% of corn replaced with soybean hulls; S14 = 14% of corn replaced with soybean hulls; S28 = 28% of corn replaced with soybean hulls

[2] ACE = acetate; PRO = propionate; BUT = butyrate; LAC = lactic acid; A:P = acetate:propionate; NH_3 = ammonia

Table 5.5 – Digestibility and microbial efficiency due to enzyme inclusion in continuous culture fermenters fed soybean hull and corn diets

	Enzyme[1]			
Item[2]	E0	E45	SEM	*P*-Value
DMd, %	78.8	77.8	0.8	0.3
OMd, %	66.7	66.9	0.6	0.8
NDFd, %	49.8	49.2	1.9	0.8
ADFd, %	82.7	80.8	1.1	0.2
Nd, %	92.6	92.7	1.5	1
MoEff[3]	31.2	30.8	0.6	0.6

[1] Enzyme: Dry fermentation product, *B. subtilis, A. oryzae* and *T. viride*, Cattlemace, R&D Life Sciences, Menomonie, WI; E45 = 0.045% enzyme included; E0 = no enzyme included

[2] DMd = DM digestibility; OMd = OM digestibility; NDFd = NDF digestibility; ADFd = ADF digestibility; Nd = nitrogen digestibility; MoEff = Microbial efficiency

[3] Grams of bacterial nitrogen per kilogram OM digested

Table 5.6 – VFA concentrations, lactic acid concentration, ammonia concentration and pH due to enzyme inclusion in continuous culture fermenters fed soybean hull and corn diets

	Enzyme[1]			
Item[2]	E0	E45	SEM	*P*-Value
ACE, m*M*	56.1	58.7	0.8	0.02
PRO, m*M*	25	23.4	0.5	0.03
BUT, m*M*	16.4	15.9	0.5	0.4
LAC, m*M*	0.58	0.53	0.08	0.3
A:P	2.31	2.58	0.04	<0.01
NH_3, mg/dl	6.65	6.72	0.14	0.8
pH	6.64	6.66	0.005	0.3

[1] Enzyme: Dry fermentation product, *B. subtilis, A. oryzae* and *T. viride*, Cattlemace, R&D Life Sciences, Menomonie, WI; E45 = 0.045% enzyme included; E0 = no enzyme included

[2] ACE = acetate; PRO = propionate; BUT = butyrate; LAC = lactic acid; A:P = acetate:propionate; NH_3 = ammonia

Table 5.7 – VFA concentrations, lactic acid concentration, ammonia concentration and pH at 0, 4 and 8 hours post-feeding in continuous culture fermenters fed soybean hull and corn diets

	Hours[1]				
Item[2]	0	4	8	SEM	*P*-Value
ACE, mM	52.2^{b}	59.4^{a}	60.5^{a}	0.9	<0.01
PRO, mM	20.8^{b}	25.5^{a}	26.3^{a}	0.5	<0.01
BUT, mM	14.8^{b}	16.6^{a}	17^{a}	0.4	<0.01
LAC, mM	0.18^{b}	0.8^{a}	0.72^{a}	0.05	<0.01
A:P	2.58^{a}	2.4^{b}	2.34^{b}	0.06	<0.01
NH_3, mg/dl	6.53^{b}	7.8^{a}	5.72^{c}	0.2	<0.01
pH	6.72^{a}	6.63^{b}	6.62^{b}	0.006	<0.01

[1] Hours post-feeding

[2] ACE = acetate; PRO = propionate; BUT = butyrate; LAC = lactic acid; A:P = acetate:propionate; NH_3 = ammonia

[a,b,c] Within row, least squares means without common superscript differ ($P<0.05$)

CHAPTER 6

Fiber and enzyme inclusion effects on diet digestibility and growth performance in beef calves consuming corn-based diets

ABSTRACT

A feedlot study was conducted to determine increasing fiber inclusion and enzyme addition effects on diet digestibility and animal performance. The hypothesis was fiber inclusion and enzyme addition would increase fiber digestibility with no negative effect on animal performance. Ten treatments (TRT) were arranged in a 5x2 factorial using five diets (DIET) and two enzyme inclusion rates (ENZ). Within DIET, four contained whole shell corn (WSC) with 0, 7, 14 or 28% SH replacing corn and the fifth contained WSC and 7% grass hay. The ENZ was a commercial proprietary mix of *B. subtilis, A. oryzae* and *T. viride* (Cattlemace, R&D Life Sciences, Menomonie, WI) included in diets at 0% (E0; S0, S7, S14, S28, H7) or 0.045% (E45; S0e, S7e, S14e, S28e, H7e). One-hundred steers (288 ± 31 kg BW) were stratified by weight and blocked into one heavy block and one light block per TRT (2 pen/TRT). Steers were fed for 70 days with titanium dioxide included in the final 14 days and fecal samples collected on day 70. Diets were balanced for post-ruminal AA and RDP based on available ME. Individual DMI was measured using a GrowSafe FI system. There was no difference (P>0.3) in initial BW, final BW or ADG. The DMI was cubic (P<0.01) due to DIET and was greatest for 7 and 28% SH. There was no difference (P>0.7) in DMI due to ENZ or DIETxENZ. Gain:feed was not different (P=0.2) due to ENZ and had a cubic response

(*P*<0.01) to DIET. There was a DIETxENZ interaction (*P*<0.01) for G:F in which S0, S0e, S14e and S28e were greatest (*P*<0.05). The S14e and S28e were greater (*P*<0.05) than S14 and S28, thus ENZ improved G:F in WSC diets with greater SH inclusion. There was no ENZ or DIET effect (*P*>0.2) on any digestibilities except ADF in which E0 was greater (*P*=0.03) than E45. The NDF digestibility tended to have a cubic response (*P*=0.1) to DIET and was greatest for 7 and 28%. The only DIETxENZ interaction for digestibilities was nitrogen (*P*=0.05). Fiber digestibility in WSC diets did not improve with SH inclusion or ENZ addition. Steers fed WSC diets containing 14-28% SH and 0.045% ENZ converted feed at the same rate as steers fed 100% WSC diets and had no decrease in ADG.

Keywords: digestibility, fermentation, soybean hulls

INTRODUCTION

Soybean hulls (SH) provide economic advantages as a dietary energy source but are not without drawbacks. As SH inclusion increases in corn-based diets, performance decreases (Ludden et al., 1995). Though ADG may remain the same, G:F was reduced by SH inclusion (Ferreira et al., 2011a). Ludden et al. (1995) reported decreases in ADG due to increasing SH as well, though ADG changes between 0 and 20% SH inclusion were minimal. Diet digestibility was typically the first consideration when determining reasons for reduced G:F as DM and OM digestibilities were reduced in corn diets with SH inclusion (Ferreira et al., 2011b). Including fibrolytic enzymes in high fiber steer diets has improved digestibility (Feng et al., 1996). Corn-based diets have not responded as favorably to fibrolytic enzyme addition though fibrous feed ingredient inclusion was limited to 4.9% (Beauchemin et al., 1997). A precursory study reported increasing SH inclusion and fibrolytic enzyme addition improved fiber fermentation in continuous culture, increasing NDF digestibility (NDFd), acetate production and acetate:propionate. The current feedlot study objective was to determine increasing fiber inclusion and enzyme addition effects on *in vivo* diet digestibility and animal performance. The hypothesis was fiber inclusion and enzyme addition would increase fiber digestibility in steers fed whole shell corn diets with no negative effect on growth performance.

MATERIALS AND METHODS

Experimental animal use was approved by the University of Missouri Animal Care and Use Committee. One-hundred crossbred steers (288 ± 31 kg BW) were fed treatment diets for 70 days following a 14-day receiving period. All calves received an electronic ID tag (Allflex US Inc., Dallas-Fort Worth Airport, TX) in the left ear to facilitate intake measurement using a GrowSafe FI system (GrowSafe Systems Ltd, Airdrie, AB, Canada) and a visual ID tag (Allflex US Inc., Dallas-Fort Worth Airport, TX) in the right ear. An injectable ivermectin (Noromectin, Norbrook Inc., USA, Lenexa, KS) was administered to control internal and external parasites. All steers received a vaccination and booster for IBR, BVD 1 & 2, PI3, BRSV & Mannheimia haemolytica (Pyramid 5 + Presponse SQ, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) as well as for clostridial infections (UltraChoice 8, Pfizer Animal Health, New York, NY).

Steers were stratified by weight and blocked into one heavy pen and one light pen per treatment (2 pens/treatment). Each pen (5m x 9.1m) had a concrete floor and access to shelter. *Ad libitum* water was provided at all times via automatic waterers (Ritchie Industries Inc., Conrad, IA). Each pen had one GrowSafe bunk that allowed one animal to eat at a time. Feed ingredients were blended in a truck-mounted mixer (Reel Auggie 3120, KUHN North America Inc., Brodhead, WI) and fed on a daily basis. Diets were mixed in a random order each day and pen feeding order per TRT was alternated each day. Initial BW (IBW) was measured for two consecutive days and calves were assigned to treatment pens following the second days weight (day 0). All steers were implanted (Revalor XS, Merck Animal Health, Summit, NJ) on day 0. Intermediate weights were measured on day 28 and day 55. Starting on day 55, diets contained 0.1% titanium dioxide to facilitate digestibility calculations. Final BW (FBW) was measured on day 69 and 70 of the study and fecal grab samples were collected. All weights were measured prior to the daily feeding.

Ten treatments (TRT) were arranged in a 5x2 factorial using five diets (DIET) and two enzyme inclusion rates (ENZ). Within DIET, four contained whole shell corn (WSC) with 0, 7, 14 or 28 % SH replacing corn and the fifth contained WSC and 7% grass hay. The ENZ was a commercial proprietary mix of *Bacillus subtilis, Aspergillus oryzae* and *Trichoderma viride* (Cattlemace, R&D Life Sciences, Menomonie, WI) included in diets at 0% (E0; S0, S7, S14, S28, H7) or 0.045% (E45; S0e, S7e, S14e, S28e, H7). Diets were formulated (Table 6.1, 6.2) based on estimated microbial efficiency (MoEff) as well as nutrient dilution rate calculations (Meng, 1999) to meet RDP and RDN requirements (Russell, 1992). Predicted gains were normalized across

TRT by meeting absorbable amino acid (AA) requirements for the lowest ME diet and adjusting greater ME diets for AA to meet but not exceed the lowest ME diet ADG. RDP, RDN, and AA requirements were met with urea, soybean meal, Amino Plus (Ag Processing Inc., Omaha, NE) and porcine bloodmeal. Mineral inclusion rates were formulated to meet NRC recommended levels for growing cattle (NRC, 2000). Rumensin 90 (200g/kg Monensin; Elanco Animal Health, Indianapolis, IN) was included in the diet to provide 27g monensin per 909 kg feed. Choice white grease was included in the pelleted supplement at 2% inclusion to improve pellet quality. All ingredients were pelleted except WSC, grass hay, and SH pellets, The WSC, grass hay, SH, and pelleted supplement were combined on farm in a truck-mounted mixer.

Feed samples were gathered on a weekly basis, ground through a 2mm screen in a Wiley Mill (Arthur H. Thomas Company, Philadelphia, PA) and analyzed for DM, OM, total N, NDF and ADF (Table 6.2). The DM was determined by drying 1g sample replicates at 105°C for 24 hours. Organic matter was determined by incinerating the DM samples at 500°C for 12 hours. Total N was determined by combustion (LECO FB-428, LECO Corporation, St. Joseph, MI). NDF and ADF were determined using the ANKOM fiber analysis method (ANKOM Technology, Macedon, NY). The fecal samples collected on day 70 were weighed and dried at 55°C. The dried samples were reweighed and ground through a 2mm screen. Fecal samples were analyzed for DM, OM, total N, NDF, ADF and titanium dioxide content. Fecal samples were prepared for titanium determination using methods outlined by Myers (2004) and were measured colorimetrically (DU-65 spectrometer, Beckman, Palo Alto, CA). Individual intake was used to determine individual titanium dioxide intake based on titanium inclusion in the feed.

PROC GLM procedure of SAS was used to separate TRT effects at $P<0.05$ (SAS 9.3, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

There was no difference ($P>0.7$) in IBW (Table 6.3). Neither ADG nor FBW differed ($P>0.3$) due to DIET, ENZ or DIETxENZ. Numerically, ADG increased as SH inclusion increased in E45 diets whereas there was no pattern in E0 diets however there was no significant interaction ($P=0.4$). Ferreira et al. (2011a) also reported no difference in ADG in lambs fed cracked corn diets with increasing SH inclusion. Though Ludden et al. (1995) reported a linear decrease in ADG as SH inclusion increased from 0 to 60%, the numerical difference in ADG between 0 and 20% SH was only 0.1 kg. Dry matter intake had a cubic response ($P<0.01$) to DIET and was greatest for S7 and S28 however there was no difference ($P>0.7$) in DMI due to ENZ or DIETxENZ interaction. Ferreira et al. (2011a) reported a linear increase in DMI due to SH whereas Ludden et al. (1995) reported linear and quadratic responses to SH inclusion. The varied results may have been due to differences in SH inclusion as Ludden et al. (1995) replaced cracked corn with SH at rates from 0 to 60% and Ferreira et al (2011a) included 0 to 45% SH in cracked corn diets.

Gain:feed was not different ($P=0.2$) due to ENZ and had a cubic response ($P<0.01$) to DIET. There was a DIETxENZ interaction ($P<0.01$) for G:F (Table 6.5). The

S0, S0e, S14e and S28e treatments were greatest and were not different (*P*<0.05). The S14e and S28e treatments were greater (*P*<0.05) than S14 and S28 for G:F. There was no difference (*P*>0.05) in G:F due to DIETxENZ for diets with lower SH or hay inclusion rates, ENZ only improved G:F in WSC diets with at least 14% SH proportion. Ludden et al. (1995) and Ferreira et al. (2011a) reported linear decreases in G:F in ruminants fed cracked corn diets with increasing SH inclusion. Based on DIET effects alone, the current study was greatest for G:F with no SH inclusion as well. The ENZ negated the SH effect on reducing G:F. Beauchemin et al. (1995) compared enzyme effects on performance in steers consuming alfalfa hay, timothy hay or barley silage and reported mixed effects on G:F as enzyme inclusion rate increased in the timothy hay diet but no effects in alfalfa and barley. However it was difficult to compare enzyme experiments because enzyme strains, activity and target substrates differ greatly. Because there was a DIETxENZ interaction but no ENZ effect on G:F, it was determined that SH inclusion must exceed 14% corn replacement in order to provide sufficient substrate for ENZ to be effective in corn-based diets balanced for RDN, RDP and AA.

There was no ENZ or DIET effect (*P*>0.2) on any measured digestibility values except ADF digestibility (ADFd). Despite the beneficial ENZ effect on G:F, ADFd for E0 was greater (*P*=0.03) than E45 (Table 6.4). This result disagreed with Giraldo et al. (2008a) in which fibrolytic enzyme inclusion had no effect on ADFd. Additionally, Feng et al. (1996) reported ADFd was greater or not different due to enzyme inclusion in steers fed grass hay diets. There was a tendency for a cubic response (*P*=0.1) in NDFd due to diet with the numerically greatest values for S7 and S28 (Table 6.3). The S7 diet was greatest for NDFd yet had the numerically poorest G:F. Ludden et al. (1995) reported a tendency in NDFd with a 4.2% numerical difference in NDFd between 0 and 20% SH whereas the current study had 12.5% numerical NDFd difference between S0 and S7. It was not clear why fiber digestibilities responded in this manner as a precursory ENZ study with the same SH:WSC proportions and ENZ inclusion rates reported a linear increase in NDFd due to SH inclusion and VFA production favoring increased fiber fermentation in E45 diets (Russell and Kerley, 2013). Nitrogen digestibility (Nd) had a DIETxENZ interaction (*P*=0.05; Table 6.5) but no ENZ or DIET response (*P*>0.5). The S14 diet was greater for Nd (*P*<0.05) than S0, H7 and S14e however the reason was unknown. Giraldo et al. (2008a) reported no difference in CP digestibility due to enzyme inclusion.

Fiber digestibility in WSC diets did not improve as SH inclusion increased. Including ENZ in the diet did not improve fiber digestibility in diets containing WSC and SH. Steers fed WSC diets containing 14-28% SH and 0.045% ENZ converted feed at the same rate as steers fed 100% WSC diets and had no decrease in ADG. The reason for the G:F improvement was unknown as no digestibility values favored 14 or 28% SH diets containing ENZ. Further work may help to elucidate the reason for the G:F improvement.

TABLES

Table 6.1 - Ingredient composition of diets (%, DM basis)

	E0 diets[1]					E45 diets[2]				
Ingredient	S0	S7	S14	S28	H7	S0e	S7e	S14e	S28e	H7e
Whole shell corn	89.02	83.99	78.38	66.63	80.42	88.97	83.94	78.33	66.58	80.42
Soybean hulls	-	6.37	12.88	25.99	-	-	6.37	12.88	25.99	-
Hay	-	-	-	-	6.07	-	-	-	-	6.07
Soybean meal	7.5	5.7	4.0	0.6	0.2	7.5	5.7	4.0	0.6	0.2
Limestone	1.5	1.4	1.3	1.1	1.4	1.5	1.4	1.3	1.1	1.4
Supplement[3]	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86
Urea	0.86	0.80	0.71	0.53	-	0.86	0.80	0.71	0.53	-
Potassium chloride	0.26	0.18	0.08	-	-	0.26	0.18	0.08	-	-
Amino Plus[4]	-	0.7	1.8	4.2	10.3	-	0.7	1.8	4.2	10.3
Dicalcium phosphate	-	-	-	0.1	-	-	-	-	0.1	-
Bloodmeal	-	-	-	-	0.70	-	-	-	-	0.70
Enzyme	-	-	-	-	0.045	0.045	0.045	0.045	0.045	0.045

[1] Diets: S0 = no soybean hulls; S7 = 7% of corn replaced with soybean hulls; S14 = 14% of corn replaced with soybean hulls; S28 = 28% of corn replaced with soybean hulls; H7 = 7% of corn replaced with hay;

[2] E45 diets: 0.045% dry fermentation product included, *B. subtilis, A. oryzae* and *T. viride*, Cattlemace, R&D Life Sciences, Menomonie, WI

[3] Composition: 46.46% Choice white grease (improved pellet quality), 19.74% Sodium chloride, 11.61% Titanium dioxide (digestibility marker), 11.61% Beef trace mineral premix (10,000 ppm Copper; 25,000 ppm Iron; 20,000 ppm Manganese; 30,000 Zinc; 100 ppm Cobalt; 100 ppm Selenium; 500 ppm Iodine), 8.59% Beef vitamin premix (3,006,302 IU/kg Vitamin A; 751,575 IU/kg Vitamin D; 75,157 IU/kg Vitamin E), 1.74% Rumensin 90 (200g/kg Monensin; Elanco Animal Health, Indianapolis, IN), 0.23% Zinc oxide.

[4] Amino Plus: Ag Processing Inc., Omaha, NE

Table 6.2 - Analytical composition of diets and hay (%, DM basis)

	E0 diets[1]					E45 diets[2]					
Item	S0	S7	S14	S28	H7	S0e	S7e	S14e	S28e	H7e	Hay
DM, as-fed basis	87.9	87.8	87.9	89.2	87.9	88.7	89.5	90.5	87.4	88.7	84.1
CP	14.1	13.6	13.5	13.0	12.4	14.5	14.0	13.8	13.1	12.3	6.6
ME, Mcal/kg	3.15	3.13	3.11	3.08	3.11	3.15	3.13	3.11	3.08	3.11	1.9
NDF	16.1	19.6	23.0	31.1	20.5	15.8	19.4	22.0	29.4	21.0	71.7
ADF	4.5	7.8	10.6	17.4	7.8	4.7	7.8	10.2	15.8	7.7	42.2

[1] Diets: S0 = no soybean hulls; S7 = 7% of corn replaced with soybean hulls; S14 = 14% of corn replaced with soybean hulls; S28 = 28% of corn replaced with soybean hulls; H7 = 7% of corn replaced with hay;

[2] E45 diets: 0.045% dry fermentation product included, *B. subtilis, A. oryzae* and *T. viride*, Cattlemace, R&D Life Sciences, Menomonie, WI

Table 6.3 - Bodyweight, growth and digestibility in steers consuming corn-based diets with increasing soybean hull replacement

	Diet[1]						P-values[2]			
Item[3]	S0	S7	S14	S28	H7	SEM	Model	Linear	Quadratic	Cubic
IBW, kg	288	283	289	289	289	7.7	1	0.8	0.8	0.7
FBW, kg	444	447	452	453	442	10.2	0.9	0.5	0.9	0.9
ADG, kg/d	2.27	2.37	2.36	2.38	2.21	0.07	0.4	0.3	0.6	0.7
DMI, kg/d	8.51	10.83	9.34	9.6	9.42	0.28	<0.01	0.2	<0.01	<0.01
G:F	0.27	0.22	0.25	0.25	0.24	0.006	<0.01	0.5	<0.01	<0.01
Digestibility										
DM, %	69	71.2	69.1	69.7	69.5	2.6	1	1	0.7	0.6
OM, %	70.7	72.4	70.6	71.3	71.5	2.6	1	1	0.8	0.6
NDF, %	41.5	54	47.8	52.1	45.6	4	0.2	0.2	0.3	0.1
ADF, %	41.6	46.9	40	42	37	4.5	0.4	0.8	0.7	0.2
Nitrogen, %	63.6	65.6	65	65.7	62.6	2.3	0.8	0.5	0.8	0.7

[1] Diets: S0 = no soybean hulls; S7 = 7% of corn replaced with soybean hulls; S14 = 14% of corn replaced with soybean hulls; S28 = 28% of corn replaced with soybean hulls; H7 = 7% of corn replaced with hay

[2] Linear, quadratic and cubic contrasts include S0, S7, S14 and S28

[3] IBW = initial BW; FBW = final BW

Table 6.4 - Bodyweight, growth and digestibility in steers consuming corn and soybean hull diets with enzyme addition

	Enzyme[1]			
Item[2]	E0	E45	SEM	*P*-value
IBW, kg	289	286	4.8	0.7
FBW, kg	446	449	6.3	0.8
ADG, kg/d	2.28	2.36	0.05	0.3
DMI, kg/d	9.6	9.5	0.2	0.9
G:F	0.24	0.25	0.004	0.2
Digestibility				
DM, %	70.2	69.2	1.6	0.6
OM, %	71.9	70.6	1.6	0.6
NDF, %	50.6	45.8	2.4	0.2
ADF, %	45.2	37.7	2.5	0.03
Nitrogen, %	64.3	64.7	1.4	0.8

[1] Enzyme: Dry fermentation product, *B. subtilis, A. oryzae* and *T. viride*, Cattlemace, R&D Life Sciences, Menomonie, WI; E45 = 0.045% enzyme included; E0 = no enzyme included

[2] IBW = initial BW; FBW = final BW

Table 6.5 - Bodyweight, growth and digestibility in steers consuming corn-based diets with increasing soybean hull replacement and enzyme addition

	E0 diets[1]					E45 diets[2]						
Item[3]	S0	S7	S14	S28	H7	S0e	S7e	S14e	S28e	H7e	SEM	*P*-value
IBW, kg	291	281	290	291	290	284	285	288	286	288	10.9	1.0
FBW, kg	445	445	450	445	447	444	449	453	461	437	14.4	0.9
ADG, kg/d	2.23	2.37	2.33	2.23	2.27	2.31	2.38	2.40	2.53	2.16	0.1	0.4
DMI, kg/d	8.2	11	9.4	9.8	9.4	8.8	10.7	9.3	9.4	9.4	0.4	0.7
G:F	0.27^{a}	0.22^{e}	0.25^{bcd}	0.23^{de}	0.24^{cde}	0.26^{abc}	0.22^{e}	0.26^{abc}	0.27^{ab}	0.23^{de}	0.009	<0.01
Digestibility												
DM, %	68.6	72.2	74.2	69.9	66.3	69.4	70.3	64.1	69.4	72.8	3.8	0.2
OM, %	70.7	73.6	75.5	71.4	68.3	70.6	71.2	65.6	71	74.6	3.8	0.2
NDF, %	49.2	56.3	53.6	52.7	41.2	33.8	51.7	41.9	51.5	50.0	5.8	0.2
ADF, %	41.6	51.3	47.5	48.9	37.1	41.6	42.5	32.4	35.1	36.9	6.7	0.5
Nitrogen, %	61.3^{b}	64.8^{ab}	70^{a}	66.1^{ab}	59.1^{b}	65.8^{ab}	66.3^{ab}	60^{b}	65.3^{ab}	66.2^{ab}	3.3	0.05

[1] Diets: S0 = no soybean hulls; S7 = 7% of corn replaced with soybean hulls; S14 = 14% of corn replaced with soybean hulls; S28 = 28% of corn replaced with soybean hulls; H7 = 7% of corn replaced with hay

[2] E45 diets: 0.045% dry fermentation product included, *B. subtilis, A. oryzae* and *T. viride*, Cattlemace, R&D Life Sciences, Menomonie, WI

[3] IBW = initial BW; FBW = final BW

[a,b,c,d,e] Least squares means without common superscript differ ($P<0.05$)

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