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I am submitting herewith a dissertation written by Howard Martin Blalock entitled "Supplementation of Purified Fiber to Tall Fescue Grass Hay Diets." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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**SUPPLEMENTATION OF PURIFIED FIBER TO TALL FESCUE GRASS HAY
DIETS**

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Howard Martin Blalock
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DEDICATION

This dissertation is dedicated to my wife, Laura Blalock, for believing in me and standing by my side throughout this process, my two boys, Hunter and Noah Blalock, for all the hugs, kisses and pure enjoyment that they brought to each and every day, my parents, Leslie Blalock and Connie Blalock, and the rest of my family, for all the support and encouragement that they have provided.

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ABSTRACT

Experiments were conducted to define the degradation kinetics of purified fiber products compared to conventional feedstuffs. *In situ* analysis was conducted on soybean hulls, dried distillers grains, dried corn gluten feed, rice mill, wheat middlings, solka floc, oat fiber (200 and 300 series), corn bran, red and white wheat bran using nylon bags. *In situ* results determined oat fiber (300) and solka floc were most similar to soybean hulls and secondly to corn gluten feed. An oat fiber: solka floc mixture was formulated for subsequent experiments. The *in vitro* experiment consisted of alfalfa hay, soybean hulls, corn gluten feed, oat fiber (300), solka floc, and 60% oat fiber: 40% solka floc. *In vitro* DM disappearance was greatest for soybean hulls followed by oat fiber, corn gluten feed, and the fiber mixture. *In vitro* gas production was greatest for soybean hulls, oat fiber, and the fiber mixture. Subsequently six cannulated steers and six wether lambs were arranged in replicated 3 x 3 Latin squares to determine effects of supplementing purified fiber to steers and lambs consuming tall fescue hay on nutrient disappearance, ruminal kinetics, and N balance. Free choice hay was supplemented with 40% solka floc: 60% oat fiber at 0, 0.25, or 0.50% of BW. Steer forage intake and total N intake were not affected by supplementation. Total intake of OM, NDF, and ADF increased with supplementation. Ruminal and total tract OM, NDF, and ADF disappearance increased with supplementation. Ruminal and total tract nitrogen disappearance were not affected by supplementation in steers. In lambs, organic matter, NDF, and ADF digested in the total tract was increased with supplementation. Total tract N digestion

decreased, however, retention was not affected. In steers, ruminal acetate concentration tended to increase, butyrate increased, and propionate not affected by supplementation. Ruminal pH increased with supplementation. Ruminal $\text{NH}_3\text{-N}$ concentrations decreased with supplementation, but a treatment x time interaction existed. These data indicate that in both steers and lambs supplementing tall fescue hay diets with digestible fiber increases ruminal and total tract fiber digestion without affecting forage intake or digestion of nitrogen while also increasing ruminally available energy.

Key Words: Supplement, Digestion, Tall Fescue

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PART 1

REVIEW OF LITERATURE

Introduction

The ruminant animal is capable of harvesting vegetation and utilizing the nutrients from lands that do not support economic crop production. The importance of this capability was described by Church (1988) where it is pointed out that rangeland constitutes approximately 40% of the earth's 34 billion acres of land. Thus, unless utilized by herbivores, such as ruminants, this portion of land would be unproductive to the human race. The ruminant's ability to efficiently utilize the nutrients from such lands is of great importance not only to the beef producer but also to consumers around the world.

Tennessee ranks 14th in total number of cattle and 9th in number of beef cows within the United States (Tennessee Agricultural Statistics Service, 2005). The primary product marketed by the industry is feeder calves produced on a forage base of 3.0 million ha of forages. The forages are predominantly cool season with tall fescue accounting for approximately one half of the total forage base.

Tall fescue has an extensive root system which improves soil condition, supports relatively higher production during summer and fall periods than other cool-season grasses and is also more resistant to harsh winters (Narasimhalu et al., 1995). These authors reported that in sheep, tall fescue hay had a greater apparent digestibility compared to timothy or reed canary grass hay. This may be explained by the report that tall fescue lignifies slowly during the growth phase and therefore its structural tissues are rapidly degraded in the rumen (Akin et al., 1987). Early regrowth of tall fescue has been reported to contain as much as

23.6% CP with the CP content declining to 14.7% after 35 d (Hitchcock et al., 1990). Dubbs et al. (2003) reported that tall fescue degradable intake protein (DIP) was between 72.4 and 74.6% of CP during April and October, when vegetative growth was occurring, and declined to 65% at forage maturity in June. McCracken et al. (1993) reported the nutrient composition of tall fescue from May through November and found that the CP content remained constant between 13.1 and 15.6% CP with NDF ranging from 63.0 to 70.5% and ADF from 39.2 to 56.5%. Acid detergent lignin increased from 6.0% in May to 7.7% in November.

According to NRC (1996), the CP in fescue hay is comprised of between 67 and 82% DIP with TDN ranging between 44 and 61% of DM. Using TDN as an estimator of whole tract degradable organic matter (DOM) and 53% TDN and 14.3% CP as an average value for tall fescue, it is calculated that tall fescue contains approximately 269 g of CP/kg of degradable organic matter (DOM; Ruminant Nitrogen Usage, 1985). A value of 210 g of CP/kg of DOM has been reported to be useful in identifying situations in which significant losses of ingested protein might occur (Poppi and McLennan, 1995) and therefore identified the need for additional DOM for the utilization of the available nitrogen.

With the growing focus on agricultural pollution and the continuing efforts to improve the efficiency of beef production, beef producers want to maximize nutrient retention as saleable beef products and to minimize nutrient excretion. Therefore, nutritional goals of animal agriculture should be to provide adequate available nutrients for maintenance and production with minimal waste. Considering these needs within the beef industry, efforts are warranted to

attempt to further define methods to optimize nutrient utilization and nutrient requirements of beef cattle. Therefore, the objectives of this research are to: (1) identify purified fiber products with degradation kinetics comparable to conventional by-product feedstuffs, (2) to determine *in vitro* fermentation parameters of comparable products, (3) to determine the effects of supplementing digestible high fiber energy on ruminal fermentation parameters, nitrogen utilization and digestion of tall fescue *in vivo*.

Microbial Reliance

The ruminant animal relies largely upon microorganisms to help meet their daily requirements for nutrients, such as CP, energy, and B-vitamins (Church, 1988). Microorganisms, as do most other living entities, have a requirement for nitrogen, amino acids, energy, and macro- and microelements. Predominantly, the digestion of fibrous feedstuffs meets these requirements with the primary contributor to this digestion being bacteria (Forsberg et al., 1999). Varga and Kolver (1997) estimated that only 10-35% of energy intake by the animal is captured as net energy due to 20 to 70% of cellulose not being digested by the microflora. Firkins (1996) described a similar scenario with nitrogen metabolism that potentially 60% of ruminally available nitrogen will not enter the small intestine as microbial protein. With nitrogen and energy being the predominant nutrients considered to affect microbial activity and growth, efforts to improve the efficiency of ruminal fiber digestion and/or nitrogen utilization are typically approached from this basis. Additionally, bacterial requirements for minerals

were demonstrated by Lamanna and Mallette (1953) with the growth of bacteria being halted in the absence of mineral salts.

Protein and Energy. Nitrogen is continually recycled to the rumen from the blood stream for re-utilization and this conservatory mechanism allows ruminants to survive on diets low in N. From 23 to 92% of plasma urea is recycled to the digestive tract, with higher values associated with lower N intake (Church, 1988). Therefore, microbial N production is an important aspect of ruminant nutrition. Microbes located within the rumen possess the ability to incorporate nitrogen from both true protein and non-protein nitrogen (NPN) into bacterial protein (BCP) to be utilized by the host animal (Church, 1988, Pond et al., 1995). An advantage of ruminants over monogastric animals is the ability of microbes to convert low quality protein or NPN into higher quality protein. Furthermore, the ruminant animal possesses the ability to survive when NPN is the sole source of N, even though the efficiency of microbial growth is enhanced by the availability of amino acids and peptides (Griswold et al., 1996). However, when cattle are maintained on a high CP forage diet, ruminal energy may limit the ability of the microorganisms to utilize the available NPN (Elizalde et al., 1998). Nitrogen excretion in feces and urine may amount to as much as 70% of daily N intake (Tamminga and Verstegen, 1996). This loss of N from ruminants can contribute to a growing worldwide problem of environmental N pollution (Castillo et al., 2001; Smith and Frost, 2000). Castillo et al. (2001) suggested that loss of N is a result of inadequate N utilization by rumen microbes. Potential

exists to significantly increase N utilization and therefore decrease N pollution through nutritional manipulations (Tamminga, 1992).

The efficiency of N incorporation into BCP is limited by the amount of energy (quantity of ATP from digestible OM) available for the bacteria and the efficiency with which they use the available energy (Church, 1988). The synthesis of BCP is dependent upon microbial growth and several factors have been described to affect growth rates. The yield of microbial biomass is related to the amount of substrate available and the energy used for maintenance, which, is a function of maintenance requirement and the growth rate (or dilution rate) (Dewhurst et al., 2000). The maintenance requirement is dependent upon the growth rate, as faster growth rates require proportionally less energy for maintenance than slower growth rates. Harmeyer (1986) described motility, cellular turnover, production of extracellular molecules, active transport, inefficient phosphorylation, uncoupling, and lysis of cells as maintenance costs of microbes important to consider. By increasing substrate availability, many of these maintenance costs can be decreased through the increase in available energy.

The importance of the rumen in degradation and utilization of N with energy supplemented animals has been demonstrated. Whitelaw et al. (1990) using female lambs conventionally fed or abomasally infused with nutrients reported an increase in urea degradation with animals conventionally fed and supplemented with cassava along with a decrease in urinary urea excretion. However, animals infused with nutrients showed no significant effects on urea

degradation by providing half the maintenance requirement of N, implying that changing the protein:energy ratio has a greater effect on N metabolism in animals with a functional rumen than in those without (Whitelaw et al., 1990). Increasing energy available to ruminal microorganisms creates a greater demand for N. In situations where energy is the limiting component, supplementation of energy has the potential to increase microbial N incorporation and decrease N excretion.

Energy Supplementation

Energy supplementation may be accomplished by increasing fermentable OM present in the rumen, allowing for greater energy release. This can be accomplished by the use of energy concentrate feedstuffs such as grains (corn, wheat, sorghum) or through the utilization of feedstuffs containing highly digestible fiber found in by-product feedstuffs such as soybean hulls, wheat middlings, and corn gluten feed. Supplementation of ruminal energy is intended to provide additional energy to the host animal as well as to the ruminal microorganisms allowing for greater microbial activity, growth, and possibly allowing for greater utilization of ammonia N present in the rumen (Church, 1988).

Forages that have high CP concentrations are often considered to adequately supply the CP required by the animal (Elizalde et al., 1998). However, according to Poppi and McLennan (1995), a substantial portion of this CP may never reach the small intestine due to high losses of ruminally-degraded protein (RDP). With the inclusion of ruminal energy supplements in a feeding

regimen, it may be possible to increase duodenal amino acid flow due to enhancing microbial CP synthesis (Elizalde et al., 1998), therefore, better utilizing N available from forages. However, if negative associative effects between the energy supplement and the forage component cause reduced forage digestion or intake, energy supplementation may not enhance ruminal energy or microbial CP flow (Horn and McCollum, 1987). Therefore, supplement type fed may play a role in forage nutrient utilization.

Associative Effects. This phenomenon refers to non-additive differences in digestibility of feedstuffs fed as components of mixed diets fed at high intakes compared to the digestibilities determined for the same feedstuffs when fed alone (Church, 1988). This can be the result of an effect on intake or could involve parameters that are much more difficult to explain such as effects on rate of passage not allowing appropriate time for complete fermentation or effects on rumen microorganism growth. Energy concentrates tend to exert negative associative effects on forage digestion when fed at high levels of the diet (Hannah et al., 1989). Positive associative effects on forage digestion may also exist between feedstuffs as reported with addition of soybean meal to a low quality forage diet (Farmer et al., 2001).

Type of Energy Supplementation

Non-fibrous Carbohydrates. Carbohydrates of non-fibrous origin are often referred to as non-structural carbohydrates (NSC) and are often represented by energy concentrate feedstuffs. This fraction of feeds include organic acids, sugars, starch and the non-starch polysaccharides such as

fructans, galactans, arabans, β -glucans and pectin (Trowell, 1974). The digestion of the NSC fractions of citrus pulp, sugar beet pulp, soybean hulls, mature and immature alfalfa has been reported to have an exponential rate of approximately 0.20 to 0.40 per hour (Hall et al., 1998). Even though a wide variety of feedstuffs contain NSC in varying amounts, corn is the most commonly used high NSC feedstuff used in the beef cattle industry. Starch, the predominant carbohydrate source in grains, including corn, is comprised of glucose molecules bound together by an α 1, 4 glucose link (Trowell, 1974). Enzymes responsible for breaking the glucose linkages in starch to release glucose molecules are present in the intestinal tract of ruminants. Also, residing within the rumen of animals consuming a high starch ration are amylolytic bacteria capable of fermenting starch for the production of propionic acid (Church, 1988).

The use of corn as an energy supplement for grazing cattle tends to decrease ruminal pH (Elizalde et al., 1998; Caton and Dhuyveter, 1997; Anderson et al., 1988) and result in detrimental effects on forage digestion. Church (1979) suggested that ruminants consuming concentrates had a ruminal pH ranging from 5.8 to 6.6. Mertens (1977) suggested that forage fiber digestion declines when ruminal pH falls below 6.7. However, Orskov (1982) reported that a ruminal pH below 6.2 resulted in reduced cellulolytic bacteria activity and digestion of straw. Even though the exact pH at which fiber digestion is affected is unclear, it appears that at some point as ruminal pH decreases, forage fiber digestion decreases.

Hannah et al. (1989) supplemented either pelleted ground corn or whole shell corn at a rate of 1% of body weight (BW) to steers and heifers grazing high quality fescue pasture and found that forage OM intake tended to be reduced and percent of forage OM digested was decreased due to supplementation while total OM intake was increased and total percent of OM digested was not different from unsupplemented animals. Howard et al. (1992) reported that providing a corn based supplement at a rate of 10% or 20% of the ad libitum hay intake to lambs grazing low quality tall fescue did not affect NDF digestion when compared to the control diet, however, NDF digestion was increased by 10% supplementation compared to 20% supplementation. Ruminal pH was decreased by supplementation with the high level of supplementation (Howard et al, 1992). Nitrogen intake and digestibility as a percent of intake was increased by supplementation while percent of N retained was not affected, however, the high level of supplementation tended to increase N retention.

Structural Carbohydrates. Structural carbohydrates are the predominant fraction of the plant cell wall. The primary components of this fraction are cellulose, hemicellulose and lignin (Trowell, 1974). Cellulose is comprised of beta 1, 4 linked glucose molecules while hemicellulose is also comprised of similar beta 1, 4 linkages with the sugar xylose being linked (Trowell, 1974). Hemicellulose is closely associated with lignin that has a strong negative influence on fiber digestion with lignin content generally increasing in advanced stages of maturity of forages (Cherney et al., 1993). Mammalian animals do not possess the enzymes necessary to degrade beta 1, 4 linkages

therefore digestion is dependent upon microorganisms which possess the necessary enzymes to degrade these bonds.

The ruminal changes brought on by feeding concentrates to ruminants has led researchers to investigate the use of high-fiber by-product feeds as sources of energy for grazing ruminants. Compared with grains, use of by-product feeds such as wheat middlings, soybean hulls or corn gluten feed as an energy supplement for cattle grazed on or fed high-roughage growing diets has generally resulted in higher ruminal pH and fewer negative associative effects on NDF digestion (Horn et al., 1995; Hannah et al., 1990). Klopfenstein et al. (1985) indicated that steers fed corn cob and alfalfa haylage based diets and wet corn fiber were 5.4% more efficient in converting feed to gain than were steers fed corn containing diets. Hsu et al. (1987) reported that lambs fed fescue hay and corn fiber performed similarly to those fed corn.

However, the use of by-product feedstuffs as a supplement has yielded inconsistent results seemingly dependent upon the quality and/or CP content of the forage. Steers grazing high quality tall fescue showed no differences in NDF digestion, duodenal nitrogen flow, microbial protein synthesis, pH or VFA production when supplemented with corn or corn gluten feed at 1.4 or 2.8 kg/d, or a cornstarch:corn gluten meal mixture supplemented at 1.4 kg/d (Elizalde et al., 1998). There was a tendency for a lower ruminal ammonia nitrogen (NH_3 N) concentration in steers supplemented with corn and corn gluten feed compared to unsupplemented animals, but the differences in mean values were small (Elizadale et al., 1998). Hannah et al. (1989) reported that supplementing either

pelleted ground corn or corn gluten feed at 1% of BW to growing steers and heifers grazing high quality tall fescue numerically reduced forage OM intake compared to non-supplemented animals (5.25 vs. 6.40 kg/d). Corn supplementation decreased forage OM digestibility 19% with no difference between corn gluten feed supplemented and control calves. Total dietary OM digestibilities were increased 14% when supplements were fed with no differences detected between corn or corn gluten feed supplementation (Hannah et al., 1989). Hannah et al. (1990) limit fed steers (2% of BW) alfalfa haylage or haylage plus corn or corn gluten feed at either 20% or 60% of the diet. They reported that as level of supplementation increased from 20 to 60% of the diet, total OM digestibility increased 6% with corn compared to 23% with corn gluten feed supplementation, however, supplementation of either supplement at 60% resulted in an increase in the rate and efficiency of gain. Hess et al. (1996) supplemented corn at 0.34% of BW or wheat bran at 0.34% or 0.48% of BW to steers grazing high quality endophyte-free fescue pasture and reported a decrease in forage intake with supplementation. The authors also reported fermentation of experimental substrates with rumen fluid from experimental animals, the control diet had a greater *in vitro* OM disappearance than the supplemented substrates. The changes described in *in vitro* OM disappearance were unexplained by the authors as dietary N and fiber content remained relatively constant across all treatment groups. Ruminal pH, total VFA and molar proportions of VFA were not affected by supplementation; however, there was a supplement X time interaction for molar proportions of acetate, butyrate, valerate

and isovalerate (Hess et al., 1996). These interactions reflected changes in the magnitude of differences, not the direction and therefore were not further considered (Hess et al., 1996). Ruminal NH_3 N concentration was greater in wheat bran supplemented steers with the difference attributed to greater N content of the wheat bran diet as compared to corn. The differences were largely explained by the quality of forage being consumed along with finding that supplementation occurred at a level at which substitution for forage was occurring.

A high fiber supplement containing soybean hulls and wheat middlings fed at a rate of 0.75% of BW to growing steers on medium quality wheat pasture resulted in increased ADG compared to steers supplemented with a starch based (corn) supplement fed at the same rate and corn supplemented steers having a similar ADG as those not supplemented (Horn et al., 1995). Garces-Yepez et al. (1997) indicated that growing cattle consuming bermudagrass hay supplemented with pelleted ground corn, soybean hulls or wheat middlings at either 0%, 25% or 50% of the estimated TDN intake had a greater ADG as supplementation rate increased, however, supplementation of soybean hulls at 50% of TDN intake resulted in a greater ADG as compared to corn supplemented at the same level. Wheat middlings supplementation resulted in an intermediate ADG response compared to corn and soybean hulls. Garces-Yepez et al. (1997) also reported that sheep consuming bermudagrass hay and supplemented as previously described displayed greater ($P < 0.01$) NDF digestion when supplemented with soybean hulls versus corn or wheat middlings at 25% or 50% of TDN intake. In

this study, NDF digestion decreased from the low to the high level in sheep receiving the corn supplement while those receiving soybean hulls showed an increase in NDF digestion from the low to high level. Galloway et al. (1993) reported that supplementing corn (0.50% of BW), soybean hulls (0.70% of BW), or corn plus soybean hulls (0.25 + 0.35% of BW) to cattle consuming bermudagrass or orchardgrass hay ad libitum depressed hay OM intake while total OM intake was increased. Supplementation of soybean hulls increased total tract NDF digestibility and OM digestibility compared to cattle supplemented with corn while mixing corn and soybean hulls resulted in a greater OM digestibility compared to corn or soybean hulls regardless of the hay source.

Supplementation increased Total N digestibility in steers consuming orchardgrass hay compared to those consuming bermudagrass hay, no effect was detected between types of supplement provided (Galloway et al., 1993).

Carey et al. (1993) reported that steers consuming medium quality ground brome hay and supplemented isocalorically with corn, barley or beet pulp to gain 0.5 kg/d resulted in forage intake to be slightly depressed by supplementation while total DMI was not affected. Total DM and OM digestibility was greatest for corn and beet pulp supplemented animals while NDF and ADF digestibility was greatest in control and beet pulp supplemented animals. Total CP digestibility was greatest in control animals, intermediate with corn and beet pulp supplementation and lowest with barley supplemented animals while ruminal NH_3 response curves revealed that beet pulp supplemented animals had a greater response ($P < 0.05$) suggesting that more ruminal NH_3 may have been used by

bacteria associated with fiber digestion in beet pulp supplemented steers (Carey et al., 1993). Interestingly, ruminal pH was lowest in beet pulp and barley supplemented animals and remained lower than 6.2 for more than half the 24 h period (Carey et al., 1993). Mertens (1977) suggested that ruminal pH concentrations of less than 6.2 could inhibit ruminal fiber digestion. Driedger et al. (2000) supplementing steers grazing tall fescue with oat fiber or corn starch reported a tendency for supplementation to decrease forage intake while total DMI was unaffected. Ruminal pH was lowest ($P < 0.10$) in animals supplemented with corn starch and ruminal NH_3 was greatest in control animals (Driedger et al., 2000). The reduction in ruminal NH_3 concentration by supplementation suggests the possibility of greater N capture through the use of supplements.

Grigsby et al. (1992) reported that in steers fed a low quality bromegrass hay diet with soybean hulls substituted at a rate of 0, 15, 30, 45 or 60% of the diet resulted in a linear increase in ruminal and total tract digestibilities of DM, OM and cell wall as level of soybean hulls substitution increased. True ruminal N digestibility was not affected by soybean hulls substitution, but total tract N digestion decreased with increasing level of soybean hull substitution. A tendency existed for microbial N flow at the duodenum to increase and ruminal NH_3 concentrations to decrease as level of substitution increased. Total VFA concentrations also increased as soybean hull substitution increased with no effect on pH. This research would suggest that substituting a higher quality feedstuff for lower quality forage increases nutrient digestion with an apparent

positive effect on the ruminal bacteria ability to utilize available N within the rumen.

Inconsistencies found in effects of supplemental by-product feedstuffs has been speculated to be at least partly attributed to differences in CP supply as well as due to replacement of forage intake by supplement, termed substitution (Caton and Dhuyvetter, 1997). Substitution is the amount of basal forage exchanged for supplemented nutrients, which can be given a value called the substitution coefficient (amount of decrease in basal forage divided by amount of supplement provided; Caton and Dhuyvetter, 1997; Minson, 1990; Horn and McCollum, 1987). Minson (1990) speculated that substitution coefficients are responsive to forage quality, in that as forage CP increases, substitution coefficients increase. Substitution is evident in trials where forage DMI is reduced by supplementation while total DMI is not affected.

Degradable intake protein (DIP) is thought to exert a positive effect on forage intake and digestion in cattle receiving low quality forage where adequate DIP is not supplied (Farmer et al., 2001). Farmer et al. (2001) reported that supplementing steers consuming prairie hay with a 43% CP supplement of which 57.5% was DIP either 2, 3, 5 or 7 d per week at a rate of 0.36% of BW resulted in forage, total and digestible OM intakes to increase linearly ($P < 0.01$) with frequency of supplementation. Organic matter and NDF digestion also increased with increased frequency of supplementation (Farmer et al., 2001). When adequate DIP is supplied by the forage, such as appears to be the case with cool season forages, energy has the potential to be the limiting factor in N utilization.

Therefore, supplementing digestible fiber without substantially increasing N, could increase N utilization. However, if the substitution coefficient is increased with forages high in CP (Minson, 1990), then supplementation with such a product may result in a net decrease in forage intake.

Overall, providing supplemental energy in the form of readily digestible fiber (by-product feedstuffs) usually has a less negative effect on forage intake than starch based supplements, and use may lead to increased total intake. It appears that use of such products in ruminants on a forage based diet yields fewer negative effects on fiber digestion than grain supplementation. It is believed that providing grain as supplemental energy tends to increase the efficiency of energy use (NRC, 1996) by shifting the VFA production so that more propionate is being produced, while the efficiencies related to the use of by-product feedstuffs are less well defined (Caton and Dhuyvetter, 1997).

Identifying Limiting Nutrients. The potential to increase microbial digestion and subsequent N utilization evolves around a concept of identifying the limiting nutrient. Olsen et al. (1999) reported that in steers fed low quality tallgrass prairie hay with no supplement or a supplement designed to contain one of three levels of starch (0, 0.15, 0.30% of BW) and one of four levels of DIP (0.03, 0.06, 0.09, 0.12% of BW) resulted in an increase in forage, total and digestible OM intake as DIP supplementation increased but a reduction in forage, total and digestible OM intake as starch supplementation increased. Ruminal NH_3 concentration was decreased linearly ($P = 0.02$) with increasing starch supplementation and increased with DIP supplementation (Olsen et al., 1999).

When supplemented with starch, increasing protein supplementation did tend to increase NDF digestion and decrease ruminal ammonia concentration as compared to protein supplementation alone (Olsen et al., 1999). The limiting nutrient for microbial production as the energy level was increased became N. Therefore, by supplementing N, to meet the demand by microorganisms allowed increased microbial activity and increased fiber digestion. Even though negative effects of starch supplementation were not completely alleviated by additional DIP, the effects were reduced. However, Ferrell et al. (1999) reported that in sheep fed low quality brome hay and supplemented with corn starch, corn starch plus urea, corn starch plus SBM, or corn starch plus feather meal and blood meal resulted in no effect on forage intake while total DMI was increased by supplementation with the feather and blood meal resulting in the greatest increase and corn starch, the lowest increase. Supplementation increased apparent DM and OM digestibility, with feather and blood meal supplementation resulting in the greatest quantity of DM and OM digested and corn starch the lowest. Apparent N digestibility tended to be less in corn starch supplemented than in control sheep, and both were approximately 50% of that in sheep supplemented with urea, SBM, or feather and blood meal (Ferrell et al., 1999). It appears that supplementation of energy (corn starch) alone to low quality forages may impair forage N digestion while supplementation with energy and N together increases apparent N digestion. Considering the greater quantity of DM, OM, and N digested with blood and feather meal supplementation compared to corn starch supplementation, N may have limited microbial activity in the latter.

Due to the energy source used by Ferrell et al. (1999) and Olsen et al. (1999) being composed primarily of starch, the negative effects that are often associated with feeding starch on forage digestion is possibly involved in the lack of response to supplementation. In the case of low quality forages, such that CP is relatively low, increasing N supply to the microbes can lead to an increase in microbial digestion (Olsen et al., 1999). However, when starch is fed as an energy supplement with these forages (Olsen et al., 1999; Ferrell et al., 1999) fiber digestion is not increased and may be reduced with a possible increase in N utilization. Cellulolytic bacteria typically prefer NH_3 as a N source while many of these species have an NH_3 requirement (Church, 1988). Therefore, In terms of increasing microbial digestion and N utilization, the use of by-product feedstuffs is further exemplified in that the possibility remains to increase N utilization by the microbes while fiber digestion could also be improved. Driedger et al. (2000) suggests that due to fiber supplementation either not affecting or increasing forage intake and digestibility and resulting in less dramatic shifts in ruminal pH, forage use may be enhanced with fiber, as compared with starch supplementation. Changes in ruminal NH_3 concentration also indicate the possibility for greater N capture with energy supplementation (Driedger et al., 2000). With ruminants grazing a fescue based forage, CP and metabolizable protein (MP) is thought to be adequate in the diet with energy being the limiting the factor (Elizalde et al., 1998). Therefore, by supplementing a fibrous feed source, ruminal energy availability may be increased causing a higher demand

for available N by the microorganisms. This increased demand for N could result in a reduction in N excretion and an increase in retention by the animal.

In Situ Analysis

The *in situ* technique has been widely utilized for many years to provide estimates of the rate and extent of nutrient disappearance (Mehrez and Orskov, 1977). This technique is commonly used to study the effects of ruminal environment on the kinetics of ruminal fermentation (Uden and Van Soest, 1984; Nocek, 1988). Feeding frequency of the host animal and pore size of nylon bags used for incubation are important factors that may affect the interpretation of results (Meyer and Mackie, 1986). Marinucci et al. (1992) concluded that *in situ* analysis was useful in determining the effects of diet of the host animal on ruminal digestibility; however, caution was expressed in evaluating data in absolute terms. Therefore, to produce a technique that yields reproducible results, standardized procedures are required, unless ruminal environmental alterations are a goal of the research (Vanzant et al., 1998).

In Situ vs In Vitro. During the early stages of *in situ* analysis, substantial error may result due to a low weight loss, and, for poor quality forages, adherence of microbes at early stages can lead to higher weights and thus distortion of results (Getachew et al., 1998). Dewhurst et al. (1995) compared the nylon bag *in situ* and the Tilley and Terry (1963) *In vitro* technique and reported that the *in situ* method overestimated the fermentation of a variety of feedstuffs. The overestimation was attributed to the proportion of rapidly fermentable carbohydrates of the feeds where, particularly at short incubation times, there

was a loss of the rapidly fermentable fraction from the bags before it was fermented. Similarly, Varel and Kreikemeier (1995) reported that the *in situ* technique resulted in greater rate and extent of digestion compared to *in vitro* analysis. Additionally, it was reported that the *in situ* method consistently had a shorter lag time than the *in vitro* method. Conversely, examining low quality forages has resulted in a possible underestimation of dry matter loss with the *in situ* compared to the *In vitro* method (Monson et al., 1969; Orskov and Ryle, 1990).

***In Vitro* Methodology**

Microbial biomass and short-chain fatty acids (SCFA) are the useful products and gases (mainly CO₂ and CH₄) are the waste products of ruminal degradation (Blummel et al., 1999). The use of *in vitro* fermentation methods are widely used in efforts to examine and optimize the utilization of fiber sources and to better identify factors that may affect fermentation (Pell and Schofield, 1993; Grant and Mertens, 1992). Many inherent interactions present in the *in vivo* system, such as those between passage rates, particle size and rate of particle size reduction make interpretation and incorporation of *in vitro* data into models of *in vivo* ruminant digestion difficult (Grant and Mertens, 1992). The lack of a standardized *in vitro* method also leads to further problems of applying results to *in vivo* systems. Grant and Mertens (1992) examined the effect of digestion vessel, gassing procedure, type of buffer used, addition of nutritive factors and the incorporation of reducing agents on the rate of *in vitro* digestion and reported that of the factors tested, only gassing technique had an affect. Continuous

gassing resulted in increased rate of digestion compared to CO₂ bubbled method (0.062 and 0.049 %/h, respectively), however, there was no difference between the prediction of indigestible residue (27.37 and 27.80%, respectively). It should be noted that the type of digestion vessel used was different between gassing methods in this portion of the experiment, so some effect of vessel may have contributed to differences reported due to method of gassing. The use of microminerals and reducing agents tended to increase rate of digestion, with the use of microminerals appearing to be the most beneficial to the digestion of low quality forages (Grant and Mertens, 1992). Martinez and Church (1970) reported that of the required microminerals, Co, I, Fe, Mn, Mo, and Zn has the ability to stimulate cellulose digestion *in vitro*.

Conventional *in vitro* systems examine either substrate degradation (Tilley and Terry, 1963) or one of the products generated after a given time of incubation in a mixed rumen suspension (Blummel et al., 1999). Taya et al. (1980) demonstrated that gas production was linearly related to cellulose digestion in pure cultures, however, it has been suggested that using a combination of gas production measurements as well residue determinations would be most indicative of the substrate truly degraded (Goering and Van Soest, 1970; Blummel et al., 1999). The residual substrate is used to determine the amount of substrate used in the fermentation while the gas measurement indicates the amount of the substrate converted into SCFA and gases (Blummel et al., 1997a). The ratio of substrate degraded to gas volume produced was termed “partitioning factor” (Blummel et al., 1999). Blummel et al. (1997b)

calculated that in the fermentation of roughages 1 mL of gas is coupled to the requirement of 2.2 mg of substrate for the production of SCFA, and fermentative CO_2 , CH_4 and H_2O . This factor was found to be in agreement with the ratio of substrate apparently degraded to volume of gas produced for two types of stover leaves with a partitioning factor of 2.1 mg/mL for each type of substrate tested (Blummel et al., 1999). It is believed that substrates with a high true degradability but low gas production should be selected to obtain feeds with high microbial efficiencies (Blummel et al., 1999). Therefore, the use of gas production measurements along with residue determinations may give insight into the efficiencies associated with fermentation under different conditions and may help in gaining an understanding of the nutritional factors that are related to ruminal fermentation.

Gas Production Method. Primarily, gas production is the result of the fermentation of carbohydrates to acetate, propionate, and butyrate (Beuvink and Spoelstra, 1992; Blummel and Orskov, 1993). Gas produced from fermentation of protein is relatively small compared to that produced from carbohydrates (Wolin, 1960) and the contribution of fat to gas production is negligible (Getachew et al., 1998). Fermentation of 200 mg of coconut, palm kernel and/or soybean oil produced less than 3 mL of gas while incubation with a similar amount of casein and cellulose produced approximately 23 and 80 mL of gas, respectively (Menke and Steingass, 1988; Getachew et al., 1997).

Gas evolved is either associated directly with fermentation or generated as a result of buffering short chain fatty acids (SCFA) which releases CO_2 from

bicarbonate buffer. Production of CO₂ has been estimated to be 0.8 – 1.0 mmol of CO₂ per mmol SCFA produced (Beuvink and Spoelstra, 1992; Blummel and Orskov, 1993). Production of 2 mole of acetate and 1 mole of butyrate results in the release of 2 mole CO₂ directly from fermentation while 2 mole and 1 mole of CO₂ released indirectly from buffering of acetate and butyrate, respectively (Hungate, 1966). However, the production of 2 mol propionate and lactate results in the indirect release of 2 mol CO₂ from buffering activity. With typical forage substrates the proportion of gas released as a result of buffering is approximately 50%; however, as the molar proportion of propionate increases the proportion of gas released from buffering of SCFA also increases (Blummel and Orskov, 1993). The gas released from the generation of propionate is only that which is associated with bicarbonate buffering. Therefore, gas is produced predominantly from the fermentation of substrate to acetate and butyrate so the utilization of substrates which increase the proportion of propionate is associated with lower gas production (Wolin et al., 1960; Van Soest, 1994).

Important factors that influence *in vitro* gas production are anaerobiosis, proper temperature, suitable pH, adequate buffer, sufficient inoculum, and sample size (Getachew et al., 1998; Pell and Schofield, 1993). Trei et al. (1970) using 3-h incubations, reported a significant decrease in gas production per gram of DM as sample size increased from 1 to 3 g accompanied by a decrease in dry matter disappearance, presumably a result of exhaustion of buffer. In order to maintain linearity between fermentation of OM and gas production, the amount of VFA produced must not exceed 4.5 mmol/60-mL of buffered rumen fluid (1:2,

rumen fluid:buffer) to avoid exhaustion of the buffer (Beuvink and Spoelstra, 1992) which leads to a reduction in pH and microbial activity. From stoichiometry of fermentation, this implies that sample weight should be limited to 400 mg fermentable OM/60 mL of buffered rumen fluid. Cone et al. (1996) reported exhaustion of buffer when more than 0.5 g corn cob mix was incubated in 60 mL of buffered inoculum containing rumen fluid and buffer in a 1:2 ratio.

Pell and Schofield (1993) using 50-mL serum bottles, reported that 45 mL of gas was produced in the fermentation of 200 mg of grass hay during 24 h, which corresponds closely to the volume available in the serum bottle containing 10 mL of liquid. As a result of the volume of gas produced, the authors chose to reduce the amount of substrate to 100 mg. This is approximately 20% of amount typically used in an *in vitro* digestion, and resulted in an increase in the significance of sampling errors (Pell and Schofield, 1993). Theodorou et al. (1994) reported that the pressure in the bottle head space should not exceed 7.0 psi, which can be controlled by altering head space volume. It is possible that accumulated pressure may negatively affect microbial fermentation and illicit changes in the solubility of gases (Getachew et al., 1998). Therefore, it is advised that pressure accumulation be monitored and controlled to ensure accurate measurements.

Statistical Modeling. The measurement of fermentative gas production as a means of describing ruminal fermentation has been utilized since the early 1940's (Getachew et al., 1998). The measurement of *in vitro* gas production became a routine method of feed evaluation after Menke et al. (1979) reported a

high correlation between gas production and *in vivo* apparent digestibility. These early systems relied upon periodic readings of gas production producing a limited number of data points which were relatively inaccurate (Cone et al., 1997). Due to the relatively low accuracy, gas production profiles can be described by simple one-phasic (France and Thornley, 1984) or exponential (Beuvink and Kogut, 1993; Cone et al., 1997) models.

The exponential model describes gas production using first order reaction kinetics with or without a lag phase. The rate of gas production is proportional to potential gas production, which is associated with the substrate concentration (Beuvink and Kogut, 1993). The logistic model, which has been used to evaluate microbial growth, has also been employed to describe *in vitro* gas production. The logistic model assumes gas production rate to be proportional to microbial activity, represented by the amount of gas produced and to substrate concentration while the Gompertz model assumes that rate of gas production is proportional to microbial activity, but the proportionality parameter decreases with time which in turn controls the shape of the curve (Beuvink and Kogut, 1993). The Gompertz model proportionality parameter describes a loss of efficiency of fermentation over time.

According to Beuvink and Kogut (1993) the exponential model, followed by the logistic model, provided the worst fit to gas production data compared to the other sigmoidal models (Gompertz, Richards, Schnute, and Modified Gompertz) compared. The shortcoming of the exponential model was explained as being due to the model assuming that after a discrete lag time the feed is

fermented instantaneously at maximum rate. However, gas production curves have a sigmoidal shape thus resembles growth curves of bacteria as gas production is proportional to the amount of metabolic end products produced during bacterial growth (Beuvink and Spoelstra, 1992). The logistic model, even though being descriptive of microbial activity, fails to account for the change in efficiency of fermentation over time. The Gompertz model, even though superior to the exponential and logistic models, underestimated the asymptote and did not give a successful fit for the exponential part of the curve. The models discussed appear to be particularly inadequate describing the gas production data which present a rapid rate of gas production during the early stage of fermentation and a slower development in the asymptotic phase (Beuvink and Kogut, 1993).

The development of more advanced equipment produced gas production profiles that could not be satisfactorily fit using the simple models (Beuvink and Kogut, 1993), due to the accuracy of description of the stages of gas production. Cone et al. (1996) described *in vitro* gas production to be multi-phasic with the first phase representing the fermentation of the soluble fraction, the second phase the non-soluble fraction, and the third phase a result of microbial turnover. The multi-phasic model describes each phase as the asymptotic phasic gas production, the time lapse at which half of the asymptote is reached, and a parameter describing the shape of the curve (Cone et al., 1997). Beuvink and Kogut (1993) developed the modified Gompertz model which describes gas production as a dual phase being comprised of a fast and a slow fermentable fraction. This model describes the maximal rate of gas production for each phase

along with its own fractional decay constant governing the rate of declining gas production within each phase. The modified Gompertz models provided a better fit to gas production data described by Beuvink and Kogut (1993) compared to the exponential, logistic, and Gompertz models. However, according to Cone et al. (1997), the more complex multi-phasic model provided a better fit compared to the modified Gompertz model. Therefore, the ultimate factor determining the choice of model appears to be the relative accuracy of describing the phases of gas production. It is also possible that this may be controlled by the type of substrate fermented as certain phases of fermentation may be more pronounced with different substrates. However, it seems clear that these more complex nonlinear models provide a better description of the gas production profiles using more sensitive measurement techniques.

Conclusion

In the state of Tennessee, forages are the predominant resource utilized for ruminant livestock production. The forage resources are composed primarily of cool season forages with tall fescue predominating. These species of forages tend to contain adequate to high levels of CP (nitrogen) and ruminally available N in relation to their levels of total digestible nutrients. Therefore, supplemental TDN may be warranted to maximize the utilization of ruminally available N. However, many conventional supplements contain high levels of starch and/or CP. Supplementation with high levels of starch may impair forage fiber utilization while using supplements with high CP content may be counter productive in terms of maximizing ruminally available N utilization. Therefore, the experiments

described in this dissertation were designed to identify purified fiber products containing very low levels of CP to be used in subsequent experiments and to examine the effects of supplementing digestible fiber to ruminants on ruminal parameters, fiber digestion, and N retention.

PART 2

***IN SITU* DEGRADATION KINETICS AND *IN VITRO* DEGRADATION AND GAS
PRODUCTION OF PURIFIED FIBER AND CONVENTIONAL BY-PRODUCT
FEEDSTUFFS**

Abstract

Experiments were conducted to define the *in situ* degradation kinetics and the *in vitro* degradation, gas production, changes in volatile fatty acid (VFA) and ammonia nitrogen ($\text{NH}_3\text{-N}$) concentrations of purified fiber products to be used in subsequent experiments in comparison to conventional by-product feedstuffs. The *in situ* experiment utilized four and the *in vitro* experiment two ruminally cannulated steers maintained on a diet containing 70% alfalfa cubes and 30% corn/vitamin/mineral mix. *In situ* analysis was conducted in nylon bags using soybean hulls (SBH), dried distillers grains, dried corn gluten feed, rice mill, wheat middlings, solka flock, oat fiber (200 and 300 series) corn bran, red and white wheat bran, cellulose acetate, and fescue hay incubated in each animal for 0, 3, 6, 9, 12, 24, 48, and 96 h. The *in situ* results indicated that oat fiber (300) and solka floc had degradation kinetics most similar to SBH and secondly to corn gluten feed. A 60% oat fiber:40% solka floc mixture was formulated for subsequent experiments. The *in vitro* experiment consisted alfalfa hay, SBH, corn gluten feed, oat fiber (300), solka floc, and 60% oat fiber:40% solka floc mix incubated in 4:1 McDougall's buffer:rumen fluid for 96 h. Dry matter (DM) disappearance was greatest for SBH followed by oat fiber, corn gluten feed, and the fiber mixture. Gas production was greatest for SBH, oat fiber, and the fiber mixture. The partitioning factor (ml gas produced/mg substrate degraded) was greatest for alfalfa and corn gluten feed. Incubation of SBH, oat fiber, solka floc, and the fiber mixture resulted in the greatest concentration of total VFA and oat fiber and the fiber mixture had the greatest concentration of propionate.

Concentrations of valerate, isovalerate, and isobutyrate decreased by incubation of purified fiber substrates compared to conventional feedstuffs. Concentrations of $\text{NH}_3\text{-N}$ were decreased in fluid incubated with all purified fiber substrates, increased with alfalfa and corn gluten feed and SBH resulted in little change from initial concentrations. Therefore, it was concluded that oat fiber and fiber mix have relatively similar fermentation profiles as SBH and have the potential to increase ruminally available organic matter (OM) to enhance nitrogen (N) utilization by steers consuming tall fescue grass hay.

Key words: *in vitro*, *in situ*, purified fiber, rumen, degradation.

Introduction

Tall fescue is typically considered to be either adequate or excessive in crude protein (CP). Based on NRC (1996), tall fescue contains approximately 269 g of CP/kg of degradable organic matter (DOM). A value of 210 g of CP/kg of DOM has been reported to indicate significant losses of ingested N (Poppi and McLennan, 1995) and a need for additional DOM (energy) to allow for greater microbial protein synthesis.

Supplementation of energy is commonly achieved through the use of grains which may negatively effect ruminal fiber digestion (Mertens and Lofton, 1980). Fibrous by-product feedstuffs are an alternative form of supplemental energy which may limit the negative effects on forage fiber digestion (Cordes et al., 1988). With the growing focus on agricultural pollution and continued efforts to improve the efficiency of beef production, beef producers want to maximize nutrient retention as saleable beef products and minimize nutrient excretion.

Therefore, efforts are necessary to define methods to maximize ruminal fermentation and ruminal nutrient utilization.

In situ degradability can be utilized effectively to rank feedstuffs based upon their rumen degradability (Mehrez and Orskov, 1977). The method provides the ability to define the degradation kinetics of different feed constituents such as CP, OM, and fiber (Melaku et al., 2003). *In vitro* gas production primarily measures gas produced from carbohydrate fermentation (Menke and Steingass, 1988). Therefore, gas produced from the fermentation of a feed source is a reflection of microbial degradation and volatile fatty acid (VFA) production.

The objectives of this experiment were to (1) identify purified fiber products with *in situ* degradation kinetics comparable to conventional by-product feedstuffs to be used to supplement tall fescue grass hay diets and (2) compare the *in vitro* fermentation parameters of the selected purified fiber products to selected by-product feedstuffs.

Materials and Methods

In Situ Experiment. Four ruminally cannulated steers were used to incubate Dacron bags for *in situ* estimation of carbohydrate (A, B and C fractions) degradation rates. Substrates incubated included soybean hulls, dried distillers grains with solubles, dried corn gluten feed, rice mill, wheat middlings, fescue hay, solka floc, Canadian Harvest[®] oat fiber 200 and 300 (Opta Food Ingredients, Inc., Bedford, MA), corn bran, stabilized red and white wheat bran (Opta Food Ingredients, Inc., Bedford, MA), cellulose acetate, and cellulose powder.

Steers were housed in individual pens within an enclosed, environmentally controlled room. Temperature was maintained at 23°C with 16 h of light and 8 h of dark. Steers were fed equal portions twice daily at 0500 and 1700 h. The diet (Table 1) consisted of 70% alfalfa cubes and 30% of a grain mixture fed at a level to meet the net energy for maintenance and exceed the CP requirement of the animals (NRC, 1996). According to NRC (1996), the diet supplied 321 and 387 g/d of DIP and MP, respectively, which exceeded requirements by 83.5 and 71 g/d, respectively. Animals had continuous access to fresh water with a complete vitamin and mineral supplement included in the diet to meet or exceed animal requirements. The diet was fed for 10 d prior to incubating samples to allow for ruminal adaptation.

In Situ Methodology. Dacron bags were labeled with a waterproof permanent marker and the weight recorded. For each animal and time point, Dacron bags (10 x 20 cm; Ankom Co., Fairport, NY) were filled with 4 g of each sample ground to pass a 2-mm screen in a Wiley Mill and heat sealed. Particle size of purified fiber substrates were 2-mm or less and, therefore, were not ground prior to incubation. Bags were then placed into a large (~45.7 cm x 38.1 cm), weighted (8 to 12 #7 or #8 rubber stoppers) polyester mesh bag and soaked in a bucket containing warm water (39°C) for 20 min immediately prior to incubation. Bags were placed within the ventral rumen at 1700 on d 0, 1700 on d 2, 1700 on d 3, and at 0500, 0800, 1100 and 1400 on d 4. Removal of all bags occurred simultaneously at 1700 d 4. Incubation times represented were 0, 3, 6, 9, 12, 24, 48 and 96 h. The 0 h bags were not placed into rumen. At

Table 1. Diet composition for *in situ* and *in vitro* degradation experiments

Item	% Diet, DM
Alfalfa Cubes	69.55
Grain mixture	
Cracked Corn	29.92
Vitamin/Mineral Supplement ^a	0.28
Molasses	0.25
Calculated chemical analysis ^b	
NE _m , Mcal/kg DM	1.57
CP, % DM	14.98
DIP, % of CP DM	51.17
Ca, % DM	1.01
P, % DM	0.28

^aCO-OP All Purpose Cattle Mineral 650, Tennessee Farmers Cooperative, Lavergne, TN; max 27% Ca, min 4.5% P, max 19.2% salt, min 0.3% Mg, min 0.1% K, min 24 ppm Co, min 1000 ppm Cu, min 36 ppm I, min 3000 ppm Mn, min 20 ppm Se, min 3500 ppm Zn, min 529,200 IU/kg Vit. A, min 99,225 IU/kg Vit. D, min 309 IU/kg Vit. E.

^bValues calculated based on NRC, 1996 .

termination of incubation, bags were rinsed in a 37.9-L container of ice cold water to stop microbial activity. Bags were then rinsed using five rinse cycles (1 min rinse and 2 min spin) in a standard top loading washing machine (Vanzant et al., 1998). Zero hour bags were rinsed prior to ruminally incubated bags. After rinsing, all bags were placed in a 55°C forced air drying oven and dried for approximately 48 h. Dried samples were allowed to air equilibrate for approximately 60 min to room temperature (AOAC, 2000) before weighing to determine residual DM. Original and incubated sample material composited across steers by incubation time were analyzed for NDF (without sodium sulfite) and ADF (Ankom Technologies, Fairport, NY) for determination of residual fiber.

Calculations. Carbohydrate digestion was partitioned into three fractions (A, B and C) based on relative susceptibility to ruminal degradation as described by the Cornell Net Carbohydrate Protein system. The A fraction or soluble fraction was that soluble in water, the B fraction described as the in-soluble but ruminally degradable fraction, and the C fraction as the ruminally non-degradable component of the feedstuff. The A fraction was determined by the nutrients removed by rinsing of 0 h bags. The C fraction was represented by the residual nutrient in the 96 h bags while the B fraction was estimated by subtraction of the C fraction from material remaining after the 0 h wash (A fraction).

Statistical Analysis. The data were used to determine the B fraction DM, NDF and ADF rate of *in situ* degradation by fitting the percentage of residue remaining to the non linear model of Mertens and Loften (1980). Computations associated with the model used in the determination of *in situ* degradation rates

were conducted using the nonlinear procedures (Marquardt method) of SAS (SAS Inst. Inc., Cary, NC).

In Vitro Experiment. Rumen fluid was collected from two steers housed and fed as described in the *in situ* experiment. The diet was fed for 14 d prior to initiation of the experiment to allow for ruminal adaptation.

Fourteen 250-mL screw cap bottles (Fisher Scientific, # 064141B) were previously prepared to contain 0.5 g of one of five products in duplicate. The products consisted of either soybean hulls, corn gluten feed, solka floc, oat fiber 300 series, or solka floc:oat fiber (40%:60%; MIX) combination ground to pass a 1-mm screen and placed into F57 filter bags (Ankom Technology, Fairport, NY). Duplicate lab controls (alfalfa hay) and blanks were also included within each of three runs. At least 12 h prior to rumen fluid collection, 80 mL of McDougall's Buffer (Goering and Van Soest, 1970) was placed into each bottle and gassed with CO₂ for 20 min, a rubber stopper was immediately inserted and the bottles allowed to stand over night. On the morning of fluid collection, bottles were pre-warmed to 39°C at least 2 h prior to collection of rumen fluid. Rumen fluid was collected from each animal two hours post AM feeding and strained through two layers of cheese cloth into a pre-warmed (39°C) thermos. After returning to the lab, rumen fluid was strained through four layers of cheesecloth and glass wool. Rumen fluid from the two animals was equally combined and 20 mL of fluid added to each bottle, previously gassed with CO₂ for 20 min. The final buffer:rumen fluid inoculum was in a 4:1 ratio for a total volume of 100-mL to ensure fermentation was not limited by buffering capacity or inoculum size (Pell

and Schofield, 1993). After mixing and gassing with CO₂, a rubber stopper was placed into the bottle. The mixture was gently swirled and placed immediately into a pre-warmed (39°C) shaking water bath. Gas pressure sensors (OneSix Analog Pressure Sensor, Point Six, Inc., Lexington, KY) were connected to each bottle using Viton[®] tubing via a 1/16 in hose barb connected to a national pipe thread (NPT) threaded fitting (Cole-Parmer Instrument Co., Vernon Hills, IL) previously threaded through the cap of the *in vitro* bottle and sealed using Rectorseal #5 pipe thread sealant (Rectorseal, Houston, TX). The screw cap was fitted with a Viton o-ring (Marco Rubber and Plastic, # V1000-218) lightly coated with vacuum grease and tightened onto the fermentation vessel. Bottles were allowed to continuously shake at 39°C in a covered orbital shaking water bath and gas pressure recorded every 15 min over 96 h.

Sensor Calibration. *In vitro* bottles, previously described, were filled with 100 mL of McDougall's buffer, gassed with CO₂, sealed, and allowed to stand over night. Bottles were pre-warmed to 39°C for 2 h and sealed using a screw cap fitted with duplicate NPT threaded fittings. The NPT fittings were connected to individual sensors, as previously described, and a 60-mL syringe filled with CO₂. Bottles were filled with 60 mL of CO₂ in 15-mL increments and the gas pressure recorded at each increment after allowing 5 min to equilibrate. The pressure reading was graphed against gas volume and the resulting slope used to convert gas pressure to gas volume (mL).

Sample Analysis. After 96 h of incubation, F57 Ankom bags were removed, rinsed with deionized water, dried at 105°C for 24 h, and weighed to

determine dry matter disappearance (DMD) not corrected for bacterial biomass. After weighing, bags were washed with neutral detergent solution (NDS) at 100°C for 75 min followed by three 5 min rinses with deionized water to completely separate microbial biomass from the undegraded feed residues (Blummel et al., 1999). Samples were then dried at 105°C for 24 h and reweighed for determination of true dry matter disappearance (TDMD). Two 5-mL samples of incubation fluid from each *in vitro* bottle were collected and stored at -20°C for analysis of VFA content (Erwin et al., 1961) and ammonia nitrogen (Broderick and Kang, 1980). Samples for ammonia nitrogen determination were mixed with 1 mL of 25% meta-phosphoric acid prior to storage. The VFA analysis was conducted using gas chromatography (Shimadzu GC-2010, column HP-FFAP 10 m x 0.53 mm x 1.0 µm, Shimadzu Scientific Instruments, Columbia, MD) utilizing helium as the carrier gas (100 ml/min) and a flame ionization detector (compressed air at 350 – 380 ml/min, hydrogen at 30 – 35 ml/min). Changes in VFA and ammonia nitrogen concentrations were determined as the difference between the concentration in fluid from bottles containing substrates and those containing no sample (blank). The partitioning factor was calculated by dividing the amount of substrate degraded by the total volume of gas produced (mg/mL).

Statistical Analysis. Prior to statistical analysis, gas production, VFA, and NH₃-N data were corrected using respective data obtained from bottles containing no sample (blanks) by subtracting the blank from the sample values. Non-linear regression analysis using the modified Gompertz model as described

by Beuvink and Kogut (1993) was used to describe the gas production profiles of each sample. The model was as follows:

$$Y = TG \exp\{-RF/DF \exp(-DF \cdot t) - RS/DS \exp(-DS \cdot t)\}$$

where: TG = total gas produced, RF = shape parameter describing the rate of gas produced from the rapidly fermentable fraction, RS = shape parameter describing the slowly fermentable fraction, DF = rate of decay in gas production associated with the rapidly fermentable fraction, DS = rate of decay associated with the slowly fermentable fraction, and t = time. All data were then analyzed as a completely randomized design with replication using the MIXED procedures of SAS. Analysis was conducted using the mean value of duplicate samples within each *in vitro* run replicated three times to account for day variation. The model included the parameters of replicate and substrate for determining differences in DM disappearance, rates of gas production, VFA and ammonia N production. Correlations were also determined using the CORR procedure of SAS for the variables TDMD, gas production, total VFA, partitioning factor, and acetate: propionate ratio.

Results

Of the products used in this trial, only corn bran, oat fiber (series 200 and 300), and solka floc possessed fiber degradation properties similar to the feedstuffs chosen (citrus pulp, distillers grains with solubles, corn gluten feed, rice mill, soybean hulls, and wheat middlings). Cellulose acetate appears to be highly non-degraded in the rumen with cellulose powder not being of appropriate particle size to be retained within Dacron bags to any measurable extent,

therefore, data are not presented for these substrates. Red and white wheat bran, though having very similar degradation properties (B-fraction degradation; 0.088 and 0.133 %/h, respectively), will not be discussed due to the nitrogen content being relatively high.

Purified fiber sources (solka floc, oat fiber 200 and 300 series, corn bran, stabilized red and white wheat bran) contained 58.0 to 90.0% NDF (Table 2) while conventional feedstuffs (citrus pulp, distillers grains with solubles, corn gluten feed, rice mill, soybean hulls, and wheat middlings) ranged from 24.4 to 66.2%. The ADF contents were within a common range from 12.3 to 49.0%. Most products tested had a high potentially degradable fraction (B fraction; Table 3) with rice mill and the oat fiber 200 series having the lowest (42.4% and 66.9%; respectively). The A fraction was variable with solka floc having a numerically higher proportion (63.4%) compared to the other products. Analysis of fiber degradation data yielded a wide array of values for rate of fiber digestion. Citrus pulp, rice mill and wheat middlings had a numerically higher rate of B-fraction and NDF degradation while the rate of ADF degradation in citrus pulp was numerically higher than all other substrates tested (0.118 %/h; Table 4)

In vitro dry matter disappearance (DMD) and true dry matter disappearance (TDMD) were greatest for soybean hulls, corn gluten feed, and oat fiber (Table 5). Oat fiber, oat fiber/solka, and soybean hull substrates produced the greatest volume of gas while gas production was least for alfalfa with corn gluten feed and solka floc substrates intermediate. Gas production associated with the fermentation of solka floc was 24 mL less than that

Table 2. Chemical composition^a of *in situ/in vitro* substrates

Substrates	NDF ^b	ADF ^c	CP ^d
	% of DM		
Conventional Feedstuffs			
Citrus pulp	24.4	18.6	7.5
Corn gluten feed	50.6	12.8	17.9
Distillers grains	59.6	21.0	30.2
Fescue hay	68.0	38.2	10.0
Rice mill	59.8	39.5	7.5
Soybean hulls	66.2	47.8	11.9
Wheat middlings	37.2	12.3	18.0
Fiber Sources			
Corn bran	76.1	19.3	4.4
Oat fiber (200)	90.0	45.2	0.9
Oat fiber (300)	73.4	49.0	0.6
Red wheat bran	60.0	17.1	18.9
Solka floc	64.0	48.1	0.1
White wheat bran	58.0	15.4	16.5

^aBased on chemical analysis.

^bNeutral detergent fiber.

^cAcid detergent fiber.

^dCrude protein.

Table 3. Readily soluble, potentially degradable, and undegradable DM of *in situ* substrates

Substrates	A-Fraction ^a	B-Fraction ^b	C-Fraction ^c
	% of DM		
Conventional Feedstuffs			
Citrus pulp	41.8	55.5	2.7
Corn gluten feed	30.6	66.0	3.4
Distillers grains	27.7	64.3	8.0
Fescue hay	18.8	50.6	30.6
Rice mill	20.9	21.5	57.6
Soybean hulls	12.6	83.9	3.5
Wheat middlings	41.6	43.8	14.6
Fiber Sources			
Corn bran	26.4	70.7	2.9
Oat fiber (200)	5.0	61.8	33.2
Oat fiber (300)	23.1	71.7	5.2
Red wheat bran	14.7	64.1	21.2
Solka floc	63.4	36.1	0.5
White wheat bran	17.7	64.4	17.8

^aReadily soluble.

^bPotentially degradable.

^cUndegradable.

Table 4. Rate of B-fraction and potentially degradable NDF and ADF disappearance of *in situ* substrates

Substrate	B-fraction ^a	NDF ^b	ADF ^c
	%/ h		
Conventional Feedstuffs			
Citrus pulp	10.9	12.5	11.8
Corn gluten feed	4.1	3.1	2.3
Distillers grains	3.8	4.9	5.0
Fescue hay	2.7	2.8	2.6
Rice mill	10.5	5.4	4.1
Soybean hulls	3.8	3.2	3.0
Wheat middlings	13.3	9.6	4.1
Fiber Sources			
Corn bran	1.6	1.5	1.6
Oat fiber (200)	1.1	1.2	1.1
Oat fiber (300)	2.3	2.4	2.3
Red wheat bran	8.8	7.5	2.7
Solka floc	4.4	4.3	4.1
White wheat bran	13.3	14.5	11.7

^aPotentially degradable fraction.

^bNeutral detergent fiber.

^cAcid detergent fiber.

Table 5. Effect of substrate on true *in vitro* DMD, gas production, rate of gas production, and partitioning factor

Item	<i>In vitro</i> parameter			
	DMD, mg	TDMD, mg ^e	Gas, mL	PF, mg/mL ^f
Alfalfa	290.38 ^{ab}	391.72 ^b	58.22 ^a	6.81 ^a
Corn gluten feed	343.50 ^{bc}	402.90 ^{bc}	75.29 ^b	5.37 ^b
Soybean hulls	379.18 ^c	446.93 ^c	113.33 ^c	3.97 ^d
Oat fiber 300	355.07 ^{bc}	408.32 ^{bc}	106.64 ^c	3.84 ^{cd}
Solka floc	268.10 ^a	315.65 ^a	82.69 ^b	3.55 ^c
Oat fiber/solka	325.72 ^{ab}	374.37 ^b	103.56 ^c	3.61 ^c
SEM ^g	19.16	18.38	4.31	0.13

^{a,b,c,d}Means in the same column with different superscript differ ($P < 0.05$).

^eBags rinsed with neutral detergent solution to remove microbial biomass.

^fPartitioning factor, mg substrate degraded/mL gas produced.

^gn = 3.

associated with oat fiber alone. Even though the oat fiber/solka contained 40% solka floc, gas production was just 3 mL less than oat fiber. The partitioning factor (mg substrate degraded/mL gas produced) was greatest for alfalfa hay followed by corn gluten feed. Oat fiber and soybean hulls had similar partitioning factor with oat fiber also being similar to the other purified substrates. The fast rate of gas production (RF) was greatest for oat fiber/solka and least for alfalfa and solka floc with corn gluten feed, soybean hulls, and oat fiber intermediate, but similar to the other products (Table 6). The slow rate of gas production (RS) was not different between substrates. The gas production model parameters were affected by run ($P < 0.05$) as run 2 was greater than run 1 and 3 for solka floc, oat fiber/solka, and soybean hulls while run 3 was greater than run 1 and 2 for oat fiber. With the high degree of sensitivity of the gas pressure sensors, great care is warranted to maintain consistency to minimize the run to run variation. Gas production profiles show an extended lag time for purified substrates (Fig. 1, 2, 3) compared to conventional feedstuffs (Fig. 4, 5, 6). There was a correlation ($P < 0.04$) between DMD and gas production as well as gas production and total VFA.

Total VFA and acetate concentrations tended to be affected by substrate ($P = 0.06$) with oat fiber, oat fiber/solka, solka floc, and soybean hulls having a greater concentration than both alfalfa hay and corn gluten feed (Table 7). Purified fiber substrates increased *in vitro* propionate concentrations with corn gluten feed and soybean hulls being intermediate. However, the acetate:propionate ratio was similar between purified fiber substrates and corn

Table 6. Model parameters describing the *in vitro* gas production profile of each sample

Item	Model Parameter ^c			
	RF	DF	RS	DS
Alfalfa	0.33 ^a	0.16 ^a	0.03	0.027 ^a
Corn gluten feed	0.65 ^{ab}	0.21 ^a	0.03	0.022 ^a
Soybean hulls	2.19 ^{ab}	0.53 ^b	0.06	0.027 ^a
Oat fiber 300	1.23 ^{ab}	0.13 ^a	0.04	0.024 ^a
Solka floc	0.48 ^a	0.06 ^a	0.02	0.009 ^b
Oat fiber/solka	3.23 ^b	0.14 ^a	0.08	0.026 ^a
SEM ^d	0.88	0.05	0.02	0.004

^{a,b}Means in the same column with different superscript differ ($P < 0.05$).

^cRF and RS = initial rate of gas production of fast and slowly fermented fraction, respectively; DF and DS = decay rate of gas production of fast and slowly fermented fraction, respectively.

^dn = 3.

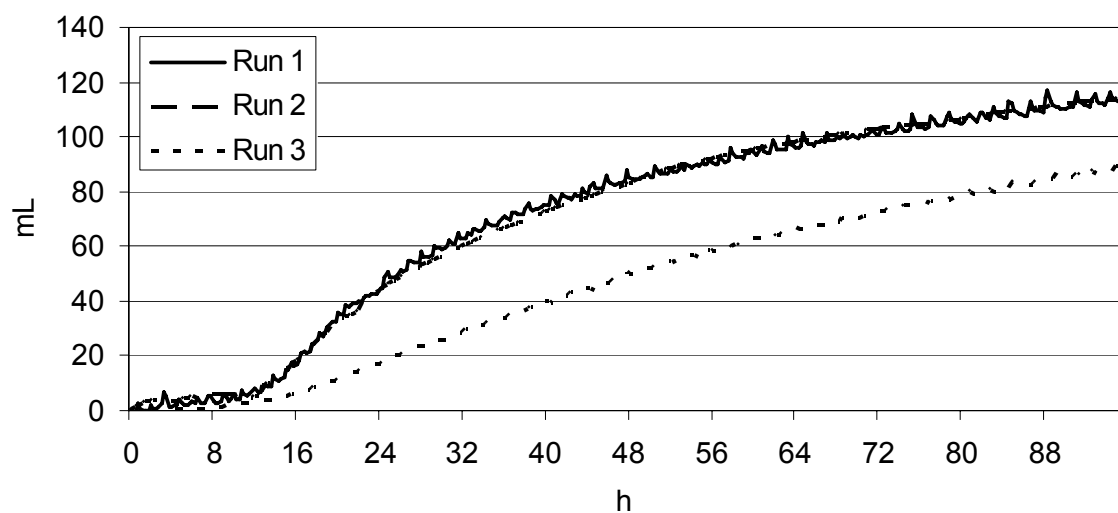


Figure 1. Gas production profile of oat fiber 300 (n=3) incubated over 96 h

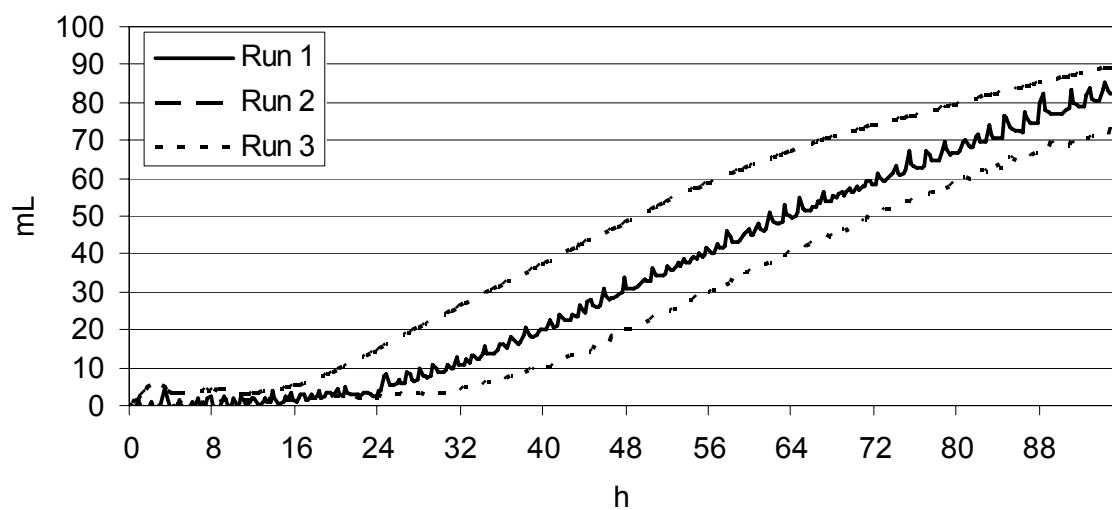


Figure 2. Gas production profile of solka floc (n=3) incubated over 96 h

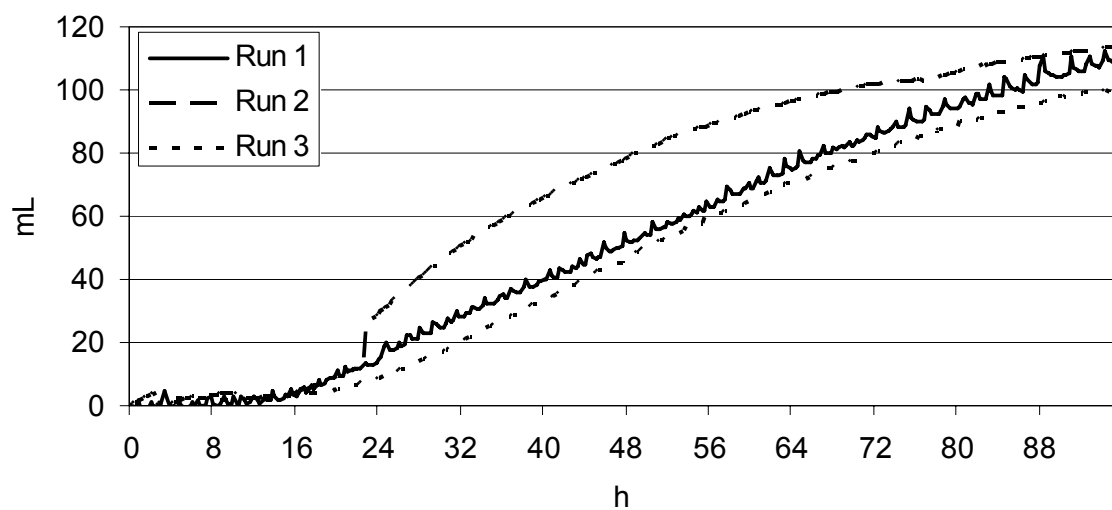


Figure 3. Gas production profile of 60% oat fiber:40% solka floc (n=3) incubated over 96 h

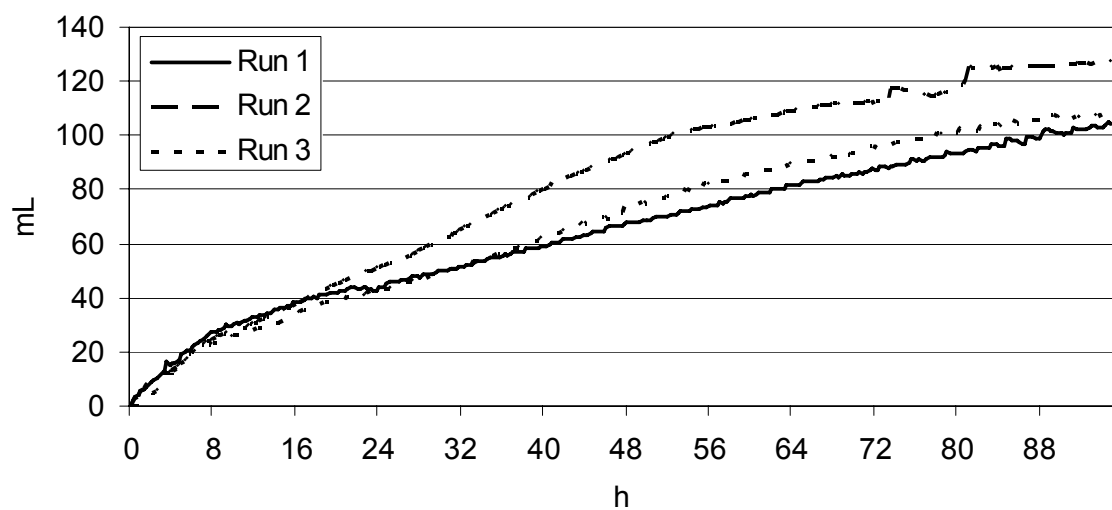


Figure 4. Gas production profile of soybean hulls (n=3) incubated over 96 h

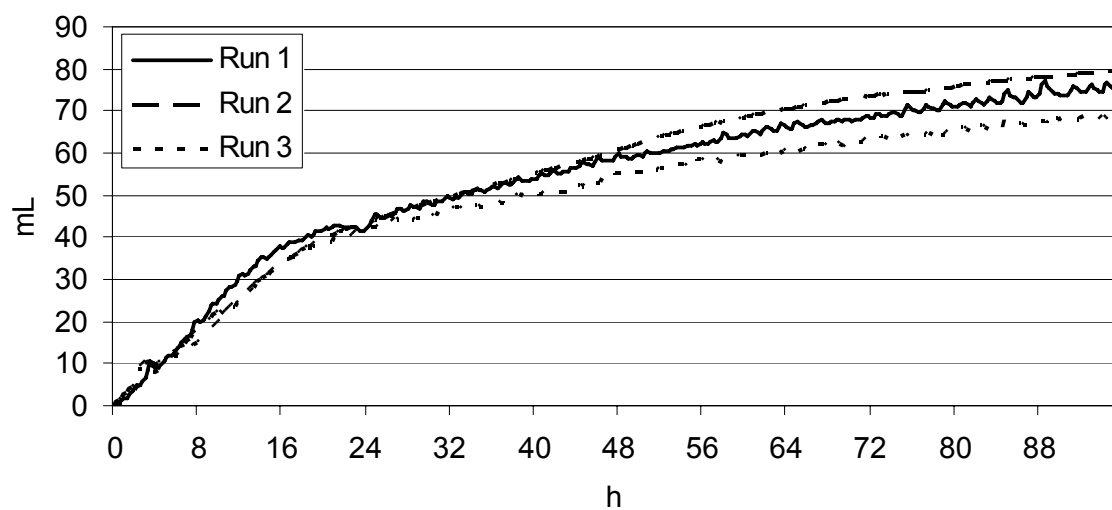


Figure 5. Gas production profile of corn gluten feed (n=3) incubated over 96 h

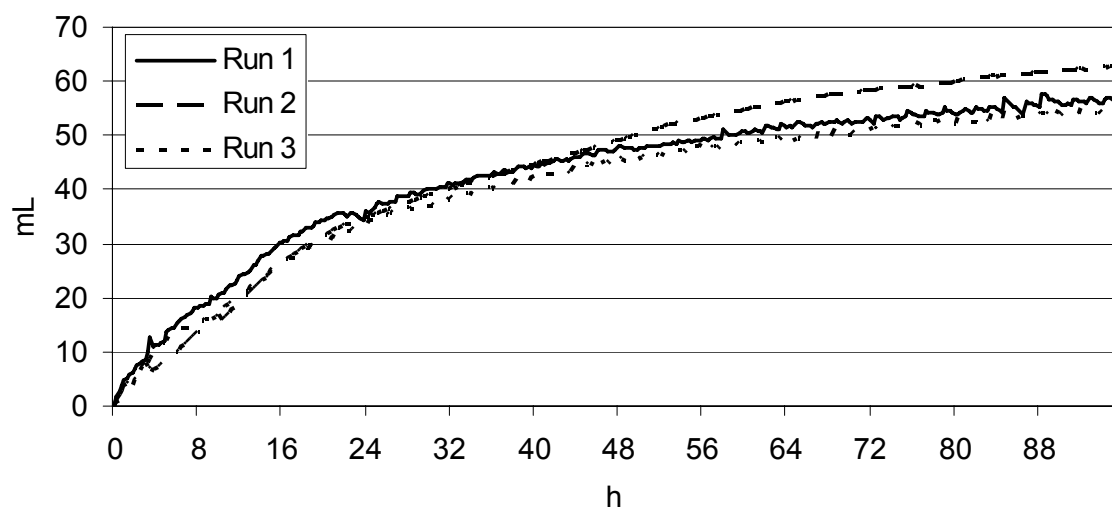


Figure 6. Gas production profile of alfalfa hay (n=3) incubated over 96 h

Table 7. Effect of substrate on *in vitro* VFA and ammonia-N concentration

Item	Alfalfa	Corn gluten feed	Soybean hulls	Oat fiber300	Solka floc	oat fiber/solka	SEM ^g
Acetate, mM	13.98 ^{ab}	11.26 ^a	16.28 ^{ab}	18.52 ^b	15.38 ^{ab}	15.83 ^{ab}	1.85
Propionate, mM	4.22 ^a	7.91 ^b	7.63 ^b	11.12 ^c	9.64 ^{bc}	10.81 ^c	0.91
Ac:Pr Ratio	3.30 ^a	1.41 ^b	2.13 ^d	1.65 ^c	1.59 ^{bc}	1.47 ^{dc}	0.07
Butyrate, mM	1.85	2.17	2.04	2.46	1.66	1.99	0.26
Valerate, mM	0.58 ^a	0.52 ^a	0.44 ^b	0.24 ^{b,e}	0.11 ^{b,f}	0.11 ^{b,f}	0.05
Isovalerate, mM	0.47 ^a	0.37 ^a	0.53 ^a	0.20 ^b	0.08 ^b	0.09 ^b	0.05
Isobutyrate, mM	0.28 ^a	0.17 ^b	0.13 ^{bc}	0.10 ^c	0.08 ^c	0.07 ^c	0.03
Total VFA, mM	21.40 ^a	22.41 ^a	27.04 ^{ab}	32.63 ^b	26.96 ^{ab}	28.89 ^{ab}	2.54
Ammonia-N, mg/dL	8.00 ^a	4.05 ^b	-0.40 ^c	-5.84 ^{d,e}	-6.19 ^d	-7.63 ^{d,f}	0.75

^{a,b,c,d}Means in the same row with different superscript differ ($P < 0.05$).

^{e,f}Means in the same row with different superscript differ ($P < 0.10$).

^gn=3.

gluten feed with alfalfa hay having the greatest ratio. Butyrate (Table 7) concentrations were not affected by type of substrate; however, *in vitro* valerate (Table 7) concentrations were decreased by incubation with purified fiber substrates. The concentration of isobutyrate and isovalerate were also reduced by purified fiber substrates, while, soybean hull isobutyrate concentrations were intermediate to oat fiber and corn gluten feed (Table 7). Substrate tended ($P < 0.10$) to affect ammonia-N concentration with the purified fiber substrates causing the greatest decrease followed by soybean hulls (Table 7). Alfalfa hay and corn gluten feed increased the *in vitro* concentration of ammonia-N with alfalfa causing the greatest increase.

Discussion

The *in situ* experiment was successful in determining potential purified fibers that would be adequate to simulate ruminal degradation kinetics of conventional by-products. A combination of solka floc and either oat fiber 200, oat fiber 300, or corn bran would be suitable in an effort to mimic the fiber degradation properties of some of the conventional feedstuffs used in this trial. Specifically, soybean hulls appears to be the feedstuff of choice in that values obtained describing the B-fraction, NDF and ADF degradation rate of soybean hulls lie between those values obtained for solka floc, oat fibers and corn bran. Corn gluten feed also possessed similar degradation kinetics as the purified fiber sources. Experimentally, oat fiber has been used to supplement beef cattle consuming forage based diets with apparent positive effects on N utilization (Driedger et al., 2000). The use of purified fiber sources provide researchers with

a source of fermentable organic matter without substantially increasing N availability allowing supplementation strategies to be examined without the inherent effect of supplemental degradable intake protein.

The differences in DMD, TDMD and total volume of gas produced resulted in distinct differences in the partitioning factor, particularly substrates containing a higher proportion of NDF tended to have a greater TDMD and gas production than those containing lower proportions of NDF. Getachew et al. (2004) and Iantcheva et al. (1999) reported a negative correlation between NDF and TDMD, however, the negative effect was much greater in grass hay compared to alfalfa. This phenomenon is probably related to feed quality and the proportion of fermentable fiber present. The use of NDF in predictive models is complicated by the fact that naturally occurring fiber is comprised of a mixture of components. For example, NDF is comprised of hemicellulose, cellulose, and lignin with each component degrading at different rates (Van Soest, 1982). Therefore, the use of NDF to describe degradability is dependent upon the proportion of these different components and their respective degradation rates.

The correlation between TDMD and gas production in the present study is similar to that reported by Apori et al. (1998) using browse leaves as the substrate. Conversely, Getachew et al. (2004) reported that TDMD and gas production were poorly correlated. This was probably a result of a greater proportion of CP and fat in their substrates as demonstrated by improved prediction of TDMD by the inclusion of the CP, fat, and non-fiber carbohydrate along with gas production in their prediction model. The greater partitioning factor

associated with alfalfa and corn gluten feed in the present study may be indicative of the CP of the substrate. It has been reported that the evolution of gas is reduced with fermentation of protein compared to carbohydrate (Menke and Steingass, 1988; Getachew et al., 1998).

Fondevila et al. (2002) reported similar rates of gas production for both starch and purified cellulose while pectin produced gas at an increased rate. Beuvink and Spoelstra (1992) observed a higher rate of gas evolution with rice starch compared to crystalline cellulose. Differences in rates of gas production between starch and cellulose seem to be largely dependent upon the structure of the cellulose potentially affecting ruminal availability. The increased rate of gas production with oat fiber compared to solka floc is probably related to the relative ruminal degradability of the cellulose and hemicellulose of the products. Gas production profiles of the conventional feedstuffs were relatively similar with only difference in rate of production being apparent. However, incubation of the purified fiber substrates produced gas production profiles with an extended lag time compared to conventional feedstuffs. The normal practice of filtering rumen fluid through several layers of cheesecloth to remove solid particles may be contributing to extended lag time in the purified fiber substrates due to fiber degrading microorganisms being typically attached to solid particles (Meyer and Mackie, 1986).

Fondevila et al. (2002) reported that purified cellulose incubation with straw increased total VFA and propionate production compared to rice starch incubation. Beuvink and Spoelstra (1992) also reported an increase in total VFA

and the proportion of propionate in *in vitro* fluid incubated with cellulose compared to starch. These findings support the changes in *in vitro* VFA concentrations reported in this experiment. However, unlike our findings, Getachew et al. (2004) reported an increase in the Ac:Pr ratio as the proportion of digestible NDF increased. The change in concentrations of valerate, isovalerate, and isobutyrate can be expected as the primary origins of these VFA are from the fermentation of protein (Allison, 1962). Therefore, the decline in concentration of these VFA with purified fiber substrates can be explained by the CP content of these products being low (< 0.50% CP). Also, net uptake of ammonia-N from the inoculum associated with the purified fiber substrate could also be expected since a substrate source of significant N was not provided in the system, therefore, the only N being added to the system would be of microbial origin.

It should be noted that the fermentation vessels were not fitted with a means of purging accumulated gas over the 96 h incubations. Therefore, it is possible that our results may have been affected by this increase in gas pressure. However, Theodorou et al. (1994) reported that *in vitro* fermentation is not affected until head space gas pressure exceeds 7.0 psi. Using the Ideal Gas Law equation, it was determined that only soybean hulls, oat fiber, and oat fiber/solka floc mix resulted in gas pressures of greater than 7.0 psi, however, this level was not exceeded until after approximately 56, 52, and 56 h of fermentation, respectively, at which time extent of digestion should have been attained.

Implications

Purified fiber substrates are viable options for incorporation into research protocols designed to increase ruminally available energy without significantly increasing N availability. With the growing demand to increase nutrient utilization and decrease N pollution, such products may be useful in targeting animal requirements and nutrient supply, especially in production scenarios where N availability exceeds the energetic ability of microorganism utilization. Also, the descriptions made of ruminal degradation characteristics of the by-products may be useful in the development of other supplementation strategies to optimize nutrient utilization.

PART 3

SUPPLEMENTATION OF PURIFIED FIBER TO STEERS AND LAMBS CONSUMING TALL FESCUE HAY: EFFECTS ON INTAKE, SITE, AND EXTENT OF NUTRIENT DIGESTION IN STEERS AND NITROGEN BALANCE IN LAMBS

Abstract

Six ruminally and duodenally cannulated steers and 6 wether lambs were arranged in a replicated 3 x 3 Latin square. Steers and lambs had free choice access to tall fescue hay and supplemented with purified fiber (60% solka floc, 40% oat fiber) at 0, 0.25 or 0.50% of BW prior to AM hay feeding. Periods were 18 d for steers and 17 d for lambs. On d 7 to 17 steers were intra-ruminally dosed with Cr_2O_3 followed by total fecal collection from d 12 to 17 and duodenal digesta sampling at 10 hr intervals on d 13 to 17 arranged to provide sample representing every other hour over a 24 h period. Steers were intra-ruminally pulse dosed with Co-EDTA on d 17 and rumen fluid sampled at 0, 3, 6, 9, 12, and 24 h post dosing for Co, VFA, and $\text{NH}_3\text{-N}$ content. On d 18 ruminal contents were evacuated, weighed and sub-samples retained for bacterial separation. On d 12 to 17 total fecal and urine collections were conducted with sub-samples retained. In steers, forage intake and total N intake were not affected by supplementation. Total intake of DM, NDF, ADF and OM was increased ($P < 0.05$) with increased supplementation. Total tract DM, NDF, ADF and OM digestion were increased ($P < 0.05$) by supplementation. Ruminal and total tract nitrogen digestion was not affected by supplementation. Total N flow at the duodenum was increased by supplementation. Ruminal acetate concentration tended to increase, butyrate increased, and propionate was not affected by supplementation. Ruminal pH increased with supplementation. Ruminal $\text{NH}_3\text{-N}$ concentrations decreased with supplementation, but a treatment x time interaction existed due to $\text{NH}_3\text{-N}$ remaining constant throughout the 24 hr period.

in steers not supplemented. In lambs, forage intake was decreased by supplementation and total OM intake increased. Organic matter, NDF, and ADF digested total tract was increased with supplementation. Total tract N digestion decreased, however, retention was not affected by supplementation. These data indicate that in both steers and lambs supplementing tall fescue hay diets with digestible fiber can increase ruminal and total tract fiber digestion without affecting forage intake or digestion of nitrogen while also increasing ruminally available energy.

Key Words: Supplements, Digestion, Tall fescue

Introduction

In the ruminant animal, energy demands for microbial and animal production are predominantly derived from microbial degradation of digestible OM. Increasing ruminally available OM can increase duodenal amino acid flow by enhancing microbial protein synthesis (Elizalde et al., 1998). This is due to fermentable OM availability being a major determinant of microbial protein synthesis (Dijkstra et al., 1998). Grain is a common source of highly fermentable DM, however, grains contain high amounts of starch which may negatively effect ruminal fiber digestion (Mertens and Loften, 1980) and forage intake (Pordomingo et al., 1991) potentially decreasing DE intake. Rapid fermentation of starch often exceeds the buffering ability of the rumen to maintain a stable pH, with low ruminal pH causing impaired function of cellulolytic bacteria, reducing the capacity for fiber digestion (Orskov and Fraser, 1975).

By-product feedstuffs offer alternatives to grain by providing energy predominantly in the form of degradable fiber therefore limiting negative effects on forage fiber digestion (Cordes et al., 1988). Steers consuming alfalfa haylage and supplemented with corn gluten feed required 2 h less for ruminal pH to recover to initial values and maintained a higher pH compared to corn supplemented steers (Hannah et al., 1990). Furthermore, steers consuming orchardgrass hay supplemented with soybean hulls and corn had similar total ruminal VFA, acetate and propionate concentrations (Galloway et al., 1993).

Energy supplementation has the potential to enhance N utilization. Furthermore, the use of high fiber energy supplements may limit the reduction in forage fiber digestion often associated with starch supplementation. Therefore, these experiments were conducted to evaluate the effects of supplemental purified fiber on ruminal parameters, site and extent of nutrient digestion in steers and nutrient digestion and nitrogen balance in lambs consuming tall fescue hay.

Material and Methods

Animals and Diets. Six steers (402.7 ± 5.5 kg initial BW), previously fitted with a ruminal cannula and a double-L shaped duodenal cannula (Streeter et al., 1991) placed 10 cm distal to the pylorus, maintained at the Johnson Animal Research and Teaching Unit at the University of Tennessee were used in a replicated 3 x 3 Latin square experiment. All surgical and animal care procedures were approved by the University of Tennessee Animal Care and Use Committee. Steers were either housed in individual pens or metabolism crates within a temperature controlled room (23°C) with 16-h of light and 8-h of dark.

Steers had free choice access to water and tall fescue hay provided in equal portions at 0500 h and 1700 h. Hay was provided at a rate of 105% of the average intake for the previous 5-d. Feed refusals were determined daily prior to the morning feeding. Additionally, steers received one of three treatments consisting of: supplementation at 0% (Control), 0.25% or 0.50% of BW (DM basis) with purified fiber. The purified fiber supplement consisted of solka floc and Canadian Harvest[®] oat fiber 300 (Opta Food Ingredients, Inc., Bedford, MA; 40:60%). This combination was chosen based on the results of an *in situ* experiment to replicate the NDF and ADF content, A and B carbohydrate fraction content and degradation rate of soybean hulls fed at a level of 0.33% and 0.66% of BW (DM basis), respectively. Individual BW was determined at the initiation of each period to calculate the amount of supplement to be provided. Fiber supplement was hydrated for approximately 2 min under continuous mixing using a fine mist sprayer connected to a garden hose and provided once daily in the morning, approximately 30 min prior to the feeding of hay. Any supplement not consumed at the time of hay feeding was placed directly into the rumen of the respective animal. Supplement was supplied at a rate of 33% (d 1 and 2) and 66% (d 3 and 4) of the total amount over the first 4-d of each experimental period to aid in adaptation. A vitamin and mineral mix (CO-OP All Purpose Cattle Mineral 650; max 27% Ca, min 4.5% P, max 19.2% salt, min 0.3% Mg, 0.1% K, min 24 ppm Co, min 1000 ppm Cu, min 36 ppm I, min 3000 ppm Mn, min 20 ppm Se, min 3500 ppm Zn, min 29,200 IU/kg Vit. A, min 99,225 IU/kg Vit. D, min 309 IU/kg Vit. E) was provided at a rate of 90.7 g•hd⁻¹•d⁻¹ to meet or exceed the

requirements (NRC, 1996) of beef cattle as part of the purified fiber supplement or in a loose form to those animals not receiving supplement.

Sampling. Experimental periods consisted of 18 d with 7 d of diet adaptation. Animals were maintained in individual pens from d 1 to 5 before being moved to metabolism crates on d 6. Gelatin capsules (Torpac capsules, size #07; Torpac Inc., Fairfield, NJ) containing 9 g of chromic oxide were intra-ruminally dosed at 0500 and 1700 on d 7 to 17 for use as an indigestible marker of digesta flow. Samples of hay, supplement, and orts were obtained on d 9 to 14. Grab samples of hay were collected from 4 separate locations from each square bale fed. Hay samples were equally composited by period after drying and grinding. On d 13 to 17, collection of approximately 200 g of duodenal digesta was conducted every 10 h, modified from Sackman et al. (2003), to represent every other hour over a 24-h period. From each sampling time, a 125 g sub-sample of duodenal digesta was composited by steer within period and stored (-20°C) for later analysis. Feces were collected twice daily on d 12 to 17. Total feces collected were weighed, manually mixed and a 2.5% sub-sample obtained and composited by steer within period and stored (-20°C) for later analysis.

On d 17, each steer was intra-ruminally pulse dosed with 12.5 g of Co-EDTA in a 100 mL aqueous solution (Uden et al., 1980). Administration of the Co-EDTA marker into the rumen was conducted by placement of a polypropylene tube connected to a stainless steel funnel through the rumen cannula into the ventral rumen. As marker was poured through the funnel, the tube was placed

into different locations within the rumen to facilitate mixing. Rumen fluid (approximately 100-mL) was collected by suction strainer from multiple locations within the ventral rumen (Raun and Burroughs, 1962) prior to dosing and at 3, 6, 9, 12 and 24-h post-dosing. Rumen fluid pH was determined immediately after collection (Accumet Basic AB15 pH Meter, Fisher Scientific). Twenty milliliters of rumen fluid was stored (-20°C) for later analysis of Co and a 5 mL sample acidified with 1-mL of 25% meta-phosphoric acid and stored (-20°C) for subsequent analysis of ammonia concentration. A separate 5-mL sample was obtained and stored (-20°C) for determination of ruminal VFA concentrations.

On d 18, rumen contents were manually evacuated from the reticulo-rumen of each steer 4-h after the morning feeding (Lesperance et al., 1960). Total rumen contents were weighed, manually mixed and triplicate sub-samples of approximately 400 g collected. An additional 2-kg sample was weighed into a container containing 2-L of cold (4°C) 0.9% NaCl. Remaining rumen contents were immediately placed back into the animal.

Chemical Analysis and Calculations. Hay, supplement, and orts samples were weighed, dried (55°C), re-weighed, and ground to pass a 1-mm screen in a Wiley Mill (Arthur H Thomas Company, Philadelphia, PA). Samples of purified fiber were not ground due to particle size being less than 1 mm in the original product. Feed samples were analyzed for DM, OM (AOAC, 2000), NDF (without sodium sulfite) and ADF (Ankom Technologies, Fairport, NY), and N via a combustion analyzer (AOAC, 2002). Duodenal composites were weighed, lyophilized, and re-weighed for determination of DM and ground through a 0.84-

mm screen using an Intermediate Wiley Mill due to limited sample size. Duodenal samples were analyzed for DM, OM, NDF (without sodium sulfite), ADF, N, Cr (Williams et al., 1962) and purine content (Zinn and Owens, 1986) using 2.0 M perchloric acid (Makkar and Becker, 1999) and centrifuged as described by Obispo and Dehority (1999). Duodenal analysis was used for determination of ruminal DM, OM, N, and fiber digestion, as well as total duodenal and bacterial N flow. Fecal composites were weighed, lyophilized, and reweighed for DM determination and ground to pass a 1-mm screen in a Wiley Mill. Fecal samples were analyzed for DM, OM, NDF (without sodium sulfite), ADF, N and Cr for determination of total tract DM, OM, N, NDF, and ADF disappearance. Digesta flow and extent of ruminal digestion of DM, OM, N, NDF and ADF was calculated using marker (Cr):nutrient ratios in duodenal samples (Hsu et al., 1987). Fecal Cr analysis was used to determine Cr recovery for validation of duodenal Cr measurements.

Frozen rumen fluid samples were thawed, centrifuged (15,000 x g; 10 min) and the supernatant collected for subsequent analysis of VFA concentration (Erwin et al., 1961) and ammonia-N concentration (Broderick and Kang, 1980). The VFA analysis was conducted using gas chromatography (Shimadzu GC-2010, column HP-FFAP 10 m x 0.53 mm x 1.0 μ m, Shimadzu Scientific Instruments, Columbia, MD) utilizing helium as the carrier gas (100 ml/min) and a flame ionization detector (compressed air at 350 – 380 ml/min, hydrogen at 30 – 35 ml/min). Cobalt concentration was determined (AOAC, 2000) by atomic

absorption using an air/acetylene flame (Unicam 969 AA Spectrometer; TJA Unicam, Cambridge, United Kingdom).

Ruminal fermentation balance was calculated using the molar proportions of acetate, propionate, and butyrate as described by Church (1988) and the data presented in Appendix A. Ruminal fluid kinetics were calculated from the regression of the natural logarithm of Co concentration on time after dosing (Uden et al., 1980). The absolute value of the slope defined as the fluid dilution rate. Fluid volume was determined by dividing the Co dose by the antilog of the marker concentration at time zero. Rate of fluid outflow was determined by dividing the fluid volume by the fluid dilution rate and rumen turnover time was calculated as the reciprocal of fluid dilution rate.

Rumen samples were weighed, dried in a forced-air oven (55°C, 96 h), and reweighed. Rumen samples were used to determine ruminal DM content. After chilling for 24 h, the 2-kg sample of rumen contents was manually mixed and homogenized in a Waring blender at high speed for 1 min and strained through four layers of cheesecloth. Bacteria were separated from protozoa and feed particles by centrifugation (800 x g; 20 min). The supernatant was decanted into 250-mL bottles and centrifuged (18,000 x g; 20 min; 4°C) to pellet bacteria and the supernatant discarded. The pellet was re-suspended using purified water and centrifuged (18,000 x g; 20 min; 4°C). This step was repeated three times and the bacteria frozen (-20°C). Bacteria were lyophilized before grinding with a

mortar and pestle. Bacteria were analyzed for purine and N content to determine nitrogen:purine ratios for estimation of duodenal bacterial N flow.

Statistical Analysis. Intake, nutrient flow, disappearance, and fluid kinetics data were analyzed as a replicated 3 x 3 Latin Square design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model included square, period(square), steer(square) and treatment. Linear and quadratic orthogonal contrasts were used to separate treatment means when a significant F-test was detected ($P < 0.05$). Statistical analysis of VFA, ammonia-N, and pH data were conducted using a repeated measures treatment design with the addition of the variable time and treatment x time interaction to the model. Linear and quadratic orthogonal contrasts were used to separate treatment means when a significant F-test was detected ($P < 0.05$). The LSD mean separation procedure of SAS was used for comparisons over time.

Lamb Nitrogen Balance. Six lambs (27.4 ± 0.6 kg initial BW) housed at the Johnson Animal Research and Teaching Unit at the University of Tennessee were used in a replicated 3 x 3 Latin square experiment. The University of Tennessee Animal Care and Use Committee approved all animal care procedures. Animals were housed in individual pens and then metabolism crates within an environmentally controlled room (23°C) with 16 h of light and 8 h of dark.

Animals had free choice access to water and tall fescue hay provided in equal portions at 0800 and 1700 h. Hay was provided at a rate of 110% of the average intake for the previous 5 d. Feed refusals were determined daily prior to

the morning feeding. Animals received one of three treatments consisting of: supplementation at 0% (Control), 0.25%, or 0.50 of BW with purified fiber mix. The basis and formulation of the purified fiber supplement provided was as described in the steer experiment. Body weight was determined prior to the initiation of each experimental period for calculation of amount of supplement to be provided. Supplements were fed in a pelleted form in an effort to enhance palatability and were fed once daily approximately 30 min prior to hay feeding. A complete vitamin/mineral supplement (CO-OP Sheep/Goat Mineral W/Zinpro #683; max 10.8% Ca, min 4.5% P, min 1.50% Mg, min 0.90% K, min 0.30% S, min 1.55% Zn, min 180 ppm I, min 2000 ppm Fe, min 4000 ppm Mn, min 60 ppm Se, min 2,205,000 IU/kg Vit. A, min 165,375 IU/kg Vit D, min 6,615 IU/kg Vit. E) was provided at a rate of $8.6 \text{ g} \cdot \text{hd}^{-1} \cdot \text{d}^{-1}$ to meet or exceed the daily requirements of growing lambs (NRC, 1985).

Experimental periods consisted of 17 d, with 9 d of diet adaptation. On d 10 to 15, samples of hay, supplement, and orts were collected and analyzed as described previously in the steer trial. On d 12 to 17, feces and urine were collected once daily. Total feces collected were weighed and a 5% sub-sample obtained and composited by animal within period. Fecal composite samples were weighed, lyophilized, re-weighed and ground to pass a 1-mm screen and analyzed for DM, OM, NDF (without sodium sulfite), ADF and N content. Urine was collected into 100-mL of 6 M sulfuric acid (Maas et al., 2001) and total urinary output determined using a graduated cylinder. Daily, a 5% subsample of

urine was retained and composited by animal within period and frozen until later analysis of N content.

Intake, nutrient disappearance, and N retention data were analyzed as a replicated 3 x 3 Latin Square design using the MIXED procedure of SAS. The statistical model included square, period(square), lamb(square) and treatment. Linear and quadratic orthogonal contrasts were used to separate treatment means when a significant ($P < 0.05$) F-test was detected.

Results

Steer Digestibility. The nutrient composition of hay and supplement, described in Table 8, were similar between experimental periods. Consumption of forage DM and OM was not affected by supplementation (Table 9 and 10), however, total DM (linear, $P = 0.01$; quadratic, $P = 0.06$) and OM (linear, $P = 0.01$; quadratic, $P = 0.05$) intake increased with increasing level of supplementation. Forage NDF and ADF intake were not affected by supplementation (Table 11 and 12), however, total NDF (linear, $P = 0.01$; quadratic, $P = 0.11$) and ADF (linear, $P = 0.01$; quadratic, $P = 0.07$) intake were increased with supplementation. The observed quadratic responses appear to be an artifact of the slight differences in forage intake between levels of supplementation. Forage and total N intake were not affected by supplementation (Table 13).

The apparent quantity of DM and OM (Table 9 and 10) disappearing ruminally increased (linear, $P = 0.01$; quadratic, $P < 0.14$) with increasing level of

Table 8. Nutrient composition^a of tall fescue grass hay and supplement fed to steers and lambs

Item	Steer	Lambs
	% of DM	
Tall fescue		
NDF ^b	68.5	67.1
ADF ^c	37.6	37.0
CP ^d	9.1	9.6
Ash	6.2	6.0
Supplement		
NDF	70.5	66.0
ADF	49.3	45.8
CP	0.3	0.3
Ash	1.7	1.6

^aBased on chemical analysis.

^bNeutral detergent fiber.

^cAcid detergent fiber.

^dCrude protein

Table 9. Apparent ruminal, intestinal, and total tract disappearance of dry matter (DM) by steers consuming tall fescue grass hay and supplemented with purified fiber

Item	Supplement, % of BW			SEM ^a	Probability	
	0	0.25	0.50		Linear	Quadratic
Intake, g/d						
Forage DM	6670	6769	6487	91	0.21	0.14
Total DM	6670	7821	8597	63	0.01	0.06
Duod flow, g/d ^b	4085	4248	4702	103	0.01	0.30
Fecal output, g/d	2395	2514	2763	86	0.03	0.57
DM disappearance, g/d						
Ruminal	2586	3573	3894	156	0.01	0.14
Intestinal	1689	1817	1940	95	0.12	0.99
Total tract	4275	5390	5834	109	0.01	0.05
DM disappearance, %						
Ruminal	38.82	45.76	45.38	1.55	0.03	0.11
Intestinal ^c	41.33	40.98	41.15	2.08	0.96	0.93
Total tract	64.03	68.91	67.93	0.95	0.03	0.05

^an=6.

^bDuodenal flow.

^cPercent of duodenal dry matter flow.

Table 10. Apparent ruminal, intestinal, and total tract disappearance of organic matter (OM) by steers consuming tall fescue grass hay and supplemented with purified fiber

	Supplement, % of BW				Probability	
Item	0	0.25	0.50	SEM ^a	Linear	Quadratic
Intake, g/d						
Forage OM	6258	6347	6083	85	0.20	0.15
Total OM	6258	7380	8155	58	0.01	0.05
Duod flow, g/d ^b	3281	3430	3834	77	0.01	0.23
Fecal output, g/d	2208	2307	2549	78	0.03	0.49
OM disappearance, g/d						
Ruminal	2976	3950	4320	126	0.01	0.10
Intestinal	1073	1123	1285	90	0.15	0.63
Total tract	4050	5073	5606	115	0.01	0.14
OM disappearance, %						
Ruminal	47.59	53.56	53.05	1.26	0.03	0.09
Intestinal ^c	32.81	32.94	33.47	2.35	0.85	0.95
Total tract	64.67	68.83	68.81	1.23	0.06	0.22

^an=6.

^bDuodenal flow.

^cPercent of duodenal organic matter flow.

Table 11. Apparent ruminal, intestinal, and total tract disappearance of neutral detergent fiber (NDF) by steers consuming tall fescue grass hay and supplemented with purified fiber

	Supplement, % of BW				Probability	
Item	0	0.25	0.50	SEM ^a	Linear	Quadratic
Intake, g/d						
Forage NDF	4596	4631	4431	70	0.15	0.22
Total NDF	4596	5373	5919	49	0.01	0.11
Duod flow, g/d ^b	1670	1756	2075	44	0.01	0.08
Fecal output, g/d	1526	1606	1817	58	0.02	0.40
NDF disappearance, g/d						
Ruminal	2926	3617	3843	76	0.01	0.05
Intestinal	144	150	259	44	0.12	0.38
Total tract	3070	3767	4102	84	0.01	0.14
NDF disappearance, %						
Ruminal	63.59	67.33	64.93	0.94	0.36	0.04
Intestinal	7.59	8.06	12.31	2.58	0.25	0.57
Total tract	66.70	70.19	69.38	1.23	0.18	0.21

^an=6.

^bDuodenal flow.

^cPercent of duodenal neutral detergent fiber flow.

Table 12. Apparent ruminal, intestinal, and total tract disappearance of acid detergent fiber (ADF) by steers consuming tall fescue grass hay and supplemented with purified fiber

	Supplement, % of BW				Probability	
Item	0	0.25	0.50	SEM ^a	Linear	Quadratic
Intake, g/d						
Forage ADF	2504	2535	2420	39	0.18	0.18
Total ADF	2504	3055	3462	26	0.01	0.07
Duod flow, g/d ^b	1091	1143	1403	32	0.01	0.04
Fecal output, g/d	913	969	1149	41	0.01	0.27
ADF disappearance, g/d						
Ruminal	1413	1912	2060	55	0.01	0.04
Intestinal	179	174	254	33	0.17	0.35
Total tract	1592	2086	2313	57	0.01	0.11
ADF disappearance, %						
Ruminal	56.42	62.61	59.60	1.26	0.13	0.03
Intestinal ^c	16.09	15.17	17.62	2.79	0.72	0.64
Total tract	63.59	68.43	66.91	1.58	0.19	0.16

^an=6.

^bDuodenal flow.

^cPercent of duodenal acid detergent flow.

Table 13. True and apparent ruminal disappearance, and apparent intestinal and total tract disappearance of nitrogen (N) by steers consuming tall fescue grass hay and supplemented with purified fiber

	Supplement, % of BW				Probability	
Item	0	0.25	0.50	SEM ^a	Linear	Quadratic
Intake, g/d						
Forage N	96.49	98.84	95.61	1.14	0.61	0.10
Total N	96.49	99.47	96.87	1.11	0.82	0.09
Bacterial N:purine	1.38	1.35	1.46	0.08	0.49	0.49
Duod N flow, g/d ^b	118.46	119.68	121.17	3.31	0.59	0.98
Bacterial N	96.77	95.88	84.73	7.50	0.30	0.60
Nonbacterial N	21.70	23.80	36.44	7.51	0.22	0.59
Fecal output, g/d	40.69	42.81	42.19	1.70	0.56	0.54
N disappearance, g/d						
Ruminal						
Apparent	-21.97	-20.21	-24.30	3.51	0.66	0.53
True ^c	74.79	75.67	60.43	7.30	0.22	0.41
Intestinal	77.77	76.87	78.97	2.75	0.77	0.68
Total tract	55.80	56.66	54.68	2.01	0.71	0.59
N disappearance, %						
Ruminal						
Apparent	-22.78	-20.48	-25.38	3.84	0.65	0.48
True ^c	77.25	77.48	61.19	7.72	0.20	0.42
Intestinal ^d	65.49	64.33	64.94	1.17	0.76	0.57
Total tract	57.72	57.02	56.25	1.73	0.58	0.99
MOEFF ^e , g/kg	32.88	24.52	19.94	2.00	0.01	0.47

^an=6.

^bDuodenal flow.

^cCorrected for microbial nitrogen flow.

^dPercent of duodenal nitrogen flow.

^eMicrobial efficiency, g microbial crude protein/kg OM apparently fermented.

supplementation. Ruminal (linear, $P = 0.03$; quadratic, $P = 0.09$) disappearance of DM and OM as a percentage of intake was also increased (linear, $P = 0.03$; quadratic, $P < 0.11$). Intestinal DM and OM disappearance was not affected ($P > 0.11$) by supplementation, however, total tract disappearance expressed quantitatively or as percent of intake was increased (linear, $P = 0.01$; quadratic, $P = 0.05$; linear, $P = 0.01$; quadratic, $P = 0.14$, respectively) with supplementation. Total tract DM disappearance as a percent of intake was also increased (linear, $P = 0.03$; quadratic, $P = 0.05$) and OM disappearance tended (linear, $P = 0.06$; quadratic, $P = 0.14$) to increase with supplementation.

The quantity of NDF (Table 11) disappearing ruminally (linear, $P < 0.01$; quadratic $P = 0.05$) and total tract (linear, $P < 0.01$; quadratic $P = 0.14$) was increased with increasing level of supplementation. The quantity of ADF disappearing from the intestine was not affected by supplementation. The percentage of NDF digested ruminally was increased (linear, $P = 0.36$; quadratic, $P = 0.04$) with increasing level of supplementation while intestinal and total tract disappearance was not affected by supplementation. The quantity of ADF disappearing ruminally (linear, $P = 0.01$; quadratic, $P = 0.04$) and total tract (linear, $P = 0.01$; quadratic, $P = 0.11$) were increased with supplementation while intestinal disappearance was not affected (Table 12). Ruminal disappearance as a percent of intake increased (linear, $P = 0.13$; quadratic, $P = 0.03$) while intestinal and total tract disappearance were not affected by supplementation.

Duodenal flow of N was not affected by supplementation (Table 13). Flow of bacterial N and non-bacterial N were also not affected by supplementation.

Apparent disappearance of N, expressed as quantity or percent of intake, from the rumen, intestine, and total tract were not affected by supplementation. True ruminal N digestion was also not affected by supplementation.

Total ruminal VFA and propionate concentrations were not affected by supplementation or time (Table 14). The concentration of acetate tended to increase (linear, $P = 0.07$; quadratic, $P = 0.89$) while the ratio of acetate:propionate increased (linear, $P = 0.01$; quadratic, $P = 0.60$) with supplementation. There tended to be a supplement x time interaction ($P = 0.08$) for the ratio of acetate:propionate. The acetate:propionate ratio of steers supplemented at 0% or 0.25% of BW exhibited a decline between 9 and 12 h post supplementation followed by an increase in 0.25% supplemented steers from 12 to 24 h while steers supplemented at 0.50% increased slightly from 9 to 24 hr (Figure 7). Ruminal butyrate concentration was increased (linear, $P = 0.01$; quadratic, $P = 0.40$) with supplementation. Butyrate was also affected by time ($P < 0.01$) as the concentration increased through 6 to 9 h post supplementation before declining to initial values by 24 h (Figure 8).

Ruminal concentration of valerate decreased (linear, $P = 0.01$; quadratic, $P = 0.22$) with increasing level of supplementation. Concentrations of isovalerate and isobutyrate were reduced (linear, $P = 0.01$; quadratic, $P < 0.03$) by supplementation (Table 14). There was a supplementation x time interaction ($P = 0.02$) for ruminal isovalerate concentration (Figure 9). The 0.25% and 0.50% of BW level of supplementation resulted in a dramatic decrease in concentration through 6 h post supplementation while the concentration remained relatively

Table 14. Ruminal VFA concentration and molar proportion of steers consuming tall fescue grass hay and supplemented with purified fiber

	Supplement, % of BW				Probability	
Item	0	0.25	0.50	SEM ^a	Linear	Quadratic
VFA, mM						
Acetate	59.88	61.16	62.83	1.13	0.07	0.89
Propionate	15.58	15.49	15.35	0.30	0.58	0.95
Butyrate ^{**}	7.22	7.83	8.77	0.16	0.01	0.40
Isobutyrate ^{**}	0.66	0.54	0.53	0.02	0.01	0.03
Valerate	0.71	0.59	0.52	0.02	0.01	0.22
Isovalerate ^{**,†}	0.86	0.62	0.49	0.02	0.01	0.02
Total	84.92	86.22	88.49	1.56	0.11	0.81
VFA Eff, mM/kg ^{b,**}	28.83	21.90	20.62	1.20	0.01	0.11
Acetate:propionate ^{**,†}	3.84	3.96	4.13	0.03	0.01	0.60
VFA, molar %						
Acetate	70.38	71.00	71.08	0.17	0.01	0.20
Propionate ^{***,†}	18.36	17.96	17.29	0.11	0.01	0.29
Butyrate ^{**}	8.60	9.00	9.88	0.11	0.01	0.07
Isobutyrate ^{**}	0.80	0.64	0.61	0.03	0.01	0.05
Valerate	0.84	0.68	0.59	0.01	0.01	0.09
Isovalerate ^{***,†}	1.02	0.72	0.57	0.03	0.01	0.02

^an=6.

^bEfficiency of VFA production, total VFA produced per kg OM disappearance.

^{**}Time effect ($P < 0.01$).

^{*}Treatment x time interaction ($P < 0.05$).

[†]Treatment x time interaction ($P < 0.10$).

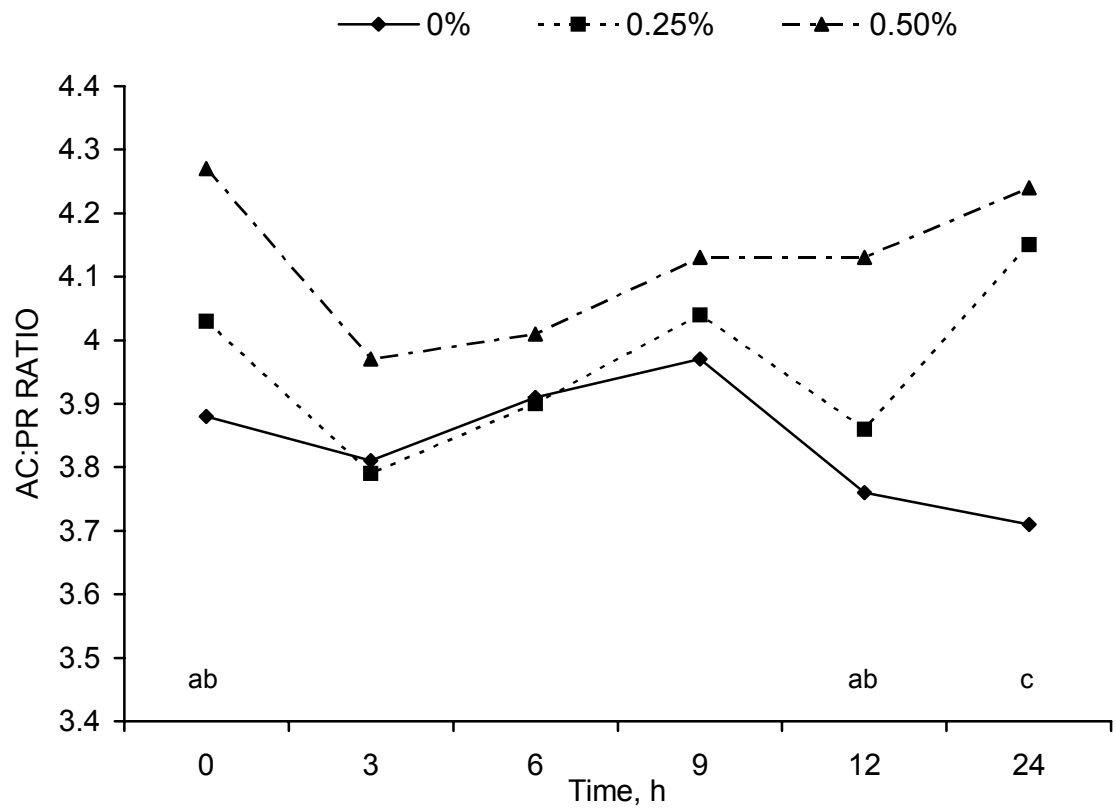


Figure 7. Influence of supplemental fiber at 0%, 0.25%, and 0.50% of BW on ruminal acetate:propionate ratio over time. (SEM = 0.08; n = 6). ^a0% vs 0.50%; $P < 0.05$, ^b0.25% vs 0.50%; $P < 0.05$, ^c0% vs 0.25% and 0.50%; $P < 0.05$ are denoted along the x-axis.

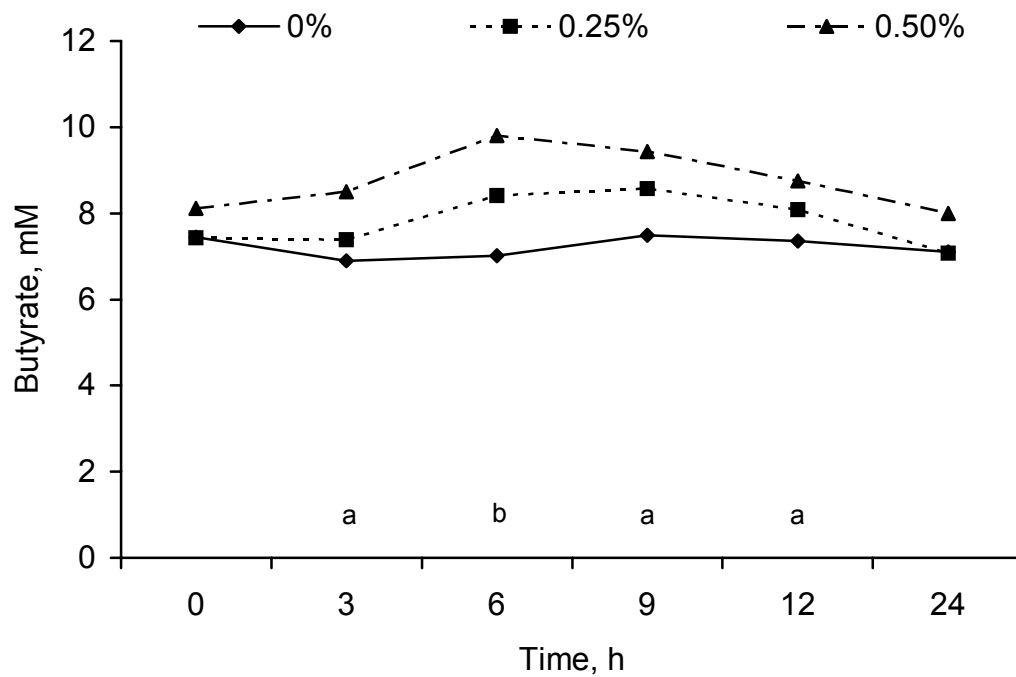


Figure 8. Influence of supplemental fiber at 0%, 0.25%, and 0.50% of BW on ruminal butyrate concentration over time. (SEM = 0.39; n = 6). ^a0% vs 0.50%; $P < 0.05$, ^b0% vs 0.25% vs 0.50%; $P < 0.05$ are denoted along the x-axis.

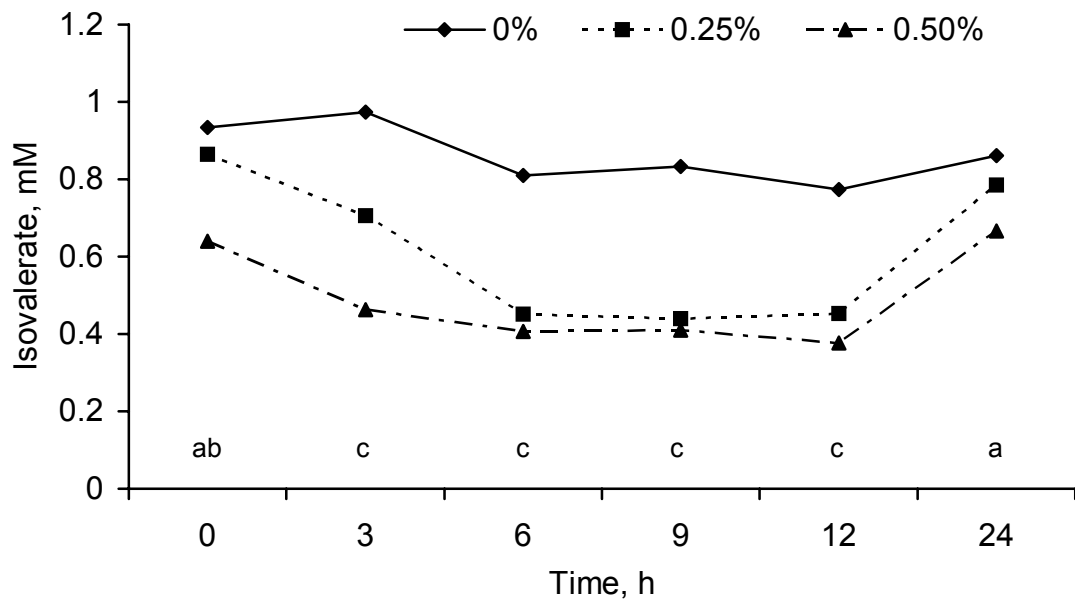


Figure 9. Influence of supplemental fiber at 0%, 0.25%, and 0.50% of BW on ruminal isovalerate concentration over time. (SEM = 0.05; n = 6). ^a0% vs 0.50%; $P < 0.05$, ^b0.25% vs 0.50%; $P < 0.05$, ^c0% vs 0.25% and 0.50%; $P < 0.05$ are denoted along the x-axis.

constant throughout the sampling period in steers not supplemented. Isobutyrate was affected by time ($P < 0.01$) as the concentration decreased through 6 to 12 h post dosing before gradually increasing to near initial concentrations by 24 hr (Figure 10).

The molar proportions of acetate (linear, $P = 0.01$; quadratic, $P = 0.20$) and butyrate (linear, $P = 0.01$; quadratic, $P = 0.07$) increased with supplementation while propionate decreased (linear, $P = 0.012$ quadratic, $P = 0.89$). Molar proportions of valerate (linear, $P < 0.01$; quadratic, $P = 0.08$), isovalerate (linear, $P < 0.01$; quadratic, $P < 0.02$), and isobutyrate (linear, $P < 0.01$; quadratic, $P < 0.05$) decreased with increasing level of supplementation.

Ruminal pH increased (linear, $P = 0.01$; quadratic, $P = 0.70$) with increasing level of supplementation (Table 15) and was affected ($P < 0.01$) by time (Figure 11). No supplementation resulted in a relatively stable pH while supplementation at 0.25% and 0.50% of BW decreased ruminal pH through 6 h post supplementation, however, this reduction was greater in 0.25% than in 0.50% of BW supplemented animals. Ruminal ammonia-N concentration was decreased (linear, $P = 0.01$; quadratic, $P = 0.03$) with supplementation.

Compared to unsupplemented steers, the ammonia-N concentration in steers receiving 0.25% and 0.50% of BW supplement decreased 47.8 and 70.1%, respectively. There was also a supplement x time interaction ($P < 0.01$) for ammonia-N concentration (Figure 12). Ruminal ammonia-N concentration in unsupplemented steers remained relatively constant throughout the sampling period. Conversely, ammonia-N concentrations decreased through 9 h

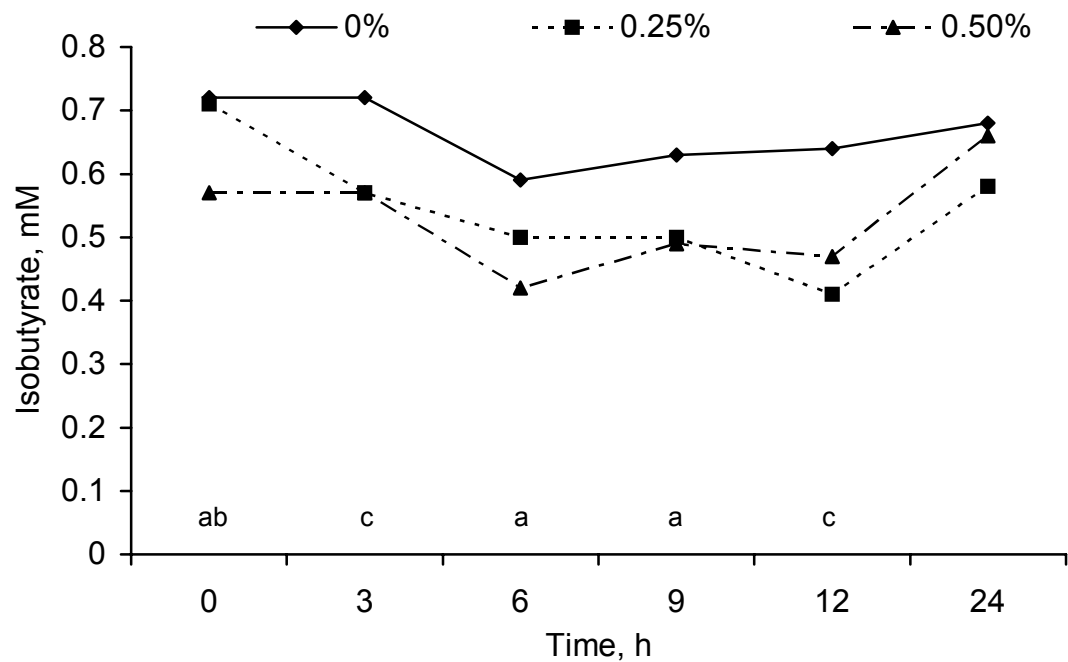


Figure 10. Influence of supplemental fiber at 0%, 0.25%, and 0.50% of BW on ruminal isobutyrate concentration over time. (SEM = 0.05; n = 6). ^a0% vs 0.50%; $P < 0.05$, ^b0.25% vs 0.50%; $P < 0.05$, ^c0% vs 0.25% and 0.50%; $P < 0.05$ are denoted along the x-axis.

Table 15. Ruminant pH, ammonia-N, and fluid kinetics of steers consuming tall fescue grass hay and supplemented with purified fiber

Item	Supplement, % of BW			SEM ^a	Probability	
	0	0.25	0.50		Linear	Quadratic
pH ^b	6.60	6.65	6.72	0.02	0.01	0.70
Ammonia-N, mM ^{bc}	2.77	1.51	0.87	0.11	0.01	0.03
Fluid passage, %/h	8.43	8.74	9.45	0.27	0.04	0.58
Rumen volume, L	78.39	79.96	75.69	2.56	0.49	0.39
Rumen turnover, h	12.14	11.66	10.67	0.38	0.04	0.60
Fluid outflow, L/h	9.74	9.53	8.15	0.59	0.11	0.45
Rumen DM fill, kg	8.07	8.12	7.95	0.19	0.65	0.65
Rumen fluid fill, L	60.55	61.54	60.77	0.75	0.85	0.38

^an = 6.

^bTime effect ($P < 0.01$).

^cTreatment x time interaction ($P < 0.01$).

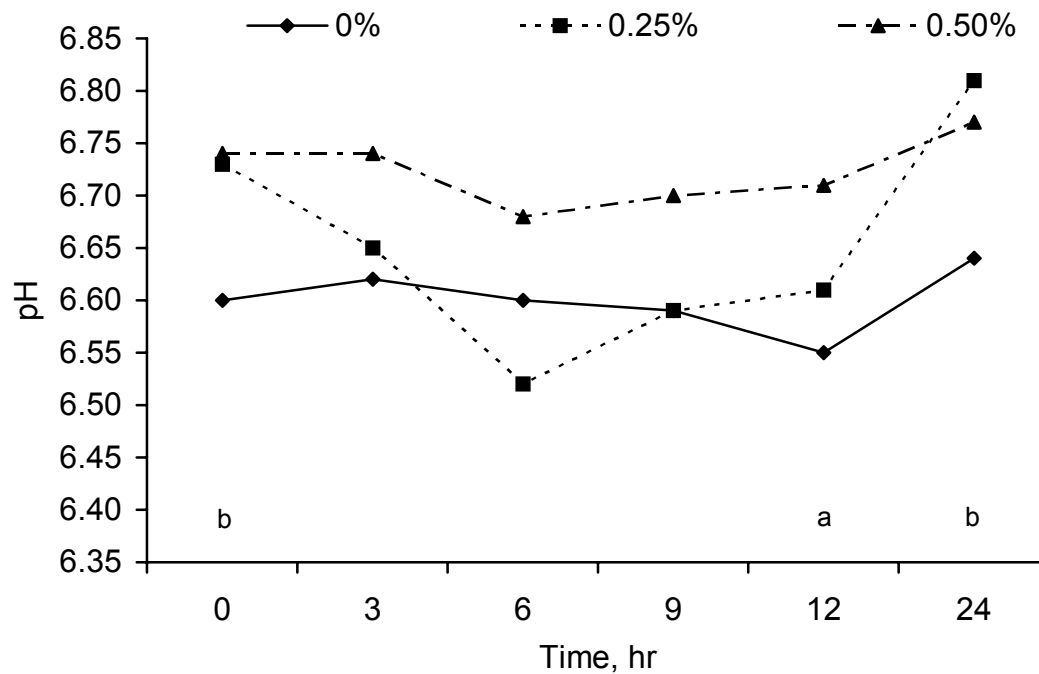


Figure 11. Influence of supplemental fiber at 0%, 0.25%, and 0.50% of BW on ruminal pH over time. (SEM = 0.05; n = 6). ^a0% vs 0.50%; $P < 0.05$, ^b0% vs 0.25% and 0.50%; $P < 0.05$ are denoted along the x-axis.

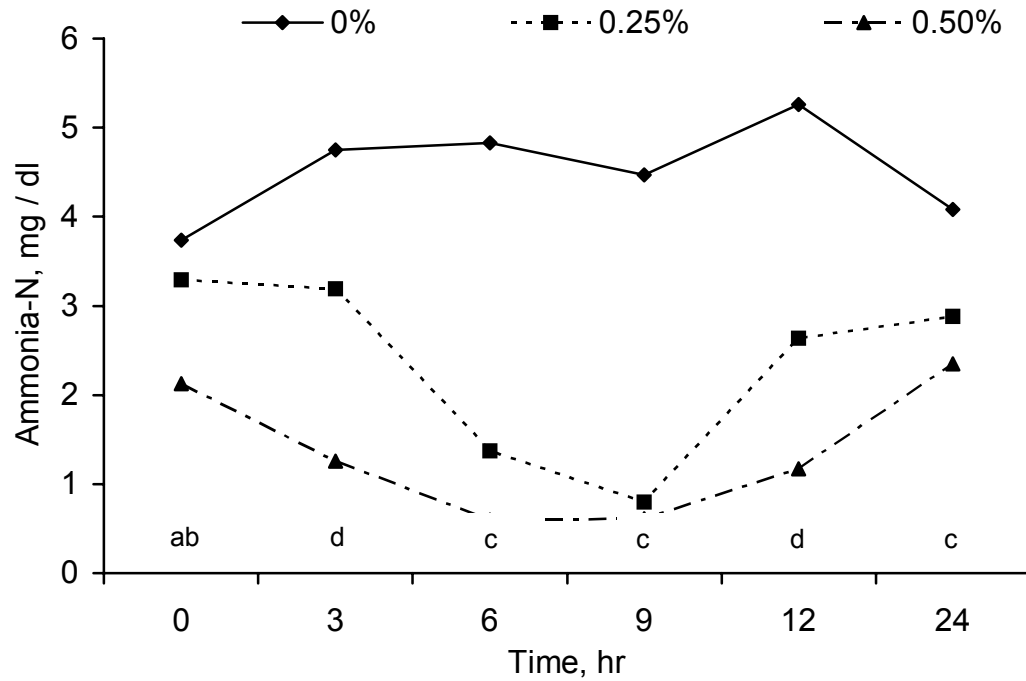


Figure 12. Influence of supplemental fiber at 0%, 0.25%, and 0.50% of BW on ruminal ammonia N concentration over time. (SEM = 0.45; n = 6). ^a0% vs 0.50%; $P < 0.05$, ^b0.25% vs 0.50%; $P < 0.05$, ^c0% vs 0.25% and 0.50%; $P < 0.05$, ^d0% vs 0.25% vs 0.50%; $P < 0.05$ are denoted along the x-axis.

post-supplementation, after which concentrations began to increase in supplemented steers. Rumen fluid dilution rate, outflow, and volume were unaffected by supplementation while fluid passage rate was increased (linear, $P = 0.04$; quadratic, $P = 0.58$) and turnover time decreased (linear, $P = 0.04$; quadratic, $P = 0.60$) with supplementation (Table 15).

Lamb Digestibility and Nitrogen Balance. Forage DM, OM, NDF, and ADF intake were reduced (linear, $P < 0.01$; quadratic, $P < 0.10$) by increasing level of supplementation (Table 16 and 17). Forage N intake was also reduced (linear, $P = 0.03$; quadratic, $P = 0.05$) by supplementation with 0.25% of BW supplementation being the lowest and 0.50% intermediate (Table 18). However, total DM, OM, NDF, and ADF intake was increased (linear, $P < 0.01$; quadratic, $P < 0.07$) by supplementation with total N intake remaining lower (linear, $P = 0.05$; quadratic, $P = 0.05$) with increasing level of supplementation.

The apparent quantity of DM and OM disappearing total tract was increased (linear, $P < 0.02$; quadratic, $P > 0.51$) with increasing level of supplementation while the percent disappearance was not affected (Table 16). The apparent quantity of NDF and ADF disappearing total tract was also increased (linear, $P = 0.01$; quadratic, $P > 0.46$) by supplementation, however, when expressed as a percent of intake, supplementation did not affect the disappearance of either fraction (Table 17). The quantity of N excreted in urine was not affected, however, fecal N excretion was increased (linear, $P = 0.04$; quadratic, $P = 0.46$) with increasing level of supplementation (Table 18). Apparent total tract N disappearance decreased (linear, $P < 0.03$; quadratic, $P >$

Table 16. Apparent total tract disappearance of dry matter (DM) and organic matter (OM) by lambs consuming tall fescue grass hay and supplemented with purified fiber

Item	Supplement, % of BW			SEM ^a	Probability	
	0	0.25	0.50		Linear	Quadratic
DM intake, g/d						
Forage	913	860	855	9	0.01	0.07
Total	913	931	996	7	0.01	0.04
Fecal output, g/d	376	374	399	10	0.15	0.29
Total tract disappearance						
DM, g/d	537	557	597	12	0.02	0.53
DM, %	58.87	59.83	59.95	1.11	0.52	0.78
OM intake, g/d						
Forage	858	808	802	8	0.01	0.07
Total	858	877	941	7	0.01	0.04
Fecal output, g/d	344	343	366	9	0.15	0.29
Total tract disappearance						
OM, g/d	513	534	575	11	0.01	0.51
OM, %	59.85	60.91	61.14	1.10	0.44	0.77

^an = 6

Table 17. Apparent total tract disappearance of neutral detergent fiber (NDF) and acid detergent fiber (ADF) by lambs consuming tall fescue grass hay and supplemented with purified fiber

	Supplement, % of BW				Probability	
Item	0	0.25	0.50	SEM ^a	Linear	Quadratic
NDF intake, g/d						
Forage	605	566	560	5	0.01	0.05
Total	605	613	653	4	0.01	0.03
Fecal output, g/d	226	223	238	5	0.16	0.20
Total tract disappearance						
NDF, g/d	379	390	415	7	0.01	0.46
NDF, %	62.70	63.67	63.53	0.94	0.56	0.65
ADF intake, g/d						
Forage	329	309	304	3	0.01	0.10
Total	329	342	369	3	0.01	0.07
Fecal output, g/d	127	127	137	3	0.08	0.19
Total tract disappearance						
ADF, g/d	202	215	232	4	0.01	0.77
ADF, %	61.31	62.92	62.83	1.03	0.34	0.53

^an = 6

Table 18. Nitrogen intake, total tract disappearance and retention by lambs consuming tall fescue grass hay and supplemented with purified fiber

	Supplement, % of BW				Probability	
Item	0	0.25	0.50	SEM ^a	Linear	Quadratic
N intake, g/d						
Forage	14.9	14.2	14.3	0.1	0.03	0.05
Total	14.9	14.3	14.4	0.1	0.05	0.05
Fecal N, g/d	6.9	7.1	7.7	0.2	0.04	0.46
Urinary N, g/d	4.9	5.0	4.7	0.1	0.29	0.19
Total tract N disappearance						
Apparent, g/d	8.0	7.2	6.8	0.3	0.02	0.56
Apparent, %	53.91	50.41	46.91	1.68	0.03	0.99
Retention						
N, g/d	3.2	2.2	2.1	0.3	0.06	0.29
N, %	21.12	15.23	14.64	2.24	0.09	0.38

^an = 6

0.56) with supplementation whether expressed as a quantity or as a percent of intake. Nitrogen retention tended ($P < 0.10$) to decrease with supplementation when expressed as total quantity (linear, $P = 0.06$; quadratic, $P = 0.29$) and as a percent of intake (linear, $P = 0.09$; quadratic, $P = 0.38$).

Discussion

In steers, forage DM intake was not different between levels of supplementation, therefore, additional ruminally degradable substrate that was available was a result of supplementation. The supplements used in the present study were designed to provide similar intakes of NDF and ADF and to have similar A and B carbohydrate fiber fractions and degradation rates as soybean hulls while providing a reduced amount of N (0.05% N) compared to soybean hulls (1.9% N). Therefore, the effects could be expected to be similar to that of soybean hull supplementation.

Some concerns surrounding the practice of energy supplementation of forage based diets are the potential to decrease forage intake and/or forage nutrient utilization. Generally, as the intake of supplemental energy increases, forage intake tends to decrease. Reductions in forage intake associated with supplementation have been commonly attributed to starch rather than fibrous by-product feedstuffs (Caton and Dhuyvetter, 1997). The depression in forage intake associated with grain supplementation is thought to either be due to reductions in ruminal pH or potentially an effect of the type of carbohydrate being degraded (Mould et al., 1983).

Our results indicate that supplementation with purified fiber does not affect forage intake of steers consuming tall fescue hay, however, forage intake in lambs was decreased. Similar to our results in steers, Driedger et al. (2000) reported no effect on forage intakes in steers grazing tall fescue and either not supplemented or supplemented with oat fiber at 0.75% of BW. However, supplementing forage fed cattle with up to 3 kg/d with soybean hulls has been reported to result in small decreases in forage intake (Martin and Hibberd, 1990). Also, Hannah et al. (1989) supplemented steers grazing tall fescue with corn gluten feed at 1% of BW reported a 14% decrease in forage OM intake. Our results produced contradicting results of forage intake between steers and lambs. This difference is potentially attributable to species dependent intake parameters associated with lambs tending to exhibit greater concentrate:forage substitution than cattle (Elliott, 1967). However, the discrepancy may have also been due to differences in forage consumption. In the present study, lamb forage intake averaged 3.2% of BW while steers consumed forage at 1.6% of BW, therefore, the effect of supplementation on forage intake in lambs may have been due to intake limitations.

As demonstrated by the current experiment, supplementation has the potential to increase total intake of ruminants fed forage-based diets dependent upon the presence or the extent of forage intake depression. Supplementation of steers and lambs in this experiment linearly increased total DM, OM, NDF and ADF intake. Even though forage intake was decreased by supplementation at 0.25 and 0.50% of BW in lambs, total intake was increased as forage intake was

only decreased 53 and 59 g, respectively. Similarly, Garces-Yepez et al. (1997) supplemented steers fed bermudagrass hay with either a corn/soybean meal mix, wheat middlings, or soybean hulls reported increased digestible OM intake with supplementation while forage OM intake was depressed due to supplementation. Carey et al. (1993) reported no effect of supplementation with either barley, beet pulp, or corn on total intake as forage intake was decreased by supplementation. Similarly, Elizalde et al. (1998) reported similar total OM intakes in steers grazing tall fescue and supplemented with corn gluten feed due to a depression in forage OM intake compared to control steers.

In contrast, reduced forage and total OM intake has been reported in steers grazing fescue pasture and supplemented with wheat bran (Hess et al., 1996). Driedger et al. (2000), also supplementing a purified fiber (oat fiber) as well as corn starch, reported that total DM intake was not affected by supplementation even though forage intake was also not affected. Forage intake in supplemented animals were slightly reduced by supplementation resulting in the lack of effect on total intake. By design, steer N intake was not affected by supplementation in the present trial due to the low N content of the supplement provided. However, as a result of decreased forage intake in lambs, N intake was decreased in that experiment. The supplement was chosen in an effort to increase the utilization of available N from tall fescue hay by increasing ruminal fiber fermentation while not substantially altering N supply.

The quantity of DM, OM, and fiber disappearing ruminally, in steers, and total tract, in steers and lambs, was increased by supplementation in this

experiment. Similarly, Grigsby et al. (1992) reported a linear increase in DM, OM, and NDF digestion in steers fed a bromegrass hay diet with soybean hulls substituted up to 60% of the total diet at 15% increments. Martin and Hibberd (1990) also reported increased OM and ADF digestion with no effect on NDF digestion when cows consuming low quality hay were supplemented with soybean hulls. Garces-Yepez et al. (1997) supplemented lambs consuming bermudagrass hay with corn/soybean meal mix, wheat middlings, or soybean hulls and reported that corn and soybean hull supplementation increased total tract OM digestion compared to wheat middlings while soybean hulls resulted in the greatest total tract NDF digestion. Also, Driedger et al. (2000) reported a tendency ($P = 0.12$) for increased DM digestibility by supplementation of oat fiber compared to corn starch in steers grazing tall fescue. However, Elizalde et al. (1998) reported no effect on OM digestibility when steers grazing tall fescue were supplemented with corn or corn gluten feed. Commonly, supplementation of forage based diets is achieved through the use of products that have no inherent similarities to the basal diet consumed. In an effort to minimize any negative associative effects on forage digestion, the current experiment was designed to supply supplemental nutrients in a completely fibrous form that would closely resemble the fiber portion of the basal diet. The net result was increased ruminal fermentation of OM and fiber with no apparent affect on the utilization of forage fiber.

The variability of results reported by others describing the effects supplemental energy on digestibility may at least be partially explained by

differences in the level of protein provided and the quality of forage. Protein supplements may be used to maintain a dietary isonitrogenous condition (Garces-Yepetz et al., 1997), yet differences in the CP content and quality of the forage used in supplementation trials may exist. Galloway et al. (1993) demonstrated that orchardgrass increased OM digestibility and had greater N digestibility compared to bermudagrass regardless of the type of supplement provided. Olson et al. (1999) also demonstrated that total tract DM, OM, and NDF digestion increased linearly with increasing level of digestible protein intake (DIP). Increased digestibility by supplementing DIP is probably the result of alleviating a deficiency in N-containing compounds as a supply of ammonia facilitates microbial fermentation (Olsen et al., 1999). Therefore, effects of energy supplementation on diet digestibility may be dependent upon protein supply. Under conditions of limited CP, energy supplementation could exacerbate a CP deficiency resulting in reduced intake, digestibility, and performance (Sanson et al., 1990). In the current experiment with steers, OM intake increased 1123 and 1897 g with 0.25% and 0.50% of BW supplementation, respectively, with a subsequent 982 and 1324 g increase in ruminal OM disappearance. Increasing level of supplementation increased NDF intake 776 and 1287 g, and ADF intake 546 and 930 g, respectively, while ruminal NDF disappearance increased 690 and 881 g and ADF disappearance increased 494 and 618 g, respectively. Therefore, supplementation at least increased the quantity of OM, NDF, and ADF digested indicating that even though ruminal ammonia concentrations were

significantly reduced, ruminal N availability did not appear to limit microbial activity.

It is not apparent that supplementation increased forage fiber digestion and it is presumed that the increase in digestibility reported is a function of the highly digestible nature of the supplement provided. However, due to the small particle size (~ 1-mm) of the fiber supplement provided it would be expected that passage from the rumen would be relatively fast compared to the passage of forage particles indicating that, based upon the increased ruminal fiber digestion, forage fiber digestion may have been increased by supplementation. This could lead to an increase in the proportion of fiber being fermented in the large intestine, as supplemental fiber escapes ruminal fermentation. This was supported in lambs, as fecal N increased with increasing level of supplementation. However, in steers, fecal N was not affected by supplementation.

Grigsby et al. (1992) reported increased duodenal flow of N in steers fed bromegrass hay substituted with soybean hulls from 0 to 60% of the diet while duodenal flow of microbial N was not affected. Galloway et al. (1993) reported no effect on total duodenal flow of N, however, microbial N flow at the duodenum was increased in steers fed two different hay types and supplemented with soybean hulls. In our experiment, bacterial and total N flow at the duodenum was not affected by supplementation of steers, potentially a result of insufficient forage N as ruminal ammonia N concentration was dramatically decreased by supplementation. Similar to the present experiment, true ruminal N digestibility

was not affected by substitution with soybean hulls into bromegrass hay diets while total tract N digestibility decreased linearly with increasing soybean hull substitution (Grigsby et al., 1992). Similarly, Nakamura and Owen (1989) reported lower total tract N digestibility with soybean hulls than with corn and suggested that due to the relatively rapid rate of ruminal passage of soybean hulls, hindgut fermentation was increased resulting in an increase in fecal excretion of microbial N. However, Galloway et al. (1993) reported no effect on total tract N digestibility by supplementation with soybean hulls. In our experiment, ruminal passage of purified fiber did not appear to dramatically alter hindgut fermentation in steers, as total tract N digestion was not affected by supplementation. This would appear to indicate that fermentation of the purified fiber may have been rapid enough to allow for near complete degradation to occur in the rumen prior to passage to the small intestine.

In lambs, the decrease in total tract nitrogen disappearance was probably a result of insufficient rumen retention of the purified fiber resulting in an increase in hindgut fermentation and the subsequent increase in fecal N excretion.

Hess et al. (1996) reported that steers consuming tall fescue and supplemented with wheat bran and corn at either 0.34% of BW or wheat bran at 0.48% of BW had increased concentrations of total VFA due to supplementation, however, type of supplement did not affect total VFA concentration. Grigsby et al. (1992) reported that steers consuming a bromegrass hay diet substituted with soybean hulls at a rate of 0 to 60% of the diet had a linear increase in total VFA with increasing level of supplementation. The current experiment did not

demonstrate any significant effect of purified fiber supplementation on total VFA concentrations. Similarly, Galloway et al. (1993) supplemented steers consuming either bermudagrass or orchardgrass hay with corn, soybean hulls or a corn-soybean hull combination at 0.5, 0.7, or 0.6% of BW, respectively. Total VFA concentrations were numerically increased by supplementation with soybean hulls to each hay type, however, the difference was not significant.

Grigsby et al. (1992) indicated that substituting soybean hulls into a bromegrass hay diet linearly increased ruminal acetate concentration and decreased propionate concentration. As a result, the acetate:propionate ratio was linearly increased as the amount of soybean hulls in the diet increased. Supplementation of corn and soybean hulls (0.5 and 0.7% of BW, respectively) to bermudagrass and orchardgrass hay diets resulted in no change in the ruminal concentrations of acetate or propionate or the acetate:propionate ratio (Galloway et al., 1993). The present findings are in agreement with previous work in that supplementation of high fiber feedstuffs tended to favor acetate production over the production of propionate compared to feedstuffs containing a greater proportion of soluble and rapidly fermentable carbohydrates. Even though only a tendency for increased ruminal acetate concentration existed and propionate was not affected, the acetate:propionate ratio was increased with increasing level of supplementation, yet, the increase was minimal despite the significance. Using continuous culture fermenters fed a mixed alfalfa and grass hay substrate and supplemented with either beet pulp, corn, or soybean hulls, Bach et al. (1999) reported acetate concentrations were greatest for pasture only and soybean hull

supplemented media and lowest for beet pulp and corn supplemented media. Propionate concentration was increased in corn supplemented compared to pasture only feeding. The shifts in ruminal VFA concentrations were an attribute of the fermentability of the carbohydrate being supplemented, demonstrating that as the proportion of fiber increased, acetate concentrations increased as well.

Galloway et al. (1993) reported an increase in butyrate concentration when steers consuming bermudagrass or orchardgrass hay were supplemented with soybean hulls. Bach et al. (1999) reported increased butyrate concentration as a result of supplementing an alfalfa and grass mixture with beet pulp, however, providing supplemental soybean hulls did not affect butyrate concentration relative to providing grass only. Substitution of soybean hulls into a bromegrass hay diet did not change the ruminal butyrate concentration when the diet contained from 0 to 60% soybean hulls. The increased butyrate concentration reported in the present experiment may be explained by an overall increase in ruminal substrate availability due to forage intake not being decreased as a result of supplementation.

The present results indicate that the concentrations of isobutyrate, valerate, and isovalerate were reduced as a result of supplementation. Compared to unsupplemented steers, isovalerate decreased 27.9 and 43.0%, respectively while isobutyrate concentration decreased 18.2 and 19.7% for 0.25 and 0.50% of BW supplementation, respectively. Russell et al. (1984) demonstrated that the branched chain fatty acids (BCFA) are essential growth factors for cellulolytic bacteria. Gorosito et al. (1985) reported that

supplementation of rumen fluid from a cow consuming timothy hay with isovalerate, isobutyrate, and 2-methylbutyrate alone and in combination increased *in vitro* fiber digestion of alfalfa hay, orchard grass, corn silage, and wheat straw. However, increased fiber digestion of timothy hay, reed canary grass, and Bermuda grass were not affected. Furthermore, the BCFA are important factors in N utilization by these bacteria as they serve as carbon skeletons for the synthesis of branched chain amino acids as well as higher chain length fatty acids (Allison et al., 1962a; Allison et al., 1962b). The ruminal production of isobutyrate and isovalerate is a function of protein degradation by other bacteria that deaminate and decarboxylate branched chain amino acids forming the BCFA (Allison et al., 1962a). Recycling of bacterial protein may also give rise to some BCFA as well (Bryant and Doestch, 1955). The rapid decline in isobutyrate and isovalerate following the consumption of the supplement indicates that either ruminal protein degradation was not substantial enough to sustain the concentrations in the presence of additional highly fermentable fiber or there was an increase in the incorporation of the products into bacterial CP. If the former is true then the inability to maintain these concentrations potentially resulted in sub maximal microbial protein production leading to a diminished response in fiber digestion. Microbial N flow to the duodenum was not affected by supplementation in the current experiment indicating that ruminal protein degradation was probably not substantial enough to maintain ruminal levels of these BCFA. Bach et al. (1999) reported increased BCFA concentration with supplementation of a mixed alfalfa and grass diet with beet pulp and soybean

hulls. Galloway et al. (1993) also reported that the isobutyrate and isovalerate concentration of animals consuming bermudagrass or orchardgrass hay and supplemented with soybean hulls was either not changed or elevated due to supplementation as the product supplemented were a source of dietary N.

Driedger et al. (2000) supplemented steers grazing tall fescue with either hydrogen peroxide treated oat fiber or corn starch. Ruminal pH was 6.62, 6.54, and 6.35 for unsupplemented, oat fiber, and corn starch supplementation, respectively. The oat fiber used by Driedger et al. (2000) was similar to that fed in this trial. Ruminal pH increased in the current experiment as a result of supplementation. Chemical treatment of the products, especially the solka floc, during production may have introduced buffers into the rumen leading to the response observed. Others have reported either similar or decreased ruminal pH due to supplementation with conventional feeds (Grigsby et al., 1992; Galloway et al., 1993; Hess et al., 1996 Elizalde et al., 1998). Orskov (1982) and Hoover (1986) reported a reduction in ruminal fiber digestion when the pH decreased below 6.2 and 6.0, respectively. In the current experiment, the pH never declined below 6.5, therefore, it is unlikely that ruminal pH diminished fiber digestion.

Grigsby et al. (1992) reported that substitution of soybean hulls into a brome grass hay diet linearly decreased ruminal ammonia concentration as the level of soybean hulls substituted increased. Driedger et al. (2000) reported that ruminal ammonia concentration decreased from 5.71 mM in unsupplemented animals to 1.66 mM in animals supplemented with oat fiber at 0.75% of BW. In the present experiment, ruminal ammonia concentration in unsupplemented

animals remained relatively constant with values never declining below 3.7 mg/dl at any time during the collection with a spike in the concentration occurring at 12 h, which also corresponded to the afternoon hay feeding. Ammonia concentration in animals provided supplement at 0.25% of BW remained constant through 3-h post supplementation and offering of morning hay, however, through 9 h the concentration rapidly declined before spiking to near initial levels (2.64 mg/dl) at 12 h. Although low, the ammonia concentration of steers supplemented at 0.25% of BW was less than 1.0 mg/dl at only 9 h with a maximum concentration of 3.29 mg/dl. The concentration did not follow the same trend with supplementation at 0.50% of BW, following the morning feed offering, ruminal ammonia concentration declined through 9 h followed by only a slight increase at 12 h. This indicates a potential lack of ruminally available N, especially in the 0.50% of BW supplemented steers where ruminal ammonia concentration was maintained below 1.0 mg/dl at 6 and 9-h post morning feeding with maximal concentrations never exceeding 2.35 mg/dl. It has been demonstrated that ruminal ammonia concentrations below 5 mg/dl limited microbial growth efficiency (Satter and Slyter, 1974). However, Petersen (1987) suggested that optimal fiber digestion was maintained at only 1 to 2 mg/dl. Furthermore, Demeyer and Van Nevel (1986) suggested that a ruminal ammonia concentration of only 0.1 mM (0.17 mg/dl) was needed to maintain some microbial growth. Based on the decline in ruminal ammonia concentration along with the decrease in ruminal BCFA concentrations, ruminal fermentation was potentially limited by ruminal N availability, however, this effect was not apparent in the digestibility data.

A major factor controlling the synthesis of microbial crude protein is the availability of energy (Dijkstra et al., 1998). Based on the fermentative end products acetate, propionate, and butyrate, supplementation did increase ruminally available energy. However, an increase in microbial protein production would also be dependent upon adequate N availability. Satter and Slyter (1974) noted that VFA production was only slightly decreased by N-limiting conditions while acid-precipitable N was decreased more than twice that of the VFA reduction. The linear decrease in total VFA concentration/kg OM fermented indicates that as supplementation level increased, the efficiency of energy capture as VFA decreased. Therefore, it is possible that ruminal VFA concentrations increased due to increased availability of fermentable OM without altering microbial protein synthesis due to decreasing ammonia-N concentrations with supplementation.

Anderson et al. (1988) reported that fluid passage rate was not affected by supplementation with soybean hulls at 0, 12.5, 25, or 50% of a corn stalk diet. In contrast, Martin and Hibberd (1990) reported that increasing level of soybean hulls supplementation to a low quality grass hay diet resulted in increased fluid passage rate. Grigsby et al. (1992) reported that fluid passage rate decreased with increasing soybean hull substitution into a brome grass hay diet. In this experiment using tall fescue hay, rumen fluid volume was not affected, however, fluid passage rate was increased. Galloway et al. (1993) reported that supplementation with corn or soybean hulls to a bermudagrass or orchardgrass hay diet did not affect rumen fluid volume, however, fluid passage rate was

decreased by supplementation in the bermudagrass diet while an increase was reported due to supplementation in the orchardgrass diet.

Implications

These results suggest that supplementation of ruminants consuming tall fescue hay with purified fiber can increase total nutrient intake without significantly affecting forage intake in cattle, however, forage intake may be reduced in lambs. As a result, digestible fiber intake can be increased causing an increase in the amount of fermentable OM present ruminally, potentially enhancing energy availability. As well, this can be accomplished without significantly increasing ruminally available protein. Even though not observed in this experiment, this could lead to greater N capture, especially under conditions of greater forage N availability. Thus, further research is warranted examining the use of purified fiber with forages containing greater concentrations of CP.

Supplemental purified fiber sources (oat fiber and solka floc) low in N decreased ruminal ammonia concentrations and the concentrations of branched chain fatty acids. A decrease in ruminal ammonia concentration can be the result of any or all of the following: decreased ammonia production, increased microbial utilization, increased passage to the duodenum, or increased uptake across the ruminal epithelium. The increased fluid passage rate may have led to an increase in ammonia-N flowing out of the rumen to the duodenum while it does not appear likely that ruminal epithelial uptake was altered as ruminal pH remained relatively high for all treatments. Furthermore, supplementation of tall fescue hay relatively low in N with high fiber energy supplements does not appear to increase

microbial N utilization. However, purified fiber does appear to possess similar energetic capacities as other traditional by-product feedstuffs as demonstrated by the slight shifts in VFA concentrations without providing an additional source of N. Additional research is needed to determine if supplemental energy alone can increase microbial N capture when N intake is greater as well as to intricately examine ruminal N flow in animals supplemented with purified fiber.

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APPENDIX

Appendix A. Ruminant fermentation balance^a of steers consuming tall fescue grass hay and supplemented with purified fiber

Item	Supplement, % of BW		
	0	0.25	0.50
Glucose used, moles	52.97	53.48	54.06
Gas production	87.58	89.00	90.85
CH ₄ , moles	34.90	35.51	36.16
CO ₂ , moles	52.68	53.48	54.68
ATP yield, moles	256.53	258.38	259.81
Energy			
Input, Mcal	35.65	35.99	36.38
Output			
VFA, Mcal	25.99	26.18	26.41
CH ₄ , Mcal	7.36	7.49	7.62
ATP, Mcal ^c	1.80	1.81	1.82
Heat, Mcal	0.51	0.52	0.53
Potential microbial yield			
Dry cells, g ^d	2565	2584	2598
Protein, g ^e	1539	1550	1559
ATP/mole glucose	4.84	4.83	4.81
Energy lost with fermentation			
CH ₄ , % of initial	20.64	20.80	20.95
ATP, % of initial	5.04	5.03	5.00
Heat, % of initial	1.42	1.44	1.46
Total, % of initial	27.10	27.26	27.41
Non-glucogenic ratio	4.78	4.97	5.27

^aCalculated from fermentation balance equations (Church, 1988).

^bn = 6.

^cBased on 7 Kcal/mole ATP.

^dBased on Y_{ATP} = 10.

^eBased on protein comprising 60% of cell DM.

VITA

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