EFFECTS OF SUPPLEMENT TYPE AND FORAGE TYPE ON RUMINAL METABOLISM AND DIET DIGESTIBILITY OF CATTLE

BY

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THESIS

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ABSTRACT

Many ruminant animal production systems still rely heavily on forages (Jung and Allen, 1995). Yet, poor quality forages, including crop residues like corn stover, do not usually meet the animal's nutrient requirements (NRC, 2000). Corn stover is the fibrous portion of the corn plant left on the field after corn is harvested (i.e. the stalks, leaves, cobs, and husks). Though it is one of the most abundant crop residues in the U.S. (Glassner et al., 1998), it contains little protein or energy (NRC, 2000). In such cases, supplementation in dry or liquid form of the deficient nutrient(s) to provide adequate nutrition is necessary to achieve optimum animal performance (Coleman and Moore, 2003). Despite the fact that liquid feeding has been in practice for over 100 years (Kunkle et al., 1997), research on the use of liquid supplements in grain-based feedlot diets, is limited. Additionally, few studies have directly compared liquid supplementation to dry supplementation and even less have used a commercial liquid supplement.

Objectives were to test the interaction of supplement type, liquid versus dry, and forage type, hay versus corn stover, on diet digestibility and ruminal metabolism of cattle. Ruminally fistulated steers were used in a replicated 4×4 Latin square with a 2×2 factorial arrangement of treatments: 1) hay with a liquid supplement (HL), 2) hay with a dry supplement (HD), 3) corn stover with a liquid supplement (SL), and 4) corn stover with a dry supplement (SD). Steers were fed once daily for ad-libitum intake. Each period began with 14 d dietary adaptation, followed by 8 d of collections (5 d digestibility collection, a 1 d rumen fluid collection, a 1 d in-situ incubation phase and Block 1 methane collection, and a 1 d Block 2 methane collection). In-situ disappearance, which measures the degradation of DM and NDF occurring in the rumen alone, was determined by placing bags, containing soybean hulls, in the rumen for 24 h. There were no interactions ($P \geq 0.25$) of supplement and forage type on DMI, apparent total tract digestibility,

or ruminal pH. Nor were there effects ($P \geq 0.12$) of supplement type on DMI, apparent total tract or in situ digestibility, or ruminal pH. However, steers fed hay had increased ($P < 0.01$) DMI and increased (trend; $P = 0.07$) apparent total tract NDF digestibility when compared to steers fed corn stover, regardless of supplement type. Although apparent total tract NDF digestibility was driven by forage type, there was a tendency ($P = 0.09$) for a forage by supplement type interaction for in situ NDF disappearance (ISNDFD). There were no differences in ISNDFD in steers fed hay; but, liquid supplementation increased ISNDFD in steers fed corn stover. At 0, 1.5, and 18 h post-feeding, ruminal pH was greater ($P \leq 0.01$) in cattle consuming corn stover when compared to those fed hay, regardless of supplement type. There was a supplement by hour interaction ($P = 0.04$) on acetate (Ac) concentrations. At 0h post-feeding, there was no effect; however, at 3 and 6 h post-feeding Ac concentrations were reduced in steers fed liquid when compared to those fed dry supplements. In addition, there was a supplement by hour ($P = 0.02$) interaction for butyrate (Bu) concentration; where, at all time points, Bu concentrations increased ($P \leq 0.01$) in steers fed liquid when compared to those fed dry supplements. Steers fed hay, regardless of supplement type, had increased ($P < 0.01$) concentrations of Ac and total VFA compared to steers fed corn stover. There was no interaction ($P \leq 0.88$) of forage type × supplement type on methane emissions. In addition, there were no main effects ($P \geq 0.24$) of forage nor supplement types on 24 h CH_4 emissions, CH_4 per kg BW, or CH_4 per kg DMI.

Hay was more digestible than corn stover, evidenced by decreased ruminal pH values, increased Ac concentrations, and greater total VFA production, all reflecting greater fermentation. Supplement type had no effect on ruminal fermentation, apparent total tract digestibility of DM, OM, or NDF, or total VFA production. Liquid supplementation tended to improve NDF degradation in the rumen of steers fed corn stover. Liquid supplementation

increased Bu concentrations which has been found to have beneficial effects on the rumen environment and the animal as a whole.

DEDICATION

For my husband, Derek. Without your love, support, and even your willingness to get some "hands on" research experience yourself -- even if it involved steers in diapers and early mornings -- I would not have able to achieve all that I have accomplished.

For my parents. Thank you for always fostering not only my education but my growth as an individual. I would not be where am in life today without your endless love and support.

And for my beautiful daughter, Rosemary. I am so blessed to have you in my life. I now truly know the unexplainable love that a mother has for her child. Though you are only a few weeks old as I write this, I hope this serves as inspiration to pursue all your dreams because I know you will make your father and I very proud.

Words cannot express the love and gratitude that I have for all of you.

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CHAPTER 1: REVIEW OF LITERATURE

INTRODUCTION

Feed costs are the largest expense to beef cattle enterprises, representing 60 to 70% of total costs, and have a great effect on profitability (Lawrence et al., 2010). Though grazing may often be the most economical way to "harvest" forages, it is not necessarily the most efficient method. About 68% of the world's 16 million square miles of agricultural land is used as permanent livestock pasture (Waters et al., 2013). The use of so much land is justified because pasture grazing decreases labor and equipment requirement associated with mechanical harvest (Horrocks and Vallentine, 1999). However, herbivores that are not grazed are fed harvested forage at some proportion of their diet. According to Wedin (1976), the percentage of tall, productive forage mixtures wasted in grazing production systems are variable. For example, cattle in a rotational grazing system trample or waste 34% of the forage; those in a daily rotational grazing system, only 25% (Wedin, 1976). However, method of delivery of stored forage can also affect feed waste. According to Nenn et al. (2016), 22.3% and 18.2% of forage was wasted when fed from a ring or fence-line bunk, respectively; yet, feeding either ground hay or a combination of dry and wet forage from a pull-type self-feeder wagon decreased DM wastage to 4 or 6.2%, respectively.

There are many reasons why producers choose to feed harvested forages, despite the increased labor and cost. Mechanical harvesting may allow for more uniform consumption, decreased waste from trampling and defecation, as well as less trampling damage to the plants and soil surface (Blaser et al., 1959; Walton, 1983; Nenn et al., 2016). Furthermore, grazing may not always be an option (e.g. during drought, summer slump, and winter). As a result, harvested forage may be heavily relied upon. Regardless of harvest method, grazing or mechanical, many

ruminant animal production systems rely heavily on forages (Jung and Allen, 1995). For instance, breeding and backgrounding programs typically feed forage-based diets (Horrocks and Valentine, 1999). Additionally, forages, as a source of dietary fiber, are important for rumen health and are included, at least in minimal amounts, in feedlot diets (Vasconcelos and Galyean, 2009). That said, forage quality plays a role in animal performance and the efficacy of feeding forages depends on many factors (Horrocks and Vallentine, 1999), like forage quality for example.

Poor quality forages do not usually meet nutrient requirements of beef cattle (NRC, 2000). In such cases, supplementation is necessary to achieve optimum animal performance (Coleman and Moore, 2003). For example, Brown (1993) found that steers fed ammoniated stargrass hay had a 31% increase in live weight gain when supplemented with 0.5 kg cottonseed meal and 1.5 kg molasses compared to steers that were not supplemented. Furthermore, McLennan et al. (1981) noted a 91% reduction in weight loss in steers fed rice straw with 60 g urea and 60 g molasses compared to steers fed rice straw without supplementation. Supplementation may not only improve animal performance (Hersom, 2008) but may also increase the digestibility of low quality forages (Galyean and Goetsch, 1993), thus creating an opportunity to feed cheap alternatives in place of more expensive feedstuffs. Supplements can be provided in both liquid and dry form. Forage quality (Galyean and Goetsch, 1993; Buxton, 1996; Buxton et al., 1996; Horrocks and Vallentine, 1999) and supplementation (Caton and Dhuyvetter, 1997; Moore et al., 1999; Hersom, 2008) have been extensively reviewed. Therefore, this review will briefly touch upon these, but will primarily focus on the interactions of forage quality and type of supplementation.

FORAGE QUALITY

Buxton (1996) best described forage quality as a function of nutritional composition, intake, digestibility, and the utilization of nutrients by the animal. Thus, when assessing forage quality in the plant itself knowing that forage composition varies with maturity, plant part, plant species, and preservation method is critical. In addition, depending on the cost of grain, feed costs may be reduced when good-quality forages are fed by decreasing the need for concentrates and supplements (Horrocks and Vallentine, 1999). For the purpose of this review, primary focus will be placed on dry, harvested forages.

The components of forage plant cells can be divided into two broad categories: cell walls and cell contents, or the lumen (Waters et al., 2013). The cell wall develops in two phases. First, during primary wall growth, the plant cell grows in size through elongation (Jung and Allen, 1995). Once this phase is complete, secondary cell wall thickening occurs. During this phase, the secondary cell wall thickens from the primary cell wall to the lumen (Jung and Allen, 1995). Lignin deposition occurs starting in the middle lamella, the intercellular space of the primary cell wall, and continues into the secondary cell wall (Terashima et al., 1993). The main components of the cell wall are carbohydrates (largely structural, discussed below) and lignin (Moore and Hatfield, 1994). They differ from the cell contents which include proteins, lipids, soluble minerals, and soluble carbohydrates (largely non-structural; Buxton et al., 1996). While cell contents are very digestible, access to these nutrients can only be achieved by breakdown of the cell wall (Buxton et al., 1996). Cell wall digestibility is variable and dependent on maturity, which affects other things (Iiyama et al., 1993; Terashima et al., 1993).

Carbohydrates of forages can be categorized as either structural or nonstructural. The primary nonstructural carbohydrates are starches and fructans (Moore and Hatfield, 1994).

Starch is present in two forms: amylose and amylopectin (Moore and Hatfield, 1994). Fructans are chains of fructose (Moore and Hatfield, 1994). Both starches and fructans are easily degraded to simple sugars and rapidly fermented by rumen microbes to produce volatile fatty acids (**VFA**; Morrison, 1979; Moore and Hatfield, 1994). These VFA are then absorbed into the blood stream and provide energy to the animal.

Structural carbohydrates which are found within the cell wall can be classified as cellulosic, hemicellulosic, or pectic (Moore and Hatfield, 1994; Buxton et al., 1996). Pectins are quickly and entirely digested in the rumen (Chesson and Forsberg, 1988; White et al., 1993). Both cellulose and hemicellulose are degraded by rumen microorganisms, primarily cellulolytic bacteria. In pure form, both cellulose and hemicellulose are completely degraded in the rumen (Van Soest, 1973; Moore and Hatfield, 1994). The final component of the cell wall, lignin, provides strength and rigidity to cell walls as well as structure for leaves and stems (Varner and Lin, 1989; Buxton and Redfearn, 1997). Furthermore, lignin helps prevent water loss and disease by decreasing cell wall permeability (Zeikus, 1980; Dean and Eriksson, 1992). However, lignin is completely indigestible to the animal and its concentration has a negative correlation with cell wall digestibility because lignin acts as a barrier to microbial degradation of hemicellulose and cellulose (Buxton et al., 1987; Jung and Deetz, 1993). That is, the cross-linkage with lignin between hemicellulose and cellulose affects the extent to which they are digested.

Methods to determine concentrations of cell wall components and cell contents have been validated. The Van Soest detergent analysis is used to differentiate the components of the cell wall separating them into neutral detergent solubles (**NDS**), neutral detergent fiber (**NDF**), and acid detergent fiber (**ADF**). NDS are comprised primarily of the highly digestible cell content components: sugars, starch, pectin, lipids, soluble carbohydrates, protein, non-protein nitrogen

(**NPN**), and water-soluble vitamins and minerals (Horrocks and Vallentine, 1999). Nonfiber carbohydrates (**NFC**) include starch, sugars, pectin, and ß-glucans (Van Soest et al., 1991). Neutral detergent fiber accounts for the primary cell wall components: hemicellulose, cellulose, and lignin. Its concentration can be negatively related to the energy availability of forage (Buxton et al., 1996). The concentration of ADF, representing cellulose and lignin, is a better correlation to poor forage digestibility than NDF (Horrocks and Vallentine, 1999). Although carbohydrates provide as much as 80% of the energy supplied to ruminant (Van Soest, 1982), only one third of the energy in the cell wall is actually used by ruminants (Buxton, 1996). There are several factors that affect the amount of energy available from a forage; and, the ability of the animal to use the energy from the forage is used as an indicator or forage quality.

Forage maturity at harvest is considered one of the most important determinants of forage quality (Buxton, 1996; Ball, et al., 2001). Maturation of most forage plants includes multiple stages of development: seedling, vegetative, and reproductive (Skinner and Moore, 2007). During the early rapid growth stages, forage plants typically contain adequate nutrient concentrations to provide for growth and production in cattle (Horrocks and Vallentine, 1999). As a plant matures, its potential to be digested decreases and nutritive value decreases. This is due to increasing fiber concentrations and decreasing cell content concentrations (Buxton and Redfearn, 1997). In fact, two-thirds of NDF and half of the structural carbohydrates within mature forage stems may be indigestible (Buxton and Casler, 1993) due to the process of lignification, or the increasing of lignin concentration, as plants mature (Cherney et al., 1993; Brink and Fairbrother, 1994; Cuomo et al., 1996; Hockensmith et al., 1997). The extent of this process varies between plant species. For instance, lignin concentrations in grasses more than double with maturity from the vegetative and reproductive stages while this increase is less

severe in legumes (Albrecht et al., 1987; Bidlack and Buxton, 1992; Brink and Fairbrother, 1994). In addition, grasses typically contain greater NDF concentrations than legumes primarily due to the NDF concentration differences in the leaves of grasses and legumes (Buxton, 1996); however, lignin, as a percentage of NDF, is greater in legumes than grasses. For example, lignin as a percentage of NDF ranges from 16.67 to 19.44% for common legume hays (birdsfoot trefoil, ladino clover hay, red clover hay, vetch hay; NRC, 2000) compared to 6.06 to 11.11% for common grass hays (KY 31 fescue hay, sorghum-sudangrass hay, bahiagrass hay; NRC, 2000). Nonetheless, this increase in lignin and overall NDF content also slows digestion rates (Ball et al., 2001). Therefore increased NDF and ADF concentrations reflect poor forage quality, not only in that they limit digestibility, but they also limit intake by increasing the bulk of the diet and contributing to physical fill (Buxton et al., 1996). Increasing fiber concentrations occur not only in the stems of the plant, but also in leaves, especially in leaves of grasses (Buxton, 1996). Relative to stems, leaves have a greater concentration of cell contents and digestibility, thus, the nutritive value of forages is dependent on leafiness (Horrocks and Vallentine, 1999). Therefore, a greater leaf:stem ratio is indicative of greater nutritive value. However, leafiness often decreases with advancing maturity. In addition, leaf:stem can be decreased by the loss of leaves during harvest (Horrocks and Vallentine, 1999).

Similar to leafiness, protein concentration also decreases with increasing forage maturity. This occurs in part because decreasing protein concentration are caused by the decrease in cell contents, in addition to decreased leaf/stem ratios. Because stems have a lower protein concentration than leaves, the overall protein in the plant is reduced (Buxton, 1996). According to Minson (1990), as maturity increased, the crude protein concentration for a variety of forages decreased 1 g kg^{-1} d^{-1} on average. Protein is a vital source of amino acids and nitrogen in feeds

(Rayburn, 1996). Protein plays an important role in not only maintaining production of the animal, but also that of the rumen environment. Ruminant protein requirements are presented in terms of crude protein (**CP**; Buxton et al., 1996). Requirements vary depending on the stage of production and physiological state of the animal (NRC, 2000). Crude protein can be divided into three classes: soluble intake protein (**SIP**) which is converted to ammonia in the rumen, degraded intake protein (**DIP**) which is utilized by rumen microbes for growth and feed digestion, and undegraded intake protein (**UIP**) which can be absorbed in the intestinal tract (Rayburn, 1996). Most forage protein is degraded in the rumen leaving only a small proportion of UIP (Titgemeyer and Löest, 2001), and this varies by plant and maturity. While the majority of CP is found within the cell contents (Horrocks and Vallentine, 1999), there is also protein present in the cell walls of plants. However, the CP in the cell walls exists in a less digestible form, due to complexes with lignin (Van Soest, 1982). This decrease in available protein and overall decrease in forage quality limits productivity of ruminants.

As previously stated, utilization of forage in ruminants depends on the voluntary intake and nutritive value of forage, and both are affected by the maturity and quality of the forage (Huhtanen and Jaakkola, 1993). The nutritive value and voluntary intake of poor quality forages do not typically allow the animal to meet nutrient requirements (NRC, 2000). To alleviate nutritional deficiency and achieve optimum animal performance, supplementation may be necessary (Coleman and Moore, 2003). Animals can be provided supplemental protein, energy, as well as vitamins and minerals. While this supplementation can be in a variety of forms, liquid supplementation is of great interest.

LIQUID SUPPLEMENTATION

Because both the CP concentration and the intake of forage decrease with increasing plant maturity, there is generally a need for protein supplementation in cattle fed mature forages. Furthermore, only seven of the 21 mineral elements required by ruminants are present in forages in great enough concentrations to meet requirements (Church and Pond, 1982; Buxton et al., 1996). Mineral deficiencies can limit organic matter (**OM**) and cell wall digestibilities (Buxton et al., 1996). Therefore, supplementation is necessary to achieve optimal animal performance in cattle consuming forages.

Williams (1995) estimated that over 1.7 million tons of liquid feed were produced during the 1994 to 1995 production year. About 45% of this liquid was used in feedlots while the remaining 55% was considered non-feedlot, presumably fed to cattle in forage-based systems (Kunkle et al., 1997). Furthermore, past estimates of the production of liquid feed tonnage have shown a 9.7% average annual growth from 1977 to 1997 (Kunkle et al., 1997).

Liquid supplements typically consist of molasses and other ingredients. With reports of liquid feeding values dating back to 1890; that is, molasses in livestock feeds is not a novel feed ingredient (Kunkle et al., 1997). Another common ingredient in liquid supplements is glycerin. Glycerin supply has increased with the expansion of the biodiesel industry (Parsons et al., 2009; Hales et al., 2013). Despite the rich history of liquid feeding, research on the use of liquid supplements in feedlots, grain-based diets, is limited. Many studies have been conducted in forage-based systems. However, very few have directly compared liquid supplementation to dry supplementation and even fewer have used a commercial liquid supplement.

Some of the benefits of liquid supplementation include improved diet palatability, dilution of unappetizing flavors, and decreased dustiness (Lahr et al., 1983). But, increasing the soluble carbohydrates in the diet can also be beneficial. Molasses has a high sugar content which

accounts for 60 to 65% of sugarcane molasses solids (Kunkle et al., 1997). Sucrose typically accounts for 65 to70% of total sugars while glucose and fructose primarily account for the remainder (Binkley and Wolfram, 1953; Chen, 1985; Curtin, 1993; Stateler, 1993). Helmer and Bartley (1971) found that the inclusion of NFC, such as the sugars in molasses, in high urea supplements improves both supplement palatability and cattle performance. Sauer et al. (1975) observed that supplements containing NFC provide readily available energy and carbon skeletons to favor the production of ruminal microbial protein from ammonia-N. Furthermore, one of the main benefits of supplementation is nutrient synchrony in the rumen, the parallel provision of energy and nitrogen sources, leading to optimized microbial efficiency (Hersom, 2007). Hemsley and Moir (1963) found that molasses may supply not only energy, but also the sulfur and branched-chain volatile fatty acids required by cellulolytic rumen microbes. While these attributes would theoretically increase dry matter intake and digestibility, results of various studies have had inconsistent results (Arroquy et al., 2004).

Arroquy et al. (2004) evaluated the effects of supplementing fistulated steers, fed poor quality hay, with either dextrose or starch with different proportions of NPN in RDP. These researchers found no supplement type by RDP source interactions for forage intake, digestion, or passage rate; in addition, supplement type did not affect intake. Organic matter and NDF digestibilities were greater when dextrose was supplemented compared to starch. Researchers attributed this improvement to increased rumen retention time seen in steers supplemented with dextrose. Ciriaco et al. (2015) found that apparent total tract digestibility of DM, organic matter, NDF, and ADF increased linearly with increasing supplementation of a 50:50 mixture of molasses:crude glycerol in steers fed Bermuda grass hay with no effect on hay intake. Yet, other studies have reported decreases in DM digestion and poor quality forage intake with the

supplementation of NFC (Rittenhouse et al., 1970; DelCurto et al., 1990; Olson et al., 1999). The effects of NFC supplementation on forage digestibility may be affected by forage quality. Moore et al. (1999) found that supplements decreased forage intake when forage TDN:CP ratio was less than seven, representing adequate amounts of CP, or when forage intake was greater than 1.75% BW. These authors suggest the decrease in poor quality forage intake may be attributed to a substitutive effect that is the animal consumes the NFC supplement in place of the forage when forage quality is poor. Souza et al. (2010) observed this effect in heifers consuming poor quality, tropical signal grass hay with starch supplementation alone in which the intake of 0.49 kg of forage DM was replaced with 0.53 kg of supplement. However, this reduction in forage intake in response to supplementation is typically greater with good quality than poor quality forages (Hyer et al., 1991). The decrease in forage DM digestion may be the result of the negative effects of decreased rumen pH seen when easily degradable feedstuffs such as NFC are fed on fibrolytic bacteria (Therion et al., 1982; Shi and Weimer, 1992). Grant and Mertens (1992) found that the NDF digestibilities of common forages decreased at pH values below 6.2 in vitro. On the other hand, amylolytic bacteria can thrive in a pH range of 5.0 to 7.0 (Royes et al., 2001). Therefore, amylolytic bacteria are able to outcompete cellulolytic bacteria for nutrients causing decreased populations of the latter and reduced fiber digestion (Royes et al., 2001). Furthermore, gram-negative, cellulolytic bacteria are also capable of fermenting simple sugars, and may be degrading the simple sugars rather than more complex carbohydrates, such as cellulose (Mould and Ørskov, 1983). Thus, the research on forage quality and liquid supplementation has been variable. One reason may be the wide range of liquid supplements evaluated.

Research evaluating the effects of liquid supplementation on the performance of growing cattle in a feedlot has also yielded mixed results. Several studies have shown increases in ADG

by 4 to 21% (Bradley et al., 1966; Brown et al., 1967; Lishman, 1967; Cooper et al., 1978) and/or improvements in feed efficiency from 7 to 14% (Lishman, 1967; Cooper et al., 1978) in finishing cattle with the inclusion of two to 10% molasses to high concentrate diets in place of various forms of corn. Similarly, Pritchard et al. (2015) evaluated the effects of feeding steers a typical feedlot diet with a dry meal-type supplement at 3.3% inclusion or liquid supplementation at 4.5% or 9.0% inclusions. They found that steers had similar dry matter intakes across all treatments; however, steers receiving 9.0% liquid supplementation tended to have improved ADG compared to those receiving the dry and 4.5% liquid supplements (1.71 kg vs. 1.63 kg and 1.62 kg, respectively). Furthermore, feed efficiency was best for steers fed the 9.0% liquid supplement and worst for those fed the meal-type supplement with those fed the 4.5% liquid supplement being intermediate. Long et al. (2015) studied the effects of replacing 0, 10, or 20% of dry-rolled corn with glycerin in growing diets fed to heifers which were later fed a common finishing diet. Though the inclusion of glycerin linearly decreased both ADG and G:F during the growing phase, heifers fed the 10% glycerin diet during the growing phase had the greatest overall ADG and final BW with those fed the 20% glycerin diet having the least and those fed the control being intermediate. They attributed this carryover response to the rapid fermentation of glycerin to butyrate and propionate which support ruminal papillae development leading to improved VFA absorption and energy utilization (Sander et al., 1959; Tamate et al., 1962; Mentschel et al., 2001). The results of these studies indicate that liquid supplementation may not only have beneficial effects in forage-based diets but also in diets with minimal forage such as a typical feedlot diet.

CONCLUSION

Whether grazed or fed as harvested forage, many ruminant animal production systems rely heavily on forages (Jung and Allen, 1995). Forage quality, determined often by maturity at harvest, plays a major role in the efficacy of feeding forages (Horrocks and Vallentine, 1999). Feeding poor forage quality may limit digestibility and intake (Buxton et al., 1996). In addition, protein concentration decreases with maturity and also plays an important role in maintaining the production of the animal and the rumen environment. As forage maturity increases, the voluntary intake of forages does not usually meet nutrient requirements of cattle (NRC, 2000), and supplementation is necessary to achieve optimum animal performance (Coleman and Moore, 2003). Liquid supplementation improved diet palatability, dilution of unappetizing flavors, and dustiness of feed when compared to dry supplementation (Lahr et al., 1983). However, feeding liquid supplements to beef cattle has produced inconsistent results (Arroquy et al., 2004). Furthermore, despite the fact that liquid feeding has been in practice for over 100 years (Kunkle et al., 1997), research on the use of liquid supplements in feedlots, grain-based diets, is limited. Research on the mechanism of action of liquid supplementation when fed with good and poor quality forages is needed.

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CHAPTER 2: EFFECTS OF THE INTERACTION OF FORAGE AND SUPPLEMENT TYPE ON DIGESTIBILITY AND RUMINAL FERMENTATION IN BEEF CATTLE

ABSTRACT

Objectives were to test the interaction of supplement type, liquid versus dry, and forage type, hay versus corn stover, on diet digestibility and ruminal metabolism of cattle. Rumen fistulated steers were used in a replicated 4×4 Latin square with a 2×2 factorial arrangement of treatments: 1) hay with a liquid supplement (HL), 2) hay with a dry supplement (HD), 3) corn stover with a liquid supplement (SL), and 4) corn stover with a dry supplement (SD). Steers were fed once daily for ad-libitum intake. Each period began with 14 d dietary adaptation, followed by 8 d of collections (5 d of fecal, 1 d rumen fluid collection, 1 d in-situ incubation phase and Block 1 methane collection, 1 d Block 2 methane collection). In-situ disappearance was determined by placing dacron bags, containing soybean hulls, in the rumen for 24 h. There were no interactions ($P \geq 0.25$) of supplement and forage type on DMI, apparent total tract digestibility, or ruminal pH. Nor were there effects ($P \geq 0.12$) of supplement type on DMI, apparent total tract or in situ digestibility, or ruminal pH. However, steers fed hay had increased ($P < 0.01$) DMI and increased (trend; $P = 0.07$) apparent total tract NDF digestibility when compared to steers fed corn stover, regardless of supplement type. Although apparent total tract NDF digestibility was driven by forage type, there was a tendency ($P = 0.09$) for a forage by supplement type interaction for in situ NDF disappearance (ISNDFD). There were no differences in ISNDFD in steers fed hay; but, liquid supplementation increased ISNDFD in steers fed corn stover. At 0, 1.5,

and 18 h post-feeding, ruminal pH was greater ($P \leq 0.01$) in cattle consuming corn stover when compared to those fed hay, regardless of supplement type. There was a supplement by hour interaction ($P = 0.04$) on acetate concentrations (Ac). At 0h post-feeding, there was no effect; however, at 3 and 6 h post-feeding Ac were reduced in steers fed liquid when compared to those fed dry supplements. In addition there was a supplement by hour ($P = 0.02$) interaction for butyrate concentration (Bu); where, at all time points, Bu increased ($P \leq 0.01$) in steers fed liquid when compared to those fed dry supplements. Steers fed hay, regardless of supplement, had increased ($P < 0.01$) concentrations of Ac and total VFA compared to steers fed corn stover. There was no interaction ($P \leq 0.88$) of forage type × supplement type on methane emissions. In addition, there were no main effects ($P \geq 0.24$) of forage nor supplement types on 24 h CH_4 emissions, CH_4 per kg BW, or CH_4 per kg DMI. Though there tended to be an increase in ISNDFD when steers were fed SL, this did not affect total tract digestibility.

Keywords: cattle, forage quality, liquid supplement, rumen metabolism

INTRODUCTION

Forage quality impacts cattle performance. Forages, as a source of dietary fiber, are important for rumen health and are included, at least in minimal amounts (Vasconcelos and Galyean, 2009). The NRC (2001) recommends a minimum of 17 to 19% of dietary NDF in lactating, dairy cattle diets come from forage sources. The 2015 feedlot consulting nutritionist survey found that 30% or more is the most common forage inclusion in receiving diets (Samuelson et al., 2016). Of the respondents, 4.7% indicated using corn stover as the primary forage in receiving diets (Samuelson et al., 2016), a value not recorded in 2007 (Vasconcelos and Galyean, 2007). Corn stover, a poor quality forage with limited feeding value, is the most abundant biomass in the U.S. with120 to 232 million metric tons produced annually (Glassner et al., 1998; Perlack et al., 2005). At ~ $60/ton (Gallagher and Baumers, 2012), corn stover would be a cheaper alternative to hay, 5 yr average costs at $173.60/ton (USDA, 2016), if feeding value could be improved.

Supplementing cattle fed poor quality forages with energy and protein improves growth performance (DelCurto et al., 1990; Köster et al., 1996; Kunkle et al., 1997; Bodine et al., 2000). Liquid supplements, in particular, have been fed to livestock for decades (Pate, 1983; Kunkle et al., 2000; Hales et al., 2013) and, in cattle, improve digestibility of poor quality forages (Kalmbacher et al., 1995; Bowman et al., 1999; Sowell et al., 2008). However, to our knowledge, there is no direct comparison of forage quality and liquid supplementation in for cattle fed harvested forages.

We hypothesized feeding liquid supplements would improve ruminal metabolism and diet digestibility when compared to dry supplements, and the magnitude of this response would be greatest in cattle fed poor quality forages. The objectives of this trial were to test the

interaction of supplement type, dry or liquid, and forage type, hay or corn stover, on ruminal metabolism and fiber digestibility in beef cattle.

MATERIALS AND METHODS

All animal procedures were approved by the University of Illinois Institute of Animal Care and Use Committee and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Animals and Management

Eight Angus × Simmental crossbred steers, previously fitted with rumen cannula, were blocked by BW into a large (average initial BW = 756 ± 150 kg; n = 4) and a small (average initial BW = 630 ± 45 kg; n = 4) block and used in a replicated 4 × 4 Latin square design, such that each BW block represented 1 square. Steers were housed in metabolism stalls at the University of Illinois Beef Cattle and Sheep Field Research Laboratory in Urbana, IL. Stalls (2.3 x 1.3 m) are equipped with individual feed bunks and non-siphoning automatic water bowls. The barn is equipped with a heating, ventilation, and air-conditioning system, providing a controlled environment set at 18.3° C. There was a 2 × 2 factorial arrangement of treatments and steers were assigned to 1 of 4 dietary treatments: (1) 30% hay with 10% dry supplement, (2) 30% hay with 10% liquid supplement, (3) 30% corn stover with 10% dry supplement, or (4) 30% corn stover with 10% liquid supplement. The hay was a mixture of brome, orchardgrass, and fescue. The supplements provided similar nutrients; however, the dry supplement was ground corn-based while the liquid supplement was molasses- and glycerin-based (Table 1). The remainder of the diet, on a DM basis, was 50 or 55% dry rolled corn, and 5 or 10% modified wet distiller's grains with solubles (**MWDGS**). In order to keep crude protein concentrations similar across treatments, the MWDGS inclusion was increased in the corn stover diets. Forage was included in

both diets at 30% in an effort to analyze the effect of supplement type particularly on forage digestibility as well as to make study applicable to both beef and dairy research. Supplementation with liquid or dry supplement occurred daily by mixing into the diet to make a total mixed ration. Dietary treatment sequence was assigned according to procedures outlined by Patterson and Lucas (1962). Cattle were fed once daily for ad-libitum intake.

Sampling and Analysis

Sampling periods were 22 d beginning with a 14 d acclimation phase followed by a 8 d collection phase which included a 5 d digestibility collection (Schroeder et al., 2014), a 1 d rumen fluid collection, a 1 d in-situ incubation phase and Block 1 methane collection, and a 1 d Block 2 methane collection. At the start of the trial, steers were brought in from pasture and rumen contents were retained (12 L) to re-inoculate the animals before each collection period, negating differences in ruminal microbial populations. After each sampling period, partial rumen evacuations (8 L) occurred and rumen fluid from each pair of steers from each treatment was composited. Both steers on each treatment had rumen fluid added back from the 2 steers that were consuming the diet to which they would be transitioned.

During the digestibility collection (d 1 to 5 of collection phase) dietary ingredient and feed refusal samples were collected and weighed daily. Individual ingredient samples were then analyzed for DM (24 h at 105°C). Wet dietary ingredient samples were composited within period and freeze-dried (FreeZone, Labconco, Kansas City, MO), then ground through a Wiley mill (1-mm screen, Arthur H. Thomas, Philadelphia, PA). Ground dietary ingredient samples were analyzed for ADF and NDF (using Ankom Technology method 5 and 6, respectively; Ankom^{200} Fiber Analyzer, Ankom Technology, Macedon, NY), CP (Leco TruMac, LECO Corporation, St. Joseph, MI), fat (Method 2; Ankom Technology), and total ash (500° C for 12 h, HotPack

Muffle Oven Model: 770750, HotPack Corp., Philadelphia, PA). The resulting values were used to calculate nutrient composition of the diets. Feces were collected in canvas bags secured by a leather harness strapped around the girth, between the hind legs, and under the neck of each steer. Bags were emptied twice daily during the 5 d period. Each time bags were emptied, a sub-sample of feces (5% as-is) was saved and subsamples were composited such that 1 sample was analyzed per steer for the period. Feed refusals (10% as-is) samples were also subsampled for 5 d of the 8 d collection phase and composited. Feed refusals and feces were analyzed for DM, NDF, ADF, and total ash as described above. Total digestible nutrients (**TDN**; Table 2) of the 2 forages was back-calculated from CP and ADF using TDN = 81.38 + (CP x 0.36) - (ADF x 0.77) for both the grass hay and corn stover using the Clemson Calculations (1996).

Apparent DM digestibility was calculated by subtracting the weight of the feces (DM basis) from the weight of the feed consumed (DM basis) and dividing the difference by the weight of the feed consumed (DM basis). The resulting value was converted to a percent basis by multiplying by 100. The following equation was used: $\left(\frac{((DM\ Offered - DM\ Refusal)}{(DM\ Offered - DM\ Refusal)}\right) * 100.$ Apparent OM digestibility was calculated in a similar manner. Apparent NDF digestibility was calculated by multiplying the weight of feed consumed (DM basis) by the percent NDF of the ration. The product was considered NDF offered. Feed refusals and feces were analyzed for NDF as described above. NDF refused was determined by multiplying the weight of the feed refused (DM basis) by the NDF content of the feed refusal. NDF output was calculated in a similar manner. Apparent NDF digestibility was calculated using the following equation: $\left(\frac{((NDF\ Offered - NDF\ Refused) - NDF\ Output)}{(NDF\ Offered - NDF\ Refused)}\right) * 100.$

During the rumen fluid collection (d 6 of collection phase), rumen pH was measured by collecting whole, mixed rumen content via rumen cannula at 0, 1.5, 3, 6, 9, and 12 h post-feeding. Sampling times were chosen to characterize ruminal pH through peak fermentation and pH recovery to pre-feeding level in cattle fed DGS-based diets (Felix et al., 2012). Rumen samples were then filtered through 2 layers of cheesecloth and immediately analyzed for pH (Metler Toledo FE20; Metler Toledo Inc., Columbus, OH).

Rumen fluid samples for VFA analysis were collected at 0, 3, and 6 h post-feeding. Samples were strained through 2 layers of cheesecloth and 50 to 75 mL of rumen fluid was mixed with 10 mL of H_3PO_4 and deionized water was added to achieve a 2:1 dilution (by weight). The mixture was then placed in a refrigerator and remixed by shaking several times per day for 2 d. Three days after collection, rumen fluid samples were removed from the refrigerator and 40 mL of diluted rumen fluid was centrifuged at 20,000 × g at 25°C for 20 min. Supernatant was filtered through a 0.45 µm filter. Filtered sample was then transferred in 1-mL aliquots to gas chromatography vials with 0.1 mL of 2-ethyl butyrate as an internal standard. Vials were then stored at -20°C until analyzed via gas-chromatography (**GC**; Model 5890A, Hewlett-Packard, Palo Alto, CA) for VFA.

During the in situ collection (d 7 of collection phase), ruminal fiber degradation was estimated by the NDF disappearance of soybean hulls (**SBH**) in situ. Four replicate dacron bags (Ankom Technology, 10 × 20 cm) containing SBH were used for incubation in the rumen. Bags were tied shut with nylon string and then placed in larger mesh sacs with weights. These larger sacs were placed in the rumen on d 7 of the collection phase. After a 24 h incubation, bags were removed, rinsed, and dried at 55°C for 3 d. Dried samples were weighed to determine DM and ground to be analyzed for NDF (using Ankom Technology method 5, referenced above). In

addition, 4 bags were used to determine the "washout" (0 h) value of SBH from the in situ bags. These bags were not placed in the rumen but were subjected to the same rinsing and drying procedures as the incubated bags. To determine in situ disappearance the following equation was used (DM basis):

$$\left(1 - \left(\frac{weight\ of\ SBH\ after\ incubation}{weight\ of\ SBH\ before\ incubation}\right) \times 100)\right) - \left(1 - \left(\frac{weight\ of\ SBH\ \ after\ washout}{weight\ of\ SBH\ \ before\ washout}\right) \times 100\right)$$

To determine NDF disappearance, weight of SBH NDF was used in the same equation.

On d 7 and 8 of the collection period, 4 steers within block were alternately placed in 4 chambers of the Ruminant Emission Measurement System (REMS), and gas exchange data were collected for the following 24 h. Feed and water were provided inside the chamber for ad libitum intake. Refusal samples were collected and analyzed for DM. Methane emissions were calculated using the following equation adapted from (Moody et al., 2008):

$$Emission\ Rate = Q \times 10^{-6} \times \left(\left(\frac{v_{out}}{v_{in}}\right) C_{CH4}^{out} - C_{CH4}^{in}\right) \times \frac{T_{STD}}{T_{in}} \times \frac{P_{barometric}}{P_{STD}}$$

Where:

$CH4\ out = concentration\ of\ methane\ leaving\ the\ chamber, ppm_v$

$C_{CH4}^{in} = concentration\ of\ methane\ from\ external\ environment, ppmv$

$Q = ventilation\ rate\ of\ recycled\ barn\ air\ entering\ the\ chamber, \frac{m^3}{s}$

$M = molar\ mass\ of\ methane = 16.01\ g/mol$

$V_m = molecular\ volume\ of\ gas\ at\ standard\ conmditons = .2241\ L/mol$

$v_{out,in} = specific\ volume\ of\ air, \frac{m^3}{kg}$

$T_{STD} = temperature\ at\ standard\ conditons = 293.15\ K$

T_{in} = $Temperature\ of\ recycled\ barn\ air\ entering\ chamber, K$

P_{STD} = $barometric\ pressure\ at\ standard\ conditons$ = 101325 Pa

$P_{barometric}$ = $barometric\ pressure\ according\ to\ ASHRAE\ model$ = Pa

The ruminant emission measurement system collects gas samples, measures environmental conditions, and calculates gas emissions. Further details on the REMS are provided by Maia et al. (2014a, 2014b). It is comprised of: 1) 6 individual positively pressurized polycarbonate chambers designed to restrain the animal's head and neck, 2) thermal environmental control and fresh air supply to maintain animal comfort, and 3) gas sampling systems that use infrared photoacoustic multi-gas technology (IR-PAS, INNOVA 1412, California Analytical, Inc., Orange, CA) to measure CH_4. The system utilized a solenoid valve multiplexer that sampled (10 consecutive samples) gas from each chamber every 50 min. Prior to data collection, recovery tests were performed. Data collection cycle was repeated from background, chamber 1 to chamber 6. At each sampling cycle, the last 5 samples were used to calculate an average value which was saved for that sampling cycle. Therefore, 1 value represented the instantaneous methane concentration for the 50min per cycle in final calculations. Missing data were omitted by entering the background value with same environmental condition to force emission rate to be zero at that time point. Then, the total time interval was adjusted, which gave an emission rate normalized to 24 hours (Ramirez, 2014). In the MATLAB code, linear interpolation was done between each background value to give a value used in the emission calculation at each time point for each chamber value. The methane concentration and other data were then processed into the emission equation and the result represented the emission for each 50min interval. For the final result presented, trapezoidal integration method was used to calculate daily emission rate (Maia et al., 2014a).

Statistical Analysis

The experimental design was a replicated 4 x 4 Latin square design with a 2×2 factorial arrangement of treatments. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The Bayesian information criterion was used to select the diagonal covariance structure. The model was:

$$Y_{ijklmn} = \mu + S_i + c_{j(i)} + p_k + F_1 + S_m + (FS)_{lm} + e_{ijklmn}$$

where, Y_{ijklmn} = response variable; μ = mean; S_i = the fixed effect of square; $c_{j(i)}$ = the random effect of steer nested within square; p_k = the random effect of period; F_1 = the fixed effect of forage type (hay or corn stover); S_m = the fixed effect of supplement type (dry or liquid); $(FS)_{lm}$ = the fixed effect of the interaction of the forage type and supplement type; and e_{ijklmn} = the experimental error. Repeated measures were used to analyze ruminal pH and VFA concentrations. Individual steer was the experimental unit. Significance was declared at $P \leq 0.05$, and trends are discussed at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

There were no interactions ($P \geq 0.17$) of forage type and supplement type on DMI, dry fecal output, OM intake, or OM output (Table 3). While supplement type had no effect ($P \geq 0.12$) on DMI, dry fecal output, or OM output, there was a tendency ($P = 0.09$) for steers fed the liquid supplement to consume less OM matter. This reflects the difference in OM content of the 2 supplements. The dry supplement had a greater OM content than the liquid supplement (87% vs 83%, respectively). Steers fed the hay diets had increased ($P < 0.01$) DMI and OM intake compared to those fed corn stover diets. We had hypothesized this would be the case. The hay

(49.21% TDN, 68.26% NDF, 46.05% ADF, 9.14% CP; Table 2) was a better quality forage than the corn stover (39.99% TDN, 79.59% NDF, 56.10% ADF, 5.02% CP). The increased NDF and ADF concentrations of the corn stover may have restricted passage and intake due to the effect of physical fill (Jung and Allen, 1995; Buxton, 1996); however, rate of passage was not determined in this trial. Steers consuming the hay diets also tended ($P \leq 0.07$) to have greater fecal output of DM and OM than steers consuming the corn stover diets. Because of the increased intake, and corresponding increased fecal output, there were no treatment differences ($P \geq 0.18$) in apparent total tract DM or OM digestibility when cattle fed hay diets were compared to those fed corn stover.

We had hypothesized liquid supplementation would increase intake, however, no corresponding increase in intake was observed with liquid supplementation compared to dry, regardless of forage type. Sowell et al. (2003) found that the supplementation of cows on poor quality winter range with a molasses-based liquid supplement increased forage DMI by approximately 22% compared to cows that were not supplemented. Additionally, they found that feeding the liquid supplement increased in situ DMD by 26 to 47%. Researchers have attributed the increased intake noted with liquid supplementation to increased digestibility (Cohen, 1974; Garg and Gupta, 1992; Bowman et al., 1999; Sowell et al., 2003). We hypothesized that liquid supplementation would improve total tract digestibility of the diet, and that the magnitude of this response would be greatest in steers fed the poor quality corn stover. However, this was not the case. In fact, the only effect on total tract digestibility was a tendency ($P = 0.07$) for decreased apparent total tract NDF digestibility for steers consuming the corn stover diets compared to those consuming the hay diets, regardless of supplement type fed. This difference in NDF digestibility can be attributed to the poor quality of the corn stover. The hay and corn stover

contributed 72.0 to 74.2% of dietary NDF. The NDF content of the corn stover used in this trial was 79.59% compared to that of the hay, 68.26% (Table 2). This increased NDF value is expected as corn stover is harvested in an advanced stage of maturity. As a plant matures, its potential to be digested decreases due to the process of lignification, increasing lignin concentration (Cherney et al., 1993; Brink and Fairbrother, 1994; Cuomo et al., 1996; Hockensmith et al., 1997). Increasing lignin concentration is directly correlated with decreased cellulose digestibility (Kamstra et al., 1958). In its advanced maturity, both cellulose and lignin increased while the hemicellulose concentrations decreased, causing a greater quantity of NDF to be less digestible than that found in the hay. Duckworth et al. (2014) found the apparent DM and ADF digestibility of a 20% corn stover diet (DM basis) to be 62.6% and 49.1%, respectively. Steers fed in the current trial had poor overall NDF digestibility by comparison, 44.05 vs. 39.35% for cattle fed the hay diets vs those fed the corn stover, respectively (Table 3). Though ADL content was not reported in either experiments, this may have been due to elevated lignin concentrations of the diets used in the current experiment though dietary with lignin acting as a barrier to microbial degradation of hemicellulose and cellulose (Buxton et al., 1987; Jung and Deetz, 1993).

Similar to the responses for total tract digestibility, there were no interactions, nor main effects, ($P \geq 0.12$) of forage and supplement type on in situ DM disappearance (Table 3). However, there tended ($P = 0.08$) to be an interaction, where: steers fed corn stover with liquid supplement had the greatest in situ ruminal degradation of NDF. These data supported our hypothesis that liquid supplementation would improve ruminal fiber digestibility when cattle were fed poor quality forages. Bowman et al. (1999) also found that liquid supplementation of cows grazing medium-quality range increased in situ forage NDF digestibility by 49% and 30%

compared to cows that did not receive supplement after 48 and 77 h of ruminal incubation, respectively. Bowman et al. (1998) and other researchers have attributed the increase in in situ NDF digestibility to the stimulation of growth of cellulolytic microorganisms in the presence of the readily available sugars found in liquid supplement (Hiltner and Dehority, 1983; Firkens et al., 1991). Again, however, the increase in ruminal in situ degradation of NDF when cattle were fed liquid supplement with corn stover, compared to other treatments, did not translate to an increase in apparent total tract NDF digestibility. Several studies have reported 6 to 36% increases in forage DM and OM digestibility with liquid supplementation to forage-based diets compared to no supplementation (Garg and Gupta, 1992; Kalmbacher et al., 1995; Bowman et al., 1999). Furthermore, Hales et al. (2013) observed a linear increase in apparent OM digestibility with increasing levels of glycerin in place of alfalfa hay in a grain-based receiving diet. However, liquid supplementation did not affect ($P \geq 0.18$) apparent total tract DM or OM digestibility in the current trial. This may be due to increased grain content of the diets and the inclusion of the positive control, dry supplements in diets not containing liquid supplement. Potentially fermentable NDF that escaped rumen fermentation may have presented a relatively more digestible fiber component to the cellulolytic microorganisms in the hindgut (Beever et al. 1981). Thus, hindgut fermentation must have masked improvement.

Ruminal pH

Ruminal pH drops with increasing fermentation (Allen and Mertens, 1988), and is often recorded as a measure of diurnal fermentation pattern (Gregorini, 2012) and ruminal health (Owens, 1998). In this trial, there was no 3-way interaction ($P = 0.90$) of forage type × supplement type × time. There also was no interaction ($P = 0.51$) of supplement type × time nor was there a main effect ($P = 0.34$) of supplement type on ruminal pH (Figure 1). Boyd et al.

(2013) also did not see an effect on pH when supplemental glycerol replaced ground corn in the corn silage-based diets of lactating dairy cows. Ruminal pH was also not affected by increasing levels of molasses (1, 2, or 3 kg DM) fed to steers consuming grass hay ad libitum (Osuji and Khalili, 1994). In contrast to these results, Khalili (1993) found that increasing levels of molasses supplementation (0, 1.5, 3, and 4.5 kg DM/d) linearly decreased ruminal pH in cows fed grass hay ad libitum. Long et al. (2015) observed a more rapid decrease in pH in steers fed glycerin in a dry, rolled corn-based diet compared with those not receiving glycerin. Wang et al. (2009) also saw a linear decrease in pH with increasing crude glycerol supplementation at 0%, 1.1%, 2.2%, and 3.3% of diet DM in cattle fed 60% corn stover, 40% concentrate diets. Yet, Parsons (2010) reported ruminal pH of cannulated steers fed finishing diets to increase as crude glycerol increased up to 4%. With supplement type having no effect on ruminal pH in the current trial, we may infer that the liquid supplement and the dry corn-based, dry supplement may have been degraded at similar rates.

Despite the lack of supplemental effect on ruminal pH, there was an interaction (P = 0.01) of forage type × time. The ruminal pH of steers consuming corn stover diets were greater ($P \leq 0.01$) than those of steers consuming the hay diets at 0, 1.5, and 18 h post-feeding, and tended ($P = 0.07$) to be greater at 12 h post-feeding. The increased ruminal pH values in steers consuming corn stover suggest that there was either less fermentation occurring at these time points in steers fed corn stover diets compared to those fed the hay diets, or more saliva production buffering the pH. Fieser and Vanzant (2004) found that ruminal pH values were lesser in cattle consuming tall fescue hay in the vegetative and boot stages compared to the heading and mature stages and attributed this difference to decrease in cellulose digestibility with advancing maturity. While pH may also have been affected by the pattern of intake or saliva

production, neither were measured in this study and relative comparisons in beef cattle fed hay and corn stover for these parameters do not exist to this authors knowledge.

VFA concentration

Similar to the pH data, there was no 3-way interaction of forage, supplement and time ($P \geq 0.49$; Table 4).There was an interaction ($P = 0.02$) of supplement type × time on ruminal acetate concentration. This was primarily driven by the decrease ($P \leq 0.04$) in acetate concentrations at 3 and 6 h post-feeding in steers fed the liquid supplement compared to those fed dry supplement (10.50% and 5.09%, respectively). In agreement with our results, Long et al. (2015) reported reductions in acetate concentrations post-feeding with increasing dietary glycerin inclusion fed to steers on a concentrate-based diet. In addition, the replacement of supplemental wheat bran with graded levels of molasses in cattle fed a basal diet of ad libitum grass hay resulted in decreased acetate (Osuji and Khalili, 1994). Thus, it appears that liquid supplementation reduces acetate concentrations in grain and forage-based diets suggesting something more than the replacement of NDF with NFC is occurring in the rumen. However, limited research on the ruminal ecology shifts when liquid supplementation replaces dry has been conducted (Abo El-Nor et al., 2010).

In addition to the supplement × time, there was a main effect of forage ($P < 0.01$) on acetate concentrations. Steers fed corn stover diets had an 11.3% decrease in acetate concentrations when compared to steers fed hay, regardless of supplement type. Fieser and Vanzant (2004) reported decreased ruminal acetate concentrations in cattle consuming tall fescue hay in the heading and mature stages compared to cattle consuming the vegetative and boot stages. This is reflective of the decrease in forage digestibility as the plant matures and quality decreases.

There was a supplement type × time interaction ($P = 0.02$) for ruminal butyrate concentrations as well. At all time-points post-feeding, steers fed liquid supplement had the greatest butyrate concentrations. Overall, liquid supplementation caused a 25.47% increase ($P < 0.01$) in butyrate concentration compared to dry supplementation. Our hypothesis, relative to the decreasing acetate concentrations and increasing butyrate concentrations, is that some of the microbes stimulated by liquid supplementation may be preferentially making butyrate over acetate. Multiple studies have noted a decrease in acetate and increase in butyrate concentrations in response to increasing levels of soluble carbohydrates in the diet (Kellogg and Owens, 1969; Khalili and Huhtanen, 1991; Khalili, 1993); and, specific to liquid supplementation, several studies have noted an increase in ruminal butyrate concentrations (Marty and Preston, 1970; Olbrich and Wayman, 1972; Ferraro et al., 2009; Wang et al., 2009; Carvalho et al., 2011), whether calves were supplemented with molasses, glycerin, or a combination of the 2. However, the mechanism of this response is still unclear.

There were no effects ($P \geq 0.29$) of treatment on ruminal propionate concentrations. However, there was a tendency ($P = 0.10$) for 9.85% decrease in ruminal propionate concentrations in steers fed corn stover diets compared to those fed hay. Because reductions in both acetate and propionate concentrations in steers fed corn stover diets compared to those fed hay, there was no main effect ($P = 0.62$) of forage on acetate:propionate (**A:P**) in these steers. Although interactions ($P < 0.01$) of both forage and supplement × time were detected, the changes in these responses were slight and not biologically relevant. The A:P across diets in our study ranged from 4.11 to 5.75 whereas most feedlot cattle have an A:P around 2:1.We included forage in both diets at 30% DMB to be able to more accurately test the effect of forage type in

the interaction. The result was an A:P that more closely resembled ratios seen in dairy cattle. These values suggest that the results of this study may be applicable to dairy.

Methane Production

There was no interaction ($P \leq 0.88$) of forage type × supplement type on methane emissions (Table 5). Furthermore, there were no main effects ($P \geq 0.24$) of forage nor supplement types on 24 h CH_4 emissions, CH_4 per kg BW, or CH_4 per kg DMI. Acetate production has been shown to favor methane production (Johnson and Johnson, 1995). While we saw a decrease in acetate concentrations in steers fed the corn stover diets, they also tended to have decreased propionate concentrations. Since propionate serves as an alternative hydrogen sink to methane (Janssen, 2010), the potential for decreased methane emissions in steers fed corn stover may have been negated. Additionally, the A:P greatly affects methane production. Wolin and Miller (1988) found that if the A:P was .5, the loss of substrate energy as methane would be 0%. They also concluded that if all carbohydrate was fermented to acetate without propionate being produced, energy loss as methane would be 33%. Therefore, without any interaction of forage × supplement type ($P \geq 0.32$) on VFA results nor any main effects of forage or supplement type on A:P, we did not expect to see any effects on methane production.

CONCLUSIONS

We had hypothesized that feeding cattle a liquid supplement would improve ruminal metabolism and digestibility of forages when compared to feeding dry supplement, and the magnitude of this response would be greatest when cattle were fed poor quality forages. As expected, hay was more digestible than corn stover which resulted in greater rates of

fermentation as reflected by decreased ruminal pH values, increased acetate concentrations, and greater total VFA production. In contrast to our hypothesis, supplement type had no effect on ruminal fermentation rates, apparent total tract digestibility of DM, OM, or NDF, or total VFA production. Yet, in comparison to dry supplementation, liquid supplementation tended to improve NDF degradation in the rumen of steers fed corn stover. Furthermore, although at the expense of acetate, liquid supplementation increased butyrate concentrations which has been found to have beneficial effects on the rumen environment and the animal as a whole. Further research may be warranted to explore the effects on liquid supplementation on ruminal NDF degradation as well as the possible benefits of increased butyrate production, particularly in growing animals.

TABLES AND FIGURES

Table 1. Composition of diets

	Hay		Corn Stover	
% Inclusion, DMB	Dry	Liquid	Dry	Liquid
Corn, Dry Rolled	55	55	50	50
MWDGS[1]	5	5	10	10
Grass Hay[2]	30	30	-	-
Corn Stover[3]	-	-	30	30
Suppl1[4]	10	-	10	-
Suppl2[5]	-	10	-	10
Analyzed Composition				
NDF	28.46	27.61	33.03	32.18
ADF	17.10	16.79	20.91	20.60
CP	10.24	11.06	10.09	10.92
Fat	3.67	3.44	3.56	3.33

[1]Archer Daniels Midland Co. (Peoria, IL)
[2]Hay was a mixture of brome, orchardgrass, and fescue.
[3]Corn stover is ground corn stalks, cobs, husks, and leaves.
[4]Supplement 1 contained (%DM basis): 83.276% ground corn, 11.250% limestone, 2.700% urea, 1.485% salt, 1.035% vitamin ADEK, 0.243% trace mineral premix; 89.63% DM, 16.8% CP, 4.5% Ca, 0.26% P. Supplement supplied by University of Illinois Feed Mill (Champaign, IL).
[5]Supplement 2 contained (%DM basis): 65.56% DM, 23.3% CP, 6.1% Ca, 0.26% P. Supplement supplied by Quality Liquid Feeds (Dodgeville, WI).

Table 2. Composition of Forages

	Forage	
Item, % DM basis	Grass Hay	Corn Stover
TDN[1]	49.21	39.99
NDF	68.26	79.59
ADF	46.05	56.10
CP	9.14	5.02
Fat	2.61	1.01
Ash	0.09	0.07

[1]Total digestible nutrients was back-calculated from CP and ADF using TDN = 81.38 + (CP x 0.36) - (ADF x 0.77) for grass hay and corn stover

Table 3. Effects of the interaction of forage type and supplement on digestibility

	Hay		Corn Stover			*P* – values[1]		
	Dry	Liquid	Dry	Liquid	SEM	F	S	F x S
Item, DM basis								
Intake, kg								
DM	15.9	14.2	12.4	12.2	0.59	<0.01	0.12	0.25
OM	14.4	12.7	11.0	10.8	0.52	<0.01	0.09	0.17
Fecal Output, kg								
DM	5.6	5.3	4.8	4.7	0.36	0.07	0.54	0.74
OM	5.1	4.7	4.3	4.1	0.33	0.04	0.43	0.75
Digestibility, %								
DM	63.0	60.7	58.8	58.8	2.20	0.18	0.62	0.62
OM	64.5	62.7	61.6	62.1	2.12	0.40	0.76	0.59
NDF	47.4	40.7	40.0	38.7	2.52	0.07	0.15	0.27
In situ Disappearance[2]								
DM, %	34.5	33.2	31.7	36.4	1.89	0.91	0.36	0.12
NDF, %	32.2	31.0	28.1	35.9	2.52	0.87	0.20	0.08

[1]F = effect of forage quality; S = effect of supplement; F x S = interaction of forage quality and supplement

[2]In situ disappearance of soybean hulls after 24-h.

Table 4. Effects of the interaction of forage type and supplement over time on VFA production

	Hay		Corn Stover			*P* – values[1]			
Item	Dry	Liquid	Dry	Liquid	SEM	F	S	F x H	S x H
n	8	8	8	8	-	-	-	-	-
Acetate, m*M*					3.43	<0.01	0.05	0.45	0.02
0[2]	70.24	69.57	56.40	60.86			0.67		
3	88.43	77.24	78.40	72.07			<0.01		
6	88.55	88.10	83.57	76.28			0.04		
Propionate, m*M*					1.89	0.10	0.83	0.29	0.76
0	13.63	13.26	10.11	11.17					
3	19.44	17.71	16.98	18.02					
6	20.30	18.91	18.34	18.46					
Butyrate, m*M*					0.73	0.14	<0.01	0.02	0.02
0	8.15	9.40	5.32	7.83		<0.01	0.01		
3	11.12	13.87	9.77	14.96		0.86	<0.01		
6	11.35	14.65	10.56	14.79		0.66	<0.01		
A:P[3]					0.28	0.62	0.31	<0.01	<0.01
0	5.17	5.34	5.75	5.52		0.16	0.92		
3	4.64	4.47	4.84	4.11		0.76	0.10		
6	4.46	4.47	4.85	4.23		0.78	0.26		
Total VFA, m*M*					5.08	<0.01	0.52	0.32	0.24
0	95.11	94.19	74.56	82.41					
3	122.65	111.63	108.19	108.28					
6	124.36	118.59	115.78	112.17					

[1]F = effect of forage; S = effect of supplement; H = effects of time, hours post-feeding ($P < 0.01$); F × S = interaction of forage and supplement ($P \geq 0.32$); F × H = interaction of forage quality and time; S × H = interaction of supplement and time; F × S × H = interaction of forage quality, supplement, and time ($P \geq 0.49$). When an interaction of F × H and S × H occurred for butyrate, acetate, and A:P ($P < 0.05$), the SLICE option (SAS Ins Inc, v. 9.4, Cary, NC, 2014) was used to compare treatments at each time point.

[2]Denotes hours post-feeding

[3]Acetate:Propionate

Table 5. Effects of the interaction of forage type and supplement on CH_4 Emissions

	Hay		Corn Stover			*P*-values[1]		
Item	Dry	Liquid	Dry	Liquid	SEM	F	S	F x S
n	8	8	8	8	-	-	-	-
CH_4 Emission[2], g/d	161.9	162.8	140.2	138.7	20.3	0.27	0.99	0.95
g CH_4/kg BW	0.23	0.24	0.20	0.21	0.03	0.24	0.75	0.88
g CH_4/kg DMI	6.97	9.20	5.82	8.50	2.45	0.71	0.33	0.93

[1]F = effect of forage quality; S = effect of supplement; F x S = interaction of forage quality and supplement

[2]Ramirez, B.C. 2014. Design and evaluation of open-circuit respiration chambers for beef cattle.

Figure 1

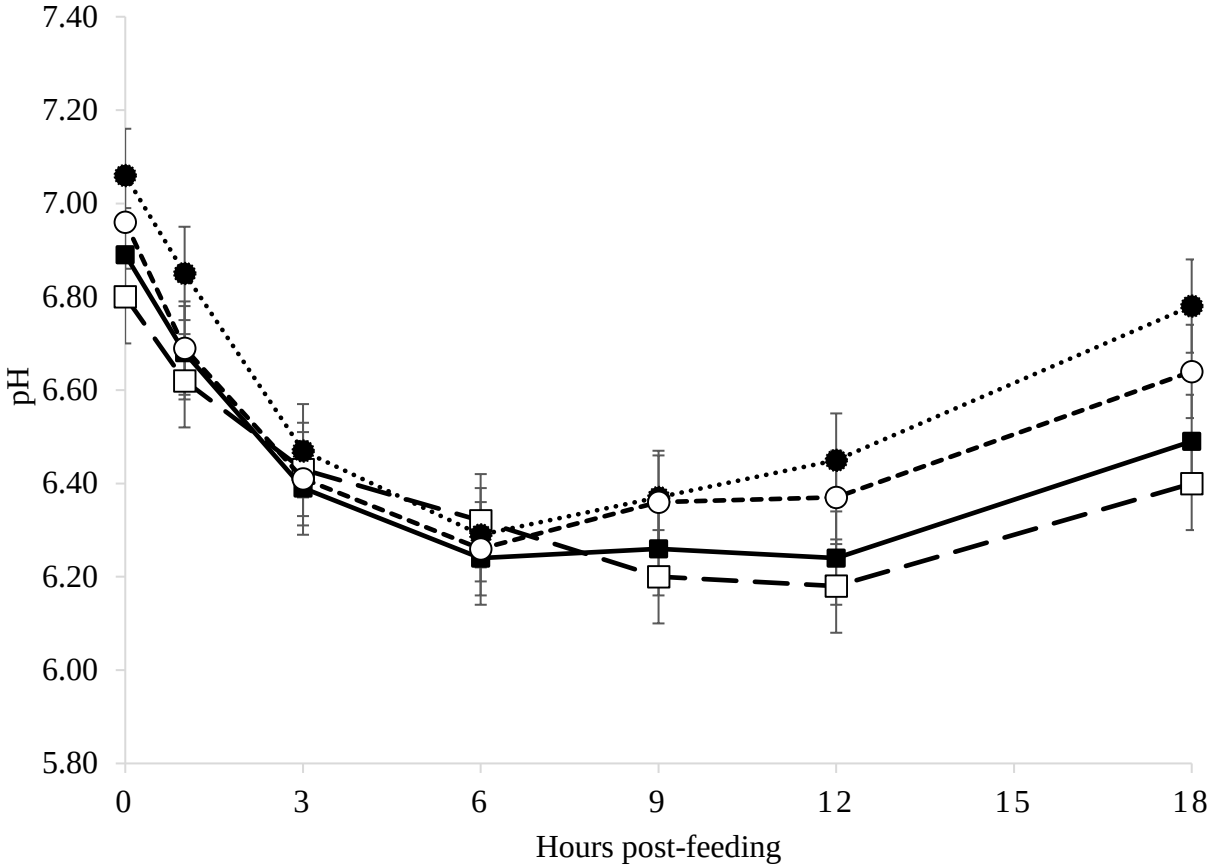

Figure 1. Effects of the interaction of forage type and supplement over time on ruminal pH. Steers were fed hay with dry supplement (■), hay with liquid supplement (□), corn stover with dry supplement (●), or corn stover with liquid supplement (○). Effects of the interaction of forage quality and supplement over time on ruminal pH. There was no interaction of forage × supplement × time, supplement × time ($P = 0.51$), or main effect of supplement ($P = 0.34$). There was an interaction of forage type × time ($P < 0.01$) and main effects of forage ($P = 0.04$) and time ($P < 0.01$). The error bars reflect the SEM associated with the interaction of forage × supplement × time (SEM = 0.10).

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APPENDIX: SUPPLEMENTAL TABLES AND FIGURES

Figure 2

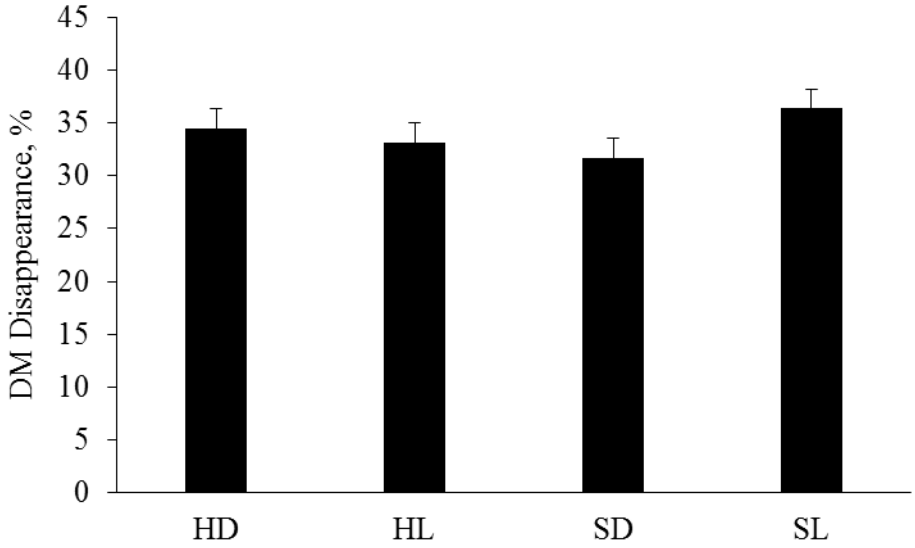

Figure 2. Effects of the interaction of forage type and supplement on in situ DM disappearance. Steers were fed hay with dry supplement (HD), hay with liquid supplement (HL), corn stover with dry supplement (SD), or corn stover with liquid supplement (SL). Effects of the interaction of forage type and supplement on in situ DM disappearance. Values represent averages of in situ DM disappearance after 24 h incubation. No forage type ($P = 0.91$), supplement type ($P = 0.36$) or forage type × supplement type interaction ($P = 0.12$) was observed. The error bars reflect the SEM associated with the interaction of forage type ×supplement type (SEM = 1.89).

Figure 3

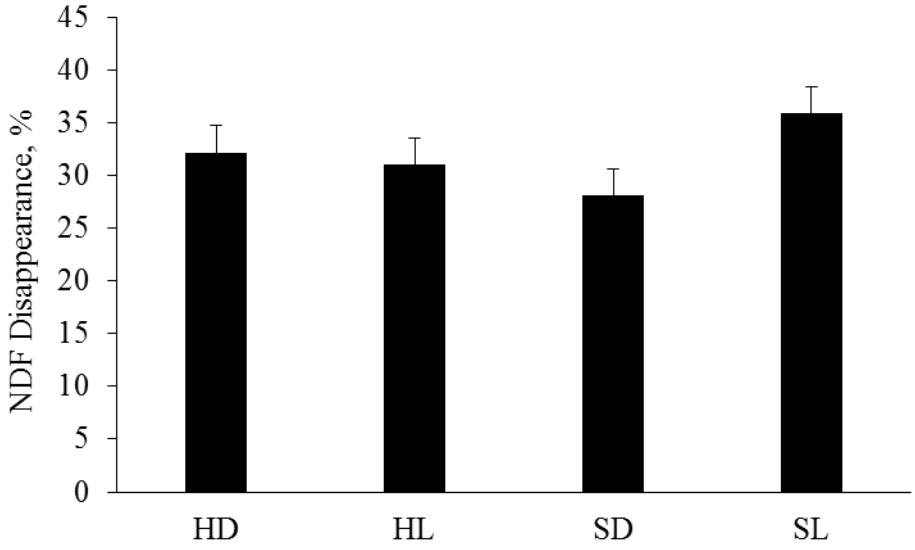

Figure 3. Effects of the interaction of forage type and supplement on in situ NDF disappearance. Steers were fed hay with dry supplement (HD), hay with liquid supplement (HL), corn stover with dry supplement (SD), or corn stover with liquid supplement (SL). Effects of the interaction of forage type and supplement on in situ NDF disappearance. Values represent averages of in situ DM disappearance after 24 h incubation. No forage type (P = 0.87) or supplement type (P = 0.20) was observed. There was a tendency for a forage type × supplement type interaction (P = 0.08) was observed. The error bars reflect the SEM associated with the interaction of forage type × supplement type (SEM = 2.52).

Table 6. Daily methane emissions (raw data)

Date[1]	Chamber	Calf ID	Diet	ER.rs(g/d)
1007	1	123	HL	164.2893601
1007	2	160	HD	291.5747829
1007	3	166	PL	160.6466437
1007	4	638	PD	168.7656344
1008	1	565	HL	110.2473322
1008	2	737	PD	56.68006983
1008	3	682	PL	162.7606115
1008	4	619	HD	205.1432523
1029	1	123	HD	196.8268336
1029	2	160	HL	290.6692969
1029	3	166	PD	160.8547527
1029	4	638	PL	141.2792043
1030	1	565	HD	138.0894025
1030	2	737	PL	67.49006307
1030	3	682	PD	170.0272141
1030	4	619	HL	258.8393394
1120	1	123	PL	188.0546994
1120	2	160	PD	208.072704
1120	3	166	HL	203.2966466
1120	4	638	HD	128.2159575
1121	1	565	PL	150.2006219
1121	2	737	HD	113.0852871
1121	3	682	PD	168.7269105
1121	4	103	HL	65.17811753
1212	1	123	PD	156.3159939
1212	2	160	PL	182.9360205
1212	3	166	HD	137.4261975
1212	4	638	HL	139.4118804
1213	1	565	PD	48.73741933
1213	2	737	HL	53.81317757
1213	3	682	HD	85.1178639
1213	4	103	PL	56.52762594

[1] Monitoring dates in 2015: October 7 and 8, October 29 and 30, November 20 and 21, December 12 and 13